National Institute for Health and Care Excellence

Final

Lyme disease: diagnosis and management

Appendix D: Evidence tables for the review on initial diagnostic tests for Lyme disease

NICE guideline 95

Diagnostic evidence review

April 2018

Final

This evidence review was developed by the National Guideline Centre

Disclaimer

The recommendations in this guideline represent the view of NICE, arrived at after careful consideration of the evidence available. When exercising their judgement, professionals are expected to take this guideline fully into account, alongside the individual needs, preferences and values of their patients or service users. The recommendations in this guideline are not mandatory and the guideline does not override the responsibility of healthcare professionals to make decisions appropriate to the circumstances of the individual patient, in consultation with the patient and, where appropriate, their carer or guardian.

Local commissioners and providers have a responsibility to enable the guideline to be applied when individual health professionals and their patients or service users wish to use it. They should do so in the context of local and national priorities for funding and developing services, and in light of their duties to have due regard to the need to eliminate unlawful discrimination, to advance equality of opportunity and to reduce health inequalities. Nothing in this guideline should be interpreted in a way that would be inconsistent with compliance with those duties.

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Appendix D: Clinical evidence tables

D.1 Initial tests

D.1.1 Cohort studies (adults)

Reference	Bil-Lula 2015[7]
Study type	Cohort study
Study methodology	Data source: serum samples from patients Recruitment: group at high risk of developing Lyme disease due to infection of Borrelia burgdorferi sensu lato
Number of patients	n = 577
Patient characteristics	Age, median (range): 45 years (20-65) Gender (male to female ratio): 491/86 Family origin: not reported Setting: not reported Country: Poland
Target condition(s)	Lyme disease (Borrelia burgdorferi sensu lato infection)
Index test(s) and reference standard	Index tests ELISA (IgM) – serum ELISA (IgG) – serum WB/IB (IgM) – serum

Reference	Bil-Lula 2015[7]				
	WB/IB (IgG) - serum Reference standard CDC recommendation: clinical diagnosis (erythema migrans, palsy of facial nerve or arthritis), medical history, assessment of risk exposure, diagnostic tests including the assessment of antibodies to Borrelia spp class IgM and IgG Time between measurement of index test and reference standard: not reported				
2×2 table [ELISA (IgM) – serum], unspec Lyme disease		Reference standard +	Reference standard −	Total	
	Index test +	6	160	166	
	Index test −	12	399	411	
	Total	18	559	577	
2×2 table [ELISA (IgG) – serum], unspec Lyme disease		Reference standard +	Reference standard −	Total	
	Index test +	7	220	227	
	Index test −	11	339	350	
	Total	18	559	577	
2×2 table [WB/IB (IgM) – serum], unspec Lyme disease		Reference standard +	Reference standard −	Total	
	Index test +	4	87	91	
	Index test −	14	472	486	
	Total	18	559	577	
2×2 table [WB/IB (IgG) – serum], unspec Lyme disease		Reference standard +	Reference standard −	Total	
	Index test +	11	309	320	
	Index test −	7	250	257	

Reference	**Bil-Lula 2015[7]**				
	Total	18	559	577	
Statistical measures	ELISA (IgM) – serum (unspec Lyme disease) Sensitivity 0.33 Specificity 0.71 ELISA (IgG) – serum (unspec Lyme disease) Sensitivity 0.39 Specificity 0.61 WB/IB (IgM) – serum (unspec Lyme disease) Sensitivity 0.22 Specificity 0.84 WB/IB (IgG) – serum (unspec Lyme disease) Sensitivity 0.61 Specificity 0.45				
Source of funding	Not reported				
Limitations	Risk of bias: patient selection, index test, reference standard Indirectness: none				

Reference	Blaauw 1999[8]				
Study type	Cohort study				
Study methodology	Data source: serum samples from patients Recruitment: Diagnosed or suspected chronic Lyme with musculoskeletal complaints				
Number of patients	n = 105				
Patient characteristics	Age, mean (range): 48.7 years (6-82) Gender (male to female ratio): 41/62 Family origin: not reported Setting: university hospital Country: Netherlands				
Target condition(s)	Lyme disease				
Index test(s) and reference standard	Index tests ELISA (IgG) – serum Reference standard Clinical diagnosis Time between measurement of index test and reference standard: not reported				
2×2 table [ELISA (IgG) – serum], unspec Lyme disease		Reference standard +	Reference standard −	Total	
	Index test +	10	12	22	
	Index test −	0	32	32	

Reference	Blaauw 1999[8]				
	Total	10	44	54	
Statistical measures	ELISA (IgG) – serum (unspec Lyme disease) Sensitivity 1.00 Specificity 0.73				
Source of funding	None declared				
Limitations	Risk of bias: index test, reference standard Indirectness: none				

Reference	Brunner 2001[12]
Study type	Cohort study
Study methodology	Data source: serum samples from patients Recruitment: patients evaluated at Lyme disease centre at Robert Wood Johnson Medical Centre (n=131), CDC prevention collection (n=38)
Number of patients	n = 169
Patient characteristics	Age, mean (SD): not reported Gender (male to female ratio): not reported Family origin: not reported Setting: Lyme disease clinic and CDC Country: USA Positive Lyme disease: RWJM: active Lyme disease (present or previous Lyme disease plus early or late dissemination, n=64) CDC: active Lyme disease (n=9) Negative Lyme disease: RWJM: previous Lyme disease (i.e. successfully treated, n=28), no Lyme disease (n=39) CDC: previous Lyme disease (n=29)
Target condition(s)	Lyme disease
Index test(s) and reference standard	Index tests ELISA (IgM/IgG) - serum ELISA (IgM) – serum

Reference	Brunner 2001[12]				
	ELISA (IgG) – serum WB/IB (IgM) – serum WB/IB (IgG) - serum Reference standard Clinical diagnosis: active Lyme disease (present or previous EM plus early or late dissemination), previous Lyme disease (successfully treated with antibiotics) Time between measurement of index test and reference standard: not reported				
2×2 table [ELISA (IgM) – serum], unspec Lyme disease (RWJM)		Reference standard +	Reference standard −	Total	
	Index test +	42	16	58	
	Index test −	22	51	73	
	Total	64	67	131	
2×2 table [ELISA (IgG) – serum], unspec Lyme disease (RWJM)		Reference standard +	Reference standard −	Total	
	Index test +	37	9	46	
	Index test −	27	58	85	
	Total	64	67	131	
2×2 table [WB/IB (IgM) – serum], unspec Lyme disease (RWJM)		Reference standard +	Reference standard −	Total	
	Index test +	37	11	48	
	Index test −	27	56	83	
	Total	64	67	131	
2×2 table		Reference standard +	Reference standard −	Total	

Reference	Brunner 2001[12]				
[WB/IB (IgG) – serum], unspec Lyme disease (RWJM)	Index test +	28	5	33	
	Index test −	36	62	98	
	Total	64	67	131	
2×2 table [ELISA (IgM) – serum], unspec Lyme disease (CDC)		Reference standard +	Reference standard −	Total	
	Index test +	7	16	23	
	Index test −	2	12	14	
	Total	9	28	37	
2×2 table [ELISA (IgG) – serum], unspec Lyme disease (CDC)		Reference standard +	Reference standard −	Total	
	Index test +	7	12	19	
	Index test −	2	16	18	
	Total	9	28	37	
2×2 table [WB/IB (IgM) – serum], unspec Lyme disease (CDC)		Reference standard +	Reference standard −	Total	
	Index test +	5	11	16	
	Index test −	4	18	22	
	Total	9	29	38	
2×2 table [WB/IB (IgG) – serum], unspec Lyme disease (CDC)		Reference standard +	Reference standard −	Total	
	Index test +	8	12	20	
	Index test −	1	17	18	
	Total	9	29	38	
2×2 table		Reference standard +	Reference standard −	Total	

Reference	Brunner 2001[12]				
[ELISA (IgM/IgG) – serum], unspec Lyme disease (CDC)	Index test +	9	24	33	
	Index test −	0	5	5	
	Total	9	29	38	
Statistical measures	ELISA (IgM) – serum (unspec Lyme disease, RWJM) Sensitivity 0.66 Specificity 0.76 ELISA (IgG) – serum (unspec Lyme disease, RWJM) Sensitivity 0.58 Specificity 0.87 WB/IB (IgM) – serum (unspec Lyme disease, RWJM) Sensitivity 0.58 Specificity 0.84 WB/IB (IgG) – serum (unspec Lyme disease, RWJM) Sensitivity 0.44 Specificity 0.93 ELISA (IgM) – serum (unspec Lyme disease, CDC) Sensitivity 0.78 Specificity 0.43 ELISA (IgG) – serum (unspec Lyme disease, CDC) Sensitivity 0.78 Specificity 0.57 WB/IB (IgM) – serum (unspec Lyme disease, CDC) Sensitivity 0.56 Specificity 0.62				

Reference	Brunner 2001[12]
	WB/IB (IgG) – serum (unspec Lyme disease, CDC) Sensitivity 0.89 Specificity 0.59 ELISA (IgM/IgG) – serum (unspec Lyme disease, CDC) Sensitivity 1.00 Specificity 0.17
Source of funding	None declared
Limitations	Risk of bias: patient selection, reference standard Indirectness: none

Reference	Gyllemark 2017[32]
Study type	Cohort study
Study methodology	Data source: CSF samples from patients Recruitment: patients investigated by lumbar puncture and blood sampling
Number of patients	n = 165
Patient characteristics	Age, median (range): Definite Lyme neuroborreliosis: 32 years (4-72 Possible Lyme neuroborreliosis with pleocytosis: 8.5 years (3-39) Possible Lyme neuroborreliosis without pleocytosis: 62 years (32-82) Non-Lyme neuroborreliosis: 23 years (1-83) Gender (male to female ratio): 85/80 Family origin: not reported Setting: not reported Country: Sweden Definite Lyme Neuroborreliosis (n=49), possible Neuroborreliosis (n=28), non-Neuroborreliosis (n=88)
Target condition(s)	Neuroborreliosis
Index test(s) and reference standard	Index tests CXCL13 – CSF Reference standard Definite Lyme neuroborreliosis: CSF pleocytosis and Borrelia-specific antibodies in CSF Possible Lyme neuroborreliosis: symptoms strongly suggestive of Neuroborreliosis, short duration of symptoms and CSF pleocytosis

Reference	Gyllemark 2017[32]				
	but not Borrelia-specific antibodies in CSF Possible Lyme neuroborreliosis: Borrelia-specific antibodies in CSF, but no pleocytosis and symptoms were less suggestive of Neuroborreliosis Time between measurement of index test and reference standard: not reported				
2×2 table [CXCL13 (cut-off >142 pg/ml) – CSF]		Reference standard +	Reference standard −	Total	
	Index test +	53	1	54	
	Index test −	10	87	97	
	Total	63	88	151	
2×2 table [CXCL13 (cut-off >250 pg/ml) – CSF]		Reference standard +	Reference standard −	Total	
	Index test +	51	0	51	
	Index test −	12	88	100	
	Total	63	88	151	
Statistical measures	CXCL13 (>142 pg/ml) – CSF Sensitivity 0.84 Specificity 0.99 CXCL13 (>250 pg/ml) – CSF Sensitivity 0.81 Specificity 1.00				
Source of funding	Government grants				
Limitations	Risk of bias: patient selection, index test, reference standard Indirectness: none				

Reference	Henningsson 2014[39]
Study type	Cohort study
Study methodology	Data source: serum samples from patients Recruitment: patients evaluated at Lyme disease centre at Robert Wood Johnson Medical Centre (n=131), CDC prevention collection (n=38)
Number of patients	n = 175
Patient characteristics	Age, median (range): Definite Lyme neuroborreliosis: 39 years (3-85) Possible Lyme neuroborreliosis: 30 years (4-49) Gender (male to female ratio): Definite Lyme neuroborreliosis: 33/19 Possible Lyme neuroborreliosis: 3/1 Family origin: not reported Setting: not reported Country: Sweden Definite Neuroborreliosis (n=52) Possible Neuroborreliosis (n=4) Healthy blood donors (n=90) Pleocytosis for other reasons (n=29)
Target condition(s)	Neuroborreliosis

Reference	Henningsson 2014[39]				
Index test(s) and reference standard	Index tests ELISA (IgM/IgG) – serum ELISA (IgM/IgG) – serum/CSF Reference standard Clinical diagnosis Time between measurement of index test and reference standard: not reported				
2×2 table [ELISA (IgM/IgG) – AI (IDEIA)], Lyme neuroborreliosis		Reference standard +	Reference standard −	Total	
	Index test +	48	1	49	
	Index test −	4	28	32	
	Total	52	29	81	
2×2 table [ELISA (IgM/IgG) – AI (recomBead)], Lyme neuroborreliosis		Reference standard +	Reference standard −	Total	
	Index test +	52	3	55	
	Index test −	0	26	26	
	Total	52	29	81	
2×2 table [ELISA (IgM/IgG) – serum (recomBead)], Lyme neuroborreliosis		Reference standard +	Reference standard −	Total	
	Index test +	45	7	52	
	Index test −	7	22	29	
	Total	52	29	81	
2×2 table		Reference standard +	Reference standard −	Total	

Reference	Henningsson 2014[39]				
[ELISA (IgM/IgG) – AI (VIDAS)], Lyme neuroborreliosis	Index test +	45	2	47	
	Index test −	7	27	34	
	Total	52	29	81	
2×2 table [ELISA (IgM/IgG) – serum (VIDAS)], Lyme neuroborreliosis		Reference standard +	Reference standard −	Total	
	Index test +	48	8	56	
	Index test −	4	21	25	
	Total	52	29	81	
Statistical measures	ELISA (IgM/IgG) – AI (Lyme neuroborreliosis) (IDEIA) Sensitivity 0.92 Specificity 0.97 ELISA (IgM/IgG) – AI (Lyme neuroborreliosis) (recomBead) Sensitivity 1.00 Specificity 0.90 ELISA (IgM/IgG) – serum (Lyme neuroborreliosis) (recomBead) Sensitivity 0.87 Specificity 0.76 ELISA (IgM/IgG) – AI (Lyme neuroborreliosis) (VIDAS) Sensitivity 0.87 Specificity 0.93 ELISA (IgM/IgG) – serum (Lyme neuroborreliosis) (VIDAS) Sensitivity 0.92 Specificity 0.72				

Reference	Henningsson 2014[39]
Source of funding	None declared
Limitations	Risk of bias: patient selection, index test, reference standard Indirectness: none

Reference	Henningsson 2016[40]
Study type	Cohort study
Study methodology	Data source: CSF samples from patients Recruitment: not reported
Number of patients	n = 132
Patient characteristics	Age, median (range): Definite Lyme neuroborreliosis: 38 years (3-72) Possible Lyme neuroborreliosis with pleocytosis: 21 years (3-55) Possible Lyme neuroborreliosis with AI: 64 years (50-81) Non-Lyme neuroborreliosis: 39 years (1-83) Gender (male to female ratio): 63/72 Family origin: not reported Setting: not reported Country: Sweden Definite Lyme Neuroborreliosis (n=35), possible Neuroborreliosis (n=43), non-Neuroborreliosis (n=83)
Target condition(s)	Neuroborreliosis
Index test(s) and reference standard	Index tests CXCL13 (Quantikine) – CSF CXCL13 (RecomBead) – CSF Reference standard Definite Lyme neuroborreliosis: according to European guidelines

Reference	Henningsson 2016[40]				
	Possible Lyme neuroborreliosis: Clinical diagnosis based on CSF pleocytosis and neurological symptoms strongly suggestive of Neuroborreliosis but normal AI Possible Lyme neuroborreliosis: based on elevated Borrelia-specific AI but no CSF pleocytosis Time between measurement of index test and reference standard: not reported				
2×2 table [CXCL13 (Quantikine) – CSF]		Reference standard +	Reference standard −	Total	
	Index test +	39	0	39	
	Index test −	4	83	87	
	Total	43	83	126	
2×2 table [CXCL13 (RecomBead) – CSF]		Reference standard +	Reference standard −	Total	
	Index test +	40	0	40	
	Index test −	3	83	86	
	Total	43	83	126	
Statistical measures	CXCL13 (Quantikine) – CSF Sensitivity 0.91 Specificity 1.00 CXCL13 (RecomBead) – CSF Sensitivity 0.93 Specificity 1.00				
Source of funding	Government and industry grants				
Limitations	Risk of bias: patient selection, index test, reference standard Indirectness: none				

Reference	Ljostad 2008[64]
Study type	Cohort study
Study methodology	Data source: CSF samples from patients Recruitment: patients included in a treatment trial for Lyme disease
Number of patients	n = 59
Patient characteristics	Age, mean (SD): not reported Gender (male to female ratio): not reported Family origin: not reported Setting: not reported Country: Norway Definite neuroborreliosis (n=37) Probable neuroborreliosis (n=7) Not neuroborreliosis (n=8)
Target condition(s)	Neuroborreliosis
Index test(s) and reference standard	Index tests CXCL13 - CSF Reference standard Clinical diagnosis based on criteria: New neurological symptoms & objective findings suggestive of Neuroborreliosis; Lymphocytic pleocytosis (>5 leucocytes/mm3); Intrarthecal Borrelia antibody production Time between measurement of index test and reference standard: not reported

Reference	**Ljostad 2008[64]**				
2×2 table [CXCL13 – CSF], Lyme neuroborreliosis		Reference standard +	Reference standard −	Total	
	Index test +	37	3	40	
	Index test −	0	5	5	
	Total	37	8	45	
Statistical measures	CXCL13 – CSF Sensitivity 1.00 Specificity 0.63				
Source of funding	Academic grants				
Limitations	Risk of bias: reference standard Indirectness: none				

Reference	Nordberg 2012[80]				
Study type	Cohort study				
Study methodology	Data source: CSF samples from patients Recruitment: patients admitted to hospital with symptoms of suspected neuroborreliosis				
Number of patients	n = 117				
Patient characteristics	Age, median (range): 65/52 Gender (male to female ratio): not reported Family origin: not reported Setting: hospital Country: Finland				
Target condition(s)	Neuroborreliosis				
Index test(s) and reference standard	Index tests ELISPOT – serum Reference standard Clinical diagnosis plus CSF lymphocytic pleocytosis ≥5 mononuclear leucocytes per µL and intrathecal production of specific anti-Borrelia IgG antibodies Time between measurement of index test and reference standard: not reported				
2×2 table [ELISPOT (cut-off 10 spots or		Reference standard +	Reference standard −	Total	
	Index test +	3	8	11	

Reference	Nordberg 2012[80]				
more) – CSF], Lyme neuroborreliosis	Index test −	11	95	106	
	Total	14	103	117	
2×2 table [ELISPOT (cut-off 5 spots or more) – CSF], Lyme neuroborreliosis		Reference standard +	Reference standard −	Total	
	Index test +	5	19	24	
	Index test −	9	84	93	
	Total	14	103	117	
Statistical measures	ELISPOT (cut-off 10 spots or more) – CSF Sensitivity 0.21 Specificity 0.92 ELISPOT (cut-off 5 spots or more) – CSF Sensitivity 0.36 Specificity 0.82				
Source of funding	Charity grants, public grants				
Limitations	Risk of bias: patient selection, index test, reference standard Indirectness: none				

Reference	Tjernberg 2011[110]				
Study type	Cohort study				
Study methodology	Data source: CSF samples from patients Recruitment: patients examined for suspected Lyme neuroborreliosis				
Number of patients	n = 261				
Patient characteristics	Age, range: 2-87 years Gender (male to female ratio): 157/104 Family origin: not reported Setting: microbiology department Country: Sweden				
Target condition(s)	Neuroborreliosis				
Index test(s) and reference standard	Index tests ELISA C6 – CSF CXCL13 – CSF Reference standard European Federation of Neurological Societies guidelines Time between measurement of index test and reference standard: not reported				
2×2 table [ELISA C6 – CSF]		Reference standard +	Reference standard −	Total	
	Index test +	117	2	119	

Reference	Tjernberg 2011[110]				
	Index test −	7	90	97	
	Total	124	92	216	
2×2 table [CXCL13 – CSF]		Reference standard +	Reference standard −	Total	
	Index test +	122	2	124	
	Index test −	2	90	92	
	Total	124	92	216	
Statistical measures	ELISA C6 – CSF Sensitivity 0.94 Specificity 0.98 CXCL13 – CSF Sensitivity 0.98 Specificity 0.98				
Source of funding	Government grant				
Limitations	Risk of bias: patient selection, reference standard Indirectness: serious				

D.1.2 Case-control studies (adults)

Reference	Ang 2015[1]
Study type	Case-control
Study methodology	Data source: validation studies for B. burgdorferi antibody assays from 8 laboratories Recruitment: not reported
Number of patients	n = 369 cases, 228 healthy controls
Patient characteristics	Age, mean (SD): not reported Gender (male to female ratio): not reported Family origin: not reported Setting: 8 laboratories Country: the Netherlands Cases: patients with typical manifestations of Lyme borreliosis (according to ESGBOR guidelines – EM clinical information or positive PCR on skin biopsy, ACA clinical information or positive PCR and matching histopathology, neuroborreliosis defined as meningoradiculitis and/or bilateral facial palsy, CSF pleocytosis and/or positive CSF PCR for Borrelia spp., arthritis defined as monoarthritis of the knee and/or positive PCR for Borrelia spp. In synovial fluid or synovial biopsy Controls : healthy controls from healthcare workers for a check on hepatitis B vaccination and from stem cell donors
Target condition(s)	Lyme disease
Index test(s) and reference standard	Index test(s) ELISA (IgM) – serum ELISA (IgM/IgG) – serum Immunoblot (IgM) – serum

Reference	Ang 2015[1]				
	Immunoblot (IgM/IgG) – serum Reference standard ESGBOR guidelines: EM clinical information or positive PCR on skin biopsy, ACA clinical information or positive PCR and matching histopathology, neuroborreliosis defined as meningoradiculitis and/or bilateral facial palsy, CSF pleocytosis and/or positive CSF PCR for Borrelia spp., arthritis defined as monoarthritis of the knee and/or positive PCR for Borrelia spp. In synovial fluid or synovial biopsy Time between measurement of index test and reference standard: not reported				
2×2 table		Reference standard +	Reference standard −	Total	EM ELISA (IgM) Diacheck Serum
	Index test +	0	0	0	
	Index test −	13	15	28	
	Total	13	15	28	
		Reference standard +	Reference standard −	Total	EM ELISA (IgM) Enzygnost Serum
	Index test +	11	10	21	
	Index test −	70	97	167	
	Total	81	107	188	
		Reference standard +	Reference standard −	Total	EM ELISA (IgM) Euroimmun Serum
	Index test +	0	0	0	
	Index test −	5	10	15	
	Total	5	10	15	
		Reference standard +	Reference standard −	Total	EM ELISA (IgM) Liaison Serum
	Index test +	5	6	11	
	Index test −	52	221	273	
	Total	57	227	284	
		Reference standard +	Reference standard −	Total	EM

Reference	Ang 2015[1]				
	Index test +	4	1	5	ELISA (IgM) Medac Serum
	Index test −	27	91	118	
	Total	31	92	123	
		Reference standard +	Reference standard −	Total	EM ELISA (IgM)
	Index test +	0	0	0	Mikrogen Serum
	Index test −	5	15	20	
	Total	5	15	20	
		Reference standard +	Reference standard −	Total	EM ELISA (IgM)
	Index test +	18	29	47	Serion Serum
	Index test −	18	77	95	
	Total	36	106	142	
		Reference standard +	Reference standard −	Total	EM ELISA (IgM)
	Index test +	1	0	1	Virotech Serum
	Index test −	12	14	26	
	Total	13	14	27	
		Reference standard +	Reference standard −	Total	EM ELISA (IgM/IgG)
	Index test +	12	1	13	Diacheck Serum
	Index test −	1	14	15	
	Total	13	15	28	
		Reference standard +	Reference standard −	Total	EM ELISA (IgM/IgG)
	Index test +	77	13	90	Enzygnost Serum
	Index test −	4	94	98	
	Total	81	107	188	
		Reference standard	Reference standard −	Total	EM

Reference	Ang 2015[1]				
		+			ELISA (IgM/IgG) Euroimmun Serum
	Index test +	4	2	6	
	Index test −	1	12	13	
	Total	5	14	19	
		Reference standard +	Reference standard −	Total	EM ELISA (IgM/IgG) Liaison Serum
	Index test +	52	19	71	
	Index test −	5	208	213	
	Total	57	227	284	
		Reference standard +	Reference standard −	Total	EM ELISA (IgM/IgG) Medac Serum
	Index test +	25	3	28	
	Index test −	6	89	95	
	Total	31	92	123	
		Reference standard +	Reference standard −	Total	EM ELISA (IgM/IgG) Mikrogen Serum
	Index test +	5	0	5	
	Index test −	0	15	15	
	Total	5	15	20	
		Reference standard +	Reference standard −	Total	EM ELISA (IgM/IgG) Serion Serum
	Index test +	30	30	60	
	Index test −	6	76	82	
	Total	36	106	142	
		Reference standard +	Reference standard −	Total	EM ELISA (IgM/IgG) Virotech Serum
	Index test +	13	0	13	
	Index test −	0	14	14	
	Total	13	14	27	

Reference	Ang 2015[1]				
		Reference standard +	Reference standard −	Total	EM Immunoblot (IgM) Mikrogen Serum
	Index test +	18	3	21	
	Index test −	65	101	166	
	Total	83	104	187	
		Reference standard +	Reference standard −	Total	EM Immunoblot (IgM/IgG) Mikrogen Serum
	Index test +	66	8	74	
	Index test −	17	96	113	
	Total	83	104	187	
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA (IgM) Diacheck Serum
	Index test +	0	0	0	
	Index test −	5	15	20	
	Total	5	15	20	
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA (IgM) Enzygnost Serum
	Index test +	5	10	15	
	Index test −	45	97	142	
	Total	50	107	157	
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA (IgM) Euroimmun Serum
	Index test +	0	0	0	
	Index test −	1	14	15	
	Total	1	14	15	
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA (IgM) Liaison Serum
	Index test +	0	6	6	
	Index test −	54	221	275	

Reference	**Ang 2015[1]**				
	Total	54	227	281	
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA (IgM)
	Index test +	1	1	2	Medac
	Index test −	24	91	115	Serum
	Total	25	92	117	
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA (IgM)
	Index test +	0	0	0	Mikrogen
	Index test −	1	15	16	Serum
	Total	1	15	16	
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA (IgM)
	Index test +	10	29	39	Serion
	Index test −	16	77	93	Serum
	Total	26	106	132	
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA (IgM)
	Index test +	0	0	0	Virotech
	Index test −	5	14	19	Serum
	Total	5	14	19	
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA (IgM/IgG)
	Index test +	4	1	5	Diacheck
	Index test −	1	14	15	Serum
	Total	5	15	20	
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA (IgM/IgG)
	Index test +	49	13	62	

Reference	**Ang 2015[1]**				
	Index test −	1	94	95	Enzygnost Serum
	Total	50	107	157	
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA (IgM/IgG)
	Index test +	1	2	3	Euroimmun Serum
	Index test −	0	12	12	
	Total	1	14	15	
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA (IgM/IgG)
	Index test +	54	19	73	Liaison Serum
	Index test −	0	208	208	
	Total	54	227	281	
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA (IgM/IgG)
	Index test +	25	3	28	Medac Serum
	Index test −	0	89	89	
	Total	25	92	117	
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA (IgM/IgG)
	Index test +	1	0	1	Mikrogen Serum
	Index test −	0	15	15	
	Total	1	15	16	
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA (IgM/IgG)
	Index test +	24	30	54	Serion Serum
	Index test −	2	76	78	
	Total	26	106	132	
		Reference standard +	Reference standard −	Total	Neuroborreliosis

Reference	**Ang 2015[1]**				
	Index test +	5	0	5	ELISA (IgM/IgG) Virotech Serum
	Index test −	0	14	14	
	Total	5	14	19	
		Reference standard +	Reference standard −	Total	Neuroborreliosis Immunoblot (IgM)
	Index test +	5	3	8	Mikrogen Serum
	Index test −	62	101	163	
	Total	67	104	171	
		Reference standard +	Reference standard −	Total	Neuroborreliosis Immunoblot (IgM/IgG)
	Index test +	65	8	73	Mikrogen Serum
	Index test −	2	96	98	
	Total	67	104	171	
		Reference standard +	Reference standard −	Total	Lyme arthritis ELISA (IgM)
	Index test +	0	0	0	Diacheck Serum
	Index test −	5	15	20	
	Total	5	15	20	
		Reference standard +	Reference standard −	Total	Lyme arthritis ELISA (IgM)
	Index test +	1	10	11	Enzygnost Serum
	Index test −	12	97	109	
	Total	13	107	120	
		Reference standard +	Reference standard −	Total	Lyme arthritis ELISA (IgM)
	Index test +	0	6	6	Liaison Serum
	Index test −	7	221	228	
	Total	7	227	234	
		Reference standard	Reference standard −	Total	Lyme arthritis

Reference	Ang 2015[1]				
		+			ELISA (IgM) Serion Serum
	Index test +	0	29	29	
	Index test −	2	77	79	
	Total	2	106	108	
		Reference standard +	Reference standard −	Total	Lyme arthritis ELISA (IgM) Virotech Serum
	Index test +	1	0	1	
	Index test −	4	14	18	
	Total	5	14	19	
		Reference standard +	Reference standard −	Total	Lyme arthritis ELISA (IgM/IgG) Diacheck Serum
	Index test +	4	1	5	
	Index test −	1	14	15	
	Total	5	15	20	
		Reference standard +	Reference standard −	Total	Lyme arthritis ELISA (IgM/IgG) Enzygnost Serum
	Index test +	13	13	26	
	Index test −	0	94	94	
	Total	13	107	120	
		Reference standard +	Reference standard −	Total	Lyme arthritis ELISA (IgM/IgG) Liaison Serum
	Index test +	7	19	26	
	Index test −	0	208	208	
	Total	7	227	234	
		Reference standard +	Reference standard −	Total	Lyme arthritis ELISA (IgM/IgG) Serion Serum
	Index test +	2	30	32	
	Index test −	0	76	76	
	Total	2	106	108	

Reference	**Ang 2015[1]**				
		Reference standard +	Reference standard −	Total	Lyme arthritis ELISA (IgM/IgG) Virotech Serum
	Index test +	5	0	5	
	Index test −	0	14	14	
	Total	5	14	19	
		Reference standard +	Reference standard −	Total	Lyme arthritis Immunoblot (IgM) Mikrogen Serum
	Index test +	0	3	3	
	Index test −	8	101	109	
	Total	8	104	112	
		Reference standard +	Reference standard −	Total	Lyme arthritis Immunoblot (IgM/IgG) Mikrogen Serum
	Index test +	8	8	16	
	Index test −	0	96	96	
	Total	8	104	112	
		Reference standard +	Reference standard −	Total	ACA ELISA (IgM) Diacheck Serum
	Index test +	0	0	0	
	Index test −	6	15	21	
	Total	6	15	21	
		Reference standard +	Reference standard −	Total	ACA ELISA (IgM) Enzygnost Serum
	Index test +	0	10	10	
	Index test −	14	97	111	
	Total	14	107	121	
		Reference standard +	Reference standard −	Total	ACA ELISA (IgM) Liaison Serum
	Index test +	0	6	6	
	Index test −	9	221	230	

Reference	**Ang 2015[1]**				
	Total	9	227	236	
		Reference standard +	Reference standard −	Total	ACA ELISA (IgM) Medac Serum
	Index test +	0	1	1	
	Index test −	2	91	93	
	Total	2	92	94	
		Reference standard +	Reference standard −	Total	ACA ELISA (IgM) Serion Serum
	Index test +	0	29	29	
	Index test −	2	77	79	
	Total	2	106	108	
		Reference standard +	Reference standard −	Total	ACA ELISA (IgM) Virotech Serum
	Index test +	0	0	0	
	Index test −	6	14	20	
	Total	6	14	20	
		Reference standard +	Reference standard −	Total	ACA ELISA (IgM/IgG) Diacheck Serum
	Index test +	6	1	7	
	Index test −	0	14	14	
	Total	6	15	21	
		Reference standard +	Reference standard −	Total	ACA ELISA (IgM/IgG) Enzygnost Serum
	Index test +	14	13	27	
	Index test −	0	94	94	
	Total	14	107	121	
		Reference standard +	Reference standard −	Total	ACA ELISA (IgM/IgG)
	Index test +	8	19	27	

Reference	**Ang 2015[1]**				
	Index test −	0	208	208	Liaison Serum
	Total	8	227	235	
		Reference standard +	Reference standard −	Total	ACA ELISA (IgM/IgG) Medac Serum
	Index test +	2	3	5	
	Index test −	0	89	89	
	Total	2	92	94	
		Reference standard +	Reference standard −	Total	ACA ELISA (IgM/IgG) Serion Serum
	Index test +	2	30	32	
	Index test −	0	76	76	
	Total	2	106	108	
		Reference standard +	Reference standard −	Total	ACA ELISA (IgM/IgG) Virotech Serum
	Index test +	6	0	6	
	Index test −	0	14	14	
	Total	6	14	20	
		Reference standard +	Reference standard −	Total	ACA Immunoblot (IgM) Mikrogen Serum
	Index test +	0	3	3	
	Index test −	11	101	112	
	Total	11	104	115	
		Reference standard +	Reference standard −	Total	ACA Immunoblot (IgM/IgG) Mikrogen Serum
	Index test +	11	8	19	
	Index test −	0	96	96	
	Total	11	104	115	
Statistical measures	Index test: ELISA IgM (Diacheck, serum) - EM Sensitivity 0.00				

Reference	Ang 2015[1]
	Specificity 1.00 Index test: ELISA IgM (Enzygnost, serum) - EM Sensitivity 0.14 Specificity 0.91 Index test: ELISA IgM (Euroimmun, serum) – EM Sensitivity 0.00 Specificity 1.00 Index test: ELISA IgM (Liaison, serum) – EM Sensitivity 0.09 Specificity 0.97 Index test: ELISA IgM (Medac, serum) – EM Sensitivity 0.13 Specificity 0.99 Index test: ELISA IgM (Mikrogen, serum) – EM Sensitivity 0.00 Specificity 1.00 Index test: ELISA IgM (Serion, serum) – EM Sensitivity 0.50 Specificity 0.73 Index test: ELISA IgM (Virotech, serum) – EM Sensitivity 0.08 Specificity 1.00 Index test: ELISA IgM/IgG (Diacheck, serum) – EM

Reference	Ang 2015[1]
	Sensitivity 0.92 Specificity 0.93 Index test: ELISA IgM/IgG (Enzygnost, serum) – EM Sensitivity 0.95 Specificity 0.88 Index test: ELISA IgM/IgG (Euroimmun, serum) – EM Sensitivity 0.80 Specificity 0.86 Index test: ELISA IgM/IgG (Liaison, serum) – EM Sensitivity 0.91 Specificity 0.92 Index test: ELISA IgM/IgG (Medac, serum) – EM Sensitivity 0.81 Specificity 0.97 Index test: ELISA IgM/IgG (Mikrogen, serum) – EM Sensitivity 1.00 Specificity 1.00 Index test: ELISA IgM/IgG (Serion, serum) – EM Sensitivity 0.83 Specificity 0.72 Index test: ELISA IgM/IgG (Virotech, serum) – EM Sensitivity 1.00 Specificity 1.00

Reference	Ang 2015[1]
	Index test: Immunoblot IgM (Mikrogen, serum) – EM Sensitivity 0.22 Specificity 0.97 Index test: Immunoblot IgM/IgG (Mikrogen, serum) – EM Sensitivity 0.80 Specificity 0.92 Index test: ELISA IgM (Diacheck, serum) – Neuroborreliosis Sensitivity 0.00 Specificity 1.00 Index test: ELISA IgM (Enzygnost, serum) – Neuroborreliosis Sensitivity 0.10 Specificity 0.91 Index test: ELISA IgM (Euroimmun, serum) – Neuroborreliosis Sensitivity 0.00 Specificity 1.00 Index test: ELISA IgM (Liaison, serum) – Neuroborreliosis Sensitivity 0.00 Specificity 0.97 Index test: ELISA IgM (Medac, serum) – Neuroborreliosis Sensitivity 0.04 Specificity 0.99 Index test: ELISA IgM (Mikrogen, serum) – Neuroborreliosis Sensitivity 0.00 Specificity 1.00

Reference	Ang 2015[1]
	Index test: ELISA IgM (Serion, serum) – Neuroborreliosis Sensitivity 0.38 Specificity 0.73 Index test: ELISA IgM (Virotech, serum) – Neuroborreliosis Sensitivity 0.00 Specificity 1.00 Index test: ELISA IgM/IgG (Diacheck, serum) – Neuroborreliosis Sensitivity 0.80 Specificity 0.93 Index test: ELISA IgM/IgG (Enzygnost, serum) – Neuroborreliosis Sensitivity 0.98 Specificity 0.88 Index test: ELISA IgM/IgG (Euroimmun, serum) – Neuroborreliosis Sensitivity 1.00 Specificity 0.86 Index test: ELISA IgM/IgG (Liaison, serum) – Neuroborreliosis Sensitivity 1.00 Specificity 0.92 Index test: ELISA IgM/IgG (Medac, serum) – Neuroborreliosis Sensitivity 1.00 Specificity 0.97 Index test: ELISA IgM/IgG (Mikrogen, serum) – Neuroborreliosis Sensitivity 1.00

Reference	Ang 2015[1]
	Specificity 1.00 Index test: ELISA IgM/IgG (Serion, serum) – Neuroborreliosis Sensitivity 0.92 Specificity 0.72 Index test: ELISA IgM/IgG (Virotech, serum) – Neuroborreliosis Sensitivity 1.00 Specificity 1.00 Index test: Immunoblot IgM (Mikrogen, serum) – Neuroborreliosis Sensitivity 0.07 Specificity 0.97 Index test: Immunoblot IgM/IgG (Mikrogen, serum) – Neuroborreliosis Sensitivity 0.97 Specificity 0.92 Index test: ELISA IgM (Diacheck, serum) – Lyme arthritis Sensitivity 0.00 Specificity 1.00 Index test: ELISA IgM (Enzygnost, serum) – Lyme arthritis Sensitivity 0.08 Specificity 0.91 Index test: ELISA IgM (Liaison, serum) – Lyme arthritis Sensitivity 0.00 Specificity 0.97 Index test: ELISA IgM (Serion, serum) – Lyme arthritis

Reference	Ang 2015[1]
	Sensitivity 0.00 Specificity 0.73 Index test: ELISA IgM (Virotech, serum) – Lyme arthritis Sensitivity 0.20 Specificity 1.00 Index test: ELISA IgM/IgG (Diacheck, serum) – Lyme arthritis Sensitivity 0.80 Specificity 0.93 Index test: ELISA IgM/IgG (Enzygnost, serum) – Lyme arthritis Sensitivity 1.00 Specificity 0.88 Index test: ELISA IgM/IgG (Liaison, serum) – Lyme arthritis Sensitivity 1.00 Specificity 0.92 Index test: ELISA IgM/IgG (Serion, serum) – Lyme arthritis Sensitivity 1.00 Specificity 0.72 Index test: ELISA IgM/IgG (Virotech, serum) – Lyme arthritis Sensitivity 1.00 Specificity 1.00 Index test: Immunoblot IgM (Mikrogen, serum) – Lyme arthritis Sensitivity 0.00 Specificity 0.97

Reference	Ang 2015[1]
	Index test: Immunoblot IgM/IgG (Mikrogen, serum) – Lyme arthritis Sensitivity 1.00 Specificity 0.92 Index test: ELISA IgM (Diacheck, serum) – ACA Sensitivity 0.00 Specificity 1.00 Index test: ELISA IgM (Enzygnost, serum) – ACA Sensitivity 0.00 Specificity 0.91 Index test: ELISA IgM (Liaison, serum) – ACA Sensitivity 0.00 Specificity 0.97 Index test: ELISA IgM (Medac, serum) – ACA Sensitivity 0.00 Specificity 0.99 Index test: ELISA IgM (Serion, serum) – ACA Sensitivity 0.00 Specificity 0.73 Index test: ELISA IgM (Virotech, serum) – ACA Sensitivity 0.00 Specificity 1.00 Index test: ELISA IgM/IgG (Diacheck, serum) – ACA Sensitivity 1.00 Specificity 0.93

Reference	Ang 2015[1]
	Index test: ELISA IgM (Enzygnost, serum) – ACA Sensitivity 1.00 Specificity 0.88 Index test: ELISA IgM/IgG (Liaison, serum) – ACA Sensitivity 1.00 Specificity 0.92 Index test: ELISA IgM/IgG (Medac, serum) – ACA Sensitivity 1.00 Specificity 0.97 Index test: ELISA IgM/IgG (Serion, serum) – ACA Sensitivity 1.00 Specificity 0.72 Index test: ELISA IgM/IgG (Virotech, serum) – ACA Sensitivity 1.00 Specificity 1.00 Index test: Immunoblot IgM (Mikrogen, serum) – ACA Sensitivity 0.00 Specificity 0.97 Index test: Immunoblot IgM/IgG (Mikrogen, serum) – ACA Sensitivity 1.00 Specificity 0.92
Source of funding	Not reported

Reference	Ang 2015[1]
Limitations	Risk of bias: selection, index test, reference standard Indirectness: none
Comments	Borderline results excluded from the analysis as the study authors did not necessarily interpret them as positive evidence of infection

Reference	Asbrink 1985[2]
Study type	Case-control
Study methodology	Data source: patients investigated at the Department of Dermatology Recruitment: not reported
Number of patients	n = 123 cases, 185 controls
Patient characteristics	Age, range: 17-80 years (control group) Gender (male to female ratio): not reported Family origin: not reported Setting: Department of Dermatology Country: Sweden Cases: 88 patients with typical, clinically uncomplicated EM, 26 patients with clinical and histopathological findings compatible with a diagnosis of ACA, 9 patients with EM-related extracutaneous manifestations (facial palsy, meningoradiculitis, arthritis, suspected cardiac involvement) Controls: patients living in the same area as cases, visiting the department with blood samples drawn for routine analysis, syphilis patients were excluded
Target condition(s)	Lyme disease
Index test(s) and reference standard	Index test(s) ELISA (IgM) - serum ELISA IgG) - serum Reference standard Clinical diagnosis

Reference	Asbrink 1985[2]				
	Time between measurement of index test and reference standard: not reported				
2×2 table		Reference standard +	Reference standard −	Total	EM ELISA (IgM) Serum
	Index test +	10	9	19	
	Index test −	78	176	254	
	Total	88	185	273	
		Reference standard +	Reference standard −	Total	EM ELISA (IgG) Serum
	Index test +	16	9	25	
	Index test −	72	176	248	
	Total	88	185	273	
		Reference standard +	Reference standard −	Total	ACA ELISA (IgM) Serum Before treatment
	Index test +	7	9	16	
	Index test −	19	176	195	
	Total	26	185	211	
		Reference standard +	Reference standard −	Total	ACA ELISA (IgM) Serum After treatment
	Index test +	4	9	13	
	Index test −	22	176	198	
	Total	26	185	211	
		Reference standard +	Reference standard −	Total	ACA ELISA (IgG) Serum Before treatment
	Index test +	26	9	35	
	Index test −	0	176	176	
	Total	26	185	211	
		Reference standard +	Reference standard −	Total	ACA ELISA (IgG) Serum After treatment
	Index test +	24	9	33	
	Index test −	2	176	178	
	Total	26	185	211	
Statistical	Index test: ELISA IgM (serum) – EM (before treatment)				

Reference	Asbrink 1985[2]
measures	Sensitivity 0.11 Specificity 0.95 Index test: ELISA IgG (serum) – EM (before treatment) Sensitivity 0.18 Specificity 0.95 Index test: ELISA IgM (serum) – ACA (before treatment) Sensitivity 0.27 Specificity 0.95 Index test: ELISA IgM (serum) – ACA (after treatment) Sensitivity 0.15 Specificity 0.95 Index test: ELISA IgG (serum) – ACA (before treatment) Sensitivity 1.00 Specificity 0.95 Index test: ELISA IgG (serum) – ACA (after treatment) Sensitivity 0.92 Specificity 0.95
Source of funding	Support from the Finsen and the Welander Foundation
Limitations	Risk of bias: selection, index test, reference standard Indirectness: none
Comments	Positive result: titer >410 (IgG) or >800 (IgM)

Reference	Bacon 2003[4]
Study type	Case-control
Study methodology	Data source: panel of 839 samples Recruitment: not reported
Number of patients	n = 280 cases, 559 controls (257 healthy controls used in the analysis)
Patient characteristics	Age, mean (SD): not reported Gender (male to female ratio): not reported Family origin: not reported Setting: not reported Country: USA Cases: patients with acute Lyme disease (80), early convalescent Lyme disease (106), early neurologic (15), early neurologic convalescent (11), arthritis (33), arthritis convalescent (24), late neurologic (11) Controls: healthy individuals (257), evidence of autoimmune disease or spirochaetal infection other than Lyme disease (302)
Target condition(s)	Lyme disease
Index test(s) and reference standard	Index test(s) ELISA (rVlsE1 IgM) – serum ELISA (pepC10 IgM) – serum ELISA (rVlsE1 IgG) – serum ELISA (C6 IgG) – serum Reference standard Clinical diagnosis (CDC criteria)

Reference	Bacon 2003[4]				
	Time between measurement of index test and reference standard: not reported				
2×2 table		Reference standard +	Reference standard −	Total	Acute EM ELISA (rVlsE1 IgM) Serum
	Index test +	3	5	8	
	Index test −	32	252	284	
	Total	35	257	292	
		Reference standard +	Reference standard −	Total	Acute EM ELISA (pepC10 IgM) Serum
	Index test +	7	0	7	
	Index test −	28	257	285	
	Total	35	257	292	
		Reference standard +	Reference standard −	Total	Convalescent EM ELISA (rVlsE1 IgM) Serum
	Index test +	24	5	29	
	Index test −	33	252	285	
	Total	57	257	314	
		Reference standard +	Reference standard −	Total	Convalescent EM ELISA (pepC10 IgM) Serum
	Index test +	23	0	23	
	Index test −	34	257	291	
	Total	57	257	314	
		Reference standard +	Reference standard −	Total	Acute EM ELISA (rVlsE1 IgG) Serum
	Index test +	7	2	9	
	Index test −	28	255	283	
	Total	35	257	292	
		Reference standard +	Reference standard −	Total	Acute EM

Reference	**Bacon 2003[4]**				
	Index test +	15	0	15	ELISA (C6 IgG) Serum
	Index test −	20	257	277	
	Total	35	257	292	
		Reference standard +	Reference standard −	Total	Convalescent EM ELISA (rVlsE1 IgG) Serum
	Index test +	25	2	27	
	Index test −	32	255	287	
	Total	57	257	314	
		Reference standard +	Reference standard −	Total	Convalescent EM ELISA (C6 IgG) Serum
	Index test +	33	0	33	
	Index test −	24	257	281	
	Total	57	257	314	
		Reference standard +	Reference standard −	Total	Convalescent neurologic ELISA (rVlsE1 IgM) Serum
	Index test +	6	5	11	
	Index test −	5	252	257	
	Total	11	257	268	
		Reference standard +	Reference standard −	Total	Convalescent neurologic ELISA (pepC10 IgM) Serum
	Index test +	4	0	4	
	Index test −	7	257	264	
	Total	11	257	268	
		Reference standard +	Reference standard −	Total	Early neurologic ELISA (rVlsE1 IgM) Serum
	Index test +	11	5	16	
	Index test −	4	252	256	
	Total	15	257	272	
		Reference standard	Reference standard −	Total	Early neurologic

Reference	**Bacon 2003[4]**				
		+			ELISA (pepC10 IgM) Serum
	Index test +	8	0	8	
	Index test −	7	257	264	
	Total	15	257	272	
		Reference standard +	Reference standard −	Total	Late neurologic ELISA (rVlsE1 IgM) Serum
	Index test +	1	5	6	
	Index test −	10	252	262	
	Total	11	257	268	
		Reference standard +	Reference standard −	Total	Late neurologic ELISA (pepC10 IgM) Serum
	Index test +	2	0	2	
	Index test −	9	257	266	
	Total	11	257	268	
		Reference standard +	Reference standard −	Total	Convalescent neurologic ELISA (rVlsE1 IgG) Serum
	Index test +	7	2	9	
	Index test −	4	255	259	
	Total	11	257	268	
		Reference standard +	Reference standard −	Total	Convalescent neurologic ELISA (C6 IgG) Serum
	Index test +	7	0	7	
	Index test −	4	257	261	
	Total	11	257	268	
		Reference standard +	Reference standard −	Total	Early neurologic ELISA (rVlsE1 IgG) Serum
	Index test +	15	2	17	
	Index test −	0	255	255	
	Total	15	257	272	

Reference	**Bacon 2003[4]**				
		Reference standard +	Reference standard −	Total	Early neurologic ELISA (C6 IgG) Serum
	Index test +	9	0	9	
	Index test −	6	257	263	
	Total	15	257	272	
		Reference standard +	Reference standard −	Total	Late neurologic ELISA (rVlsE1 IgG) Serum
	Index test +	11	2	13	
	Index test −	0	255	255	
	Total	11	257	268	
		Reference standard +	Reference standard −	Total	Late neurologic ELISA (C6 IgG) Serum
	Index test +	8	0	8	
	Index test −	3	257	260	
	Total	11	257	268	
		Reference standard +	Reference standard −	Total	Arthritis ELISA (rVlsE1 IgM) Serum
	Index test +	13	5	18	
	Index test −	20	252	272	
	Total	33	257	290	
		Reference standard +	Reference standard −	Total	Arthritis ELISA (pepC10 IgM) Serum
	Index test +	3	0	3	
	Index test −	30	257	287	
	Total	33	257	290	
		Reference standard +	Reference standard −	Total	Convalescent arthritis ELISA (rVlsE1 IgM) Serum
	Index test +	10	5	15	
	Index test −	14	252	266	

Reference	**Bacon 2003[4]**				
	Total	24	257	281	
		Reference standard +	Reference standard −	Total	Convalescent arthritis ELISA (pepC10 IgM) Serum
	Index test +	2	0	2	
	Index test −	22	257	279	
	Total	24	257	281	
		Reference standard +	Reference standard −	Total	Arthritis ELISA (rVlsE1 IgG) Serum
	Index test +	32	2	34	
	Index test −	1	255	256	
	Total	33	257	290	
		Reference standard +	Reference standard −	Total	Arthritis ELISA (C6 IgG) Serum
	Index test +	31	0	31	
	Index test −	2	257	259	
	Total	33	257	290	
		Reference standard +	Reference standard −	Total	Convalescent arthritis ELISA (rVlsE1 IgG) Serum
	Index test +	21	2	23	
	Index test −	3	255	258	
	Total	24	257	281	
		Reference standard +	Reference standard −	Total	Convalescent arthritis ELISA (C6 IgG) Serum
	Index test +	21	0	21	
	Index test −	3	257	260	
	Total	24	257	281	
Statistical measures	Index test: ELISA IgM rVlsE1 (serum) – acute EM Sensitivity 0.09 Specificity 0.98				

Reference	Bacon 2003[4]
	Index test: ELISA IgM pepC10 (serum) – acute EM Sensitivity 0.20 Specificity 1.00 Index test: ELISA IgM rVlsE1 (serum) – convalescent EM Sensitivity 0.42 Specificity 0.98 Index test: ELISA IgM pepC10 (serum) – convalescent EM Sensitivity 0.40 Specificity 1.00 Index test: ELISA IgG rVlsE1 (serum) – acute EM Sensitivity 0.20 Specificity 0.99 Index test: ELISA IgG C6 (serum) – acute EM Sensitivity 0.43 Specificity 1.00 Index test: ELISA IgG rVlsE1 (serum) – convalescent EM Sensitivity 0.44 Specificity 0.99 Index test: ELISA IgG C6 (serum) – convalescent EM Sensitivity 0.58 Specificity 1.00 Index test: ELISA rVlsE1 IgM (serum) - convalescent neurologic Sensitivity 0.55

Reference	Bacon 2003[4]
	Specificity 0.98 Index test: ELISA pepC10 IgM (serum) - convalescent neurologic Sensitivity 0.36 Specificity 1.00 Index test: ELISA rVlsE1 IgM (serum) - early neurologic Sensitivity 0.73 Specificity 0.98 Index test: ELISA pepC10 IgM (serum) - early neurologic Sensitivity 0.53 Specificity 1.00 Index test: ELISA rVlsE1 IgM (serum) - late neurologic Sensitivity 0.09 Specificity 0.98 Index test: ELISA pepC10 IgM (serum) - late neurologic Sensitivity 0.18 Specificity 1.00 Index test: ELISA rVlsE1 IgG (serum) - convalescent neurologic Sensitivity 0.64 Specificity 0.99 Index test: ELISA C6 IgG (serum) - convalescent neurologic Sensitivity 0.64 Specificity 1.00 Index test: ELISA rVlsE1 IgG (serum) - early neurologic

Reference	Bacon 2003[4]
	Sensitivity 1.00 Specificity 0.99 Index test: ELISA C6 IgG (serum) - early neurologic Sensitivity 0.60 Specificity 1.00 Index test: ELISA rVlsE1 IgG (serum) - late neurologic Sensitivity 1.00 Specificity 0.99 Index test: ELISA C6 IgG (serum) - late neurologic Sensitivity 0.73 Specificity 1.00 Index test: ELISA rVlsE1 IgM (serum) – arthritis Sensitivity 0.39 Specificity 0.98 Index test: ELISA pepC10 IgM (serum) – arthritis Sensitivity 0.09 Specificity 1.00 Index test: ELISA rVlsE1 IgM (serum) - convalescent arthritis Sensitivity 0.42 Specificity 0.98 Index test: ELISA pepC10 IgM (serum) - convalescent arthritis Sensitivity 0.08 Specificity 1.00

Reference	Bacon 2003[4]
	Index test: ELISA rVlsE1 IgG (serum) – arthritis Sensitivity 0.97 Specificity 0.99 Index test: ELISA C6 IgG (serum) – arthritis Sensitivity 0.94 Specificity 1.00 Index test: ELISA rVlsE1 IgG (serum) - convalescent arthritis Sensitivity 0.88 Specificity 0.99 Index test: ELISA C6 IgG (serum) - convalescent arthritis Sensitivity 0.88 Specificity 1.00
Source of funding	National Center for Research Resources, NIH, CDC
Limitations	Risk of bias: selection, index test, reference standard Indirectness: none

Reference	Branda 2010[9]
Study type	Case-control
Study methodology	Data source: patients meeting the CDC criteria for Lyme disease, healthy controls and disease controls Recruitment: not reported
Number of patients	n = 162 cases, 269 controls (166 healthy controls used in analysis)
Patient characteristics	Age, mean (SD): not reported Gender (male to female ratio): not reported Family origin: not reported Setting: 2 medical centres Country: USA Cases: patients meeting the CDC criteria for Lyme disease – culture confirmed EM (106), acute neuritis/carditis (27), arthritis/late neuritis (29) Controls: healthy controls (166), other illnesses (103)
Target condition(s)	Lyme disease
Index test(s) and reference standard	Index test(s) ELISA (IgM/IgG) Immunoblot (IgM) Immunoblot (IgG) Immunoblot (IgM/IgG) Reference standard Clinical diagnosis (CDC criteria)

Reference	Branda 2010[9]				
	Time between measurement of index test and reference standard: not reported				
2×2 table		Reference standard +	Reference standard −	Total	Acute phase EM ELISA (IgM/IgG) Serum
	Index test +	46	8	54	
	Index test −	60	187	247	
	Total	106	195	301	
		Reference standard +	Reference standard −	Total	Convalescent EM ELISA (IgM/IgG) Serum
	Index test +	97	8	105	
	Index test −	9	187	196	
	Total	106	195	301	
		Reference standard +	Reference standard −	Total	Acute phase EM Immunoblot (IgM) Serum
	Index test +	32	0	32	
	Index test −	74	195	269	
	Total	106	195	301	
		Reference standard +	Reference standard −	Total	Convalescent EM Immunoblot (IgM) Serum
	Index test +	64	0	64	
	Index test −	42	195	237	
	Total	106	195	301	
		Reference standard +	Reference standard −	Total	Acute phase EM Immunoblot (IgG) Serum
	Index test +	6	1	7	
	Index test −	100	194	294	
	Total	106	195	301	
		Reference standard +	Reference standard −	Total	Convalescent EM

Reference	**Branda 2010[9]**				
	Index test +	12	1	13	Immunoblot (IgG) Serum
	Index test −	94	194	288	
	Total	106	195	301	
		Reference standard +	Reference standard −	Total	Acute phase EM Immunoblot (IgM/IgG) Serum
	Index test +	36	1	37	
	Index test −	70	194	264	
	Total	106	195	301	
		Reference standard +	Reference standard −	Total	Convalescent EM Immunoblot (IgM/IgG) Serum
	Index test +	70	1	71	
	Index test −	36	194	230	
	Total	106	195	301	
		Reference standard +	Reference standard −	Total	Unspecified Lyme disease ELISA (IgM/IgG) Serum
	Index test +	56	8	64	
	Index test −	0	187	187	
	Total	56	195	251	
		Reference standard +	Reference standard −	Total	Unspecified Lyme disease Immunoblot (IgM) Serum
	Index test +	28	0	28	
	Index test −	28	195	223	
	Total	56	195	251	
		Reference standard +	Reference standard −	Total	Unspecified Lyme disease Immunoblot (IgG) Serum
	Index test +	48	1	49	
	Index test −	8	194	202	
	Total	56	195	251	
		Reference standard	Reference standard −	Total	Unspecified Lyme disease

Reference	Branda 2010[9]				
		+			Immunoblot (IgM/IgG) Serum
	Index test +	55	1	56	
	Index test −	1	194	195	
	Total	56	195	251	
Statistical measures	Index test: ELISA IgM/IgG (serum) – acute phase EM Sensitivity 0.43 Specificity 0.96 Index test: ELISA IgM/IgG (serum) – convalescent EM Sensitivity 0.92 Specificity 0.96 Index test: Immunoblot IgM (serum) – acute phase EM Sensitivity 0.30 Specificity 1.00 Index test: Immunoblot IgM (serum) – convalescent EM Sensitivity 0.60 Specificity 1.00 Index test: Immunoblot IgG (serum) – acute phase EM Sensitivity 0.06 Specificity 0.99 Index test: Immunoblot IgG (serum) – convalescent EM Sensitivity 0.11 Specificity 0.99 Index test: Immunoblot IgM/IgG (serum) – acute phase EM Sensitivity 0.34 Specificity 0.99				

Reference	Branda 2010[9]
	Index test: Immunoblot IgM/IgG (serum) – convalescent EM Sensitivity 0.66 Specificity 0.99 Index test: ELISA IgM/IgG (serum) – unspecified Lyme disease Sensitivity 1.00 Specificity 0.96 Index test: Immunoblot IgM (serum) – unspecified Lyme disease Sensitivity 0.50 Specificity 1.00 Index test: Immunoblot IgG (serum) – unspecified Lyme disease Sensitivity 0.86 Specificity 0.99 Index test: Immunoblot IgM/IgG (serum) – unspecified Lyme disease Sensitivity 0.98 Specificity 0.99
Source of funding	Research grant from Viramed Biotech
Limitations	Risk of bias: selection, reference standard Indirectness: none

Reference	Branda 2011[10]
Study type	Case-control
Study methodology	Data source: Phase 1 - well-characterised Lyme disease patients and symptomatic controls who had been evaluated by Lyme disease experts Phase 2 – serum samples submitted for Lyme disease testing with review of medical records and healthy controls during routine visits Recruitment: not reported
Number of patients	n = 169 cases, 1300 controls
Patient characteristics	Age, mean (SD): not reported Gender (male to female ratio): not reported Family origin: not reported Setting: general hospital Country: USA Cases: EM (114), acute neuritis or carditis (26), arthritis or late neuritis (29) Controls : symptomatic patients not meeting CDC criteria for Lyme disease (54), healthy controls during routine visits (1246)
Target condition(s)	Lyme disease
Index test(s) and reference standard	Index test(s) ELISA (IgM/IgG) – serum Reference standard Clinical diagnosis (CDC surveillance criteria)

Reference	Branda 2011[10]				
	Time between measurement of index test and reference standard: not reported				
2×2 table		Reference standard +	Reference standard −	Total	EM ELISA (IgM/IgG) Serum
	Index test +	64	21	85	
	Index test −	50	1279	1329	
	Total	114	1300	1414	
		Reference standard +	Reference standard −	Total	Unspecified early disseminated Lyme disease ELISA (IgM/IgG) Serum
	Index test +	26	21	47	
	Index test −	0	187	187	
	Total	26	208	234	
		Reference standard +	Reference standard −	Total	Unspecified late Lyme disease ELISA (IgM/IgG) Serum
	Index test +	29	21	50	
	Index test −	0	1279	1279	
	Total	29	1300	1329	
Statistical measures	Index test: ELISA IgM/IgG (serum) - EM Sensitivity 0.56 Specificity 0.98 Index test: ELISA IgM/IgG (serum) - unspecified early disseminated Lyme disease Sensitivity 1.00 Specificity 0.98 Index test: ELISA IgM/IgG (serum) - unspecified late Lyme disease Sensitivity 1.00 Specificity 0.98				
Source of	CDC, the English, Bonter, Mitchell Foundation, the Eshe Fund and the Lyme Disease and Arthritis Research Fund at Massachusetts				

Reference	Branda 2011[10]
funding	General Hospital
Limitations	Risk of bias: selection, index test, reference standard Indirectness: none

Reference	Branda 2013[11]
Study type	Case-control
Study methodology	Data source: patients evaluated at a Lyme borreliosis outpatient clinic meeting European criteria for Lyme disease and blood donors. Recruitment: not reported
Number of patients	n = 64 cases, 100 controls
Patient characteristics	Age, mean (SD): not reported Gender (male to female ratio): not reported Family origin: not reported Setting: Lyme borreliosis outpatient clinic Country: Slovenia Cases: patients meeting European criteria for Lyme disease: EM (20, 15 with positive cultures), neuroborreliosis with CSF pleocytosis, concomitant EM isolation of B. burgdorferi spirochetes from CSF or demonstration of intrathecal synthesis of specific antibodies (15), arthritis with swelling in 1 or more joints and specific antibodies in serum without alternative explanation (15), ACA with characteristic clinical picture, supportive histologic findings and high specific serum IgG antibody levels (14) Controls: 100 healthy blood donors in New Zealand, a non-endemic region
Target condition(s)	Lyme disease
Index test(s) and reference standard	Index test(s) ELISA (IgM) ELISA (IgG) ELISA (IgM/IgG) Immunoblot (IgM) Immunoblot (IgG)

Reference	Branda 2013[11]				
	Immunoblot (IgM/IgG) Reference standard Clinical diagnosis (European Lyme disease criteria) Time between measurement of index test and reference standard:				
2×2 table		Reference standard +	Reference standard −	Total	EM ELISA (IgM) European test Serum
	Index test +	8	2	10	
	Index test −	12	98	110	
	Total	20	100	120	
		Reference standard +	Reference standard −	Total	EM ELISA (IgG) European test Serum
	Index test +	13	2	15	
	Index test −	7	98	105	
	Total	20	100	120	
		Reference standard +	Reference standard −	Total	EM ELISA (IgM/IgG) European test Serum
	Index test +	15	4	19	
	Index test −	5	96	111	
	Total	20	100	120	
		Reference standard +	Reference standard −	Total	EM ELISA (IgM/IgG) USA test Serum
	Index test +	14	3	17	
	Index test −	6	97	103	
	Total	20	100	120	
		Reference standard +	Reference standard −	Total	EM ELISA (IgM/IgG)
	Index test +	14	0	14	

Reference	**Branda 2013[11]**				
	Index test −	6	100	106	USA C6 test Serum
	Total	20	100	120	
		Reference standard +	Reference standard −	Total	EM Immunoblot (IgM) European test Serum
	Index test +	7	9	16	
	Index test −	13	91	104	
	Total	20	100	120	
		Reference standard +	Reference standard −	Total	EM Immunoblot (IgM) USA test Serum
	Index test +	2	0	2	
	Index test −	18	100	118	
	Total	20	100	120	
		Reference standard +	Reference standard −	Total	EM Immunoblot (IgG) European test Serum
	Index test +	7	0	7	
	Index test −	13	100	113	
	Total	20	100	120	
		Reference standard +	Reference standard −	Total	EM Immunoblot (IgG) USA test Serum
	Index test +	2	0	2	
	Index test −	18	100	118	
	Total	20	100	120	
		Reference standard +	Reference standard −	Total	EM Immunoblot (IgM/IgG) European test Serum
	Index test +	11	9	20	
	Index test −	9	91	100	
	Total	20	100	120	
		Reference standard +	Reference standard −	Total	EM

Reference	**Branda 2013[11]**				
	Index test +	4	0	4	Immunoblot (IgM/IgG) USA test Serum
	Index test −	16	100	116	
	Total	20	100	120	
		Reference standard +	Reference standard −	Total	ACA ELISA (IgM) European test Serum
	Index test +	10	4	14	
	Index test −	2	98	100	
	Total	12	102	114	
		Reference standard +	Reference standard −	Total	ACA ELISA (IgG) European test Serum
	Index test +	14	2	16	
	Index test −	0	98	98	
	Total	14	100	114	
		Reference standard +	Reference standard −	Total	ACA ELISA (IgM/IgG) European test Serum
	Index test +	14	4	18	
	Index test −	0	96	96	
	Total	14	100	114	
		Reference standard +	Reference standard −	Total	ACA ELISA (IgM/IgG) USA test Serum
	Index test +	14	3	17	
	Index test −	0	97	97	
	Total	14	100	114	
		Reference standard +	Reference standard −	Total	ACA ELISA (IgM/IgG) USA C6 test Serum
	Index test +	14	0	14	
	Index test −	0	100	100	
	Total	14	100	114	
		Reference standard	Reference standard −	Total	ACA

Reference	**Branda 2013[11]**				
		+			Immunoblot (IgM) European test Serum
	Index test +	5	9	14	
	Index test −	9	91	100	
	Total	14	100	114	
		Reference standard +	Reference standard −	Total	ACA Immunoblot (IgM) USA test Serum
	Index test +	4	0	4	
	Index test −	10	100	110	
	Total	14	100	114	
		Reference standard +	Reference standard −	Total	ACA Immunoblot (IgG) European test Serum
	Index test +	14	0	14	
	Index test −	0	100	100	
	Total	14	100	114	
		Reference standard +	Reference standard −	Total	ACA Immunoblot (IgG) USA test Serum
	Index test +	14	0	14	
	Index test −	0	100	100	
	Total	14	100	114	
		Reference standard +	Reference standard −	Total	ACA Immunoblot (IgM/IgG) European test Serum
	Index test +	14	9	23	
	Index test −	0	91	91	
	Total	14	100	114	
		Reference standard +	Reference standard −	Total	ACA Immunoblot (IgM/IgG) USA test Serum
	Index test +	14	0	14	
	Index test −	0	100	100	
	Total	14	100	114	

Reference	**Branda 2013[11]**				
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA (IgM) European test Serum
	Index test +	12	2	14	
	Index test −	3	98	101	
	Total	15	100	115	
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA (IgG) European test Serum
	Index test +	13	2	15	
	Index test −	2	98	100	
	Total	15	100	115	
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA (IgM/IgG) European test Serum
	Index test +	15	4	19	
	Index test −	0	96	96	
	Total	15	100	115	
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA (IgM/IgG) USA test Serum
	Index test +	13	3	16	
	Index test −	2	97	99	
	Total	15	100	115	
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA (IgM/IgG) USA C6 test Serum
	Index test +	13	0	13	
	Index test −	2	100	102	
	Total	15	100	115	
		Reference standard +	Reference standard −	Total	Neuroborreliosis Immunoblot (IgM) European test Serum
	Index test +	12	9	21	
	Index test −	3	91	94	

Reference	**Branda 2013[11]**				
	Total	15	100	115	
		Reference standard +	Reference standard −	Total	Neuroborreliosis Immunoblot (IgM) USA test Serum
	Index test +	6	0	6	
	Index test −	9	100	109	
	Total	15	100	115	
		Reference standard +	Reference standard −	Total	Neuroborreliosis Immunoblot (IgG) European test Serum
	Index test +	9	0	9	
	Index test −	6	100	106	
	Total	15	100	115	
		Reference standard +	Reference standard −	Total	Neuroborreliosis Immunoblot (IgG) USA test Serum
	Index test +	6	0	6	
	Index test −	9	100	109	
	Total	15	100	115	
		Reference standard +	Reference standard −	Total	Neuroborreliosis Immunoblot (IgM/IgG) European test Serum
	Index test +	13	9	22	
	Index test −	2	91	93	
	Total	15	100	115	
		Reference standard +	Reference standard −	Total	Neuroborreliosis Immunoblot (IgM/IgG) USA test Serum
	Index test +	8	0	8	
	Index test −	7	100	107	
	Total	115	100	115	
		Reference standard +	Reference standard −	Total	Arthritis ELISA (IgM)
	Index test +	9	2	11	

Reference	**Branda 2013[11]**				
	Index test −	6	98	104	European test Serum
	Total	15	100	115	
		Reference standard +	Reference standard −	Total	Arthritis ELISA (IgG) European test Serum
	Index test +	15	2	17	
	Index test −	0	98	98	
	Total	15	100	115	
		Reference standard +	Reference standard −	Total	Arthritis ELISA (IgM/IgG) European test Serum
	Index test +	15	4	19	
	Index test −	0	96	96	
	Total	15	100	115	
		Reference standard +	Reference standard −	Total	Arthritis ELISA (IgM/IgG) USA test Serum
	Index test +	15	0	15	
	Index test −	0	100	100	
	Total	15	100	115	
		Reference standard +	Reference standard −	Total	Arthritis ELISA (IgM/IgG) USA C6 test Serum
	Index test +	14	3	17	
	Index test −	1	97	98	
	Total	15	100	115	
		Reference standard +	Reference standard −	Total	Arthritis Immunoblot (IgM) European test Serum
	Index test +	10	9	19	
	Index test −	5	91	96	
	Total	15	100	115	
		Reference standard +	Reference standard −	Total	Arthritis

Reference	**Branda 2013[11]**				
	Index test +	4	0	4	Immunoblot (IgM) USA test Serum
	Index test −	11	100	111	
	Total	15	100	115	
		Reference standard +	Reference standard −	Total	Arthritis Immunoblot (IgG) European test Serum
	Index test +	13	0	13	
	Index test −	2	100	102	
	Total	15	100	115	
		Reference standard +	Reference standard −	Total	Arthritis Immunoblot (IgG) USA test Serum
	Index test +	10	0	10	
	Index test −	5	100	105	
	Total	115	100	115	
		Reference standard +	Reference standard −	Total	Arthritis Immunoblot (IgM/IgG) European test Serum
	Index test +	14	9	23	
	Index test −	1	91	92	
	Total	15	100	115	
		Reference standard +	Reference standard −	Total	Arthritis Immunoblot (IgM/IgG) USA test Serum
	Index test +	11	0	11	
	Index test −	4	100	104	
	Total	15	100	115	
Statistical measures	Index test: ELISA European IgM (serum) - EM Sensitivity 0.40 Specificity 0.98 Index test: ELISA European IgG (serum) - EM Sensitivity 0.65				

Reference	Branda 2013[11]
	Specificity 0.98 Index test: ELISA European IgM/IgG (serum) - EM Sensitivity 0.75 Specificity 0.96 Index test: ELISA USA IgM/IgG (serum) - EM Sensitivity 0.70 Specificity 0.97 Index test: ELISA USA C6 IgM/IgG (serum) - EM Sensitivity 0.70 Specificity 1.00 Index test: Immunoblot European IgM (serum) - EM Sensitivity 0.35 Specificity 0.91 Index test: Immunoblot USA IgM (serum) - EM Sensitivity 0.10 Specificity 1.00 Index test: Immunoblot European IgG (serum) - EM Sensitivity 0.35 Specificity 1.00 Index test: Immunoblot USA IgG (serum) - EM Sensitivity 0.10 Specificity 1.00 Index test: Immunoblot European IgM/IgG (serum) - EM

Reference	Branda 2013[11]
	Sensitivity 0.55 Specificity 0.91 Index test: Immunoblot USA IgM/IgG (serum) - EM Sensitivity 0.20 Specificity 1.00 Index test: ELISA European IgM (serum) - ACA Sensitivity 0.83 Specificity 0.96 Index test: ELISA European IgG (serum) - ACA Sensitivity 1.00 Specificity 0.98 Index test: ELISA European IgM/IgG (serum) - ACA Sensitivity 1.00 Specificity 0.96 Index test: ELISA USA IgM/IgG (serum) - ACA Sensitivity 1.00 Specificity 1.00 Index test: ELISA USA C6 IgM/IgG (serum) - ACA Sensitivity 1.00 Specificity 0.97 Index test: Immunoblot European IgM (serum) - ACA Sensitivity 0.36 Specificity 0.91

Reference	Branda 2013[11]
	Index test: Immunoblot USA IgM (serum) - ACA Sensitivity 0.29 Specificity 1.00 Index test: Immunoblot European IgG (serum) - ACA Sensitivity 1.00 Specificity 1.00 Index test: Immunoblot USA IgG (serum) - ACA Sensitivity 1.00 Specificity 1.00 Index test: Immunoblot European IgM/IgG (serum) - ACA Sensitivity 1.00 Specificity 0.91 Index test: Immunoblot USA IgM/IgG (serum) - ACA Sensitivity 1.00 Specificity 1.00 Index test: ELISA European IgM (serum) – neuroborreliosis Sensitivity 0.80 Specificity 0.98 Index test: ELISA European IgG (serum) - neuroborreliosis Sensitivity 0.87 Specificity 0.98 Index test: ELISA European IgM/IgG (serum) - neuroborreliosis Sensitivity 1.00 Specificity 0.96

Reference	Branda 2013[11]
	Index test: ELISA USA IgM/IgG (serum) - neuroborreliosis Sensitivity 0.87 Specificity 0.97 Index test: ELISA USA C6 IgM/IgG (serum) - neuroborreliosis Sensitivity 0.87 Specificity 1.00 Index test: Immunoblot European IgM (serum) - neuroborreliosis Sensitivity 0.80 Specificity 0.91 Index test: Immunoblot USA IgM (serum) - neuroborreliosis Sensitivity 0.40 Specificity 1.00 Index test: Immunoblot European IgG (serum) - neuroborreliosis Sensitivity 0.60 Specificity 1.00 Index test: Immunoblot USA IgG (serum) - neuroborreliosis Sensitivity 0.40 Specificity 1.00 Index test: Immunoblot European IgM/IgG (serum) - neuroborreliosis Sensitivity 0.87 Specificity 0.91 Index test: Immunoblot USA IgM/IgG (serum) - neuroborreliosis Sensitivity 0.53 Specificity 1.00

Reference	Branda 2013[11]
	Index test: ELISA European IgM (serum) - arthritis Sensitivity 0.60 Specificity 0.98 Index test: ELISA European IgG (serum) - arthritis Sensitivity 1.00 Specificity 0.98 Index test: ELISA European IgM/IgG (serum) - arthritis Sensitivity 1.00 Specificity 0.96 Index test: ELISA USA IgM/IgG (serum) - arthritis Sensitivity 0.93 Specificity 0.97 Index test: ELISA USA C6 IgM/IgG (serum) - arthritis Sensitivity 1.00 Specificity 1.00 Index test: Immunoblot European IgM (serum) - arthritis Sensitivity 0.67 Specificity 0.91 Index test: Immunoblot USA IgM (serum) - arthritis Sensitivity 0.27 Specificity 1.00 Index test: Immunoblot European IgG (serum) - arthritis Sensitivity 0.87

Reference	Branda 2013[11]
	Specificity 1.00 Index test: Immunoblot USA IgG (serum) - arthritis Sensitivity 0.67 Specificity 1.00 Index test: Immunoblot European IgM/IgG (serum) - arthritis Sensitivity 0.93 Specificity 0.91 Index test: Immunoblot USA IgM/IgG (serum) - arthritis Sensitivity 0.73 Specificity 1.00
Source of funding	Austin L. Vickery Jr award from the Department of Pathology, Massachusetts General Hospital, Boston
Limitations	Risk of bias: selection, index test, reference standard Indirectness: none

Reference	**Callister 2002[13]**				
Study type	Case-control				
Study methodology	Data source: patients screened for Lyme disease at a single medical centre. Recruitment: not reported				
Number of patients	n = 34 cases, 34 controls				
Patient characteristics	Age, mean (SD): not reported Gender (male to female ratio): not reported Family origin: not reported Setting: single medical centre Country: USA Cases: patients with early Lyme disease (22 single EM, 12 multiple EM) Controls : no evidence of tick exposure and with symptoms unrelated to Lyme disease				
Target condition(s)	Lyme disease				
Index test(s) and reference standard	Index test(s) Immunoblot (IgM/IgG) Reference standard Clinical diagnosis Time between measurement of index test and reference standard: not reported				
2×2 table		Reference standard	Reference standard −	Total	Single EM

Reference	**Callister 2002[13]**				
		+			Immunoblot (IgM/IgG) Serum
	Index test +	12	3	15	
	Index test −	10	31	41	
	Total	22	34	56	
		Reference standard +	Reference standard −	Total	Multiple EM Immunoblot (IgM/IgG) Serum
	Index test +	10	3	13	
	Index test −	2	31	33	
	Total	12	34	46	
Statistical measures	Index test: Immunoblot IgM/IgG (serum) – single EM Sensitivity 0.55 Specificity 0.91 Index test: Immunoblot IgM/IgG (serum) – multiple EM Sensitivity 0.83 Specificity 0.91				
Source of funding	Gundersen Lutheran Medical Foundation				
Limitations	Risk of bias: selection, reference standard Indirectness: none				

Reference	**Cerar 2006[15]**				
Study type	Case-control				
Study methodology	Data source: patients presenting at the department of infectious diseases and healthy blood donors Recruitment: not reported				
Number of patients	n = 383 cases, 49 controls				
Patient characteristics	Age, mean (SD): not reported Gender (male to female ratio): not reported Family origin: not reported Setting: Department of infectious diseases Country: Slovenia Cases: Lyme suspected (198) EM (76), neuroborreliosis (28), early Lyme <6 months (60), chronic Lyme >6 months (21) Controls: healthy blood donors (49)				
Target condition(s)	Lyme disease				
Index test(s) and reference standard	Index test(s) Immunofluorescence assay (IgM) Immunofluorescence assay (IgM) Reference standard Clinical diagnosis Time between measurement of index test and reference standard: not reported				
2×2 table		Reference standard	Reference standard −	Total	EM

Reference	**Cerar 2006[15]**				
		+			IFA (IgM) Serum
	Index test +	3	0	3	
	Index test −	73	49	122	
	Total	76	49	125	
		Reference standard +	Reference standard −	Total	EM IFA (IgG) Serum
	Index test +	25	9	34	
	Index test −	51	40	91	
	Total	76	49	125	
		Reference standard +	Reference standard −	Total	Neuroborreliosis IFA (IgM) Serum
	Index test +	2	0	2	
	Index test −	26	49	75	
	Total	28	49	77	
		Reference standard +	Reference standard −	Total	Neuroborreliosis IFA (IgG) Serum
	Index test +	21	9	30	
	Index test −	7	40	47	
	Total	28	49	77	
		Reference standard +	Reference standard −	Total	Unspecified early Lyme disease IFA (IgM) Serum
	Index test +	6	0	6	
	Index test −	54	49	103	
	Total	60	49	109	
		Reference standard +	Reference standard −	Total	Unspecified chronic Lyme disease IFA (IgM) Serum
	Index test +	3	0	3	
	Index test −	18	49	67	
	Total	21	49	70	

Reference	Cerar 2006[15]				
		Reference standard +	Reference standard −	Total	Unspecified early Lyme disease IFA (IgG) Serum
	Index test +	35	9	44	
	Index test −	25	40	65	
	Total	60	49	109	
		Reference standard +	Reference standard −	Total	Unspecified chronic Lyme disease IFA (IgG) Serum
	Index test +	21	9	30	
	Index test −	0	40	40	
	Total	21	49	70	
Statistical measures	Index test: Immunofluorescence assay IgM (serum) - EM Sensitivity 0.04 Specificity 1.00 Index test: Immunofluorescence assay IgG (serum) - EM Sensitivity 0.33 Specificity 0.82 Index test: Immunofluorescence assay IgM (serum) - neuroborreliosis Sensitivity 0.07 Specificity 1.00 Index test: Immunofluorescence assay IgG (serum) - neuroborreliosis Sensitivity 0.75 Specificity 0.82 Index test: Immunofluorescence assay IgM (serum) – unspecified early Lyme disease Sensitivity 0.10 Specificity 1.00 Index test: Immunofluorescence assay IgM (serum) – unspecified chronic Lyme disease				

Reference	Cerar 2006[15]
	Sensitivity 0.14 Specificity 1.00 Index test: Immunofluorescence assay IgG (serum) – unspecified early Lyme disease Sensitivity 0.58 Specificity 0.82 Index test: Immunofluorescence assay IgG (serum) – unspecified chronic Lyme disease Sensitivity 1.00 Specificity 0.82
Source of funding	Not reported
Limitations	Risk of bias: selection, index test, reference standard Indirectness: none
Comments	Suspected Lyme disease patients not included in analysis

Reference	Cerar 2010[14]
Study type	Case-control
Study methodology	Data source: patients presenting at the department of infectious diseases Recruitment: not reported
Number of patients	n = 105 cases, 90 controls
Patient characteristics	Age, median (range): evident Lyme neuroborreliosis 56 years (18-77), suspected Lyme neuroborreliosis 52 years (28-70), tick-borne encephatlitis 54 years (19-78) Gender (male to female ratio): evident Lyme 19:15, suspected Lyme 10:17, tick-borne encephatlitis 20:12 Family origin: not reported Setting: Department of infectious diseases Country: Slovenia Cases: working clinical diagnosis of evident Lyme neuroborreliosis - EM within 4 months before neurological symptoms and/or signs including radiculoneuritic pain and/or peripheral facial palsy and pleocytosis (34), working clinical diagnosis of suspected Lyme neuroborreliosis – EM within 4 months before neurological symptoms and/or signs but no pleocytosis (27) Controls: tick-borne encephalitis (32)
Target condition(s)	Lyme disease
Index test(s) and reference standard	Index test(s) ELISA (IgM) ELISA (IgG) Reference standard Clinical diagnosis

Reference	Cerar 2010[14]				
	Time between measurement of index test and reference standard: not reported				
2×2 table		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA (IgM) CSF
	Index test +	7	0	7	
	Index test −	27	32	59	
	Total	34	32	66	
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA (IgM) Serum
	Index test +	16	1	17	
	Index test −	18	31	49	
	Total	34	32	66	
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA (IgG) CSF
	Index test +	14	1	15	
	Index test −	20	31	51	
	Total	34	32	66	
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA (IgG) Serum
	Index test +	24	16	40	
	Index test −	10	16	26	
	Total	34	32	66	
Statistical measures	Index test: ELISA IgM (CSF) – neuroborreliosis Sensitivity 0.21 Specificity 1.00 Index test: ELISA IgM (serum) – neuroborreliosis Sensitivity 0.47 Specificity 0.97				

Reference	Cerar 2010[14]
	Index test: ELISA IgG (CSF) - neuroborreliosis Sensitivity 0.41 Specificity 0.97 Index test: ELISA IgG (serum) - neuroborreliosis Sensitivity 0.71 Specificity 0.50
Source of funding	Not reported
Limitations	Risk of bias: selection, index test, reference standard Indirectness: none
Comments	Only confirmed neuroborreliosis cases included in the analysis; borderline results were excluded; no patients received antibiotics

Reference	Christova 2003[16]
Study type	Case-control
Study methodology	Data source: not reported Recruitment: not reported
Number of patients	n = 105 cases, 90 controls
Patient characteristics	Age, mean (SD): not reported Gender (male to female ratio): not reported Family origin: not reported Setting: Microbiology department, National Centre of Infectious and Parasitic Diseases Country: Bulgaria Cases: patients with EM lesions (105) Controls: healthy blood donors (90)
Target condition(s)	Lyme disease
Index test(s) and reference standard	Index test(s) ELISA (IgM) ELISA (IgG) Reference standard Clinical diagnosis Time between measurement of index test and reference standard:

Reference	**Christova 2003**[16]				
2×2 table		Reference standard +	Reference standard −	Total	EM ELISA (IgM) Serum
	Index test +	51	6	57	
	Index test −	54	84	138	
	Total	105	90	195	
		Reference standard +	Reference standard −	Total	EM ELISA (IgG) Serum
	Index test +	18	3	21	
	Index test −	87	87	174	
	Total	105	90	195	
Statistical measures	Index test: ELISA IgM (serum) - EM Sensitivity 0.49 Specificity 0.93 Index test: ELISA IgG (serum) - EM Sensitivity 0.17 Specificity 0.97				
Source of funding	Not reported				
Limitations	Risk of bias: selection, index test, reference standard Indirectness: none				

Reference	**Cinco 2006[17]**				
Study type	Case-control				
Study methodology	Data source: not reported Recruitment: not reported				
Number of patients	n = 76 cases, 59 controls				
Patient characteristics	Age, mean (SD): not reported Gender (male to female ratio): not reported Family origin: not reported Setting: not reported Country: Italy Cases: culture-confirmed EM (54), Lyme arthritis (15), neuroborreliosis (6) Controls: blood donors (26 non-endemic area, 33 endemic region)				
Target condition(s)	Lyme disease				
Index test(s) and reference standard	Index test(s) ELISA (C6) Reference standard Culture (EM), clinical diagnosis Time between measurement of index test and reference standard: not reported				
2×2 table		Reference standard	Reference standard −	Total	EM

Reference	Cinco 2006[17]				
		+			ELISA (C6) Serum
	Index test +	34	0	34	
	Index test −	20	24	44	
	Total	54	24	78	
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA (C6) Serum
	Index test +	6	0	6	
	Index test −	0	24	24	
	Total	6	24	30	
		Reference standard +	Reference standard −	Total	Lyme arthritis ELISA (C6) Serum
	Index test +	16	0	16	
	Index test −	0	24	24	
	Total	16	24	40	
Statistical measures	Index test: ELISA C6 (serum) - EM Sensitivity 0.63 Specificity 1.00 Index test: ELISA C6 (serum) - neuroborreliosis Sensitivity 1.00 Specificity 1.00 Index test: ELISA C6 (serum) – arthritis Sensitivity 1.00 Specificity 1.00				
Source of funding	Murst fund				
Limitations	Risk of bias: selection, index test, reference standard Indirectness: none				

Reference	Cinco 2006[17]
Comments	Control samples from non-endemic area used in analysis

Reference	Coyle 1993[18]
Study type	Case-control
Study methodology	Data source: patients from an endemic region presenting with clinical or laboratory evidence of B. burgdorferi infection and neurological complaints Recruitment: not reported
Number of patients	n = 77 cases, 34 controls
Patient characteristics	Age, mean (range): 34 years (3 – 84) Gender (male to female ratio): Lyme disease 33:44 Family origin: not reported Setting: Department of Neurology Country: USA Cases: clinical/laboratory evidence of B. burgdorferi infection and neurological complaints, only patients who underwent lumbar puncture as part of a work-up for neurologic Lyme disease and in whom sufficient CSF was collected, 24 had received prior antibiotic treatment but had persistent symptoms, 5 were currently receiving antibiotics Controls: other neurological diseases
Target condition(s)	Lyme disease
Index test(s) and reference standard	Index test(s) ELISA (IgM/IgG) Reference standard Clinical diagnosis

Reference	Coyle 1993[18]				
	Time between measurement of index test and reference standard: not reported				
2×2 table		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA (IgM/IgG) CSF
	Index test +	38	1	39	
	Index test −	39	33	72	
	Total	77	34	111	
Statistical measures	Index test: ELISA IgM/IgG (CSF) - neuroborreliosis Sensitivity 0.49 Specificity 0.97				
Source of funding	NIH grants, New York State grant for Lyme disease research, East End Lyme foundation				
Limitations	Risk of bias: selection, index test, reference standard Indirectness: serious (adults and children)				

Reference	**D'Arco 2017[19]**				
Study type	Case-control study				
Study methodology	Data source: serum samples from patients Recruitment: patients presenting to medical clinics				
Number of patients	n = 171				
Patient characteristics	Age, mean (SD): not reported Gender (male to female ratio): not reported Family origin: not reported Setting: medical centres, multi-centre Country: USA Cases: early Lyme disease (EM, n=152), Lyme arthritis (n=19) Controls: healthy individuals (n=139)				
Target condition(s)	EM, Lyme arthritis				
Index test(s) and reference standard	Index tests ELISA C6 (IgA) - serum Reference standard Clinical diagnosis Time between measurement of index test and reference standard: not reported				
2×2 table		Reference standard	Reference standard −	Total	EM

Reference	**D'Arco 2017[19]**				
		+			ELISA C6 (IgA) Serum
	Index test +	43	2	45	
	Index test −	98	133	231	
	Total	141	135	276	
2×2 table		Reference standard +	Reference standard −	Total	LA ELISA C6 (IgA) Serum
	Index test +	3	2	5	
	Index test −	14	133	147	
	Total	17	135	152	
Statistical measures	ELISA C6 (IgA) – serum (EM) Sensitivity 0.30 Specificity 0.99 ELISA C6 (IgA) – serum (LA) Sensitivity 0.18 Specificity 0.99				
Source of funding	Supported by government funding, authors hold shares in companies providing samples and/or diagnostic tests				
Limitations	Risk of bias: patient selection, index test, reference standard Indirectness: none				

Reference	Dessau 2010[20]
Study type	Case-control
Study methodology	Data source: Recruitment: consecutive (cases)
Number of patients	n = 117 cases, 815 controls
Patient characteristics	Age, median (range): cases 50 years (3-87) - 33 children, 26 adults up to 50 years, 57 adults above 50 years Gender (male to female ratio): cases 55:62, controls not reported Family origin: not reported Setting: not reported Country: Denmark Cases: neuroborreliosis defined by having a positive test for intrathecal antibody production and a leucocyte count in CSF of 5x10^6/L Controls: healthy blood donors (3.6% had ever been treated for Lyme disease)
Target condition(s)	Lyme disease
Index test(s) and reference standard	Index test(s) ELISA (IgM) ELISA (IgG) Reference standard Clinical diagnosis, positive test for intrathecal antibody production, leucocyte count in CSF of 5x10^6/L Time between measurement of index test and reference standard: serum and CSF samples taken on the same day

Reference	**Dessau 2010[20]**				
2×2 table		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA (IgM) Serum
	Index test +	64	26	90	
	Index test −	53	789	842	
	Total	117	815	932	
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA (IgG) Serum
	Index test +	51	14	65	
	Index test −	66	801	867	
	Total	117	815	932	
Statistical measures	Index test: ELISA IgM (serum) - neuroborreliosis Sensitivity 0.55 Specificity 0.97 Index test: ELISA IgG (serum) - neuroborreliosis Sensitivity 0.44 Specificity 0.98				
Source of funding	Not reported				
Limitations	Risk of bias: index test, reference standard Indirectness: none				

Reference	Dressler 1993[21]
Study type	Case-control
Study methodology	Data source: retrospective study – frozen samples from a serum bank, prospective study – patients evaluated in a Lyme disease clinic Recruitment: retrospective study – first 25 patients with EM, meningitis, arthritis, encephalopathy or polyneuropathy due to Lyme disease in alphabetical order (cases; control recruitment not described) Prospective study – consecutive
Number of patients	n = 154 cases (100 retrospective study, 54 prospective study), 264 controls (125 retrospective study, 139 prospective study)
Patient characteristics	Age, mean (SD): not reported Gender (male to female ratio): not reported Family origin: not reported Setting: Lyme disease clinic Country: USA Cases: retrospective study – EM (25), meningitis (25), arthritis (25), encephalopathy or polyneuropathy due to Lyme disease (25), those with EM, meningitis and arthritis had not received antibiotic therapy, half of those with encephalopathy or polyneuropathy had previously had antibiotics Prospective study: those meeting clinical criteria for Lyme disease, Lyme arthritis – brief attacks of oligoarticular arthritis in a few large joints not caused by other known types of arthritis in a person from an endemic area with objective evidence of joint inflammation at the time of evaluation (25), neuroborreliosis – meningeal signs, memory impairment or sensory abnormalities accompanied by CSF pleocytosis, increased CSF protein or electromyographic evidence of an axonal polyneuropathy not caused by other known diseases in a person from an endemic area (29) Controls: retrospective study – patients who had participated in a flu vaccination program (25), multiple sclerosis (15), amyotrophic lateral sclerosis (10), rheumatoid arthritis (15), systemic lupus erythematosus (10), chronic fatigue syndrome (25), syphilis (25) Prospective study: those not meeting clinical criteria for Lyme disease, fibromyalgia (32), other rheumatic illnesses (62), other neurologic illnesses (45)

Reference	Dressler 1993[21]				
Target condition(s)	Lyme disease				
Index test(s) and reference standard	Index test(s) Immunoblot (IgM) Immunoblot (IgG) ELISA (IgG) Reference standard Clinical diagnosis Time between measurement of index test and reference standard: not reported				
2×2 table		Reference standard +	Reference standard −	Total	EM (acute) Immunoblot (IgM) Serum Retrospective study
	Index test +	10	1	11	
	Index test −	15	124	139	
	Total	25	125	150	
		Reference standard +	Reference standard −	Total	EM (convalescent) Immunoblot (IgM) Serum Retrospective study
	Index test +	15	1	16	
	Index test −	10	124	134	
	Total	25	125	150	
		Reference standard +	Reference standard −	Total	EM (acute) Immunoblot (IgG) Serum Retrospective study
	Index test +	0	0	0	
	Index test −	25	125	150	
	Total	25	125	150	
		Reference standard +	Reference standard −	Total	EM (convalescent) Immunoblot (IgG)
	Index test +	4	0	4	

Reference	**Dressler 1993[21]**				
	Index test −	21	125	146	Serum Retrospective study
	Total	25	125	150	
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA (IgG)
	Index test +	17	5	22	Serum Prospective study
	Index test −	12	134	146	
	Total	29	139	168	
		Reference standard +	Reference standard −	Total	Neuroborreliosis Immunoblot (IgG)
	Index test +	21	7	28	Serum Prospective study
	Index test −	8	132	140	
	Total	29	139	168	
		Reference standard +	Reference standard −	Total	Arthritis ELISA (IgG)
	Index test +	22	5	27	Serum Prospective study
	Index test −	3	134	137	
	Total	25	139	164	
		Reference standard +	Reference standard −	Total	Arthritis Immunoblot (IgG)
	Index test +	24	7	31	Serum Prospective study
	Index test −	1	132	133	
	Total	25	139	164	
Statistical measures	Index test: Immunoblot IgM (serum) – acute EM (retrospective study) Sensitivity 0.40 Specificity 0.99 Index test: Immunoblot IgM (serum) – convalescent EM (retrospective study) Sensitivity 0.60 Specificity 0.99				

Reference	Dressler 1993[21]
	Index test: Immunoblot IgG (serum) – acute EM (retrospective study) Sensitivity 0.00 Specificity 1.00 Index test: Immunoblot IgG (serum) – convalescent EM (retrospective study) Sensitivity 0.16 Specificity 1.00 Index test: ELISA IgG (serum) – neuroborreliosis (prospective study) Sensitivity 0.59 Specificity 0.96 Index test: Immunoblot IgG (serum) – neuroborreliosis (prospective study) Sensitivity 0.72 Specificity 0.95 Index test: ELISA IgG (serum) –arthritis (prospective study) Sensitivity 0.88 Specificity 0.96 Index test: Immunoblot IgG (serum) –arthritis (prospective study) Sensitivity 0.96 Specificity 0.95
Source of funding	National Institutes of Health, Esche fund, Deutsche Forschungsgemeinschaft, Beckton Dickinson
Limitations	Risk of bias: selection, index test, reference standard Indirectness: none
Comments	Time point: mean 8 days after onset of symptoms for EM patients

Reference	Fallon 2014[22]
Study type	Case-control
Study methodology	Data source: specimens obtained during the conduct of 2 research protocols on individuals with post-treatment Lyme syndrome and controls Recruitment: not reported
Number of patients	n = 37 cases, 40 controls
Patient characteristics	Age, mean (SD): cases 46.5 years (10.5), controls 43.9 years (11.7) Gender (male to female ratio): cases 13:24, controls 16:24 Family origin: not reported Setting: 4 laboratories Country: USA Cases: patients with post-treatment Lyme syndrome with historical evidence meeting CDC criteria for Lyme disease Controls: no history of prior diagnosis or treatment for Lyme disease, no history of Lyme-like symptoms or illness, no history of another major neurologic or medical disorder, residence in non-endemic area and no recent exposure to a highly Lyme endemic area
Target condition(s)	Lyme disease
Index test(s) and reference standard	Index test(s) ELISA (IgM/IgG) Immunoblot (IgM) Immunoblot (IgG) Reference standard Clinical diagnosis (CDC criteria)

Reference	Fallon 2014[22]				
	Time between measurement of index test and reference standard: not reported				
2×2 table		Reference standard +	Reference standard −	Total	PTLDS ELISA (IgM/IgG) Serum Commercial lab
	Index test +	25	3	28	
	Index test −	12	37	49	
	Total	37	40	77	
		Reference standard +	Reference standard −	Total	PTLDS ELISA (IgM/IgG) Serum Speciality lab A
	Index test +	25	1	26	
	Index test −	12	39	51	
	Total	37	40	77	
		Reference standard +	Reference standard −	Total	PTLDS ELISA (IgM/IgG) Serum Speciality lab B
	Index test +	25	3	28	
	Index test −	12	37	49	
	Total	37	40	77	
		Reference standard +	Reference standard −	Total	PTLDS ELISA (IgM/IgG) Serum University reference lab
	Index test +	23	5	28	
	Index test −	14	35	39	
	Total	37	40	77	
		Reference standard +	Reference standard −	Total	PTLDS Immunoblot (IgM) Serum Commercial lab
	Index test +	6	0	6	
	Index test −	31	40	71	
	Total	37	40	77	
		Reference standard +	Reference standard −	Total	PTLDS

Reference	**Fallon 2014[22]**				
	Index test +	1	1	2	Immunoblot (IgM) Serum Speciality lab A
	Index test −	36	39	75	
	Total	37	40	77	
		Reference standard +	Reference standard −	Total	PTLDS Immunoblot (IgM)
	Index test +	16	8	24	Serum Speciality lab B
	Index test −	21	32	53	
	Total	37	40	77	
		Reference standard +	Reference standard −	Total	PTLDS Immunoblot (IgM)
	Index test +	8	5	13	Serum University reference lab
	Index test −	29	35	64	
	Total	37	40	77	
		Reference standard +	Reference standard −	Total	PTLDS Immunoblot (IgG)
	Index test +	16	0	16	Serum Commercial lab
	Index test −	21	40	61	
	Total	37	40	77	
		Reference standard +	Reference standard −	Total	PTLDS Immunoblot (IgG)
	Index test +	16	0	16	Serum Speciality lab A
	Index test −	21	40	61	
	Total	37	40	77	
		Reference standard +	Reference standard −	Total	PTLDS Immunoblot (IgG)
	Index test +	18	3	21	Serum Speciality lab B
	Index test −	19	37	56	
	Total	37	40	77	
		Reference standard	Reference standard −	Total	PTLDS

Reference	Fallon 2014[22]				
		+			Immunoblot (IgG) Serum University reference lab
	Index test +	21	1	22	
	Index test −	16	39	55	
	Total	37	40	77	
Statistical measures	Index test: ELISA IgM/IgG (serum) – PTLDS (commercial lab) Sensitivity 0.68 Specificity 0.93 Index test: ELISA IgM/IgG (serum) – PTLDS (speciality lab A) Sensitivity 0.68 Specificity 0.97 Index test: ELISA IgM/IgG (serum) – PTLDS (speciality lab B) Sensitivity 0.68 Specificity 0.93 Index test: ELISA IgM/IgG (serum) – PTLDS (university reference lab) Sensitivity 0.62 Specificity 0.88 Index test: Immunoblot IgM (serum) – PTLDS (commercial lab) Sensitivity 0.16 Specificity 1.00 Index test: Immunoblot IgM (serum) – PTLDS (speciality lab A) Sensitivity 0.03 Specificity 0.97 Index test: Immunoblot IgM (serum) – PTLDS (speciality lab B) Sensitivity 0.43 Specificity 0.80				

Reference	Fallon 2014[22]
	Index test: Immunoblot IgM (serum) – PTLDS (university reference lab) Sensitivity 0.22 Specificity 0.88 Index test: Immunoblot IgG (serum) – PTLDS (commercial lab) Sensitivity 0.43 Specificity 1.00 Index test: Immunoblot IgG (serum) – PTLDS (speciality lab A) Sensitivity 0.43 Specificity 1.00 Index test: Immunoblot IgG (serum) – PTLDS (speciality lab B) Sensitivity 0.49 Specificity 0.93 Index test: Immunoblot IgG (serum) – PTLDS (university reference lab) Sensitivity 0.57 Specificity 0.97
Source of funding	Lyme Research Alliance Inc, the Lyme Disease association Inc, the Lyme and Tick-borne Diseases Research Center
Limitations	Risk of bias: selection, reference standard Indirectness: none
Comments	Borderline results counted as positive

Reference	Flisiak 1996[23]
Study type	Case-control
Study methodology	Data source: patients of Department of Infectious Diseases and healthy volunteers Recruitment: not reported
Number of patients	n = 42 cases, 27 controls
Patient characteristics	Age, mean (SD): not reported Gender (male to female ratio): not reported Family origin: not reported Setting: Department of Infectious Diseases Country: Poland Cases: diagnosed Lyme disease – EM (18), arthritis (7), neuroborreliosis (17) Controls: healthy volunteers without clinical signs of Lyme disease
Target condition(s)	Lyme disease
Index test(s) and reference standard	Index test(s) ELISA (IgM) ELISA (IgG) ELISA (IgM/IgG) ELFA Reference standard Clinical diagnosis (CDC criteria)

	Time between measurement of index test and reference standard: not reported				
2×2 table		Reference standard +	Reference standard −	Total	EM ELISA (IgM) (flagella) Serum
	Index test +	11	4	15	
	Index test −	7	23	30	
	Total	18	27	45	
		Reference standard +	Reference standard −	Total	EM ELISA (IgM) (recombinant) Serum
	Index test +	7	8	15	
	Index test −	11	19	30	
	Total	18	27	45	
		Reference standard +	Reference standard −	Total	EM ELISA (IgG) (flagella) Serum
	Index test +	2	0	2	
	Index test −	16	27	43	
	Total	18	27	45	
		Reference standard +	Reference standard −	Total	EM ELISA (IgG) (recombinant) Serum
	Index test +	6	0	6	
	Index test −	12	27	39	
	Total	18	27	45	
		Reference standard +	Reference standard −	Total	EM ELISA (IgM/IgG) (flagella) Serum
	Index test +	12	4	16	
	Index test −	6	23	29	
	Total	18	27	45	
		Reference standard +	Reference standard −	Total	EM ELISA (IgM/IgG) (recombinant) Serum
	Index test +	13	8	21	
	Index test −	5	19	24	
	Total	18	27	45	
		Reference standard +	Reference standard −	Total	EM ELFA Serum
	Index test +	11	2	13	
	Index test −	7	25	32	

	Total	18	27	45	
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA (IgM) (flagella) Serum
	Index test +	12	4	16	
	Index test −	5	23	28	
	Total	17	27	44	
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA (IgM) (recombinant) Serum
	Index test +	12	8	20	
	Index test −	5	19	24	
	Total	17	27	44	
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA (IgG) (flagella) Serum
	Index test +	6	0	6	
	Index test −	11	27	38	
	Total	17	27	44	
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA (IgG) (recombinant) Serum
	Index test +	5	0	5	
	Index test −	12	27	39	
	Total	17	27	44	
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA (IgM/IgG) (flagella) Serum
	Index test +	15	4	19	
	Index test −	2	23	25	
	Total	17	27	44	
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA (IgM/IgG) (recombinant) Serum
	Index test +	14	8	22	
	Index test −	3	19	22	
	Total	17	27	44	
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELFA Serum
	Index test +	16	2	18	
	Index test −	1	25	26	
	Total	17	27	44	

		Reference standard +	Reference standard −	Total	Arthritis ELISA (IgM) (flagella) Serum
	Index test +	5	4	9	
	Index test −	2	23	25	
	Total	7	27	34	
		Reference standard +	Reference standard −	Total	Arthritis ELISA (IgM) (recombinant) Serum
	Index test +	7	8	15	
	Index test −	0	19	19	
	Total	7	27	34	
		Reference standard +	Reference standard −	Total	Arthritis ELISA (IgG) (flagella) Serum
	Index test +	1	0	1	
	Index test −	6	27	33	
	Total	7	27	34	
		Reference standard +	Reference standard −	Total	Arthritis ELISA (IgG) (recombinant) Serum
	Index test +	0	0	0	
	Index test −	7	27	34	
	Total	7	27	34	
		Reference standard +	Reference standard −	Total	Arthritis ELISA (IgM/IgG) (flagella) Serum
	Index test +	5	4	9	
	Index test −	2	23	25	
	Total	7	27	34	
		Reference standard +	Reference standard −	Total	Arthritis ELISA (IgM/IgG) (recombinant) Serum
	Index test +	7	8	15	
	Index test −	0	19	19	
	Total	7	27	34	
		Reference standard +	Reference standard −	Total	Arthritis ELFA Serum
	Index test +	6	2	8	
	Index test −	1	25	26	
	Total	7	27	34	
		Reference standard +	Reference standard −	Total	Unspecified Lyme disease

	Index test +	27	4	31	ELISA (IgM) (flagella) Serum
	Index test −	15	23	38	
	Total	42	27	69	
		Reference standard +	Reference standard −	Total	Unspecified Lyme disease ELISA (IgM) (recombinant) Serum
	Index test +	25	8	33	
	Index test −	17	19	36	
	Total	42	27	69	
		Reference standard +	Reference standard −	Total	Unspecified Lyme disease ELISA (IgG) (flagella) Serum
	Index test +	10	0	10	
	Index test −	32	27	59	
	Total	42	27	69	
		Reference standard +	Reference standard −	Total	Unspecified Lyme disease ELISA (IgG) (recombinant) Serum
	Index test +	12	0	12	
	Index test −	30	27	57	
	Total	42	27	69	
		Reference standard +	Reference standard −	Total	Unspecified Lyme disease ELISA (IgM/IgG) (flagella) Serum
	Index test +	32	4	36	
	Index test −	10	23	33	
	Total	42	27	69	
		Reference standard +	Reference standard −	Total	Unspecified Lyme disease ELISA (IgM/IgG) (recombinant) Serum
	Index test +	34	8	42	
	Index test −	8	19	27	
	Total	42	27	69	
		Reference standard +	Reference standard −	Total	Unspecified Lyme disease ELFA Serum
	Index test +	33	2	35	
	Index test −	9	25	34	
	Total	42	27	69	
Statistical measures	Index test: ELISA IgM (flagella) (serum) - EM Sensitivity 0.61 Specificity 0.85				

	Index test: ELISA IgM (recombinant) (serum) - EM Sensitivity 0.39 Specificity 0.70 Index test: ELISA IgG (flagella) (serum) - EM Sensitivity 0.11 Specificity 1.00 Index test: ELISA IgG (recombinant) (serum) - EM Sensitivity 0.33 Specificity 1.00 Index test: ELISA IgM/IgG (flagella) (serum) - EM Sensitivity 0.67 Specificity 0.85 Index test: ELISA IgM/IgG (recombinant) (serum) - EM Sensitivity 0.72 Specificity 0.70 Index test: ELFA (serum) - EM Sensitivity 0.61 Specificity 0.93 Index test: ELISA IgM (flagella) (serum) - neuroborreliosis Sensitivity 0.71 Specificity 0.85 Index test: ELISA IgM (recombinant) (serum) - neuroborreliosis Sensitivity 0.71 Specificity 0.70

	Index test: ELISA IgG (flagella) (serum) - neuroborreliosis Sensitivity 0.35 Specificity 1.00 Index test: ELISA IgG (recombinant) (serum) - neuroborreliosis Sensitivity 0.29 Specificity 1.00 Index test: ELISA IgM/IgG (flagella) (serum) - neuroborreliosis Sensitivity 0.88 Specificity 0.85 Index test: ELISA IgM/IgG (recombinant) (serum) - neuroborreliosis Sensitivity 0.82 Specificity 0.70 Index test: ELFA (serum) - neuroborreliosis Sensitivity 0.94 Specificity 0.93 Index test: ELISA IgM (flagella) (serum) - arthritis Sensitivity 0.71 Specificity 0.85 Index test: ELISA IgM (recombinant) (serum) - arthritis Sensitivity 1.00 Specificity 0.70 Index test: ELISA IgG (flagella) (serum) - arthritis Sensitivity 0.14 Specificity 1.00 Index test: ELISA IgG (recombinant) (serum) - arthritis

	Sensitivity 0.00 Specificity 1.00 Index test: ELISA IgM/IgG (flagella) (serum) - arthritis Sensitivity 0.71 Specificity 0.85 Index test: ELISA IgM/IgG (recombinant) (serum) - arthritis Sensitivity 1.00 Specificity 0.70 Index test: ELFA (serum) - arthritis Sensitivity 0.86 Specificity 0.93 Index test: ELISA IgM (flagella) (serum) – unspecified Lyme disease Sensitivity 0.64 Specificity 0.85 Index test: ELISA IgM (recombinant) (serum) - unspecified Lyme disease Sensitivity 0.60 Specificity 0.70 Index test: ELISA IgG (flagella) (serum) - unspecified Lyme disease Sensitivity 0.24 Specificity 1.00 Index test: ELISA IgG (recombinant) (serum) - unspecified Lyme disease Sensitivity 0.29 Specificity 1.00 Index test: ELISA IgM/IgG (flagella) (serum) - unspecified Lyme disease Sensitivity 0.76

	Specificity 0.85 Index test: ELISA IgM/IgG (recombinant) (serum) - unspecified Lyme disease Sensitivity 0.81 Specificity 0.70 Index test: ELFA (serum) - unspecified Lyme disease Sensitivity 0.79 Specificity 0.93
Source of funding	Not reported
Limitations	Risk of bias: selection, index test, reference standard Indirectness:

Reference	Flisiak 1998[24]
Study type	Case-control
Study methodology	Data source: patients of the Department of Infectious Diseases and healthy volunteers Recruitment: not reported
Number of patients	n = 48 cases, 26 controls
Patient characteristics	Age, mean (SD): not reported Gender (male to female ratio): cases 23:25, controls 8:18 Family origin: not reported Setting: Department of Infectious Diseases Country: Poland Cases: diagnosed Lyme disease – EM (19), Lyme arthritis (21), early neuroborreliosis (8) Controls: healthy volunteers from the same region
Target condition(s)	Lyme disease
Index test(s) and reference standard	Index test(s) ELISA (IgM) ELISA (IgG) ELISA (IgM/IgG) ELFA Immunoblot (IgG) Reference standard Clinical diagnosis (CDC criteria)

Reference	Flisiak 1998[24]				
	Time between measurement of index test and reference standard: diagnosis at the time of blood collection				
2×2 table		Reference standard +	Reference standard −	Total	Unspecified Lyme disease ELISA (IgM) Serum
	Index test +	28	3	31	
	Index test −	20	23	43	
	Total	48	26	74	
		Reference standard +	Reference standard −	Total	Unspecified Lyme disease ELISA (IgG) Serum
	Index test +	22	0	22	
	Index test −	26	26	52	
	Total	48	26	74	
		Reference standard +	Reference standard −	Total	Unspecified Lyme disease ELISA (IgM/IgG) Serum
	Index test +	37	3	40	
	Index test −	11	23	34	
	Total	48	26	74	
		Reference standard +	Reference standard −	Total	Unspecified Lyme disease ELFA Serum
	Index test +	39	2	41	
	Index test −	9	24	33	
	Total	48	26	74	
		Reference standard +	Reference standard −	Total	Unspecified Lyme disease Immunoblot (IgG) Serum
	Index test +	24	0	24	
	Index test −	24	26	50	
	Total	48	26	74	
Statistical measures	Index test: ELISA IgM (serum) – unspecified Lyme disease				

Reference	Flisiak 1998[24]
	Sensitivity 0.58 Specificity 0.88 Index test: ELISA IgG (serum) – unspecified Lyme disease Sensitivity 0.46 Specificity 1.00 Index test: ELISA IgM/IgG (serum) – unspecified Lyme disease Sensitivity 0.77 Specificity 0.88 Index test: ELFA (serum) – unspecified Lyme disease Sensitivity 0.81 Specificity 0.92 Index test: Immunoblot IgG (serum) – unspecified Lyme disease Sensitivity 0.50 Specificity 1.00
Source of funding	Not reported
Limitations	Risk of bias: selection, index test, reference standard Indirectness:

Reference	Fung 1994[25]
Study type	Case-control
Study methodology	Data source: serum bank containing samples from patients with Lyme disease Recruitment: alphabetical order
Number of patients	n = 189 cases, 106 controls
Patient characteristics	Age, mean (SD): not reported Gender (male to female ratio): not reported Family origin: not reported Setting: Lyme disease clinic Country: USA Cases: patients with Lyme disease - EM (75), meningitis/facial palsy (40), arthritis (49), chronic neuroborreliosis (25) Controls: patients who had recently had influenza vaccinations (15), multiple sclerosis (12), amyotrophic lateral sclerosis (9), rheumatoid arthritis (12), systemic lupus erythematosus (9), chronic fatigue syndrome (19), syphilis (30)
Target condition(s)	Lyme disease
Index test(s) and reference standard	Index test(s) ELISA (IgM) ELISA (IgG) ELISA (IgM/IgG) Immunoblot (IgM) Immunoblot (IgG) Immunoblot (IgM/IgG)

Reference	Fung 1994[25]				
	Reference standard Clinical diagnosis Time between measurement of index test and reference standard: not reported				
2×2 table		Reference standard +	Reference standard −	Total	EM (acute disseminated) ELISA (IgM) Serum
	Index test +	36	2	38	
	Index test −	23	104	127	
	Total	59	106	165	
		Reference standard +	Reference standard −	Total	EM (acute localised) ELISA (IgM) Serum
	Index test +	4	2	6	
	Index test −	12	104	116	
	Total	16	106	122	
		Reference standard +	Reference standard −	Total	EM (convalescent disseminated) ELISA (IgM) Serum
	Index test +	47	2	49	
	Index test −	12	104	116	
	Total	59	106	165	
		Reference standard +	Reference standard −	Total	EM (convalescent localised) ELISA (IgM) Serum
	Index test +	8	2	10	
	Index test −	8	104	112	
	Total	16	106	122	
		Reference standard +	Reference standard −	Total	EM (acute disseminated) ELISA (IgG) Serum
	Index test +	20	15	35	
	Index test −	39	91	130	
	Total	59	106	165	

Reference	Fung 1994[25]				
		Reference standard +	Reference standard −	Total	EM (acute localised) ELISA (IgG) Serum
	Index test +	5	15	20	
	Index test −	11	91	102	
	Total	16	106	122	
		Reference standard +	Reference standard −	Total	EM (convalescent disseminated) ELISA (IgG) Serum
	Index test +	30	15	45	
	Index test −	29	91	120	
	Total	59	106	165	
		Reference standard +	Reference standard −	Total	EM (convalescent localised) ELISA (IgG) Serum
	Index test +	7	15	22	
	Index test −	9	91	100	
	Total	16	106	122	
		Reference standard +	Reference standard −	Total	EM (acute disseminated) ELISA (IgM/IgG) Serum
	Index test +	36	16	52	
	Index test −	23	90	113	
	Total	59	106	165	
		Reference standard +	Reference standard −	Total	EM (acute localised) ELISA (IgM/IgG) Serum
	Index test +	6	16	22	
	Index test −	10	90	100	
	Total	16	106	122	
		Reference standard +	Reference standard −	Total	EM (convalescent disseminated) ELISA (IgM/IgG) Serum
	Index test +	48	16	64	
	Index test −	11	90	101	

Reference	**Fung 1994[25]**				
	Total	59	106	165	
		Reference standard +	Reference standard −	Total	EM (convalescent localised) ELISA (IgM/IgG) Serum
	Index test +	10	16	26	
	Index test −	6	90	96	
	Total	16	106	122	
		Reference standard +	Reference standard −	Total	EM (acute) Immunoblot (IgM) Serum
	Index test +	44	2	46	
	Index test −	31	104	135	
	Total	75	106	181	
		Reference standard +	Reference standard −	Total	EM (convalescent) Immunoblot (IgM) Serum
	Index test +	55	2	57	
	Index test −	20	104	124	
	Total	75	106	181	
		Reference standard +	Reference standard −	Total	EM (acute) Immunoblot (IgG) Serum
	Index test +	35	6	41	
	Index test −	40	100	140	
	Total	75	106	181	
		Reference standard +	Reference standard −	Total	EM (convalescent) Immunoblot (IgG) Serum
	Index test +	43	6	49	
	Index test −	32	100	132	
	Total	75	106	181	
		Reference standard +	Reference standard −	Total	EM (acute) Immunoblot (IgM/IgG)
	Index test +	49	8	57	

Reference	**Fung 1994[25]**				
	Index test −	26	98	124	Serum
	Total	75	106	181	
		Reference standard +	Reference standard −	Total	EM (convalescent) Immunoblot (IgM/IgG)
	Index test +	60	8	68	Serum
	Index test −	15	98	113	
	Total	75	106	181	
		Reference standard +	Reference standard −	Total	Chronic neuroborreliosis ELISA (IgM)
	Index test +	5	2	7	Serum
	Index test −	20	104	124	
	Total	25	106	131	
		Reference standard +	Reference standard −	Total	Meningitis/facial palsy ELISA (IgM)
	Index test +	29	2	31	Serum
	Index test −	11	104	115	
	Total	40	106	146	
		Reference standard +	Reference standard −	Total	Chronic neuroborreliosis ELISA (IgG)
	Index test +	9	15	24	Serum
	Index test −	16	91	107	
	Total	25	106	131	
		Reference standard +	Reference standard −	Total	Meningitis/facial palsy ELISA (IgG)
	Index test +	26	15	41	Serum
	Index test −	14	91	105	
	Total	40	106	146	
		Reference standard +	Reference standard −	Total	Chronic neuroborreliosis

Reference	**Fung 1994[25]**				
	Index test +	12	16	28	ELISA (IgM/IgG) Serum
	Index test −	13	90	103	
	Total	25	106	131	
		Reference standard +	Reference standard −	Total	Meningitis/facial palsy ELISA (IgM/IgG)
	Index test +	35	16	51	Serum
	Index test −	5	90	95	
	Total	40	106	146	
		Reference standard +	Reference standard −	Total	Arthritis ELISA (IgM)
	Index test +	22	2	24	Serum
	Index test −	27	104	131	
	Total	49	106	155	
		Reference standard +	Reference standard −	Total	Arthritis ELISA (IgG)
	Index test +	41	15	56	Serum
	Index test −	8	91	99	
	Total	49	106	155	
		Reference standard +	Reference standard −	Total	Arthritis ELISA (IgM/IgG)
	Index test +	43	16	59	Serum
	Index test −	6	90	96	
	Total	49	106	155	
Statistical measures	Index test: ELISA IgM (serum) – EM (acute disseminated) Sensitivity 0.61 Specificity 0.98 Index test: ELISA IgM (serum) – EM (acute localised) Sensitivity 0.25				

Reference	Fung 1994[25]
	Specificity 0.98 Index test: ELISA IgM (serum) – EM (convalescent disseminated) Sensitivity 0.80 Specificity 0.98 Index test: ELISA IgM (serum) – EM (convalescent localised) Sensitivity 0.50 Specificity 0.98 Index test: ELISA IgG (serum) – EM (acute disseminated) Sensitivity 0.34 Specificity 0.86 Index test: ELISA IgG (serum) – EM (acute localised) Sensitivity 0.31 Specificity 0.86 Index test: ELISA IgG (serum) – EM (convalescent disseminated) Sensitivity 0.51 Specificity 0.86 Index test: ELISA IgG (serum) – EM (convalescent localised) Sensitivity 0.44 Specificity 0.86 Index test: ELISA IgM/IgG (serum) – EM (acute disseminated) Sensitivity 0.61 Specificity 0.85 Index test: ELISA IgM/IgG (serum) – EM (acute localised)

Reference	Fung 1994[25]
	Sensitivity 0.38 Specificity 0.85 Index test: ELISA IgM/IgG (serum) – EM (convalescent disseminated) Sensitivity 0.81 Specificity 0.85 Index test: ELISA IgM/IgG (serum) – EM (convalescent localised) Sensitivity 0.63 Specificity 0.85 Index test: Immunoblot IgM (serum) – EM (acute) Sensitivity 0.59 Specificity 0.98 Index test: Immunoblot IgM (serum) – EM (convalescent) Sensitivity 0.73 Specificity 0.98 Index test: Immunoblot IgG (serum) – EM (acute) Sensitivity 0.47 Specificity 0.94 Index test: Immunoblot IgG (serum) – EM (convalescent) Sensitivity 0.57 Specificity 0.94 Index test: Immunoblot IgM/IgG (serum) – EM (acute) Sensitivity 0.65 Specificity 0.92

Reference	Fung 1994[25]
	Index test: Immunoblot IgM/IgG (serum) – EM (convalescent) Sensitivity 0.80 Specificity 0.92 Index test: ELISA IgM (serum) – chronic neuroborreliosis Sensitivity 0.20 Specificity 0.98 Index test: ELISA IgM (serum) – meningitis/facial palsy Sensitivity 0.72 Specificity 0.98 Index test: ELISA IgG (serum) – chronic neuroborreliosis Sensitivity 0.36 Specificity 0.86 Index test: ELISA IgG (serum) – meningitis/facial palsy Sensitivity 0.65 Specificity 0.86 Index test: ELISA IgM/IgG (serum) – chronic neuroborreliosis Sensitivity 0.48 Specificity 0.85 Index test: ELISA IgM/IgG (serum) – meningitis/facial palsy Sensitivity 0.88 Specificity 0.85 Index test: ELISA IgM (serum) – arthritis Sensitivity 0.45 Specificity 0.98

Reference	Fung 1994[25]
	Index test: ELISA IgG (serum) – arthritis Sensitivity 0.84 Specificity 0.86 Index test: ELISA IgM/IgG (serum) – arthritis Sensitivity 0.88 Specificity 0.85
Source of funding	National Institutes of Health, the Eshe Fund
Limitations	Risk of bias: selection, index test, reference standard Indirectness: none

Reference	Goettner 2005[27]
Study type	Case-control
Study methodology	Data source: clinically well-defined cases – EM patients enrolled in a borreliosis treatment study, source of patients with other presentations not reported, blood donors Recruitment: not reported
Number of patients	n = 85 cases, 110 controls
Patient characteristics	Age, mean (SD): not reported Gender (male to female ratio): not reported Family origin: not reported Setting: not reported Country: Germany Cases: clinically well-defined cases – EM (15), neuroborreliosis with intrathecal antibody production (50), ACA (10), arthritis (10) Controls: blood donors without actual or anamnestic dermatological, rheumatological or neurological disease or history of a tick bite (60), syphilis patients (10), patients who were positive for rheumatoid factor (10), patients with fever of unknown origin (30)
Target condition(s)	Lyme disease
Index test(s) and reference standard	Index test(s) Immunoblot (IgM) Immunoblot (IgG) Reference standard Clinical diagnosis

Reference	Goettner 2005[27]				
	Time between measurement of index test and reference standard: median time period between appearance of EM and collection of serum was 8 weeks (range 3-24 weeks), other presentations not reported				
2×2 table		Reference standard +	Reference standard −	Total	EM Immunoblot (IgM) line blot Serum
	Index test +	11	1	12	
	Index test −	4	109	113	
	Total	15	110	125	
		Reference standard +	Reference standard −	Total	EM Immunoblot (IgM) line blot plus additional antigens Serum
	Index test +	13	2	15	
	Index test −	2	108	110	
	Total	15	110	125	
		Reference standard +	Reference standard −	Total	EM Immunoblot (IgM) western blot Serum
	Index test +	6	2	8	
	Index test −	9	108	117	
	Total	15	110	125	
		Reference standard +	Reference standard −	Total	EM Immunoblot (IgG) line blot Serum
	Index test +	7	0	7	
	Index test −	8	110	118	
	Total	15	110	125	
		Reference standard +	Reference standard −	Total	EM Immunoblot (IgG) line blot plus additional antigens Serum
	Index test +	8	1	9	
	Index test −	7	109	116	
	Total	15	110	125	
		Reference standard	Reference standard −	Total	EM

Reference	**Goettner 2005**[27]				
		+			Immunoblot (IgG) western blot Serum
	Index test +	5	1	6	
	Index test −	10	109	119	
	Total	15	110	125	
		Reference standard +	Reference standard −	Total	ACA Immunoblot (IgG) line blot Serum
	Index test +	10	0	10	
	Index test −	0	110	110	
	Total	10	110	120	
		Reference standard +	Reference standard −	Total	ACA Immunoblot (IgG) line blot plus additional antigens Serum
	Index test +	10	1	11	
	Index test −	0	109	109	
	Total	10	110	120	
		Reference standard +	Reference standard −	Total	ACA Immunoblot (IgG) western blot Serum
	Index test +	9	1	10	
	Index test −	1	109	110	
	Total	10	110	120	
		Reference standard +	Reference standard −	Total	Neuroborreliosis Immunoblot (IgM) line blot Serum
	Index test +	23	1	24	
	Index test −	27	109	136	
	Total	50	110	160	
		Reference standard +	Reference standard −	Total	Neuroborreliosis Immunoblot (IgM) line blot plus additional antigens Serum
	Index test +	35	2	37	
	Index test −	15	108	123	
	Total	50	110	160	

Reference	Goettner 2005[27]				
		Reference standard +	Reference standard −	Total	Neuroborreliosis Immunoblot (IgM) western blot Serum
	Index test +	20	2	22	
	Index test −	30	108	138	
	Total	50	110	160	
		Reference standard +	Reference standard −	Total	Neuroborreliosis Immunoblot (IgG) line blot Serum
	Index test +	43	0	43	
	Index test −	7	110	117	
	Total	50	110	160	
		Reference standard +	Reference standard −	Total	Neuroborreliosis Immunoblot (IgG) line blot plus additional antigens Serum
	Index test +	44	1	45	
	Index test −	6	109	115	
	Total	50	110	160	
		Reference standard +	Reference standard −	Total	Neuroborreliosis Immunoblot (IgG) western blot Serum
	Index test +	36	1	37	
	Index test −	14	109	123	
	Total	50	110	160	
		Reference standard +	Reference standard −	Total	Arthritis Immunoblot (IgG) line blot Serum
	Index test +	9	0	9	
	Index test −	1	110	111	
	Total	10	110	120	
		Reference standard +	Reference standard −	Total	Arthritis Immunoblot (IgG) line blot plus additional antigens Serum
	Index test +	10	1	11	
	Index test −	0	109	109	

Reference	**Goettner 2005[27]**				
	Total	10	110	120	
		Reference standard +	Reference standard −	Total	Arthritis Immunoblot (IgG) western blot Serum
	Index test +	10	1	11	
	Index test −	0	109	109	
	Total	10	110	120	
		Reference standard +	Reference standard −	Total	Unspecified Lyme disease Immunoblot (IgG) line blot Serum
	Index test +	69	0	69	
	Index test −	16	110	126	
	Total	85	110	195	
		Reference standard +	Reference standard −	Total	Unspecified Lyme disease Immunoblot (IgG) line blot plus additional antigens Serum
	Index test +	72	1	73	
	Index test −	13	109	122	
	Total	85	110	195	
		Reference standard +	Reference standard −	Total	Unspecified Lyme disease Immunoblot (IgG) western blot Serum
	Index test +	60	1	61	
	Index test −	25	109	134	
	Total	85	110	195	
Statistical measures	Index test: Immunoblot IgM line blot (serum) - EM Sensitivity 0.73 Specificity 0.99 Index test: Immunoblot IgM line blot plus (serum) - EM Sensitivity 0.87 Specificity 0.98 Index test: Immunoblot IgM western blot (serum) – EM				

Reference	Goettner 2005[27]
	Sensitivity 0.40 Specificity 0.98 Index test: Immunoblot IgG line blot (serum) - EM Sensitivity 0.47 Specificity 1.00 Index test: Immunoblot IgG line blot plus (serum) - EM Sensitivity 0.53 Specificity 0.99 Index test: Immunoblot IgG western blot (serum) – EM Sensitivity 0.33 Specificity 0.99 Index test: Immunoblot IgG line blot (serum) - ACA Sensitivity 1.00 Specificity 1.00 Index test: Immunoblot IgG line blot plus (serum) - ACA Sensitivity 1.00 Specificity 0.99 Index test: Immunoblot IgG western blot (serum) – ACA Sensitivity 0.90 Specificity 0.99 Index test: Immunoblot IgM line blot (serum) - neuroborreliosis Sensitivity 0.46 Specificity 0.99

Reference	Goettner 2005[27]
	Index test: Immunoblot IgM line blot plus (serum) - neuroborreliosis Sensitivity 0.70 Specificity 0.98 Index test: Immunoblot IgM western blot (serum) – neuroborreliosis Sensitivity 0.40 Specificity 0.98 Index test: Immunoblot IgG line blot (serum) - neuroborreliosis Sensitivity 0.86 Specificity 1.00 Index test: Immunoblot IgG line blot plus (serum) - neuroborreliosis Sensitivity 0.88 Specificity 0.99 Index test: Immunoblot IgG western blot (serum) – neuroborreliosis Sensitivity 0.72 Specificity 0.99 Index test: Immunoblot IgG line blot (serum) - arthritis Sensitivity 0.90 Specificity 1.00 Index test: Immunoblot IgG line blot plus (serum) – arthritis Sensitivity 1.00 Specificity 0.99 Index test: Immunoblot IgG western blot (serum) –arthritis Sensitivity 1.00 Specificity 0.99

Reference	Goettner 2005[27]
	Index test: Immunoblot IgG line blot (serum) – unspecified Lyme disease Sensitivity 0.81 Specificity 1.00 Index test: Immunoblot IgG line blot plus (serum) – unspecified Lyme disease Sensitivity 0.85 Specificity 0.99 Index test: Immunoblot IgG western blot (serum) –unspecified Lyme disease Sensitivity 0.71 Specificity 0.99
Source of funding	Not reported
Limitations	Risk of bias: selection, index test, reference standard Indirectness: none

Reference	Gomes-Solecki 2001 [28]
Study type	Case-control
Study methodology	Data source: bank of sera obtained from patients with well-characterised clinical disease Recruitment: not reported
Number of patients	n = 120 cases, 100 controls
Patient characteristics	Age, mean (SD): not reported Gender (male to female ratio): not reported

Reference	Gomes-Solecki 2001 [28]				
	Family origin: not reported Setting: not reported Country: USA Cases: clinically and laboratory well-characterised Lyme disease - early localised infections typified by the presence of well-defined EM in patients from an endemic area (30), early disseminated infections typified by EM and one or more of the following: additional EM lesions, atrioventricular block, neurologic abnormalities or meningitis(60), late Lyme disease occurring more than 3 months after onset with neurologic or arthritic manifestations and positive serology test by CDC criteria (30) Controls: healthy controls from an endemic area				
Target condition(s)	Lyme disease				
Index test(s) and reference standard	Index test(s) ELISA (IgM/IgG) Recombinant rapid assay Reference standard Clinical diagnosis (culture isolation from the skin lesion or photographic documentation required for EM) Time between measurement of index test and reference standard: time after onset of symptoms at time of index test <1 month - >1 year				
2×2 table		Reference standard +	Reference standard −	Total	Unspecified Lyme disease ELISA (IgM/IgG) whole cell Serum
	Index test +	85	5	90	
	Index test −	35	95	130	
	Total	120	100	220	
		Reference standard +	Reference standard −	Total	Unspecified Lyme disease Recombinant rapid assay
	Index test +	87	3	90	

Reference	Gomes-Solecki 2001 [28]				
	Index test −	33	97	130	Serum
	Total	120	100	220	
Statistical measures	Index test: ELISA IgM/IgG (serum) whole cell – unspecified Lyme disease Sensitivity 0.71 Specificity 0.95 Index test: recombinant rapid assay (serum) – unspecified Lyme disease Sensitivity 0.72 Specificity 0.97				
Source of funding	National Institutes of Health, CDC, New York State legislative initiative in Lyme disease				
Limitations	Risk of bias: selection, index test, reference standard Indirectness: none				

Reference	Goossens 1999[29]/Goossens 2000[30]
Study type	Case-control
Study methodology	Data source: not reported Recruitment: not reported
Number of patients	n = 39 cases, 190 controls
Patient characteristics	Age, mean (SD): not reported Gender (male to female ratio): not reported Family origin: not reported Setting: not reported

Reference	Goossens 1999[29]/Goossens 2000[30]				
	Country: The Netherlands Cases: early Lyme borreliosis presenting with EM and a history of tick bite and/isolation of B. burgdorferi from their skin lesion (26), late Lyme borreliosis presenting with ACA/neuroborreliosis (13) Controls: healthy controls with no history of Lyme borreliosis and tick exposure				
Target condition(s)	Lyme disease				
Index test(s) and reference standard	Index test(s) ELISA (IgM) ELISA (IgG) ELISA (IgM/IgG) Immunoblot (IgM) Immunoblot (IgG) Reference standard Clinical diagnosis Time between measurement of index test and reference standard: not reported				
2×2 table		Reference standard +	Reference standard −	Total	EM ELISA (IgM) Serum Behring
	Index test +	20	1	21	
	Index test −	6	61	67	
	Total	26	62	88	
		Reference standard +	Reference standard −	Total	EM ELISA (IgM) Serum Boehringer
	Index test +	9	0	9	
	Index test −	17	62	79	
	Total	26	62	88	

Reference	**Goossens 1999[29]/Goossens 2000[30]**				
		Reference standard +	Reference standard −	Total	EM ELISA (IgM) Serum Dako
	Index test +	17	3	20	
	Index test −	9	59	68	
	Total	26	62	88	
		Reference standard +	Reference standard −	Total	EM ELISA (IgM) Serum Genzyme Virotech
	Index test +	21	1	22	
	Index test −	5	61	66	
	Total	26	62	88	
		Reference standard +	Reference standard −	Total	EM ELISA (IgM) Serum IBL
	Index test +	17	6	23	
	Index test −	9	56	65	
	Total	26	62	88	
		Reference standard +	Reference standard −	Total	EM ELISA (IgG) Serum Behring
	Index test +	18	9	27	
	Index test −	8	53	61	
	Total	26	62	88	
		Reference standard +	Reference standard −	Total	EM ELISA (IgG) Serum Boehringer
	Index test +	10	7	17	
	Index test −	16	55	71	
	Total	26	62	88	
		Reference standard +	Reference standard −	Total	EM ELISA (IgG) Serum Dako
	Index test +	13	2	15	
	Index test −	13	60	73	

Reference	**Goossens 1999[29]/Goossens 2000[30]**				
	Total	26	62	88	
		Reference standard +	Reference standard −	Total	EM ELISA (IgG) Serum Genzyme Virotech
	Index test +	14	4	18	
	Index test −	12	58	70	
	Total	26	62	88	
		Reference standard +	Reference standard −	Total	EM ELISA (IgG) Serum IBL
	Index test +	12	8	20	
	Index test −	14	54	68	
	Total	26	62	88	
		Reference standard +	Reference standard −	Total	EM ELISA (IgM/IgG) Serum Milenia
	Index test +	8	3	11	
	Index test −	18	59	77	
	Total	26	62	88	
		Reference standard +	Reference standard −	Total	EM Immunoblot (IgM) Serum Genzyme Virotech
	Index test +	13	7	20	
	Index test −	13	55	68	
	Total	26	62	88	
		Reference standard +	Reference standard −	Total	EM Immunoblot (IgM) Serum MRL
	Index test +	12	1	13	
	Index test −	14	61	75	
	Total	26	62	88	
		Reference standard +	Reference standard −	Total	EM Immunoblot (IgG)
	Index test +	7	11	18	

Reference	**Goossens 1999[29]/Goossens 2000[30]**				
	Index test −	19	51	70	Serum Genzyme Virotech
	Total	26	62	88	
		Reference standard +	Reference standard −	Total	EM Immunoblot (IgG)
	Index test +	1	2	3	Serum MRL
	Index test −	25	60	85	
	Total	26	62	88	
		Reference standard +	Reference standard −	Total	Unspecified Lyme disease (late) ELISA (IgM)
	Index test +	8	1	9	Serum Behring
	Index test −	5	61	66	
	Total	13	62	75	
		Reference standard +	Reference standard −	Total	Unspecified Lyme disease (late) ELISA (IgM)
	Index test +	6	0	6	Serum Boehring
	Index test −	7	62	69	
	Total	13	62	75	
		Reference standard +	Reference standard −	Total	Unspecified Lyme disease (late) ELISA (IgM)
	Index test +	9	3	12	Serum Dako
	Index test −	4	59	63	
	Total	13	62	75	
		Reference standard +	Reference standard −	Total	Unspecified Lyme disease (late) ELISA (IgM)
	Index test +	8	1	9	Serum Genzyme Virotech
	Index test −	5	61	66	
	Total	13	62	75	
		Reference standard +	Reference standard −	Total	Unspecified Lyme disease (late)

Reference	**Goossens 1999[29]/Goossens 2000[30]**				
	Index test +	8	6	14	ELISA (IgM) Serum IBL
	Index test −	5	56	61	
	Total	13	62	75	
		Reference standard +	Reference standard −	Total	Unspecified Lyme disease (late) ELISA (IgG)
	Index test +	12	9	21	Serum Behring
	Index test −	1	53	54	
	Total	13	62	75	
		Reference standard +	Reference standard −	Total	Unspecified Lyme disease (late) ELISA (IgG)
	Index test +	7	7	14	Serum Boehring
	Index test −	6	55	61	
	Total	13	62	75	
		Reference standard +	Reference standard −	Total	Unspecified Lyme disease (late) ELISA (IgG)
	Index test +	10	2	12	Serum Dako
	Index test −	3	60	63	
	Total	13	62	75	
		Reference standard +	Reference standard −	Total	Unspecified Lyme disease (late) ELISA (IgG)
	Index test +	12	4	16	Serum Genzyme Virotech
	Index test −	1	58	59	
	Total	13	62	75	
		Reference standard +	Reference standard −	Total	Unspecified Lyme disease (late) ELISA (IgG)
	Index test +	9	8	17	Serum IBL
	Index test −	4	54	58	
	Total	13	62	75	

Reference	**Goossens 1999[29]/Goossens 2000[30]**				
		Reference standard +	Reference standard −	Total	Unspecified Lyme disease (late) ELISA (IgM/IgG) Serum Milenia
	Index test +	9	3	12	
	Index test −	4	59	63	
	Total	13	62	75	
		Reference standard +	Reference standard −	Total	Unspecified Lyme disease (late) Immunoblot (IgM) Serum Genzyme Virotech
	Index test +	8	7	15	
	Index test −	5	55	60	
	Total	13	62	75	
		Reference standard +	Reference standard −	Total	Unspecified Lyme disease (late) Immunoblot (IgM) Serum MRL
	Index test +	7	1	8	
	Index test −	6	61	67	
	Total	13	62	75	
		Reference standard +	Reference standard −	Total	Unspecified Lyme disease (late) Immunoblot (IgG) Serum Genzyme Virotech
	Index test +	6	11	17	
	Index test −	7	51	58	
	Total	13	62	75	
		Reference standard +	Reference standard −	Total	Unspecified Lyme disease (late) Immunoblot (IgG) Serum MRL
	Index test +	6	2	8	
	Index test −	7	60	67	
	Total	13	62	75	
Statistical measures	Index test: ELISA IgM Behring (serum) - EM Sensitivity 0.77 Specificity 0.98 Index test: ELISA IgM Boehringer (serum) - EM				

Reference	Goossens 1999[29]/Goossens 2000[30]
	Sensitivity 0.35 Specificity 1.00 Index test: ELISA IgM Dako (serum) - EM Sensitivity 0.65 Specificity 0.95 Index test: ELISA IgM Genzyme Virotech (serum) - EM Sensitivity 0.81 Specificity 0.98 Index test: ELISA IgM IBL (serum) - EM Sensitivity 0.65 Specificity 0.90 Index test: ELISA IgG Behring (serum) - EM Sensitivity 0.69 Specificity 0.85 Index test: ELISA IgG Boehringer (serum) - EM Sensitivity 0.38 Specificity 0.89 Index test: ELISA IgG Dako (serum) - EM Sensitivity 0.50 Specificity 0.97 Index test: ELISA IgG Genzyme Virotech (serum) - EM Sensitivity 0.54 Specificity 0.94

Reference	Goossens 1999[29]/Goossens 2000[30]
	Index test: ELISA IgG IBL (serum) - EM Sensitivity 0.46 Specificity 0.87 Index test: ELISA IgM/IgG Milenia (serum) - EM Sensitivity 0.31 Specificity 0.95 Index test: Immunoblot IgM Genzyme Virotech (serum) - EM Sensitivity 0.50 Specificity 0.89 Index test: Immunoblot IgM Genzyme MRL (serum) - EM Sensitivity 0.46 Specificity 0.98 Index test: Immunoblot IgG Genzyme Virotech (serum) - EM Sensitivity 0.27 Specificity 0.82 Index test: Immunoblot IgG MRL (serum) - EM Sensitivity 0.04 Specificity 0.97 Index test: ELISA IgM Behring (serum) – unspecified (late) Lyme disease Sensitivity 0.62 Specificity 0.98 Index test: ELISA IgM Boehringer (serum) – unspecified (late) Lyme disease Sensitivity 0.46 Specificity 1.00

Reference	Goossens 1999[29]/Goossens 2000[30]
	Index test: ELISA IgM Dako (serum) – unspecified (late) Lyme disease Sensitivity 0.69 Specificity 0.95 Index test: ELISA IgM Genzyme Virotech (serum) – unspecified (late) Lyme disease Sensitivity 0.62 Specificity 0.98 Index test: ELISA IgM IBL (serum) – unspecified (late) Lyme disease Sensitivity 0.62 Specificity 0.90 Index test: ELISA IgG Behring (serum) – unspecified (late) Lyme disease Sensitivity 0.92 Specificity 0.85 Index test: ELISA IgG Boehringer (serum) – unspecified (late) Lyme disease Sensitivity 0.54 Specificity 0.89 Index test: ELISA IgG Dako (serum) – unspecified (late) Lyme disease Sensitivity 0.77 Specificity 0.97 Index test: ELISA IgG Genzyme Virotech (serum) – unspecified (late) Lyme disease Sensitivity 0.92 Specificity 0.94 Index test: ELISA IgG IBL (serum) – unspecified (late) Lyme disease Sensitivity 0.69

Reference	Goossens 1999[29]/Goossens 2000[30]
	Specificity 0.87 Index test: ELISA IgM/IgG Milenia (serum) – unspecified (late) Lyme disease Sensitivity 0.69 Specificity 0.95 Index test: Immunoblot IgM Genzyme Virotech (serum) - unspecified (late) Lyme disease Sensitivity 0.62 Specificity 0.89 Index test: Immunoblot IgM Genzyme MRL (serum) - unspecified (late) Lyme disease Sensitivity 0.54 Specificity 0.98 Index test: Immunoblot IgG Genzyme Virotech (serum) - unspecified (late) Lyme disease Sensitivity 0.46 Specificity 0.82 Index test: Immunoblot IgG MRL (serum) - unspecified (late) Lyme disease Sensitivity 0.46 Specificity 0.97
Source of funding	Not reported
Limitations	Risk of bias: selection, index test, reference standard Indirectness: None
Comments	Healthy subjects used as control group in analysis, borderline results considered positive

Reference	Grodzicki 1988[31]
Study type	Case-control
Study methodology	Data source: patients who had participated in a prospective study of early Lyme disease Recruitment: not reported
Number of patients	n = 30 cases, 64 controls
Patient characteristics	Age, mean (SD): not reported Gender (male to female ratio): not reported Family origin: not reported Setting: not reported Country: USA Cases: Early Lyme disease – localised to the skin (EM) or to regional lymph nodes (8), clinical evidence of disseminated infection in multiple organ systems (22) Controls: healthy subjects (20), patients hospitalised with a wide range of diagnoses (23), patients with Rocky Mountain spotted fever (12), patients with syphilis (9)
Target condition(s)	Lyme disease
Index test(s) and reference standard	Index test(s) ELISA (IgM/IgG) Immunoblot (IgM/IgG) Reference standard Clinical diagnosis

Reference	Grodzicki 1988[31]				
	Time between measurement of index test and reference standard: acute phase samples taken within 31 days of onset of EM, convalescence samples taken 2-4 weeks later				
2×2 table		Reference standard +	Reference standard −	Total	Unspecified (acute) Lyme disease ELISA (IgM/IgG) sonicated Serum
	Index test +	9	0		
	Index test −	21	20		
	Total	30	20		
		Reference standard +	Reference standard −	Total	Unspecified (convalescent) Lyme disease ELISA (IgM/IgG) sonicated Serum
	Index test +	18	0		
	Index test −	12	20		
	Total	30	20		
		Reference standard +	Reference standard −	Total	Unspecified (acute) Lyme disease Immunoblot (IgM/IgG) Serum
	Index test +	16	0	16	
	Index test −	14	20	34	
	Total	30	20	50	
		Reference standard +	Reference standard −	Total	Unspecified (convalescent) Lyme disease Immunoblot (IgM/IgG) Serum
	Index test +	25	0	25	
	Index test −	5	20	25	
	Total	30	20	50	
Statistical measures	Index test: ELISA IgM/IgG sonicated (serum) – unspecified acute Lyme disease Sensitivity 0.30 Specificity 1.00 Index test: ELISA IgM/IgG sonicated (serum) – unspecified convalescent Lyme disease Sensitivity 0.60 Specificity 1.00				

Reference	Grodzicki 1988[31]
	Index test: Immunoblot IgM/IgG (serum) – unspecified acute Lyme disease Sensitivity 0.53 Specificity 1.00 Index test: Immunoblot IgM/IgG (serum) – unspecified convalescent Lyme disease Sensitivity 0.83 Specificity 1.00
Source of funding	National Institute of Arthritis, Musculoskeletal and Skin Diseases and Division of Research Resources, National Institutes of Health
Limitations	Risk of bias: selection, index test, reference standard Indirectness: none
Comments	Healthy controls used in analysis

Reference	Hanrahan 1984[33]
Study type	Case-control
Study methodology	Data source: cases reported by a physician (case report forms) and control sera from syphilis screening Recruitment: not reported
Number of patients	n = 207 cases, 329 controls
Patient characteristics	Age, mean (range): cases 28 years (1-79), controls not reported Gender (male to female ratio): cases 99:108, controls not reported Family origin: not reported Setting: not reported Country: USA Cases: any person reported by a physician to have Lyme disease who had at least one of the following manifestations: EM, aseptic meningitis, facial nerve palsy and/or large joint arthritis Controls: healthy controls from a non-endemic area, Lyme disease clinical histories not known
Target condition(s)	Lyme disease
Index test(s) and reference standard	Index test(s) Immunofluorescence assay (IgG) Reference standard Clinical diagnosis Time between measurement of index test and reference standard: not reported

Reference	**Hanrahan 1984[33]**				
2×2 table		Reference standard +	Reference standard −	Total	Unspecified Lyme disease IFA (IgG) Serum titer 1:128
	Index test +	88	1	89	
	Index test −	72	328	400	
	Total	160	329	489	
		Reference standard +	Reference standard −	Total	Unspecified Lyme disease IFA (IgG) Serum titer 1:256
	Index test +	57	0	57	
	Index test −	103	329	432	
	Total	160	329	489	
		Reference standard +	Reference standard −	Total	Unspecified Lyme disease IFA (IgG) Serum titer 1:64
	Index test +	112	10	122	
	Index test −	48	319	367	
	Total	160	329	489	
Statistical measures	Index test: Immunofluorescence assay IgG titer 1:128 (serum) – unspecified Lyme disease Sensitivity 0.55 Specificity 1.00 Index test: Immunofluorescence assay IgG titer 1:256 (serum) – unspecified Lyme disease Sensitivity 0.36 Specificity 1.00 Index test: Immunofluorescence assay IgG titer 1:64 (serum) – unspecified Lyme disease Sensitivity 0.70 Specificity 0.97				
Source of funding	Not reported				
Limitations	Risk of bias: selection, index test, reference standard				

Reference	Hanrahan 1984[33]
	Indirectness: serious (cases included adults and children)

Reference	Hansen 1988[35]
Study type	Case-control
Study methodology	Data source: patients hospitalised with lymphocytic meningoradiculitis following Lyme disease Recruitment: consecutive
Number of patients	n = 56 cases, 329 controls
Patient characteristics	Age, median (range): cases 51 years (6-74) Gender (male to female ratio): cases 34:22 Family origin: not reported Setting: not reported Country: Denmark Cases: patients hospitalised with lymphocytic meningoradiculitis following Lyme disease based on clinical evidence – typical painful sensory radiculitis and lymphocytic pleocytosis in CSF Controls: healthy controls (200), aseptic meningitis (13), Guillain-Barre (14) encephalitis (11), syphilis (69), leptospirosis (22)
Target condition(s)	Lyme disease
Index test(s) and reference standard	Index test(s) ELISA (IgM) ELISA (IgG)

Reference	Hansen 1988[35]				
	Reference standard Clinical diagnosis Time between measurement of index test and reference standard: not reported				
2×2 table		Reference standard +	Reference standard −	Total	Neuroborreliosis <6 weeks ELISA (IgM) flagellum CSF
	Index test +	38	0	38	
	Index test −	5	106	111	
	Total	43	106	149	
		Reference standard +	Reference standard −	Total	Neuroborreliosis <6 weeks ELISA (IgM) flagellum Serum
	Index test +	22	10	32	
	Index test −	21	305	326	
	Total	43	315	358	
		Reference standard +	Reference standard −	Total	Neuroborreliosis <6 weeks ELISA (IgM) sonic extract CSF
	Index test +	35	0	35	
	Index test −	8	106	114	
	Total	43	106	149	
		Reference standard +	Reference standard −	Total	Neuroborreliosis <6 weeks ELISA (IgM) sonic extract Serum
	Index test +	17	10	27	
	Index test −	26	305	331	
	Total	43	315	358	
		Reference standard +	Reference standard −	Total	Neuroborreliosis <6 weeks ELISA (IgG) flagellum CSF
	Index test +	25	0	25	
	Index test −	18	106	124	
	Total	43	106	149	

Reference	**Hansen 1988[35]**				
		Reference standard +	Reference standard −	Total	Neuroborreliosis <6 weeks ELISA (IgG) flagellum Serum
	Index test +	30	9	39	
	Index test −	13	306	319	
	Total	43	315	358	
		Reference standard +	Reference standard −	Total	Neuroborreliosis <6 weeks ELISA (IgG) sonic extract CSF
	Index test +	22	0	22	
	Index test −	21	106	127	
	Total	43	106	149	
		Reference standard +	Reference standard −	Total	Neuroborreliosis <6 weeks ELISA (IgG) sonic extract Serum
	Index test +	12	13	25	
	Index test −	31	302	333	
	Total	43	315	358	
		Reference standard +	Reference standard −	Total	Neuroborreliosis 6 weeks – 6 months ELISA (IgM) flagellum CSF
	Index test +	11	0	11	
	Index test −	2	106	108	
	Total	13	106	119	
		Reference standard +	Reference standard −	Total	Neuroborreliosis 6 weeks – 6 months ELISA (IgM) flagellum Serum
	Index test +	6	10	16	
	Index test −	7	305	312	
	Total	13	315	328	
		Reference standard +	Reference standard −	Total	Neuroborreliosis 6 weeks – 6 months ELISA (IgM) sonic extract CSF
	Index test +	12	0	12	
	Index test −	1	106	107	

Reference	**Hansen 1988[35]**				
	Total	13	106	119	
		Reference standard +	Reference standard −	Total	Neuroborreliosis 6 weeks – 6 months ELISA (IgM) sonic extract Serum
	Index test +	3	10	13	
	Index test −	10	305	315	
	Total	13	315	328	
		Reference standard +	Reference standard −	Total	Neuroborreliosis 6 weeks – 6 months ELISA (IgG) flagellum CSF
	Index test +	12	0	12	
	Index test −	1	106	107	
	Total	13	106	119	
		Reference standard +	Reference standard −	Total	Neuroborreliosis 6 weeks – 6 months ELISA (IgG) flagellum Serum
	Index test +	13	9	22	
	Index test −	0	306	306	
	Total	13	315	328	
		Reference standard +	Reference standard −	Total	Neuroborreliosis 6 weeks – 6 months ELISA (IgG) sonic extract CSF
	Index test +	13	0	13	
	Index test −	0	106	106	
	Total	13	106	119	
		Reference standard +	Reference standard −	Total	Neuroborreliosis 6 weeks – 6 months ELISA (IgG) sonic extract Serum
	Index test +	11	13	24	
	Index test −	2	302	304	
	Total	13	315	328	
Statistical measures	Index test: ELISA IgM flagellum (CSF) – neuroborreliosis <6 weeks Sensitivity 0.88 Specificity 1.00				

Reference	Hansen 1988[35]
	Index test: ELISA IgM flagellum (serum) – neuroborreliosis <6 weeks Sensitivity 0.51 Specificity 0.97 Index test: ELISA IgM sonic extract (CSF) – neuroborreliosis <6 weeks Sensitivity 0.81 Specificity 1.00 Index test: ELISA IgM sonic extract (serum) – neuroborreliosis <6 weeks Sensitivity 0.40 Specificity 0.97 Index test: ELISA IgG flagellum (CSF) – neuroborreliosis <6 weeks Sensitivity 0.58 Specificity 1.00 Index test: ELISA IgG flagellum (serum) – neuroborreliosis <6 weeks Sensitivity 0.70 Specificity 0.97 Index test: ELISA IgG sonic extract (CSF) – neuroborreliosis <6 weeks Sensitivity 0.51 Specificity 1.00 Index test: ELISA IgG sonic extract (serum) – neuroborreliosis <6 weeks Sensitivity 0.28 Specificity 0.96 Index test: ELISA IgM flagellum (CSF) – neuroborreliosis 6 weeks – 6 months Sensitivity 0.85

Reference	Hansen 1988[35]
	Specificity 1.00 Index test: ELISA IgM flagellum (serum) – neuroborreliosis 6 weeks – 6 months Sensitivity 0.46 Specificity 0.97 Index test: ELISA IgM sonic extract (CSF) – neuroborreliosis 6 weeks – 6 months Sensitivity 0.92 Specificity 1.00 Index test: ELISA IgM sonic extract (serum) – neuroborreliosis 6 weeks – 6 months Sensitivity 0.23 Specificity 0.97 Index test: ELISA IgG flagellum (CSF) – neuroborreliosis 6 weeks – 6 months Sensitivity 0.92 Specificity 1.00 Index test: ELISA IgG flagellum (serum) – neuroborreliosis 6 weeks – 6 months Sensitivity 1.00 Specificity 0.97 Index test: ELISA IgG sonic extract (CSF) – neuroborreliosis 6 weeks – 6 months Sensitivity 1.00 Specificity 1.00 Index test: ELISA IgG sonic extract (serum) – neuroborreliosis 6 weeks – 6 months Sensitivity 0.85 Specificity 0.96
Source of	University of Copenhagen and the Thorvald Madsen foundation

Reference	Hansen 1988[35]
funding	
Limitations	Risk of bias: index test, reference standard Indirectness: none

Reference	Hansen 1989[34]
Study type	Case-control
Study methodology	Data source: patients presenting with EM/ACA Recruitment: not reported
Number of patients	n = 157 cases, 298 controls
Patient characteristics	Age, median (range): EM 54 years (6-83), ACA 61 years (28-89), controls not reported Gender (male to female ratio): EM 26:81, ACA 16:34, controls not reported Family origin: not reported Setting: not reported Country: Sweden, Denmark Cases: patients presenting with typical, clinically uncomplicated EM (107 - solitary n=91, multiple n=16), patients with ACA (50), diagnosis based on clinical evidence, diagnosis of ACA confirmed by histopathology Controls: healthy Danish controls (200), patients with various dermatological disorders without clinical evidence of B. burgdorferi infection
Target condition(s)	Lyme disease
Index test(s)	Index test(s)

Reference	**Hansen 1989[34]**				
and reference standard	ELISA (IgM) ELISA (IgG) Reference standard Clinical diagnosis Time between measurement of index test and reference standard: onset of EM to blood sampling median 3.5 weeks (range 2 days-12 months), median duration of ACA 2.5 years				
2×2 table		Reference standard +	Reference standard −	Total	EM (multiple) ELISA (IgM) flagellum Serum
	Index test +	11	10	21	
	Index test −	5	190	195	
	Total	16	200	216	
		Reference standard +	Reference standard −	Total	EM ELISA (IgM) flagellum Serum
	Index test +	48	10	58	
	Index test −	59	190	259	
	Total	107	200	307	
		Reference standard +	Reference standard −	Total	EM (single) ELISA (IgM) flagellum Serum
	Index test +	36	10	46	
	Index test −	55	190	245	
	Total	91	200	291	
		Reference standard +	Reference standard −	Total	EM ELISA (IgM) sonic Serum
	Index test +	18	11	29	
	Index test −	89	189	278	
	Total	107	200	307	
		Reference standard	Reference standard −	Total	EM (multiple)

Reference	**Hansen 1989**[34]				
		+			ELISA (IgG) flagellum Serum
	Index test +	6	8	14	
	Index test −	10	192	202	
	Total	16	200	216	
		Reference standard +	Reference standard −	Total	EM ELISA (IgG) flagellum Serum
	Index test +	38	8	46	
	Index test −	69	192	261	
	Total	107	200	307	
		Reference standard +	Reference standard −	Total	EM (single) ELISA (IgG) flagellum Serum
	Index test +	33	8	41	
	Index test −	58	192	250	
	Total	91	200	291	
		Reference standard +	Reference standard −	Total	EM ELISA (IgG) sonic Serum
	Index test +	12	12	24	
	Index test −	95	188	283	
	Total	107	200	307	
		Reference standard +	Reference standard −	Total	ACA ELISA (IgM) flagellum Serum
	Index test +	6	10	16	
	Index test −	44	190	234	
	Total	50	200	250	
		Reference standard +	Reference standard −	Total	ACA ELISA (IgM) sonic Serum
	Index test +	11	11	22	
	Index test −	39	189	228	
	Total	50	200	250	

Reference	**Hansen 1989[34]**				
		Reference standard +	Reference standard −	Total	ACA ELISA (IgG) flagellum Serum
	Index test +	50	8	58	
	Index test −	0	192	192	
	Total	50	200	250	
		Reference standard +	Reference standard −	Total	ACA ELISA (IgG) sonic Serum
	Index test +	49	12	61	
	Index test −	1	188	189	
	Total	50	200	250	
Statistical measures	Index test: ELISA IgM flagellum (serum) – EM (multiple) Sensitivity 0.69 Specificity 0.95 Index test: ELISA IgM flagellum (serum) – EM Sensitivity 0.45 Specificity 0.95 Index test: ELISA IgM flagellum (serum) – EM (single) Sensitivity 0.40 Specificity 0.95 Index test: ELISA IgM sonic (serum) – EM Sensitivity 0.17 Specificity 0.94 Index test: ELISA IgG flagellum (serum) – EM (multiple) Sensitivity 0.38 Specificity 0.96 Index test: ELISA IgG flagellum (serum) – EM				

Reference	Hansen 1989[34]
	Sensitivity 0.36 Specificity 0.96 Index test: ELISA IgG flagellum (serum) – EM (single) Sensitivity 0.36 Specificity 0.96 Index test: ELISA IgG sonic (serum) – EM Sensitivity 0.11 Specificity 0.94 Index test: ELISA IgM flagellum (serum) – ACA Sensitivity 0.12 Specificity 0.95 Index test: ELISA IgM sonic (serum) – ACA Sensitivity 0.22 Specificity 0.94 Index test: ELISA IgG flagellum (serum) – ACA Sensitivity 1.00 Specificity 0.96 Index test: ELISA IgG sonic (serum) – ACA Sensitivity 0.98 Specificity 0.94
Source of funding	University of Copenhagen, the Mauritzen la Fontaine foundation and the Swedish Medical Research Council
Limitations	Risk of bias: selection, index test, reference standard Indirectness: none

Reference	Hansen 1989[34]
Comments	Healthy controls used in analysis

Reference	Hansen 1991[37]
Study type	Case-control
Study methodology	Data source: patients with active untreated Lyme borreliosis Recruitment: not reported
Number of patients	n = 198 cases, 270 controls
Patient characteristics	Age, median (range): EM 45 years (6-71), neuroborreliosis 47 years (5-74), ACA 54 years (17-80), healthy controls 40 years (16-71), disease controls not reported Gender (male to female ratio): EM 9:41, neuroborreliosis 61:39, ACA 15:33, controls not reported Family origin: not reported Setting: not reported Country: Sweden, Denmark Cases: EM (50), patients hospitalised with neuroborreliosis (100), dermatologist diagnosed ACA (48) Controls: healthy controls (200), syphilis (40), leptospirosis (15), infectious mononucleosis (15)
Target condition(s)	Lyme disease
Index test(s) and reference standard	Index test(s) ELISA (IgM) ELISA (IgG) Reference standard Clinical diagnosis Time between measurement of index test and reference standard: EM duration median 3 weeks (1-12), other presentations not reported

Reference	Hansen 1991[37]				
	(duration from symptom onset is reported)				
2×2 table		Reference standard +	Reference standard −	Total	EM ELISA (IgM) Serum
	Index test +	32	0	32	
	Index test −	18	200	218	
	Total	50	200	250	
		Reference standard +	Reference standard −	Total	EM ELISA (IgG) Serum
	Index test +	28	0	28	
	Index test −	22	200	222	
	Total	50	200	250	
		Reference standard +	Reference standard −	Total	ACA ELISA (IgM) Serum
	Index test +	5	0	5	
	Index test −	43	200	243	
	Total	48	200	248	
		Reference standard +	Reference standard −	Total	ACA ELISA (IgG) Serum
	Index test +	48	0	48	
	Index test −	0	200	200	
	Total	48	200	248	
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA (IgM) Serum
	Index test +	37	0	37	
	Index test −	63	200	263	
	Total	100	200	300	
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA (IgG)
	Index test +	84	0	84	

Reference	**Hansen 1991[37]**				
	Index test −	16	200	216	Serum
	Total	100	200	300	
		Reference standard +	Reference standard −	Total	EM <6 weeks ELISA (IgM)
	Index test +	13	0	13	Serum
	Index test −	24	200	224	
	Total	37	200	237	
		Reference standard +	Reference standard −	Total	Neuroborreliosis <6 weeks ELISA (IgM)
	Index test +	32	0	32	Serum
	Index test −	38	200	238	
	Total	70	200	270	
		Reference standard +	Reference standard −	Total	EM <6 weeks ELISA (IgG)
	Index test +	8	0	8	Serum
	Index test −	29	200	229	
	Total	37	200	237	
		Reference standard +	Reference standard −	Total	Neuroborreliosis <6 weeks ELISA (IgG)
	Index test +	54	0	54	Serum
	Index test −	16	200	216	
	Total	70	200	270	
		Reference standard +	Reference standard −	Total	EM 6 weeks – 6 months ELISA (IgM)
	Index test +	3	0	3	Serum
	Index test −	10	200	210	
	Total	13	200	213	
		Reference standard +	Reference standard −	Total	Neuroborreliosis 6 weeks – 6 months

Reference	**Hansen 1991[37]**				
	Index test +	5	0	5	ELISA (IgM) Serum
	Index test −	25	200	225	
	Total	30	200	230	
		Reference standard +	Reference standard −	Total	EM 6 weeks – 6 months ELISA (IgG) Serum
	Index test +	6	0	6	
	Index test −	7	200	207	
	Total	13	200	213	
		Reference standard +	Reference standard −	Total	Neuroborreliosis 6 weeks – 6 months ELISA (IgG) Serum
	Index test +	30	0	30	
	Index test −	0	200	200	
	Total	30	200	230	
Statistical measures	Index test: ELISA IgM (serum) - EM Sensitivity 0.64 Specificity 1.00 Index test: ELISA IgG (serum) - EM Sensitivity 0.56 Specificity 1.00 Index test: ELISA IgM (serum) - ACA Sensitivity 0.10 Specificity 1.00 Index test: ELISA IgG (serum) - ACA Sensitivity 1.00 Specificity 1.00 Index test: ELISA IgM (serum) - neuroborreliosis				

Reference	Hansen 1991[37]
	Sensitivity 0.37 Specificity 1.00 Index test: ELISA IgG (serum) - neuroborreliosis Sensitivity 0.84 Specificity 1.00 Index test: ELISA IgM (serum) – EM <6 weeks Sensitivity 0.35 Specificity 1.00 Index test: ELISA IgM (serum) – neuroborreliosis <6 weeks Sensitivity 0.46 Specificity 1.00 Index test: ELISA IgG (serum) – EM <6 weeks Sensitivity 0.22 Specificity 1.00 Index test: ELISA IgG (serum) – neuroborreliosis <6 weeks Sensitivity 0.77 Specificity 1.00 Index test: ELISA IgM (serum) – EM 6 weeks – 6 months Sensitivity 0.23 Specificity 1.00 Index test: ELISA IgM (serum) – neuroborreliosis 6 weeks – 6 months Sensitivity 0.17 Specificity 1.00

Reference	Hansen 1991[37]
	Index test: ELISA IgG (serum) – EM 6 weeks – 6 months Sensitivity 0.46 Specificity 1.00 Index test: ELISA IgG (serum) – neuroborreliosis 6 weeks – 6 months Sensitivity 1.00 Specificity 1.00
Source of funding	University of Copenhagen, Danish Medical Research Council and Thorvald Madsen's Legat and Dakopatts
Limitations	Risk of bias: selection, index test, reference standard, flow and timing Indirectness: none
Comments	Healthy controls used in the analysis

Reference	Hansen 1991a[36]
Study type	Case-control
Study methodology	Data source: patients hospitalised with neuroborreliosis Recruitment: not reported
Number of patients	n = 100 cases, 35 controls
Patient characteristics	Age, mean (SD): not reported Gender (male to female ratio): not reported Family origin: not reported Setting: not reported

Reference	Hansen 1991a[36]				
	Country: Denmark Cases: patients hospitalised with neuroborreliosis – second stage lymphocytic meningoradiculitis (91), third stage chronic progressive encephalomyelitis (9) Controls: multiple sclerosis (17), Guillian-Barre syndrome (8), neurosyphilis (4), ACA (6)				
Target condition(s)	Lyme disease				
Index test(s) and reference standard	Index test(s) ELISA (IgM) ELISA (IgG) Reference standard Clinical diagnosis Time between measurement of index test and reference standard: not reported				
2×2 table		Reference standard +	Reference standard −	Total	Neuroborreliosis <6 weeks ELISA (IgM) CSF
	Index test +	50	0	50	
	Index test −	20	29	49	
	Total	70	29	99	
		Reference standard +	Reference standard −	Total	Neuroborreliosis <6 weeks ELISA (IgM) Serum
	Index test +	43	0	43	
	Index test −	27	29	56	
	Total	70	29	99	
		Reference standard +	Reference standard −	Total	Neuroborreliosis <6 weeks ELISA (IgG)
	Index test +	59	2	61	

Reference	**Hansen 1991a[36]**				
	Index test −	11	27	38	CSF
	Total	70	29	99	
		Reference standard +	Reference standard −	Total	Neuroborreliosis <6 weeks ELISA (IgG) Serum
	Index test +	54	1	55	
	Index test −	16	28	44	
	Total	70	29	99	
		Reference standard +	Reference standard −	Total	Neuroborreliosis 6 weeks – 6 months ELISA (IgM) CSF
	Index test +	16	0	16	
	Index test −	3	29	32	
	Total	19	29	48	
		Reference standard +	Reference standard −	Total	Neuroborreliosis 6 weeks – 6 months ELISA (IgM) Serum
	Index test +	11	0	11	
	Index test −	8	29	37	
	Total	19	29	48	
		Reference standard +	Reference standard −	Total	Neuroborreliosis 6 weeks – 6 months ELISA (IgG) CSF
	Index test +	19	2	21	
	Index test −	0	27	27	
	Total	19	29	48	
		Reference standard +	Reference standard −	Total	Neuroborreliosis 6 weeks – 6 months ELISA (IgG) Serum
	Index test +	19	1	20	
	Index test −	0	28	28	
	Total	19	29	48	
		Reference standard +	Reference standard −	Total	Neuroborreliosis >6 months

Reference	**Hansen 1991a[36]**				
	Index test +	1	0	1	ELISA (IgM) CSF
	Index test −	10	29	39	
	Total	11	29	40	
		Reference standard +	Reference standard −	Total	Neuroborreliosis >6 months ELISA (IgM) Serum
	Index test +	2	0	2	
	Index test −	9	29	38	
	Total	11	29	40	
		Reference standard +	Reference standard −	Total	Neuroborreliosis > 6 months ELISA (IgG) CSF
	Index test +	11	2	13	
	Index test −	0	27	27	
	Total	11	29	40	
		Reference standard +	Reference standard −	Total	Neuroborreliosis > 6 months ELISA (IgG) Serum
	Index test +	11	1	12	
	Index test −	0	28	28	
	Total	11	29	40	
Statistical measures	Index test: ELISA IgM (CSF) – neuroborreliosis <6 weeks Sensitivity 0.71 Specificity 1.00 Index test: ELISA IgM (serum) – neuroborreliosis <6 weeks Sensitivity 0.61 Specificity 1.00 Index test: ELISA IgG (CSF) – neuroborreliosis <6 weeks Sensitivity 0.84 Specificity 0.93				

Reference	Hansen 1991a[36]
	Index test: ELISA IgG (serum) – neuroborreliosis <6 weeks Sensitivity 0.77 Specificity 0.97 Index test: ELISA IgM (CSF) – neuroborreliosis 6 weeks – 6 months Sensitivity 0.84 Specificity 1.00 Index test: ELISA IgM (serum) – neuroborreliosis 6 weeks – 6 months Sensitivity 0.58 Specificity 1.00 Index test: ELISA IgG (CSF) – neuroborreliosis 6 weeks – 6 months Sensitivity 1.00 Specificity 0.93 Index test: ELISA IgG (serum) – neuroborreliosis 6 weeks – 6 months Sensitivity 1.00 Specificity 0.97 Index test: ELISA IgM (CSF) – neuroborreliosis >6 months Sensitivity 0.09 Specificity 1.00 Index test: ELISA IgM (serum) – neuroborreliosis >6 months Sensitivity 0.18 Specificity 1.00 Index test: ELISA IgG (CSF) – neuroborreliosis >6 months Sensitivity 1.00 Specificity 0.93

Reference	Hansen 1991a[36]
	Index test: ELISA IgG (serum) – neuroborreliosis >6 months Sensitivity 1.00 Specificity 0.97
Source of funding	University of Copenhagen, Danish Medical Research Council and Thorvald Madsen's Legat and Dakopatts
Limitations	Risk of bias: selection, index test, reference standard Indirectness: none
Comments	ACA controls not included in analysis

Reference	Hernandez-Novoa 2003[41]
Study type	Case-control
Study methodology	Data source: patients diagnosed with Lyme disease from 2 hospitals Recruitment: not reported
Number of patients	n = 42 cases, 129 controls
Patient characteristics	Age, mean (SD): not reported Gender (male to female ratio): not reported Family origin: not reported Setting: 2 hospitals Country: Spain Cases: patients diagnosed with Lyme disease from 2 hospitals – localised early stage (EM 24), disseminated early stage (EM 7,

Reference	Hernandez-Novoa 2003[41]				
	neuroborreliosis 11) Controls: healthy controls (53), patients with other infectious diseases (76)				
Target condition(s)	Lyme disease				
Index test(s) and reference standard	Index test(s) ELISA (IgM) ELISA (IgG) ELISA (IgM/IgG) Reference standard Clinical diagnosis (CDC criteria) Time between measurement of index test and reference standard: not reported				
2×2 table		Reference standard +	Reference standard −	Total	EM (localised) ELISA (IgM) Serum
	Index test +	9	7	16	
	Index test −	15	122	137	
	Total	24	129	153	
		Reference standard +	Reference standard −	Total	EM (localised) ELISA (IgG) Serum
	Index test +	5	55	60	
	Index test −	19	74	93	
	Total	24	129	153	
		Reference standard +	Reference standard −	Total	EM (localised) ELISA (IgM/IgG) Serum
	Index test +	12	0	12	
	Index test −	12	0	12	
	Total	24	0	24	

Reference	Hernandez-Novoa 2003[41]				
		Reference standard +	Reference standard −	Total	Unspecified disseminated Lyme disease ELISA (IgM) Serum
	Index test +	12	7	19	
	Index test −	6	122	128	
	Total	18	129	147	
		Reference standard +	Reference standard −	Total	Unspecified disseminated Lyme disease ELISA (IgG) Serum
	Index test +	4	55	59	
	Index test −	14	74	88	
	Total	18	129	147	
		Reference standard +	Reference standard −	Total	Unspecified disseminated Lyme disease ELISA (IgM/IgG) Serum
	Index test +	14	0	14	
	Index test −	4	0	4	
	Total	18	0	18	
Statistical measures	Index test: ELISA IgM (serum) – EM (localised) Sensitivity 0.38 Specificity 0.95 Index test: ELISA IgG (serum) – EM (localised) Sensitivity 0.21 Specificity 0.57 Index test: ELISA IgM/IgG (serum) – EM (localised) Sensitivity 0.50 Specificity not estimable Index test: ELISA IgM (serum) – unspecified disseminated Lyme disease Sensitivity 0.67 Specificity 0.95				

Reference	Hernandez-Novoa 2003[41]
	Index test: ELISA IgG (serum) – unspecified disseminated Lyme disease Sensitivity 0.22 Specificity 0.57 Index test: ELISA IgM/IgG (serum) – unspecified disseminated Lyme disease Sensitivity 0.78 Specificity not estimable
Source of funding	Not reported
Limitations	Risk of bias: selection, index test, reference standard Indirectness: none

Reference	Hunfeld 2002[42]
Study type	Case-control
Study methodology	Data source: patients diagnosed with Lyme disease by experienced physicians, blood donors, patients with other infectious or autoimmune diseases Recruitment: not reported
Number of patients	n = 226 cases, 1382 controls
Patient characteristics	Age, mean (SD): not reported Gender (male to female ratio): not reported Family origin: not reported Setting: not reported

Reference	Hunfeld 2002[42]				
	Country: Germany Cases: patients diagnosed with Lyme disease - EM (148), neuroborreliosis (35), ACA and Lyme arthritis (43) Controls: randomly chosen healthy blood-donor sera from individuals without a known history of tick bite or any clinical manifestation of Lyme disease in their medical history (1107), patients with other spirochaetal infections, infections other than Lyme disease or autoimmune diseases (275)				
Target condition(s)	Lyme disease				
Index test(s) and reference standard	Index test(s) ELISA (IgM) ELISA (IgG) ELISA (IgM/IgG) Reference standard Clinical diagnosis Time between measurement of index test and reference standard: not reported				
2×2 table		Reference standard +	Reference standard −	Total	EM ELISA (IgM) Serum
	Index test +	91	87	178	
	Index test −	57	1020	1077	
	Total	148	1107	1255	
		Reference standard +	Reference standard −	Total	EM ELISA (IgG) Serum
	Index test +	33	60	93	
	Index test −	115	1047	1162	
	Total	148	1107	1255	
		Reference standard	Reference standard −	Total	EM

Reference	**Hunfeld 2002[42]**				
		+			ELISA (IgM/IgG) Serum
	Index test +	100	0	100	
	Index test −	48	0	48	
	Total	148	0	148	
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA (IgM) Serum
	Index test +	26	87	113	
	Index test −	9	1020	1029	
	Total	35	1107	1142	
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA (IgG) Serum
	Index test +	18	60	78	
	Index test −	17	1047	1064	
	Total	35	1107	1142	
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA (IgM/IgG) Serum
	Index test +	30	0	30	
	Index test −	5	0	5	
	Total	35	0	35	
		Reference standard +	Reference standard −	Total	Unspecified (chronic) Lyme disease ELISA (IgM) Serum
	Index test +	4	87	91	
	Index test −	39	1020	1059	
	Total	43	1107	1150	
		Reference standard +	Reference standard −	Total	Unspecified (chronic) Lyme disease ELISA (IgG) Serum
	Index test +	40	60	100	
	Index test −	3	1047	1050	
	Total	43	1107	1150	

Reference	Hunfeld 2002[42]				
		Reference standard +	Reference standard −	Total	Unspecified (chronic) Lyme disease ELISA (IgM/IgG) Serum
	Index test +	41	0	41	
	Index test −	2	0	2	
	Total	43	0	43	
Statistical measures	Index test: ELISA IgM (serum) - EM Sensitivity 0.61 Specificity 0.92 Index test: ELISA IgG (serum) - EM Sensitivity 0.22 Specificity 0.95 Index test: ELISA IgM/IgG (serum) - EM Sensitivity 0.68 Specificity not estimable Index test: ELISA IgM (serum) – neuroborreliosis Sensitivity 0.74 Specificity 0.92 Index test: ELISA IgG (serum) - neuroborreliosis Sensitivity 0.51 Specificity 0.95 Index test: ELISA IgM/IgG (serum) - neuroborreliosis Sensitivity 0.86 Specificity not estimable Index test: ELISA IgM (serum) – unspecified (chronic) Lyme disease Sensitivity 0.09				

Reference	Hunfeld 2002[42]
	Specificity 0.92 Index test: ELISA IgG (serum) - unspecified (chronic) Lyme disease Sensitivity 0.93 Specificity 0.95 Index test: ELISA IgM/IgG (serum) - unspecified (chronic) Lyme disease Sensitivity 0.95 Specificity not estimable
Source of funding	Not reported
Limitations	Risk of bias: selection, index test, reference standard Indirectness: none
Comments	Healthy controls used in the analysis

Reference	Jaulhac 1996[43]
Study type	Case-control
Study methodology	Data source: patients with clinically evident Lyme arthritis Recruitment: cases consecutive, controls not reported
Number of patients	n = 12 cases, 29 controls
Patient characteristics	Age, mean (range): cases 44 years (7-71), controls not reported Gender (male to female ratio): cases 10:2, controls not reported Family origin: not reported Setting: not reported Country: France Cases: patients with clinically evident Lyme arthritis (typical case defined as fulfilling the CDC criteria or a case of objective joint swelling in 1 or a few large joints following a recent and well-documented EM), all patients had been bitten by ticks and had a previous history of EM Controls: patients with rheumatoid arthritis (11), osteoarthritis (11), septic arthritis (3), psoriatic arthritis (3), Sjögren's syndrome (1)
Target condition(s)	Lyme disease
Index test(s) and reference standard	Index test(s) PCR Reference standard Clinical diagnosis (CDC criteria or objective joint swelling in 1 or a few large joints following a recent and well-documented EM) Time between measurement of index test and reference standard: not reported

Reference	Jaulhac 1996[43]				
2×2 table		Reference standard +	Reference standard −	Total	Arthritis PCR Synovial fluid
	Index test +	5	0	5	
	Index test −	7	29	36	
	Total	12	29	41	
Statistical measures	Index test: PCR (synovial fluid) – arthritis Sensitivity 0.42 Specificity 1.00				
Source of funding	National Institute of Health and Medical Research and the Medical School of Strasbourg				
Limitations	Risk of bias: selection, index test, reference standard Indirectness: none				

Reference	Johnson 1996[44]
Study type	Case-control
Study methodology	Data source: serum samples contributed by physicians with extensive experience in the clinical diagnosis of Lyme disease, healthy blood donors, patients with other illnesses Recruitment: not reported
Number of patients	n = 111 cases, 224 controls
Patient characteristics	Age, mean (SD): not reported Gender (male to female ratio): not reported Family origin: not reported

Reference	Johnson 1996[44]				
	Setting: not reported Country: USA Cases: EM (58), early neurologic (3), Lyme arthritis (36), late neurologic (14) Controls: healthy blood donors (113), autoimmune disorders, leptospirosis, periodontitis, relapsing fever, syphilis, tularemia and other illnesses (111)				
Target condition(s)	Lyme disease				
Index test(s) and reference standard	Index test(s) ELISA (IgM/IgG) Reference standard Clinical diagnosis Time between measurement of index test and reference standard: not reported				
2×2 table		Reference standard +	Reference standard −	Total	EM ELISA (IgM/IgG) Serum
	Index test +	27	5	32	
	Index test −	31	108	139	
	Total	58	113	171	
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA (IgM/IgG) Serum
	Index test +	16	5	21	
	Index test −	1	108	109	
	Total	17	113	130	
		Reference standard +	Reference standard −	Total	Arthritis ELISA (IgM/IgG)
	Index test +	32	5	37	

Reference	**Johnson 1996[44]**				
	Index test −	4	108	112	Serum
	Total	36	113	149	
		Reference standard +	Reference standard −	Total	Unspecified Lyme disease ELISA (IgM/IgG) Serum
	Index test +	75	5	80	
	Index test −	36	108	144	
	Total	111	113	224	
Statistical measures	Index test: ELISA IgM/IgG (serum) - EM Sensitivity 0.47 Specificity 0.96 Index test: ELISA IgM/IgG (serum) - neuroborreliosis Sensitivity 0.94 Specificity 0.96 Index test: ELISA IgM/IgG (serum) - arthritis Sensitivity 0.89 Specificity 0.96 Index test: ELISA IgM/IgG (serum) – unspecified Lyme disease Sensitivity 0.68 Specificity 0.96				
Source of funding	Not reported				
Limitations	Risk of bias: selection, reference standard Indirectness: none				
Comments	Healthy controls used in the analysis				

Reference	Jovicic 2003[45]
Study type	Case-control
Study methodology	Data source: patients with Lyme disease, blood donors from endemic and non-endemic areas, patients with other conditions Recruitment: not reported
Number of patients	n = 94 cases, 120 controls
Patient characteristics	Age, mean (SD): not reported Gender (male to female ratio): not reported Family origin: not reported Setting: not reported Country: Serbia Cases: patients diagnosed with Lyme disease - EM (40), Lyme carditis (4), neuroborreliosis or Lyme arthritis (50) Controls: healthy blood donors with no history of disease from an endemic area (50) and a non-endemic area (30), patients with syphilis, rheumatoid arthritis and systemic lupus erythematosus (40)
Target condition(s)	Lyme disease
Index test(s) and reference standard	Index test(s) ELISA (IgM/IgG) Immunoblot (IgM/IgG) Immunofluorescence assay (IgM/IgG) Reference standard Clinical diagnosis

Reference	Jovicic 2003[45]				
	Time between measurement of index test and reference standard: not reported (EM - 31 samples collected 2-6 weeks, 9 samples collected 2-6 months after tick bite)				
2×2 table		Reference standard +	Reference standard −	Total	Unspecified Lyme disease ELISA (IgM/IgG) Serum
	Index test +	63	8	71	
	Index test −	31	112	143	
	Total	94	120	214	
		Reference standard +	Reference standard −	Total	Unspecified Lyme disease Immunoblot (IgM/IgG) Serum
	Index test +	87	5	92	
	Index test −	7	115	122	
	Total	94	120	214	
		Reference standard +	Reference standard −	Total	Unspecified Lyme disease Immunofluorescence assay (IgM/IgG) Serum
	Index test +	34	13	47	
	Index test −	60	107	167	
	Total	94	120	214	
Statistical measures	Index test: ELISA IgM/IgG (serum) - unspecified Lyme disease Sensitivity 0.67 Specificity 0.93 Index test: Immunoblot IgM/IgG (serum) - unspecified Lyme disease Sensitivity 0.93 Specificity 0.96 Index test: Immunofluorescence assay IgM/IgG (serum) - unspecified Lyme disease Sensitivity 0.36 Specificity 0.89				

Reference	Jovicic 2003[45]
Source of funding	Not reported
Limitations	Risk of bias: selection, index test, reference standard Indirectness: none

Reference	Kaiser 1998[46]
Study type	Case-control
Study methodology	Data source: patients with neuroborreliosis admitted to the Department of Neurology and patients with neurosyphilis Recruitment: not reported
Number of patients	n = 67 cases, 14 controls
Patient characteristics	Age, mean (SD): not reported Gender (male to female ratio): not reported Family origin: not reported Setting: Department of Neurology, Department of Immunology Country: Germany Cases: patients with neuroborreliosis – acute duration of symptoms ≤6 months (52), chronic duration of symptoms >6 months (15) Controls: patients with neurosyphilis
Target condition(s)	Lyme disease
Index test(s) and reference	Index test(s) ELISA (IgM)

Reference	Kaiser 1998[46]				
standard	ELISA (IgG) Reference standard Clinical diagnosis (based on clinical symptoms, lymphocytic pleocytosis in CSF and assessment of specific IgM and/or IgG antibodies in serum) Time between measurement of index test and reference standard: not reported				
2×2 table		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA IgM recombinant CSF
	Index test +	28	0	28	
	Index test −	39	14	53	
	Total	67	14	81	
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA IgM recombinant Serum
	Index test +	53	0	53	
	Index test −	14	14	28	
	Total	67	14	81	
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA IgM sonicated CSF
	Index test +	5	0	5	
	Index test −	62	14	76	
	Total	67	14	81	
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA IgM sonicated Serum
	Index test +	29	0	29	
	Index test −	38	14	52	
	Total	67	14	81	
		Reference standard +	Reference standard −	Total	Neuroborreliosis

Reference	**Kaiser 1998[46]**				
	Index test +	39	0	39	ELISA IgG recombinant CSF
	Index test −	28	14	42	
	Total	67	14	81	
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA IgG recombinant Serum
	Index test +	43	0	43	
	Index test −	24	14	38	
	Total	67	14	81	
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA IgG sonicated CSF
	Index test +	21	10	31	
	Index test −	46	4	50	
	Total	67	14	81	
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA IgG sonicated Serum
	Index test +	62	10	72	
	Index test −	5	4	9	
	Total	67	14	81	
Statistical measures	Index test: ELISA IgM recombinant (serum) - neuroborreliosis Sensitivity 0.42 Specificity 1.00 Index test: ELISA IgM recombinant (CSF) - neuroborreliosis Sensitivity 0.79 Specificity 1.00 Index test: ELISA IgM sonicated (serum) - neuroborreliosis Sensitivity 0.07 Specificity 1.00				

Reference	Kaiser 1998[46]
	Index test: ELISA IgM sonicated (CSF) - neuroborreliosis Sensitivity 0.43 Specificity 1.00 Index test: ELISA IgG recombinant (serum) - neuroborreliosis Sensitivity 0.58 Specificity 1.00 Index test: ELISA IgG recombinant (CSF) - neuroborreliosis Sensitivity 0.64 Specificity 1.00 Index test: ELISA IgG sonicated (serum) - neuroborreliosis Sensitivity 0.31 Specificity 0.29 Index test: ELISA IgG sonicated (CSF) - neuroborreliosis Sensitivity 0.93 Specificity 0.29
Source of funding	Federal Ministry of Education and Research
Limitations	Risk of bias: selection, index test, reference standard Indirectness: none
Comments	Leukocytes pro microliter in CSF (median): Acute neuroborreliosis: 246 (7-600) Chronic neuroborreliosis: 60 (10-135)

Reference	Kaiser 1999[47]
Study type	Case-control
Study methodology	Data source: patients with neuroborreliosis admitted to the Department of Neurology, healthy persons from an endemic area positive in routine serological tests, patients with other diseases Recruitment: not reported
Number of patients	n = 96 cases, 120 controls
Patient characteristics	Age, mean (SD): not reported Gender (male to female ratio): not reported Family origin: not reported Setting: Department of Neurology, Department of Immunology Country: Germany Cases: patients with neuroborreliosis – acute duration of symptoms ≤6 months (81), chronic duration of symptoms >6 months (15) Controls: people living in an endemic area who were found to be positive in routine serological tests (80), patients with neurosyphilis (20), patients with Epstein-Barr virus (20)
Target condition(s)	Lyme disease
Index test(s) and reference standard	Index test(s) ELISA (IgM) ELISA (IgG) Reference standard Clinical diagnosis

Reference	Kaiser 1999[47]				
	Time between measurement of index test and reference standard: not reported				
2×2 table		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA (IgM) recombinant Serum
	Index test +	81	6	87	
	Index test −	15	74	89	
	Total	96	80	176	
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA (IgM) whole-cell Serum
	Index test +	51	8	59	
	Index test −	45	72	117	
	Total	96	80	176	
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA (IgG) recombinant Serum
	Index test +	77	14	91	
	Index test −	19	66	85	
	Total	96	80	176	
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA (IgG) whole-cell Serum
	Index test +	75	80	155	
	Index test −	21	0	21	
	Total	96	80	176	
		Reference standard +	Reference standard −	Total	Neuroborreliosis <6 months ELISA (IgM) Serum
	Index test +	49	8	57	
	Index test −	32	72	104	
	Total	81	80	161	
		Reference standard +	Reference standard −	Total	Neuroborreliosis <6 months

Reference	**Kaiser 1999[47]**				
	Index test +	35	26	61	ELISA (IgG) Serum
	Index test −	46	54	100	
	Total	81	80	161	
		Reference standard +	Reference standard −	Total	Neuroborreliosis >6 months ELISA (IgM)
	Index test +	2	8	10	Serum
	Index test −	13	72	85	
	Total	15	80	95	
		Reference standard +	Reference standard −	Total	Neuroborreliosis >6 months ELISA (IgG)
	Index test +	15	26	41	Serum
	Index test −	0	54	54	
	Total	15	80	95	
Statistical measures	Index test: ELISA IgM recombinant (serum) - neuroborreliosis Sensitivity 0.84 Specificity 0.93 Index test: ELISA IgM whole-cell (serum) - neuroborreliosis Sensitivity 0.53 Specificity 0.90 Index test: ELISA IgG recombinant (serum) - neuroborreliosis Sensitivity 0.80 Specificity 0.82 Index test: ELISA IgG whole-cell (serum) - neuroborreliosis Sensitivity 0.78 Specificity 0.00 Index test: ELISA IgM (serum) – neuroborreliosis <6 months				

Reference	Kaiser 1999[47]
	Sensitivity 0.60 Specificity 0.90 Index test: ELISA IgG (serum) – neuroborreliosis <6 months Sensitivity 0.43 Specificity 0.68 Index test: ELISA IgM (serum) – neuroborreliosis >6 months Sensitivity 0.13 Specificity 0.90 Index test: ELISA IgG (serum) – neuroborreliosis > 6months Sensitivity 1.00 Specificity 0.68
Source of funding	Federal Ministry of Education and Research
Limitations	Risk of bias: selection, index test, reference standard Indirectness: none
Comments	Leukocytes pro microliter in CSF (median): acute neuroborreliosis: 172 (7-600), chronic neuroborreliosis: 60 (10-135) Total protein in CSF mg/L (median): acute neuroborreliosis: 1300 (460-3600), chronic neuroborreliosis: 2700 (500-7500)

Reference	Karlsson 1989[49]
Study type	Case-control
Study methodology	Data source: patients with neuroborreliosis and patients with meningitis/encephalitis Recruitment: not reported
Number of patients	n = 68 cases, 44 controls
Patient characteristics	Age, median (range): cases 46 years (6-73), controls 34 years (14-79) Gender (male to female ratio): cases 27:41, controls 21:23 Family origin: not reported Setting: not reported Country: Sweden Cases: patients with neuroborreliosis (fulfilling at least one of the following criteria: pleocytosis with a predominance of lymphocytes in the CSF together with neurological signs and symptoms and/or general symptoms compatible with neuroborreliosis, or neurological signs and symptoms following EM within 3 months) Controls: patients with meningitis/encephalitis of non-borrelia etiology (44)
Target condition(s)	Lyme disease
Index test(s) and reference standard	Index test(s) ELISA (IgM) ELISA (IgG) ELISA (IgM/IgG) Immunoblot (IgM) Immunoblot (IgG) Immunoblot (IgM/IgG)

Reference	Karlsson 1989[49]				
	Reference standard Clinical diagnosis Time between measurement of index test and reference standard: not reported				
2×2 table		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA (IgM) CSF
	Index test +	39	1	40	
	Index test −	29	43	72	
	Total	68	44	112	
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA (IgM) Serum
	Index test +	23	1	24	
	Index test −	45	43	88	
	Total	68	44	112	
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA (IgG) CSF
	Index test +	39	2	41	
	Index test −	29	42	71	
	Total	68	44	112	
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA (IgG) Serum
	Index test +	26	3	29	
	Index test −	42	41	83	
	Total	68	44	112	
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA (IgM/IgG) CSF
	Index test +	44	0	44	
	Index test −	24	0	24	

Reference	**Karlsson 1989[49]**				
	Total	68	0	68	
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA (IgM/IgG) Serum
	Index test +	40	4	44	
	Index test −	28	40	68	
	Total	68	44	112	
		Reference standard +	Reference standard −	Total	Neuroborreliosis Immunoblot (IgM) Serum
	Index test +	46	5	51	
	Index test −	22	39	61	
	Total	68	44	112	
		Reference standard +	Reference standard −	Total	Neuroborreliosis Immunoblot (IgG) Serum
	Index test +	44	5	49	
	Index test −	24	39	63	
	Total	68	44	112	
		Reference standard +	Reference standard −	Total	Neuroborreliosis Immunoblot (IgM/IgG) Serum
	Index test +	53	8	61	
	Index test −	15	36	51	
	Total	68	44	112	
		Reference standard +	Reference standard −	Total	Neuroborreliosis <6 weeks ELISA (IgM) Serum
	Index test +	20	1	21	
	Index test −	35	43	78	
	Total	55	44	99	
		Reference standard +	Reference standard −	Total	Neuroborreliosis <6 weeks ELISA (IgG)
	Index test +	15	3	18	

Reference	**Karlsson 1989[49]**				
	Index test −	40	41	81	Serum
	Total	55	44	99	
		Reference standard +	Reference standard −	Total	Neuroborreliosis <6 weeks ELISA (IgM/IgG)
	Index test +	28	4	32	Serum
	Index test −	27	40	67	
	Total	55	44	99	
		Reference standard +	Reference standard −	Total	Neuroborreliosis <6 weeks Immunoblot (IgM)
	Index test +	37	5	42	Serum
	Index test −	18	39	57	
	Total	55	44	99	
		Reference standard +	Reference standard −	Total	Neuroborreliosis <6 weeks Immunoblot (IgG)
	Index test +	32	5	37	Serum
	Index test −	23	39	62	
	Total	55	44	99	
		Reference standard +	Reference standard −	Total	Neuroborreliosis <6 weeks Immunoblot (IgM/IgG)
	Index test +	41	8	49	Serum
	Index test −	14	36	50	
	Total	55	44	99	
		Reference standard +	Reference standard −	Total	Neuroborreliosis >6 weeks ELISA (IgM)
	Index test +	3	1	4	Serum
	Index test −	10	43	53	
	Total	13	44	57	
		Reference standard +	Reference standard −	Total	Neuroborreliosis >6 weeks

Reference	**Karlsson 1989[49]**				
	Index test +	11	3	14	ELISA (IgG) Serum
	Index test −	2	41	43	
	Total	13	44	57	
		Reference standard +	Reference standard −	Total	Neuroborreliosis >6 weeks ELISA (IgM/IgG)
	Index test +	12	4	16	Serum
	Index test −	1	40	41	
	Total	13	44	57	
		Reference standard +	Reference standard −	Total	Neuroborreliosis >6 weeks Immunoblot (IgM)
	Index test +	9	5	14	Serum
	Index test −	4	39	43	
	Total	13	44	57	
		Reference standard +	Reference standard −	Total	Neuroborreliosis >6 weeks Immunoblot (IgG)
	Index test +	12	5	17	Serum
	Index test −	1	39	40	
	Total	13	44	57	
		Reference standard +	Reference standard −	Total	Neuroborreliosis >6 weeks Immunoblot (IgM/G)
	Index test +	12	8	20	Serum
	Index test −	1	36	37	
	Total	13	44	57	
Statistical measures	Index test: ELISA IgM (CSF) - neuroborreliosis Sensitivity 0.57 Specificity 0.98 Index test: ELISA IgM (serum) - neuroborreliosis Sensitivity 0.34				

Reference	Karlsson 1989[49]
	Specificity 0.98 Index test: ELISA IgG (CSF) - neuroborreliosis Sensitivity 0.57 Specificity 0.95 Index test: ELISA IgG (serum) - neuroborreliosis Sensitivity 0.38 Specificity 0.93 Index test: ELISA IgM/IgG (CSF) - neuroborreliosis Sensitivity 0.65 Specificity not estimable Index test: ELISA IgM/IgG (serum) - neuroborreliosis Sensitivity 0.59 Specificity 0.91 Index test: Immunoblot IgM (serum) - neuroborreliosis Sensitivity 0.68 Specificity 0.89 Index test: Immunoblot IgG (serum) - neuroborreliosis Sensitivity 0.65 Specificity 0.89 Index test: Immunoblot IgM/IgG (serum) - neuroborreliosis Sensitivity 0.78 Specificity 0.82 Index test: ELISA IgM (serum) – neuroborreliosis <6 weeks

Reference	Karlsson 1989[49]
	Sensitivity 0.36 Specificity 0.98 Index test: ELISA IgG (serum) – neuroborreliosis <6 weeks Sensitivity 0.27 Specificity 0.93 Index test: ELISA IgM/IgG (serum) – neuroborreliosis <6 weeks Sensitivity 0.51 Specificity 0.91 Index test: Immunoblot IgM (serum) – neuroborreliosis <6 weeks Sensitivity 0.67 Specificity 0.89 Index test: Immunoblot IgG (serum) – neuroborreliosis <6 weeks Sensitivity 0.58 Specificity 0.89 Index test: Immunoblot IgM/IgG (serum) – neuroborreliosis <6 weeks Sensitivity 0.75 Specificity 0.82 Index test: ELISA IgM (serum) – neuroborreliosis >6 weeks Sensitivity 1.00 Specificity 0.97 Index test: ELISA IgG (serum) – neuroborreliosis >6 weeks Sensitivity 0.85 Specificity 0.93

Reference	Karlsson 1989[49]
	Index test: ELISA IgM/IgG (serum) – neuroborreliosis >6 weeks Sensitivity 0.92 Specificity 0.91 Index test: Immunoblot IgM (serum) – neuroborreliosis >6 weeks Sensitivity 0.69 Specificity 0.89 Index test: Immunoblot IgG (serum) – neuroborreliosis >6 weeks Sensitivity 0.92 Specificity 0.89 Index test: Immunoblot IgM/IgG (serum) – neuroborreliosis >6 weeks Sensitivity 0.92 Specificity 0.82
Source of funding	Swedish Medical Research Council
Limitations	Risk of bias: selection, index test, reference standard Indirectness: none

Reference	Karlsson 1989a[48]
Study type	Case-control
Study methodology	Data source: patients with Lyme disease and patients with other illnesses Recruitment: not reported
Number of patients	n = 77 cases, 73 controls
Patient characteristics	Age, mean (SD): not reported Gender (male to female ratio): not reported Family origin: not reported Setting: not reported Country: Sweden Cases: patients with Lyme disease - EM (30), neuroborreliosis defined as pleocytosis with lymphocytic predominance in CSF together with neurological signs and symptoms and/or general symptoms compatible with neuroborreliosis, or neurological signs and symptoms within 3 months after onset of EM (37), ACA confirmed by histology (10) Controls: patients with meningitis/encephalitis of non-borrelia aetiology (35), multiple sclerosis (8), syphilis (10), Epstein-Barr virus infection (10), rheumatoid factor positive arthritis (10)
Target condition(s)	Lyme disease
Index test(s) and reference standard	Index test(s) ELISA (IgM) Reference standard Clinical diagnosis

Reference	Karlsson 1989a[48]				
	Time between measurement of index test and reference standard: not reported (sera drawn median 3 weeks range a few days – 2 months after onset of EM, median 3 weeks range a few days – 11 months after onset of neurological symptoms and median 2 years range 6 months – 20 years after onset of symptoms for ACA)				
2×2 table		Reference standard +	Reference standard −	Total	EM ELISA (IgM) capture Serum
	Index test +	10	2	12	
	Index test −	20	71	91	
	Total	30	73	103	
		Reference standard +	Reference standard −	Total	EM ELISA (IgM) indirect Serum
	Index test +	8	7	15	
	Index test −	22	66	88	
	Total	30	73	103	
		Reference standard +	Reference standard −	Total	ACA ELISA (IgM) capture Serum
	Index test +	0	2	2	
	Index test −	10	71	81	
	Total	10	73	83	
		Reference standard +	Reference standard −	Total	ACA ELISA (IgM) indirect Serum
	Index test +	3	7	10	
	Index test −	7	66	73	
	Total	10	73	83	
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA (IgM) capture Serum
	Index test +	20	2	22	
	Index test −	17	71	88	
	Total	37	73	110	
		Reference standard	Reference standard −	Total	Neuroborreliosis

Reference	**Karlsson 1989a**[48]				
		+			ELISA (IgM) indirect Serum
	Index test +	14	7	21	
	Index test −	23	66	89	
	Total	37	73	110	
		Reference standard +	Reference standard −	Total	Unspecified Lyme disease ELISA (IgM) capture Serum
	Index test +	30	2	32	
	Index test −	47	71	118	
	Total	77	73	150	
		Reference standard +	Reference standard −	Total	Unspecified Lyme disease ELISA (IgM) indirect Serum
	Index test +	24	7	31	
	Index test −	53	66	119	
	Total	77	73	150	
		Reference standard +	Reference standard −	Total	EM <6 weeks ELISA (IgM) capture Serum
	Index test +	8	2	10	
	Index test −	20	71	91	
	Total	28	73	101	
		Reference standard +	Reference standard −	Total	EM <6 weeks ELISA (IgM) indirect Serum
	Index test +	7	7	14	
	Index test −	21	66	87	
	Total	28	73	101	
		Reference standard +	Reference standard −	Total	Neuroborreliosis <6 weeks ELISA (IgM) capture Serum
	Index test +	17	2	19	
	Index test −	10	71	81	
	Total	27	73	100	

Reference	**Karlsson 1989a[48]**				
		Reference standard +	Reference standard −	Total	Neuroborreliosis <6 weeks ELISA (IgM) indirect Serum
	Index test +	12	7	19	
	Index test −	15	66	81	
	Total	27	73	100	
		Reference standard +	Reference standard −	Total	EM 6 weeks – 6 months ELISA (IgM) capture Serum
	Index test +	2	2	4	
	Index test −	0	71	71	
	Total	2	73	75	
		Reference standard +	Reference standard −	Total	EM 6 weeks – 6 months ELISA (IgM) indirect Serum
	Index test +	1	7	8	
	Index test −	1	66	67	
	Total	2	73	75	
		Reference standard +	Reference standard −	Total	Neuroborreliosis 6 weeks – 6 months ELISA (IgM) capture Serum
	Index test +	3	2	5	
	Index test −	7	71	78	
	Total	10	73	83	
		Reference standard +	Reference standard −	Total	Neuroborreliosis 6 weeks – 6 months ELISA (IgM) indirect Serum
	Index test +	2	7	9	
	Index test −	8	66	74	
	Total	10	73	83	
Statistical measures	Index test: ELISA IgM capture (serum) - EM Sensitivity 0.33 Specificity 0.97 Index test: ELISA IgM indirect (serum) - EM				

Reference	Karlsson 1989a[48]
	Sensitivity 0.27 Specificity 0.90 Index test: ELISA IgM capture (serum) - ACA Sensitivity 0.00 Specificity 0.97 Index test: ELISA IgM indirect (serum) - ACA Sensitivity 0.30 Specificity 0.90 Index test: ELISA IgM capture (serum) - neuroborreliosis Sensitivity 0.54 Specificity 0.97 Index test: ELISA IgM indirect (serum) - neuroborreliosis Sensitivity 0.38 Specificity 0.90 Index test: ELISA IgM capture (serum) – unspecified Lyme disease Sensitivity 0.39 Specificity 0.97 Index test: ELISA IgM indirect (serum) – unspecified Lyme disease Sensitivity 0.31 Specificity 0.90 Index test: ELISA IgM capture (serum) – EM <6 weeks Sensitivity 0.29 Specificity 0.97

Reference	Karlsson 1989a[48]
	Index test: ELISA IgM indirect (serum) – EM <6 weeks Sensitivity 0.25 Specificity 0.90 Index test: ELISA IgM capture (serum) – neuroborreliosis <6 weeks Sensitivity 0.63 Specificity 0.97 Index test: ELISA IgM indirect (serum) – neuroborreliosis <6 weeks Sensitivity 0.44 Specificity 0.90 Index test: ELISA IgM capture (serum) – EM 6 weeks – 6 months Sensitivity 1.00 Specificity 0.97 Index test: ELISA IgM indirect (serum) – EM 6 weeks – 6 months Sensitivity 0.50 Specificity 0.90 Index test: ELISA IgM capture (serum) – neuroborreliosis 6 weeks – 6 months Sensitivity 0.30 Specificity 0.97 Index test: ELISA IgM indirect (serum) – neuroborreliosis 6 weeks – 6 months Sensitivity 0.20 Specificity 0.90
Source of funding	'Förenade Liv' Mutual Group Life Insurance Company and Leila and Bertil Ehrengrens Memory foundation, Swedish Society for Medical Research
Limitations	Risk of bias: selection, index test, reference standard

Reference	Karlsson 1989a[48]
	Indirectness: none

Reference	Klempner 2001[50]
Study type	Case-control study
Study methodology	Data source: serum samples from patients Recruitment: not reported
Number of patients	n = 21
Patient characteristics	Age, mean (SD): not reported Gender (male to female ratio): not reported Family origin: not reported Setting: not reported Country: USA Cases: acute Lyme disease (n=21) Controls: healthy persons (n=10)
Target condition(s)	Lyme disease
Index test(s) and reference standard	Index test WB/IB (IgG) - serum Reference standard

Reference	Klempner 2001[50]				
	Clinical diagnosis based on CDC criteria Time between measurement of index test and reference standard: not reported				
2×2 table		Reference standard +	Reference standard −	Total	Unspecified Lyme disease WB/IB (IgG) Serum
	Index test +	14	0	14	
	Index test −	7	10	17	
	Total	21	10	31	
Statistical measures	WB/IB (IgG) - serum Sensitivity 0.67 Specificity 1.00				
Source of funding	Not reported				
Limitations	Risk of bias: patient selection, index test, reference standard Indirectness: none				

Reference	Lahey 2015[52]
Study type	Case-control study
Study methodology	Data source: serum samples from patients Recruitment: not reported
Number of patients	n = 84
Patient characteristics	Age, mean: not reported Gender (male to female ratio): not reported Family origin: not reported Setting: not reported Country: USA Cases: early Lyme disease (n=79), late Lyme disease (n=5) Controls: healthy controls (n=26)
Target condition(s)	EM, Lyme arthritis
Index test(s) and reference standard	Index tests ELISA (IgM/IgG) – serum Reference standard Clinical diagnosis Time between measurement of index test and reference standard: not reported

Reference	Lahey 2015[52]				
2×2 table		Reference standard +	Reference standard −	Total	EM ELISA (IgM/IgG) Serum
	Index test +	41	1	42	
	Index test −	38	25	63	
	Total	79	26	105	
2×2 table		Reference standard +	Reference standard −	Total	LA ELISA (IgM/IgG) Serum
	Index test +	5	1	6	
	Index test −	0	25	25	
	Total	5	26	31	
Statistical measures	ELISA (IgM/IgG) – serum (EM) Sensitivity 0.52 Specificity 0.96 ELISA (IgM/IgG) – serum (LA) Sensitivity 1.00 Specificity 0.96				
Source of funding	Supported by government grants, authors supported by industry				
Limitations	Risk of bias: patient selection, index test, reference standard Indirectness: none				

Reference	Lange 1992[53]
Study type	Case-control study
Study methodology	Data source: serum samples from patients Recruitment: not reported
Number of patients	n = 36
Patient characteristics	Age, mean: not reported Gender (male to female ratio): not reported Family origin: not reported Setting: not reported Country: Germany Cases: EM Controls: Blood donors (n=100)
Target condition(s)	EM
Index test(s) and reference standard	Index tests WB/IB (IgM) – serum ELISA (IgM, flagellum) – serum ELISA (IgM, sonicated) – serum Reference standard Clinical diagnosis

Reference	Lange 1992[53]				
	Time between measurement of index test and reference standard: not reported				
2×2 table		Reference standard +	Reference standard −	Total	EM ELISA (IgM, flagellum) Serum
	Index test +	12	6	18	
	Index test −	24	94	118	
	Total	36	100	136	
2×2 table		Reference standard +	Reference standard −	Total	EM ELISA (IgM, sonicated) Serum
	Index test +	10	4	14	
	Index test −	26	96	122	
	Total	36	100	136	
2×2 table		Reference standard +	Reference standard −	Total	EM WB/IB (IgM) Serum
	Index test +	29	0	29	
	Index test −	7	100	107	
	Total	36	100	136	
Statistical measures	ELISA (IgM, flagellum) – serum (EM) Sensitivity 0.33 Specificity 0.94 ELISA (IgM, sonicated) – serum (EM) Sensitivity 0.28 Specificity 0.96 WB/IB (IgM) – serum (EM)				

Reference	Lange 1992[53]
	Sensitivity 0.81 Specificity 1.00
Source of funding	Supported by government grants
Limitations	Risk of bias: patient selection, index test, reference standard Indirectness: none

Reference	Lawrenz 199[54]
Study type	Case-control study
Study methodology	Data source: serum samples from patients Recruitment: not reported
Number of patients	n = 81
Patient characteristics	Age, mean: not reported Gender (male to female ratio): not reported Family origin: not reported Setting: multiple laboratories Country: USA Cases: EM (n=41), Acute Neuroborreliosis (n=17), Lyme arthritis (n=23) Controls: none Lyme (n=50)
Target condition(s)	EM, Neuroborreliosis, Lyme arthritis
Index test(s) and reference standard	Index tests ELISA (IgM/IgG, recombinant) – serum ELISA (IgM/IgG, whole cell) – serum Reference standard Clinical diagnosis (EM patients were culture confirmed) Time between measurement of index test and reference standard: not reported

Reference	Lawrenz 199[54]				
2×2 table		Reference standard +	Reference standard −	Total	EM ELISA (IgM/IgG, recombinant) Serum
	Index test +	26	1	27	
	Index test −	15	49	64	
	Total	41	50	91	
2×2 table		Reference standard +	Reference standard −	Total	Lyme neuroborreliosis ELISA (IgM/IgG, recombinant) Serum
	Index test +	17	1	18	
	Index test −	0	49	49	
	Total	17	50	67	
2×2 table		Reference standard +	Reference standard −	Total	LA ELISA (IgM/IgG, recombinant) Serum
	Index test +	20	1	21	
	Index test −	3	49	52	
	Total	23	50	73	
2×2 table		Reference standard +	Reference standard −	Total	EM ELISA (IgM/IgG, whole cell) Serum
	Index test +	25	3	28	
	Index test −	16	47	63	
	Total	41	50	91	
2×2 table		Reference standard +	Reference standard −	Total	Lyme neuroborreliosis ELISA (IgM/IgG, whole cell) Serum
	Index test +	17	3	20	
	Index test −	0	47	47	
	Total	17	50	67	

Reference	**Lawrenz 199[54]**				
2×2 table		Reference standard +	Reference standard −	Total	LA ELISA (IgM/IgG, whole cell) Serum
	Index test +	22	3	25	
	Index test −	1	47	48	
	Total	23	50	73	
Statistical measures	ELISA (IgM/IgGm, recombinant) – serum (EM) Sensitivity 0.63 Specificity 0.98 ELISA (IgM/IgG, recombinant) – serum (Lyme neuroborreliosis) Sensitivity 1.00 Specificity 0.98 ELISA (IgM/IgG, recombinant) – serum (LA) Sensitivity 0.87 Specificity 0.98 ELISA (IgM/IgGm, whole cell) – serum (EM) Sensitivity 0.61 Specificity 0.94 ELISA (IgM/IgG, whole cell) – serum (Lyme neuroborreliosis) Sensitivity 1.00 Specificity 0.94 ELISA (IgM/IgG, whole cell) – serum (LA) Sensitivity 0.96 Specificity 0.94				

Reference	Lawrenz 199[54]
Source of funding	Supported by government grants
Limitations	Risk of bias: patient selection, index test, reference standard Indirectness: none

Reference	Lebech 1992[56]
Study type	Case-control study
Study methodology	Data source: urine and CSF samples from patients Recruitment: not reported
Number of patients	n = 10
Patient characteristics	Age, mean: 41.5 years (SD 24) Gender (male to female ratio): 5/5 Family origin: not reported Setting: not reported Country: Denmark Cases: Neuroborreliosis (n=10) Controls: Healthy controls (n=25), Urinary tract infections (n=10), Multiple sclerosis (n=5), Central nervous system infections (n=10)
Target condition(s)	Neuroborreliosis
Index test(s) and reference standard	Index tests PCR – urine PCR - CSF Reference standard Clinical diagnosis Time between measurement of index test and reference standard: not reported

Reference	Lebech 1992[56]				
2×2 table		Reference standard +	Reference standard −	Total	Lyme neuroborreliosis PCR Urine
	Index test +	9	0	9	
	Index test −	1	35	36	
	Total	10	35	45	
2×2 table		Reference standard +	Reference standard −	Total	Lyme neuroborreliosis PCR CSF
	Index test +	2	0	2	
	Index test −	8	15	23	
	Total	10	15	25	
Statistical measures	PCR – urine Sensitivity 0.90 Specificity 1.00 PCR – CSF Sensitivity 0.20 Specificity 1.00				
Source of funding	Supported by government and industry grants				
Limitations	Risk of bias: patient selection, index test, reference standard Indirectness: none				

Reference	Lebech 1998[55]
Study type	Case-control study
Study methodology	Data source: CSF samples from patients Recruitment: consecutive patients
Number of patients	n = 150
Patient characteristics	Age, mean: 46 years (5-87) Gender (male to female ratio): not reported Family origin: not reported Setting: not reported Country: Denmark Cases: early Neuroborreliosis (n=148), chronic Neuroborreliosis (n=2) Controls: Other neurologic diseases without clinical suspicion of Lyme disease (n=70)
Target condition(s)	Neuroborreliosis
Index test(s) and reference standard	Index tests PCR - CSF Reference standard Clinical diagnosis Time between measurement of index test and reference standard: not reported

Reference	**Lebech 1998[55]**				
2×2 table		Reference standard +	Reference standard −	Total	Lyme neuroborreliosis PCR CSF
	Index test +	31	1	32	
	Index test −	119	69	188	
	Total	150	70	220	
Statistical measures	PCR – CSF Sensitivity 0.21 Specificity 0.99				
Source of funding	Supported by government and industry grants				
Limitations	Risk of bias: patient selection, index test, reference standard Indirectness: serious				

Reference	Lebech 2000[57]
Study type	Case-control study
Study methodology	Data source: skin and CSF samples from patients Recruitment: not reported
Number of patients	n = 61
Patient characteristics	Age, median: EM: 51 years (18-91) Lyme neuroborreliosis: 47 years (2-79) Gender (male to female ratio): 39/22 Family origin: not reported Setting: not reported Country: Denmark Cases: EM (n=31), Neuroborreliosis (n=30) Controls: Healthy controls (n=7), Other neurological diseases (n=20), High-dose antibiotic treatment for other infectious diseases (n=6)
Target condition(s)	EM, Neuroborreliosis
Index test(s) and reference standard	Index tests PCR – CSF PCR – skin Reference standard Clinical diagnosis

Reference	Lebech 2000[57]				
	Time between measurement of index test and reference standard: not reported				
2×2 table		Reference standard +	Reference standard −	Total	EM PCR Skin
	Index test +	22	0	22	
	Index test −	9	38	47	
	Total	31	38	69	
2×2 table		Reference standard +	Reference standard −	Total	Lyme neuroborreliosis PCR CSF
	Index test +	5	0	5	
	Index test −	25	20	45	
	Total	30	20	50	
Statistical measures	PCR – skin Sensitivity 0.71 Specificity 1.00 PCR – CSF Sensitivity 0.17 Specificity 1.00				
Source of funding	None declared				
Limitations	Risk of bias: patient selection, index test, reference standard Indirectness: serious (Lyme neuroborreliosis only)				

Reference	Ledue 2008[58]
Study type	Case-control study
Study methodology	Data source: serum samples from patients Recruitment: not reported
Number of patients	n = 60
Patient characteristics	Age, mean: not reported Gender (male to female ratio): not reported Family origin: not reported Setting: regional clinical laboratory Country: USA Cases: Early localised (EM, n=19), Early disseminated (Multiple EM, arthritis, arthralgia, abdominal pain, generalised lymphadenopathy, CNS involvement, n=41) Controls: Healthy donors (n=600), Other infectious diseases (n=196), LYMErix vaccine (n=11)
Target condition(s)	EM, Lyme disease
Index test(s) and reference standard	Index tests ELISA (IgM/IgG) – serum CLIA (IgM/IgG) - serum Reference standard Culture

Reference	Ledue 2008[58]				
	Time between measurement of index test and reference standard: not reported				
2×2 table		Reference standard +	Reference standard −	Total	EM ELISA (IgM/IgG) Serum
	Index test +	11	17	28	
	Index test −	8	790	798	
	Total	19	807	826	
2×2 table		Reference standard +	Reference standard −	Total	EM CLIA (IgM/IgG) Serum
	Index test +	13	16	29	
	Index test −	6	791	798	
	Total	19	807	826	
2×2 table		Reference standard +	Reference standard −	Total	Early disseminated Lyme disease ELISA (IgM/IgG) Serum
	Index test +	33	17	50	
	Index test −	8	790	798	
	Total	41	807	848	
2×2 table		Reference standard +	Reference standard −	Total	Early disseminated Lyme disease CLIA (IgM/IgG) Serum
	Index test +	31	16	47	
	Index test −	10	791	801	
	Total	41	807	848	
Statistical measures	ELISA (IgM/IgG) – serum (EM) Sensitivity 0.58 Specificity 0.98				

Reference	Ledue 2008[58]
	CLIA (IgM/IgG) – serum (EM) Sensitivity 0.68 Specificity 0.98 ELISA (IgM/IgG) – serum (early disseminated) Sensitivity 0.80 Specificity 0.98 CLIA (IgM/IgG) – serum (early disseminated) Sensitivity 0.76 Specificity 0.98
Source of funding	Not reported
Limitations	Risk of bias: patient selection, index test, reference standard Indirectness: none

Reference	Lencakova 2008[59]
Study type	Case-control study
Study methodology	Data source: serum samples from patients before treatment Recruitment: not reported
Number of patients	n = 74
Patient characteristics	Age, mean: not reported Gender (male to female ratio): not reported Family origin: not reported Setting: local hospital Country: Slovakia Cases: Skin manifestations (n=54), Lyme neuroborreliosis (n=7), LA (n=13) Controls: Healthy persons (n=40), Rheumatoid factor (n=10), Fever (n=10)
Target condition(s)	EM, Neuroborreliosis, Lyme arthritis
Index test(s) and reference standard	Index tests ELISA (IgM/IgG) – serum ELISA (IgM) – serum ELISA (IgG) – serum WB/IB (IgM/IgG) – serum WB/IB (IgM) – serum WB/IB (IgG) – serum IFA (IgM/IgG) – serum

Reference	Lencakova 2008[59]				
	IFA (IgM) – serum IFA (IgG) – serum Reference standard Clinical diagnosis Time between measurement of index test and reference standard: not reported				
2×2 table		Reference standard +	Reference standard −	Total	EM ELISA (IgM) Serum
	Index test +	34	1	35	
	Index test −	20	59	79	
	Total	54	60	114	
2×2 table		Reference standard +	Reference standard −	Total	EM ELISA (IgG) Serum
	Index test +	23	1	24	
	Index test −	31	59	90	
	Total	54	60	114	
2×2 table		Reference standard +	Reference standard −	Total	EM ELISA (IgM/IgG) Serum
	Index test +	48	2	50	
	Index test −	6	58	64	
	Total	54	60	114	
2×2 table		Reference standard +	Reference standard −	Total	EM WB/IB (IgM) Serum
	Index test +	33	1	34	
	Index test −	21	59	80	

Reference	Lencakova 2008[59]				
	Total	54	60	114	
2×2 table		Reference standard +	Reference standard −	Total	EM WB/IB (IgG) Serum
	Index test +	29	0	29	
	Index test −	25	60	85	
	Total	54	60	114	
2×2 table		Reference standard +	Reference standard −	Total	EM WB/IB (IgM/IgG) Serum
	Index test +	50	1	51	
	Index test −	4	59	63	
	Total	54	60	114	
2×2 table		Reference standard +	Reference standard −	Total	EM IFA (IgM) Serum
	Index test +	20	1	21	
	Index test −	34	59	93	
	Total	54	60	114	
2×2 table		Reference standard +	Reference standard −	Total	EM IFA (IgG) Serum
	Index test +	24	1	25	
	Index test −	30	59	89	
	Total	54	60	114	
2×2 table		Reference standard +	Reference standard −	Total	EM IFA (IgM/IgG) Serum
	Index test +	36	1	37	
	Index test −	18	59	77	

Reference	Lencakova 2008[59]				
	Total	54	60	114	
2×2 table		Reference standard +	Reference standard −	Total	Lyme neuroborreliosis ELISA (IgM) Serum
	Index test +	3	1	4	
	Index test −	4	59	63	
	Total	7	60	67	
2×2 table		Reference standard +	Reference standard −	Total	Lyme neuroborreliosis ELISA (IgG) Serum
	Index test +	4	1	5	
	Index test −	3	59	62	
	Total	7	60	67	
2×2 table		Reference standard +	Reference standard −	Total	Lyme neuroborreliosis ELISA (IgM/IgG) Serum
	Index test +	7	2	9	
	Index test −	0	58	58	
	Total	7	60	67	
2×2 table		Reference standard +	Reference standard −	Total	Lyme neuroborreliosis WB/IB (IgM) Serum
	Index test +	2	1	3	
	Index test −	5	59	64	
	Total	7	60	67	
2×2 table		Reference standard +	Reference standard −	Total	Lyme neuroborreliosis WB/IB (IgG) Serum
	Index test +	4	0	4	
	Index test −	3	60	63	

Reference	Lencakova 2008[59]				
	Total	7	60	67	
2×2 table		Reference standard +	Reference standard −	Total	Lyme neuroborreliosis WB/IB (IgM/IgG) Serum
	Index test +	6	1	7	
	Index test −	1	59	60	
	Total	7	60	67	
2×2 table		Reference standard +	Reference standard −	Total	Lyme neuroborreliosis IFA (IgM) Serum
	Index test +	1	1	2	
	Index test −	6	59	65	
	Total	7	60	67	
2×2 table		Reference standard +	Reference standard −	Total	Lyme neuroborreliosis IFA (IgG) Serum
	Index test +	1	1	2	
	Index test −	6	59	65	
	Total	7	60	67	
2×2 table		Reference standard +	Reference standard −	Total	Lyme neuroborreliosis IFA (IgM/IgG) Serum
	Index test +	2	1	3	
	Index test −	5	59	64	
	Total	7	60	67	
2×2 table		Reference standard +	Reference standard −	Total	LA ELISA (IgM) Serum
	Index test +	0	1	1	
	Index test −	13	59	72	

Reference	Lencakova 2008[59]				
	Total	13	60	73	
2×2 table		Reference standard +	Reference standard −	Total	LA ELISA (IgG) Serum
	Index test +	12	1	13	
	Index test −	1	59	60	
	Total	13	60	73	
2×2 table		Reference standard +	Reference standard −	Total	LA ELISA (IgM/IgG) Serum
	Index test +	12	2	14	
	Index test −	1	59	59	
	Total	13	61	73	
2×2 table		Reference standard +	Reference standard −	Total	LA WB/IB (IgM) Serum
	Index test +	0	1	1	
	Index test −	13	59	72	
	Total	13	60	73	
2×2 table		Reference standard +	Reference standard −	Total	LA WB/IB (IgG) Serum
	Index test +	13	0	13	
	Index test −	0	60	60	
	Total	13	60	73	
2×2 table		Reference standard +	Reference standard −	Total	LA WB/IB (IgM/IgG) Serum
	Index test +	13	1	14	
	Index test −	0	59	59	

Reference	Lencakova 2008[59]				
	Total	13	60	73	
2×2 table		Reference standard +	Reference standard −	Total	LA IFA (IgM) Serum
	Index test +	0	1	1	
	Index test −	13	59	72	
	Total	13	60	73	
2×2 table		Reference standard +	Reference standard −	Total	LA IFA (IgG) Serum
	Index test +	10	1	11	
	Index test −	3	59	62	
	Total	13	60	73	
2×2 table		Reference standard +	Reference standard −	Total	LA IFA (IgM/IgG) Serum
	Index test +	10	1	11	
	Index test −	3	59	62	
	Total	13	60	73	
Statistical measures	ELISA (IgM) – serum (EM) Sensitivity 0.63 Specificity 0.98 ELISA (IgG) – serum (EM) Sensitivity 0.43 Specificity 0.98 ELISA (IgM/IgG) – serum (EM) Sensitivity 0.89				

Reference	Lencakova 2008[59]
	Specificity 0.97 WB/IB (IgM) – serum (EM) Sensitivity 0.61 Specificity 0.98 WB/IB (IgG) – serum (EM) Sensitivity 0.54 Specificity 1.00 WB/IB (IgM/IgG) – serum (EM) Sensitivity 0.93 Specificity 0.98 IFA (IgM) – serum (EM) Sensitivity 0.37 Specificity 0.98 IFA (IgG) – serum (EM) Sensitivity 0.44 Specificity 0.98 IFA (IgM/IgG) – serum (EM) Sensitivity 0.67 Specificity 0.98 ELISA (IgM) – serum (Lyme neuroborreliosis) Sensitivity 0.43 Specificity 0.98 ELISA (IgG) – serum (Lyme neuroborreliosis)

Reference	Lencakova 2008[59]
	Sensitivity 0.57 Specificity 0.98 ELISA (IgM/IgG) – serum (Lyme neuroborreliosis) Sensitivity 1.00 Specificity 0.97 WB/IB (IgM) – serum (Lyme neuroborreliosis) Sensitivity 0.29 Specificity 0.98 WB/IB (IgG) – serum (Lyme neuroborreliosis) Sensitivity 0.57 Specificity 1.00 WB/IB (IgM/IgG) – serum (Lyme neuroborreliosis) Sensitivity 0.86 Specificity 0.98 IFA (IgM) – serum (Lyme neuroborreliosis) Sensitivity 0.14 Specificity 0.98 IFA (IgG) – serum (Lyme neuroborreliosis) Sensitivity 0.14 Specificity 0.98 IFA (IgM/IgG) – serum (Lyme neuroborreliosis) Sensitivity 0.29 Specificity 0.98

Reference	Lencakova 2008[59]
	ELISA (IgM) – serum (LA) Sensitivity 0.00 Specificity 0.98 ELISA (IgG) – serum (LA) Sensitivity 0.92 Specificity 0.98 ELISA (IgM/IgG) – serum (LA) Sensitivity 0.92 Specificity 0.97 WB/IB (IgM) – serum (LA) Sensitivity 0.00 Specificity 0.98 WB/IB (IgG) – serum (LA) Sensitivity 1.00 Specificity 1.00 WB/IB (IgM/IgG) – serum (LA) Sensitivity 1.00 Specificity 0.98 IFA (IgM) – serum (LA) Sensitivity 0.00 Specificity 0.98 IFA (IgG) – serum (LA) Sensitivity 0.77 Specificity 0.98

Reference	Lencakova 2008[59]
	IFA (IgM/IgG) – serum (LA) Sensitivity 0.77 Specificity 0.98
Source of funding	Supported by government and industry grants
Limitations	Risk of bias: patient selection, reference standard Indirectness: none

Reference	Leung 1989[60]
Study type	Case-control study
Study methodology	Data source: serum samples from patients Recruitment: not reported
Number of patients	n = 10
Patient characteristics	Age, mean: not reported Gender (male to female ratio): not reported Family origin: not reported Setting: not reported Country: USA Cases: Lyme disease Controls: Syphilis (n=14), Infectious mononucleosis (n=4), Rheumatoid factor (n=11)
Target condition(s)	EM, Lyme disease
Index test(s) and reference standard	Index tests ELISA (IgM/IgG) – serum Reference standard Clinical diagnosis Time between measurement of index test and reference standard: not reported

Reference	**Leung 1989[60]**				
2×2 table		Reference standard +	Reference standard −	Total	EM ELISA (IgM/IgG, colorimetric) Serum
	Index test +	7	16	23	
	Index test −	3	13	16	
	Total	10	29	39	
2×2 table		Reference standard +	Reference standard −	Total	EM ELISA (IgM/IgG, sonicated) Serum
	Index test +	9	7	16	
	Index test −	1	22	23	
	Total	10	29	39	
Statistical measures	ELISA (IgM/IgG, colorimetric) – serum (EM) Sensitivity 0.70 Specificity 0.45 ELISA (IgM/IgG, sonicated) – serum (EM) Sensitivity 0.90 Specificity 0.76				
Source of funding	Not reported				
Limitations	Risk of bias: patient selection, index test, reference standard Indirectness: none				

Reference	Liebling 1993[61]
Study type	Case-control study
Study methodology	Data source: serum, CSF, synovial fluid and urine samples from patients Recruitment: not reported
Number of patients	n = 44
Patient characteristics	Age, mean: not reported Gender (male to female ratio): not reported Family origin: not reported Setting: university hospital Country: USA Cases: Lyme disease Controls: Other inflammatory, autoimmune or infectious diseases (n=47)
Target condition(s)	Lyme disease
Index test(s) and reference standard	Index tests PCR (serum) PCR (urine) PCR (CSF) PCR (synovial fluid) Reference standard Clinical diagnosis

Reference	Liebling 1993[61]				
	Time between measurement of index test and reference standard: not reported				
2×2 table		Reference standard +	Reference standard −	Total	Lyme disease PCR Serum
	Index test +	13	0	13	
	Index test −	9	6	15	
	Total	22	6	28	
2×2 table		Reference standard +	Reference standard −	Total	Lyme disease PCR CSF
	Index test +	13	1	14	
	Index test −	0	14	14	
	Total	13	15	28	
2×2 table		Reference standard +	Reference standard −	Total	Lyme disease PCR Urine
	Index test +	3	1	4	
	Index test −	0	12	12	
	Total	3	13	16	
2×2 table		Reference standard +	Reference standard −	Total	Lyme disease PCR SF
	Index test +	4	0	4	
	Index test −	1	22	23	
	Total	5	22	27	
Statistical measures	PCR – serum Sensitivity 0.59 Specificity 1.00				

Reference	Liebling 1993[61]
	PCR – CSF Sensitivity 1.00 Specificity 0.93 PCR – urine Sensitivity 1.00 Specificity 0.92 PCR – SF Sensitivity 0.80 Specificity 1.00
Source of funding	Not reported
Limitations	Risk of bias: patient selection, reference standard Indirectness: none

Reference	Liu 2013[63]
Study type	Case-control study
Study methodology	Data source: serum samples from patients Recruitment: not reported
Number of patients	n = 159
Patient characteristics	Age, mean: not reported Gender (male to female ratio): not reported Family origin: not reported Setting: regional clinical laboratory Country: China Cases: EM (n=52), Neuroborreliosis (n=65), ACA (n=28), Lyme arthritis (n=14) Controls (n=292): Healthy blood donors (n=105), Syphilis (n=58), Leptospirosis (n=75), RA (n=54)
Target condition(s)	EM, Neuroborreliosis
Index test(s) and reference standard	Index tests ELISA (IgM) – serum ELISA (IgG) – serum WB/IB (IgM) – serum WB/IB (IgG) - serum Reference standard Clinical diagnosis

Reference	Liu 2013[63]				
	Time between measurement of index test and reference standard: not reported				
2×2 table [ELISA (IgM) – serum], EM		Reference standard +	Reference standard −	Total	
	Index test +	30	58	88	
	Index test −	22	234	256	
	Total	52	292	344	
2×2 table [ELISA (IgG) – serum], EM		Reference standard +	Reference standard −	Total	
	Index test +	41	67	108	
	Index test −	11	225	236	
	Total	52	292	344	
2×2 table [WB/IB (IgM) – serum], EM		Reference standard +	Reference standard −	Total	
	Index test +	24	17	41	
	Index test −	28	275	303	
	Total	52	292	344	
2×2 table [WB/IB (IgG) – serum], EM		Reference standard +	Reference standard −	Total	
	Index test +	35	5	40	
	Index test −	17	287	304	
	Total	52	292	344	
2×2 table [ELISA (IgM) – serum], Lyme neuroborreliosi		Reference standard +	Reference standard −	Total	
	Index test +	40	58	98	
	Index test −	25	234	259	

Reference	Liu 2013[63]				
s	Total	65	292	357	
2×2 table [ELISA (IgG) – serum], Lyme neuroborreliosis		Reference standard +	Reference standard −	Total	
	Index test +	55	67	122	
	Index test −	10	225	235	
	Total	65	292	357	
2×2 table [WB/IB (IgM) – serum], Lyme neuroborreliosis		Reference standard +	Reference standard −	Total	
	Index test +	32	17	49	
	Index test −	33	275	308	
	Total	65	292	357	
2×2 table [WB/IB (IgG) – serum], Lyme neuroborreliosis		Reference standard +	Reference standard −	Total	
	Index test +	45	5	50	
	Index test −	20	287	307	
	Total	65	292	357	
Statistical measures	ELISA (IgM) – serum (EM) Sensitivity 0.58 Specificity 0.80 ELISA (IgG) – serum (EM) Sensitivity 0.79 Specificity 0.77 WB/IB (IgM) – serum (EM) Sensitivity 0.46				

Reference	Liu 2013[63]
	Specificity 0.94 WB/IB (IgG) – serum (EM) Sensitivity 0.67 Specificity 0.98 ELISA (IgM) – serum (Lyme neuroborreliosis) Sensitivity 0.62 Specificity 0.80 ELISA (IgG) – serum (Lyme neuroborreliosis) Sensitivity 0.85 Specificity 0.77 WB/IB (IgM) – serum (Lyme neuroborreliosis) Sensitivity 0.49 Specificity 0.94 WB/IB (IgG) – serum (Lyme neuroborreliosis) Sensitivity 0.69 Specificity 0.98
Source of funding	Not reported
Limitations	Risk of bias: patient selection, index test, reference standard Indirectness: none

Reference	Magnarelli 1988[65]
Study type	Case-control study
Study methodology	Data source: not reported Recruitment: not reported
Number of patients	n = 102
Patient characteristics	Age, mean (SD): not reported Gender (male to female ratio): not reported Family origin: not reported Setting: not reported Country: USA Cases: EM plus later manifestations Controls: Syphilis (n=15), yaws (n=8), louse-borne relapsing fever (n=11), tick-borne relapsing fever (n=8), leptospirosis (n=12), Rocky Mountain spotted fever (n=16), RA (=7)
Target condition(s)	EM plus later manifestations
Index test(s) and reference standard	Index tests ELISA (IgM) - serum ELISA (IgG) - serum Reference standard Clinical diagnosis Time between measurement of index test and reference standard: not reported

Reference	Magnarelli 1988[65]				
2×2 table [ELISA IgM – serum]		Reference standard +	Reference standard −	Total	
	Index test +	86	32	118	
	Index test −	16	45	61	
	Total	102	77	179	
2×2 table [ELISA IgG – serum]		Reference standard +	Reference standard −	Total	
	Index test +	73	17	90	
	Index test −	22	60	82	
	Total	95	77	172	
Statistical measures	ELISA IgM (serum) Sensitivity 0.84 Specificity 0.58 ELISA IgG serum) Sensitivity 0.77 Specificity 0.78				
Source of funding	Not reported				
Limitations	Risk of bias: patient selection, index test, reference standard Indirectness: none				

Reference	Magnarelli 1992[66]
Study type	Case-control study
Study methodology	Data source: serum samples previously used Recruitment: not reported
Number of patients	n = 53
Patient characteristics	Age, mean (SD): not reported Gender (male to female ratio): not reported Family origin: not reported Setting: tick-infested area Country: USA Cases: EM with antibodies (n=17), EM without antibodies (n=36) Controls: healthy persons (n=40)
Target condition(s)	EM with or without antibodies
Index test(s) and reference standard	Index tests ELISA (IgG, biotin, recombinant) – serum ELISA (IgG, biotin, whole-cell) - serum ELISA (IgG, unabsorbed, recombinant) - serum ELISA (IgG, unabsorbed, whole-cell) - serum Reference standard Clinical diagnosis

Reference	Magnarelli 1992[66]				
	Time between measurement of index test and reference standard: not reported				
2×2 table [ELISA (IgG, biotin, recombinant) – serum]		Reference standard +	Reference standard −	Total	
	Index test +	19	0	19	
	Index test −	34	40	74	
	Total	53	40	93	
2×2 table [ELISA (IgG, biotin, whole-cell) – serum]		Reference standard +	Reference standard −	Total	
	Index test +	20	0	20	
	Index test −	33	40	73	
	Total	53	40	93	
2×2 table [ELISA (IgG, unabsorbed, recombinant) – serum]		Reference standard +	Reference standard −	Total	
	Index test +	18	0	18	
	Index test −	35	40	75	
	Total	53	40	93	
2×2 table [ELISA (IgG, unabsorbed, whole-cell) – serum]		Reference standard +	Reference standard −	Total	
	Index test +	17	0	17	
	Index test −	36	40	76	
	Total	53	40	93	
Statistical measures	ELISA (IgG, biotin, recombinant) - serum Sensitivity 0.36 Specificity 1.00				

Reference	Magnarelli 1992[66]
	ELISA (IgG, biotin, whole-cell) - serum Sensitivity 0.38 Specificity 1.00 ELISA (IgG, unabsorbed, recombinant) - serum Sensitivity 0.34 Specificity 1.00 ELISA (IgG, unabsorbed, whole-cell) - serum Sensitivity 0.32 Specificity 1.00
Source of funding	Supported by CDC grants and grants from the National Institutes of Health and the Mathers Foundation.
Limitations	Risk of bias: patient selection, index test, reference standard Indirectness: none

Reference	Marangoni 2005[68]
Study type	Case-control study
Study methodology	Data source: serum samples Recruitment: not reported
Number of patients	n = 45
Patient characteristics	Age, mean (range): 42 years (29-65) Gender (male to female ratio): 31:14 Family origin: not reported Setting: endemic area Country: Italy Cases: EM Controls: healthy blood donors (n=234)
Target condition(s)	EM
Index test(s) and reference standard	Index tests ELISA (IgM, Enzygnost) – serum ELISA (IgG, Enzygnost) – serum ELISA (IgM/IgG, Enzygnost) – serum ELISA (IgM/IgG, Quick C6) – serum ELISA (IgM, RecomWell) – serum ELISA (IgG, RecomWell) - serum ELISA (IgM/IgG, RecomWell) - serum

Reference	Marangoni 2005[68]				
	Reference standard Culture Time between measurement of index test and reference standard: not reported				
2×2 table [ELISA (IgM, Enzygnost) – serum]		Reference standard +	Reference standard −	Total	
	Index test +	67	9	76	
	Index test −	28	225	253	
	Total	95	234	329	
2×2 table [ELISA (IgG, Enzygnost) – serum]		Reference standard +	Reference standard −	Total	
	Index test +	35	27	62	
	Index test −	60	207	267	
	Total	95	234	329	
2×2 table [ELISA (IgM/IgG, Enzygnost) – serum]		Reference standard +	Reference standard −	Total	
	Index test +	74	36	110	
	Index test −	21	198	219	
	Total	95	234	329	
2×2 table [ELISA (IgM/IgG, Quick C6) – serum]		Reference standard +	Reference standard −	Total	
	Index test +	59	8	67	
	Index test −	36	226	262	
	Total	95	234	329	

Reference	Marangoni 2005[68]				
2×2 table [ELISA (IgM, RecomWell) – serum]		Reference standard +	Reference standard −	Total	
	Index test +	53	0	53	
	Index test −	42	234	276	
	Total	95	234	329	
2×2 table [ELISA (IgG, RecomWell) – serum]		Reference standard +	Reference standard −	Total	
	Index test +	55	7	62	
	Index test −	40	227	267	
	Total	95	234	329	
2×2 table [ELISA (IgM/IgG, RecomWell) – serum]		Reference standard +	Reference standard −	Total	
	Index test +	70	7	77	
	Index test −	25	227	252	
	Total	95	234	329	
Statistical measures	ELISA (IgM, Enzygnost) - serum Sensitivity 0.71 Specificity 0.96 ELISA (IgG, Enzygnost) - serum Sensitivity 0.37 Specificity 0.88 ELISA (IgM/IgG, Enzygnost) - serum Sensitivity 0.78 Specificity 0.85				

Reference	Marangoni 2005[68]
	ELISA (IgM/IgG, Quick C6) - serum Sensitivity 0.62 Specificity 0.97 ELISA (IgM, RecomWell) - serum Sensitivity 0.56 Specificity 1.00 ELISA (IgG, RecomWell) - serum Sensitivity 0.58 Specificity 0.97 ELISA (IgM/IgG, RecomWell) - serum Sensitivity 0.74 Specificity 0.97
Source of funding	Supported by government grants
Limitations	Risk of bias: patient selection, reference standard Indirectness: none

Reference	Marangoni 2008[67]
Study type	Case-control study
Study methodology	Data source: serum samples Recruitment: not reported
Number of patients	n = 66
Patient characteristics	Age, mean: 45.3 years Gender (male to female ratio): 29:37 Family origin: not reported Setting: endemic area Country: Italy Cases: EM Controls: blood bank in Bologna (n=300)
Target condition(s)	EM
Index test(s) and reference standard	Index tests ELISA (IgM) – serum ELISA (IgG) – serum CLIA (IgM) – serum CLIA (IgG) - serum Reference standard Culture-confirmed EM

Reference	Marangoni 2008[67]				
	Time between measurement of index test and reference standard: not reported				
2×2 table [ELISA (IgM) – serum]		Reference standard +	Reference standard −	Total	
	Index test +	36	10	46	
	Index test −	30	290	320	
	Total	66	300	366	
2×2 table [ELISA (IgG) – serum]		Reference standard +	Reference standard −	Total	
	Index test +	37	5	42	
	Index test −	29	295	324	
	Total	66	300	366	
2×2 table [CLIA (IgM) – serum]		Reference standard +	Reference standard −	Total	
	Index test +	16	19	35	
	Index test −	50	281	331	
	Total	66	300	366	
2×2 table [CLIA (IgG) – serum]		Reference standard +	Reference standard −	Total	
	Index test +	26	9	35	
	Index test −	40	291	331	
	Total	66	300	366	
Statistical measures	ELISA (IgM) - serum Sensitivity 0.55 Specificity 0.97				

Reference	Marangoni 2008[67]
	ELISA (IgG) - serum Sensitivity 0.56 Specificity 0.98 CLIA (IgM) - serum Sensitivity 0.24 Specificity 0.94 CLIA (IgG) - serum Sensitivity 0.39 Specificity 0.97
Source of funding	Not reported
Limitations	Risk of bias: patient selection, index test, reference standard Indirectness: none

Reference	Mathiesen 1996[69]
Study type	Case-control study
Study methodology	Data source: serum samples Recruitment: not reported
Number of patients	n = 117
Patient characteristics	Age, mean (SD): not reported Gender (male to female ratio): not reported Family origin: not reported Setting: endemic area Country: Italy Cases: EM (n=47), Neuroborreliosis (n=60), ACA (n=20) Controls: blood donors (n=100) Disease duration (median): EM: 3 weeks (<1 week to 1 year) Lyme neuroborreliosis: 3 weeks (1 week to 1.5 years after onset of neurological symptoms) ACA: 4 years (8 months to 10 years)
Target condition(s)	EM, Neuroborreliosis, ACA
Index test(s) and reference standard	Index tests ELISA (IgM) – serum ELISA (IgG) – serum WB/IB (IgM) – serum

Reference	Mathiesen 1996[69]				
	WB/IB (IgM) – serum Reference standard Clinical diagnosis, Culture-confirmed EM Time between measurement of index test and reference standard: not reported				
2×2 table [ELISA (IgM) – serum], EM		Reference standard +	Reference standard −	Total	
	Index test +	19	1	20	
	Index test −	28	99	127	
	Total	47	100	147	
2×2 table [ELISA (IgG) – serum], EM		Reference standard +	Reference standard −	Total	
	Index test +	14	0	14	
	Index test −	33	100	133	
	Total	47	100	147	
2×2 table [WB/IB (IgM) – serum], EM		Reference standard +	Reference standard −	Total	
	Index test +	17	1	18	
	Index test −	30	99	129	
	Total	47	100	147	
2×2 table [WB/IB (IgG) – serum], EM		Reference standard +	Reference standard −	Total	
	Index test +	12	4	16	
	Index test −	35	96	131	
	Total	47	100	147	

Reference	Mathiesen 1996[69]				
2×2 table [ELISA (IgM) – serum], Lyme neuroborreliosis		Reference standard +	Reference standard −	Total	
	Index test +	3	1	4	
	Index test −	17	99	116	
	Total	20	100	120	
2×2 table [ELISA (IgG) – serum], Lyme neuroborreliosis		Reference standard +	Reference standard −	Total	
	Index test +	20	0	20	
	Index test −	0	100	100	
	Total	20	100	120	
2×2 table [WB/IB (IgM) – serum], Lyme neuroborreliosis		Reference standard +	Reference standard −	Total	
	Index test +	2	1	3	
	Index test −	18	99	117	
	Total	20	100	120	
2×2 table [WB/IB (IgG) – serum], Lyme neuroborreliosis		Reference standard +	Reference standard −	Total	
	Index test +	1	4	5	
	Index test −	19	96	115	
	Total	20	100	120	
2×2 table [ELISA (IgM) – serum], ACA		Reference standard +	Reference standard −	Total	
	Index test +	33	1	34	
	Index test −	17	99	116	
	Total	50	100	150	

Reference	Mathiesen 1996[69]				
2×2 table [ELISA (IgG) – serum], ACA		Reference standard +	Reference standard −	Total	
	Index test +	21	0	21	
	Index test −	29	100	129	
	Total	50	100	150	
2×2 table [WB/IB (IgM) – serum], ACA		Reference standard +	Reference standard −	Total	
	Index test +	30	1	31	
	Index test −	20	99	119	
	Total	50	100	150	
2×2 table [WB/IB (IgG) – serum], ACA		Reference standard +	Reference standard −	Total	
	Index test +	23	4	27	
	Index test −	27	96	123	
	Total	50	100	150	
Statistical measures	ELISA (IgM) – serum (EM) Sensitivity 0.40 Specificity 0.99 ELISA (IgG) – serum (EM) Sensitivity 0.30 Specificity 1.00 WB/IB (IgM) – serum (EM) Sensitivity 0.36 Specificity 0.99				

Reference	Mathiesen 1996[69]
	WB/IB (IgG) – serum (EM) Sensitivity 0.26 Specificity 0.96 ELISA (IgM) – serum (Lyme neuroborreliosis) Sensitivity 0.15 Specificity 0.99 ELISA (IgG) – serum (Lyme neuroborreliosis) Sensitivity 1.00 Specificity 1.00 WB/IB (IgM) – serum (Lyme neuroborreliosis) Sensitivity 0.10 Specificity 0.99 WB/IB (IgG) – serum (Lyme neuroborreliosis) Sensitivity 0.05 Specificity 0.96 ELISA (IgM) – serum (ACA) Sensitivity 0.66 Specificity 0.99 ELISA (IgG) – serum (ACA) Sensitivity 0.42 Specificity 1.00 WB/IB (IgM) – serum (ACA) Sensitivity 0.60

Reference	Mathiesen 1996[69]
	Specificity 0.99 WB/IB (IgG) – serum (ACA) Sensitivity 0.46 Specificity 0.96
Source of funding	Kit provided by external source
Limitations	Risk of bias: patient selection, reference standard Indirectness: none

Reference	Merljak Skocir 2008[70]
Study type	Case-control study
Study methodology	Data source: patients diagnosed at outpatient clinic Recruitment: not reported
Number of patients	n = 50
Patient characteristics	Age, mean (SD): not reported Gender (male to female ratio): not reported Family origin: not reported Setting: outpatient clinic, medical university Country: Slovenia Cases: EM Controls: blood donors (n=50)
Target condition(s)	EM
Index test(s) and reference standard	Index tests WB/IB (IgM) – serum WB/IB (IgG) - serum Reference standard Clinical diagnosis Time between measurement of index test and reference standard: not reported

Reference	Merljak Skocir 2008[70]				
2×2 table [WB/IB (IgM) – serum]		Reference standard +	Reference standard −	Total	
	Index test +	4	0	4	
	Index test −	21	26	47	
	Total	25	26	51	
2×2 table [WB/IB (IgG) – serum]		Reference standard +	Reference standard −	Total	
	Index test +	8	7	15	
	Index test −	17	19	36	
	Total	25	26	51	
Statistical measures	WB/IB (IgM) - serum Sensitivity 0.16 Specificity 1.00 WB/IB (IgG) - serum Sensitivity 0.32 Specificity 0.73				
Source of funding	Not reported				
Limitations	Risk of bias: patient selection, index test, reference standard Indirectness: none				

Reference	Mitchell 1994[71]
Study type	Case-control study
Study methodology	Data source: serum samples Recruitment: not reported
Number of patients	n = 51
Patient characteristics	Age, range: 2-76 years Gender (male to female ratio): 32:19 Family origin: not reported Setting: not reported Country: USA Cases: EM Controls: healthy persons (n=16)
Target condition(s)	EM
Index test(s) and reference standard	Index tests ELISA (IgM/IgG) – serum ELFA – serum IFA (IgM) – serum Reference standard Culture Time between measurement of index test and reference standard: not reported

Reference	Mitchell 1994[71]				
2×2 table [ELISA (IgM/IgG) – serum], multiple EM		Reference standard +	Reference standard −	Total	
	Index test +	0	0	0	
	Index test −	32	16	48	
	Total	32	16	48	
2×2 table [ELFA – serum], multiple EM		Reference standard +	Reference standard −	Total	
	Index test +	18	0	18	
	Index test −	14	16	30	
	Total	32	16	48	
2×2 table [IFA (IgM) – serum], multiple EM		Reference standard +	Reference standard −	Total	
	Index test +	32	0	32	
	Index test −	0	16	16	
	Total	32	16	48	
2×2 table [ELISA (IgM/IgG) – serum], single EM		Reference standard +	Reference standard −	Total	
	Index test +	1	0	1	
	Index test −	18	16	34	
	Total	19	16	35	
2×2 table [ELFA – serum], single EM		Reference standard +	Reference standard −	Total	
	Index test +	5	0	5	
	Index test −	14	16	30	
	Total	19	16	35	

Reference	Mitchell 1994[71]				
2×2 table [IFA (IgM) – serum], single EM		Reference standard +	Reference standard −	Total	
	Index test +	8	0	8	
	Index test −	11	16	27	
	Total	19	16	35	
Statistical measures	ELISA (IgM/IgG) – serum (multiple EM) Sensitivity 0.00 Specificity 1.00 ELFA – serum (multiple EM) Sensitivity 0.56 Specificity 1.00 IFA (IgM) – serum (multiple EM) Sensitivity 1.00 Specificity 1.00 ELISA (IgM/IgG) – serum (single EM) Sensitivity 0.05 Specificity 1.00 ELFA – serum (single EM) Sensitivity 0.26 Specificity 1.00 IFA (IgM) – serum (single EM) Sensitivity 0.42 Specificity 1.00				

Reference	Mitchell 1994[71]
Source of funding	Grants by the CDC and Marshfield Medical Foundation
Limitations	Risk of bias: patient selection, index test, reference standard Indirectness: none

Reference	Molins 2014[75]
Study type	Case-control study
Study methodology	Data source: not reported Recruitment: not reported
Number of patients	n = 124
Patient characteristics	Age, mean (SD): not reported Gender (male to female ratio): not reported Family origin: not reported Setting: not reported Country: USA Cases: Early Lyme disease with EM acute phase (n=40) Early Lyme disease with EM convalescent phase (n=38) Early disseminated Lyme carditis (n=7) Early disseminated Lyme neuroborreliosis (n=10) Late Lyme disease, LA (29) Controls: healthy persons (n=203)

Reference	Molins 2014[75]				
	Standard CDC algorithm used for ELISA (IgM and IgG) + IB (IgM and IgG) – IgG used only after 1 month				
Target condition(s)	EM, Neuroborreliosis, Lyme carditis, Lyme arthritis, unspecified Lyme disease				
Index test(s) and reference standard	Index tests ELISA (IgM/IgG) – serum WB/IB (IgM) – serum WB/IB (IgG) – serum WB/IB (IgM/IgG) – serum PCR – blood and skin PCR – blood, skin and heart tissue PCR – synovial fluid Culture – blood and skin Culture – blood, skin and heart tissue Reference standard Clinical diagnosis Time between measurement of index test and reference standard: not reported				
2×2 table [ELISA (IgM/IgG) – serum], acute Lyme disease		Reference standard +	Reference standard −	Total	
	Index test +	27	14	41	
	Index test −	13	189	202	
	Total	40	203	243	
2×2 table [WB/IB (IgM) – serum], acute Lyme disease		Reference standard +	Reference standard −	Total	
	Index test +	14	4	18	
	Index test −	26	199	225	

Reference	Molins 2014[75]				
	Total	40	203	243	
2×2 table [WB/IB (IgG) – serum], acute Lyme disease		Reference standard +	Reference standard −	Total	
	Index test +	8	2	10	
	Index test −	32	201	233	
	Total	40	203	243	
2×2 table [WB/IB (IgMIgG) – serum], acute Lyme disease		Reference standard +	Reference standard −	Total	
	Index test +	18	6	24	
	Index test −	0	197	197	
	Total	18	203	221	
2×2 table [PCR – blood and skin], EM		Reference standard +	Reference standard −	Total	
	Index test +	24	0	24	
	Index test −	15	0	15	
	Total	39	0	39	
2×2 table [Culture – blood and skin], EM		Reference standard +	Reference standard −	Total	
	Index test +	17	0	17	
	Index test −	22	0	22	
	Total	39	0	39	
2×2 table [PCR – blood and skin], Lyme		Reference standard +	Reference standard −	Total	
	Index test +	2	0	2	
	Index test −	6	0	6	

Reference	Molins 2014[75]				
neuroborreliosis	Total	8	0	8	
2×2 table [Culture – blood and skin], Lyme neuroborreliosis		Reference standard +	Reference standard −	Total	
	Index test +	2	0	2	
	Index test −	4	0	4	
	Total	6	0	6	
2×2 table [PCR – blood, skin and heart], carditis		Reference standard +	Reference standard −	Total	
	Index test +	2	0	2	
	Index test −	5	0	5	
	Total	7	0	7	
2×2 table [Culture – blood, skin and heart], carditis		Reference standard +	Reference standard −	Total	
	Index test +	0	0	0	
	Index test −	4	0	4	
	Total	4	0	4	
2×2 table [ELISA (IgM/IgG) – serum], conval Lyme disease		Reference standard +	Reference standard −	Total	
	Index test +	34	14	48	
	Index test −	4	189	193	
	Total	38	203	241	
2×2 table [WB/IB (IgM) – serum], conval Lyme disease		Reference standard +	Reference standard −	Total	
	Index test +	20	4	24	
	Index test −	18	199	217	

Reference	Molins 2014[75]				
	Total	38	203	241	
2×2 table [WB/IB (IgG) – serum], conval Lyme disease		Reference standard +	Reference standard −	Total	
	Index test +	14	2	16	
	Index test −	24	201	225	
	Total	38	203	241	
2×2 table [WB/IB (IgMIgG) – serum], conval Lyme disease		Reference standard +	Reference standard −	Total	
	Index test +	27	6	33	
	Index test −	22	197	219	
	Total	49	203	252	
2×2 table [ELISA (IgM/IgG) – serum], Lyme neuroborreliosis		Reference standard +	Reference standard −	Total	
	Index test +	9	14	23	
	Index test −	1	189	190	
	Total	10	203	213	
2×2 table [WB/IB (IgM) – serum], Lyme neuroborreliosis		Reference standard +	Reference standard −	Total	
	Index test +	10	4	14	
	Index test −	0	199	199	
	Total	10	203	213	
2×2 table [WB/IB (IgG) – serum], Lyme neuroborreliosi		Reference standard +	Reference standard −	Total	
	Index test +	3	2	5	
	Index test −	7	201	208	

Reference	Molins 2014[75]				
s	Total	10	203	213	
2×2 table [WB/IB (IgMIgG) – serum], Lyme neuroborreliosis		Reference standard +	Reference standard −	Total	
	Index test +	10	6	16	
	Index test −	0	197	197	
	Total	10	203	213	
2×2 table [ELISA (IgM/IgG) – serum], LA		Reference standard +	Reference standard −	Total	
	Index test +	29	9	38	
	Index test −	0	194	194	
	Total	29	203	232	
2×2 table [WB/IB (IgM) – serum], LA		Reference standard +	Reference standard −	Total	
	Index test +	9	4	13	
	Index test −	20	199	219	
	Total	29	203	232	
2×2 table [WB/IB (IgG) – serum], LA		Reference standard +	Reference standard −	Total	
	Index test +	29	2	31	
	Index test −	0	201	201	
	Total	29	203	232	
2×2 table [WB/IB (IgMIgG) – serum], LA		Reference standard +	Reference standard −	Total	
	Index test +	29	6	37	
	Index test −	0	197	197	

Reference	Molins 2014[75]				
	Total	29	203	232	
2×2 table [ELISA (IgM/IgG) – serum], Carditis		Reference standard +	Reference standard −	Total	
	Index test +	7	9	16	
	Index test −	0	194	194	
	Total	7	203	210	
2×2 table [WB/IB (IgM) – serum], Carditis		Reference standard +	Reference standard −	Total	
	Index test +	4	4	8	
	Index test −	3	199	202	
	Total	7	203	210	
2×2 table [WB/IB (IgG) – serum], Carditis		Reference standard +	Reference standard −	Total	
	Index test +	4	2	6	
	Index test −	3	201	204	
	Total	7	203	210	
2×2 table [WB/IB (IgMIgG) – serum], Carditis		Reference standard +	Reference standard −	Total	
	Index test +	6	6	12	
	Index test −	1	197	198	
	Total	7	203	210	
2×2 table [ELISA (IgM/IgG) – serum], unspec		Reference standard +	Reference standard −	Total	
	Index test +	106	14	120	
	Index test −	18	189	207	

Reference	Molins 2014[75]				
Lyme disease	Total	124	203	327	
2×2 table [WB/IB (IgM) – serum], unspec Lyme disease		Reference standard +	Reference standard −	Total	
	Index test +	57	4	61	
	Index test −	67	199	266	
	Total	124	203	327	
2×2 table [WB/IB (IgG) – serum], unspec Lyme disease		Reference standard +	Reference standard −	Total	
	Index test +	58	2	60	
	Index test −	66	201	267	
	Total	124	203	327	
2×2 table [WB/IB (IgMIgG) – serum], unspec Lyme disease		Reference standard +	Reference standard −	Total	
	Index test +	90	6	96	
	Index test −	34	197	231	
	Total	124	203	327	
2×2 table [PCR – synovial fluid], LA		Reference standard +	Reference standard −	Total	
	Index test +	7	0	7	
	Index test −	11	0	11	
	Total	18	0	18	
Statistical measures	ELISA (IgM/IgG) – serum (acute Lyme disease) Sensitivity 0.68 Specificity 0.93				

Reference	Molins 2014[75]
	WB/IB (IgM) – serum (acute Lyme disease) Sensitivity 0.35 Specificity 0.98 WB/IB (IgG) – serum (acute Lyme disease) Sensitivity 0.20 Specificity 0.99 WB/IB (IgM/IgG) – serum (acute Lyme disease) Sensitivity 1.00 Specificity 0.97 PCR – blood and skin (EM) Sensitivity 0.62 Specificity N/A Culture – blood and skin (EM) Sensitivity 0.44 Specificity N/A PCR – blood and skin (Lyme neuroborreliosis) Sensitivity 0.25 Specificity N/A Culture – blood and skin (Lyme neuroborreliosis) Sensitivity 0.33 Specificity N/A PCR – blood, skin and heart (Carditis) Sensitivity 0.29 Specificity N/A

Reference	Molins 2014[75]
	Culture – blood, skin and heart (Carditis) Sensitivity 0.00 Specificity N/A ELISA (IgM/IgG) – serum (conval Lyme disease) Sensitivity 0.89 Specificity 0.93 WB/IB (IgM) – serum (conval Lyme disease) Sensitivity 0.53 Specificity 0.98 WB/IB (IgG) – serum (conval Lyme disease) Sensitivity 0.37 Specificity 0.99 WB/IB (IgM/IgG) – serum (conval Lyme disease) Sensitivity 0.55 Specificity 0.97 ELISA (IgM/IgG) – serum (Lyme neuroborreliosis) Sensitivity 0.90 Specificity 0.93 WB/IB (IgM) – serum (Lyme neuroborreliosis) Sensitivity 1.00 Specificity 0.98 WB/IB (IgG) – serum (Lyme neuroborreliosis) Sensitivity 0.30

Reference	Molins 2014[75]
	Specificity 0.99 WB/IB (IgM/IgG) – serum (Lyme neuroborreliosis) Sensitivity 1.00 Specificity 0.97 ELISA (IgM/IgG) – serum (LA) Sensitivity 1.00 Specificity 0.96 WB/IB (IgM) – serum (LA) Sensitivity 0.31 Specificity 0.98 WB/IB (IgG) – serum (LA) Sensitivity 1.00 Specificity 0.99 WB/IB (IgM/IgG) – serum (LA) Sensitivity 1.00 Specificity 0.97 ELISA (IgM/IgG) – serum (Carditis) Sensitivity 1.00 Specificity 0.96 WB/IB (IgM) – serum (Carditis) Sensitivity 0.57 Specificity 0.98 WB/IB (IgG) – serum (Carditis)

Reference	Molins 2014[75]
	Sensitivity 0.57 Specificity 0.99 WB/IB (IgM/IgG) – serum (Carditis) Sensitivity 0.86 Specificity 0.97 ELISA (IgM/IgG) – serum (unspec Lyme disease) Sensitivity 0.85 Specificity0.93 WB/IB (IgM) – serum (unspec Lyme disease) Sensitivity 0.46 Specificity 0.98 WB/IB (IgG) – serum (unspec Lyme disease) Sensitivity 0.47 Specificity 0.99 WB/IB (IgM/IgG) – serum (unspec Lyme disease) Sensitivity 0.73 Specificity 0.97 PCR – synovial fluid (LA) Sensitivity 0.39 Specificity N/A
Source of funding	Supported by a CDC grant
Limitations	Risk of bias: patient selection, reference standard Indirectness: none

Reference	Molins 2015[72]
Study type	Case-control study
Study methodology	Data source: serum samples from patients before treatment Recruitment: not reported
Number of patients	n = 202
Patient characteristics	Age, range: 9-83 years Gender (male to female ratio): not reported Family origin: not reported Setting: clinic Country: USA Cases: early Lyme disease Controls: healthy endemic (n=64), healthy nonendemic (94)
Target condition(s)	Early Lyme disease
Index test(s) and reference standard	Index tests ELISA (IgM/IgG) – serum WB/IB (IgM) – serum WB/IB (IgG) – serum WB/IB (IgM/IgG) - serum CLIA (IgM/IgG) - serum Reference standard

Reference	Molins 2015[72]				
	At least 1 EM present in initial clinic visit or clinical diagnosis (majority had positive culture/PCR test) Time between measurement of index test and reference standard: not reported				
2×2 table [ELISA (IgM/IgG) – serum]		Reference standard +	Reference standard −	Total	
	Index test +	101	2	103	
	Index test −	79	156	235	
	Total	180	158	338	
2×2 table [WB/IB (IgM) – serum]		Reference standard +	Reference standard −	Total	
	Index test +	60	5	65	
	Index test −	120	153	273	
	Total	180	158	338	
2×2 table [WB/IB (IgG) – serum]		Reference standard +	Reference standard −	Total	
	Index test +	7	0	7	
	Index test −	173	158	331	
	Total	180	158	338	
2×2 table [WB/IB (IgM/IgG) – serum]		Reference standard +	Reference standard −	Total	
	Index test +	15	0	15	
	Index test −	165	158	323	
	Total	180	158	338	
2×2 table		Reference standard +	Reference standard −	Total	

Reference	Molins 2015[72]				
[CLIA (IgM/IgG) – serum]	Index test +	110	14	124	
	Index test −	70	144	214	
	Total	180	158	338	
Statistical measures	ELISA (IgM/IgG) - serum Sensitivity 0.56 Specificity 0.99 WB/IB (IgM) - serum Sensitivity 0.33 Specificity 0.97 WB/IB (IgG) - serum Sensitivity 0.04 Specificity 1.00 WB/IB (IgM/IgG) – serum Sensitivity 0.08 Specificity 1.00 CLIA (IgM/IgG) - serum Sensitivity 0.61 Specificity 0.91				
Source of funding	Supported by government grants				
Limitations	Risk of bias: patient selection, index test, reference standard Indirectness: none				

Reference	Molins 2016[74]
Study type	Case-control study
Study methodology	Data source: serum samples from CDC Recruitment: not reported
Number of patients	n = 124
Patient characteristics	Age, mean (SD): not reported Gender (male to female ratio): not reported Family origin: not reported Setting: not reported Country: USA Cases: Acute and convalescent stage (n=78), Lyme neuroborreliosis (n=10), Lyme carditis (n=7), LA (n=29) Controls: healthy donors (n=203)
Target condition(s)	EM, Neuroborreliosis, Lyme carditis, Lyme arthritis, unspecified Lyme disease
Index test(s) and reference standard	Index tests ELISA (IgM/IgG) – serum WB/IB (IgM) – serum WB/IB (IgG) – serum WB/IB (IgM/IgG) – serum Reference standard Clinical diagnosis

Reference	Molins 2016[74]				
	Time between measurement of index test and reference standard: not reported				
2×2 table [ELISA (IgM/IgG) – serum], acute Lyme disease		Reference standard +	Reference standard −	Total	
	Index test +	23	5	28	
	Index test −	17	198	215	
	Total	40	203	243	
2×2 table [WB/IB (IgM) – serum], acute Lyme disease		Reference standard +	Reference standard −	Total	
	Index test +	21	12	33	
	Index test −	19	191	210	
	Total	40	203	243	
2×2 table [WB/IB (IgG) – serum], acute Lyme disease		Reference standard +	Reference standard −	Total	
	Index test +	5	3	8	
	Index test −	35	200	235	
	Total	40	203	243	
2×2 table [WB/IB (IgMIgG) – serum], acute Lyme disease		Reference standard +	Reference standard −	Total	
	Index test +	22	3	8	
	Index test −	18	188	235	
	Total	40	203	243	
2×2 table [ELISA (IgM/IgG) – serum], conval Lyme disease		Reference standard +	Reference standard −	Total	
	Index test +	32	5	37	
	Index test −	6	198	204	
	Total	38	203	241	

Reference	Molins 2016[74]				
2×2 table [WB/IB (IgM) – serum], conval Lyme disease		Reference standard +	Reference standard −	Total	
	Index test +	29	12	41	
	Index test −	9	191	200	
	Total	38	203	241	
2×2 table [WB/IB (IgG) – serum], conval Lyme disease		Reference standard +	Reference standard −	Total	
	Index test +	11	3	14	
	Index test −	27	200	227	
	Total	38	203	241	
2×2 table [WB/IB (IgMIgG) – serum], conval Lyme disease		Reference standard +	Reference standard −	Total	
	Index test +	30	15	45	
	Index test −	8	188	196	
	Total	38	203	241	
2×2 table [ELISA (IgM/IgG) – serum], Lyme neuroborreliosis		Reference standard +	Reference standard −	Total	
	Index test +	10	5	15	
	Index test −	0	198	198	
	Total	10	203	213	
2×2 table [WB/IB (IgM) – serum], Lyme neuroborreliosis		Reference standard +	Reference standard −	Total	
	Index test +	9	12	21	
	Index test −	1	191	192	
	Total	10	203	213	

Reference	Molins 2016[74]				
2×2 table [WB/IB (IgG) – serum], Lyme neuroborreliosis		Reference standard +	Reference standard −	Total	
	Index test +	4	3	7	
	Index test −	6	200	206	
	Total	10	203	213	
2×2 table [WB/IB (IgMIgG) – serum], Lyme neuroborreliosis		Reference standard +	Reference standard −	Total	
	Index test +	9	15	24	
	Index test −	1	188	189	
	Total	10	203	213	
2×2 table [ELISA (IgM/IgG) – serum], LA		Reference standard +	Reference standard −	Total	
	Index test +	29	5	34	
	Index test −	0	198	198	
	Total	29	203	232	
2×2 table [WB/IB (IgM) – serum], LA		Reference standard +	Reference standard −	Total	
	Index test +	17	12	29	
	Index test −	12	191	203	
	Total	29	203	232	
2×2 table [WB/IB (IgG) – serum], LA		Reference standard +	Reference standard −	Total	
	Index test +	28	3	31	
	Index test −	1	200	201	
	Total	29	203	232	

Reference	Molins 2016[74]				
2×2 table [WB/IB (IgMIgG) – serum], LA		Reference standard +	Reference standard −	Total	
	Index test +	28	15	43	
	Index test −	1	188	189	
	Total	29	203	232	
2×2 table [ELISA (IgM/IgG) – serum], Carditis		Reference standard +	Reference standard −	Total	
	Index test +	6	5	11	
	Index test −	1	198	199	
	Total	7	203	210	
2×2 table [WB/IB (IgM) – serum], Carditis		Reference standard +	Reference standard −	Total	
	Index test +	5	12	17	
	Index test −	2	191	193	
	Total	7	203	210	
2×2 table [WB/IB (IgG) – serum], Carditis		Reference standard +	Reference standard −	Total	
	Index test +	5	3	8	
	Index test −	2	200	202	
	Total	7	203	210	
2×2 table [WB/IB (IgMIgG) – serum], Carditis		Reference standard +	Reference standard −	Total	
	Index test +	7	15	22	
	Index test −	0	188	188	
	Total	7	203	210	

Reference	Molins 2016[74]				
2×2 table [ELISA (IgM/IgG) – serum], unspec Lyme disease		Reference standard +	Reference standard −	Total	
	Index test +	100	5	105	
	Index test −	24	198	222	
	Total	124	203	327	
2×2 table [WB/IB (IgM) – serum], unspec Lyme disease		Reference standard +	Reference standard −	Total	
	Index test +	81	5	86	
	Index test −	43	191	234	
	Total	124	203	327	
2×2 table [WB/IB (IgG) – serum], unspec Lyme disease		Reference standard +	Reference standard −	Total	
	Index test +	53	3	56	
	Index test −	71	200	271	
	Total	124	203	327	
2×2 table [WB/IB (IgMIgG) – serum], unspec Lyme disease		Reference standard +	Reference standard −	Total	
	Index test +	96	15	111	
	Index test −	28	188	216	
	Total	124	203	327	
Statistical measures	ELISA (IgM/IgG) – serum (acute Lyme disease) Sensitivity 0.57 Specificity 0.98 WB/IB (IgM) – serum (acute Lyme disease)				

Reference	Molins 2016[74]
	Sensitivity 0.53 Specificity 0.94 WB/IB (IgG) – serum (acute Lyme disease) Sensitivity 0.13 Specificity 0.99 WB/IB (IgM/IgG) – serum (acute Lyme disease) Sensitivity 0.55 Specificity 0.93 ELISA (IgM/IgG) – serum (conval Lyme disease) Sensitivity 0.84 Specificity 0.98 WB/IB (IgM) – serum (conval Lyme disease) Sensitivity 0.76 Specificity 0.94 WB/IB (IgG) – serum (conval Lyme disease) Sensitivity 0.29 Specificity 0.99 WB/IB (IgM/IgG) – serum (conval Lyme disease) Sensitivity 0.79 Specificity 0.93 ELISA (IgM/IgG) – serum (Lyme neuroborreliosis) Sensitivity 1.00 Specificity 0.98

Reference	Molins 2016[74]
	WB/IB (IgM) – serum (Lyme neuroborreliosis) Sensitivity 0.90 Specificity 0.94 WB/IB (IgG) – serum (Lyme neuroborreliosis) Sensitivity 0.40 Specificity 0.99 WB/IB (IgM/IgG) – serum (Lyme neuroborreliosis) Sensitivity 0.90 Specificity 0.93 ELISA (IgM/IgG) – serum (LA) Sensitivity 1.00 Specificity 0.98 WB/IB (IgM) – serum (LA) Sensitivity 0.59 Specificity 0.94 WB/IB (IgG) – serum (LA) Sensitivity 0.97 Specificity 0.99 WB/IB (IgM/IgG) – serum (LA) Sensitivity 0.97 Specificity 0.93 ELISA (IgM/IgG) – serum (Carditis) Sensitivity 0.86 Specificity 0.98

Reference	Molins 2016[74]
	WB/IB (IgM) – serum (Carditis) Sensitivity 0.71 Specificity 0.94 WB/IB (IgG) – serum (Carditis) Sensitivity 0.71 Specificity 0.99 WB/IB (IgM/IgG) – serum (Carditis) Sensitivity 1.00 Specificity 0.93 ELISA (IgM/IgG) – serum (unspec Lyme disease) Sensitivity 0.81 Specificity0.98 WB/IB (IgM) – serum (unspec Lyme disease) Sensitivity 0.65 Specificity 0.94 WB/IB (IgG) – serum (unspec Lyme disease) Sensitivity 0.43 Specificity 0.99 WB/IB (IgM/IgG) – serum (unspec Lyme disease) Sensitivity 0.77 Specificity 0.93
Source of funding	None

Reference	Molins 2016[74]
Limitations	Risk of bias: patient selection, reference standard Indirectness: none

Reference	Molins 2017[73]
Study type	Case-control study
Study methodology	Data source: serum samples from patients Recruitment: not reported
Number of patients	n = 124
Patient characteristics	Age, mean (SD): not reported Gender (male to female ratio): not reported Family origin: not reported Setting: not reported Country: USA Cases: acute EM (n=40), convalescent EM (n=38), Lyme neuroborreliosis (n=10), Lyme carditis (n=7), Lyme arthritis (n=29) Controls: healthy persons (n=203)
Target condition(s)	EM, Neuroborreliosis, Lyme arthritis, Lyme carditis
Index test(s) and reference	Index tests ELISA (IgM/IgG) – serum

Reference	Molins 2017[73]				
standard	ELISA (IgM) – serum ELISA (IgG) – serum ELISA C6 – serum Reference standard Clinical diagnosis Time between measurement of index test and reference standard: not reported				
2×2 table [ELISA (IgM) – serum], acute EM		Reference standard +	Reference standard −	Total	
	Index test +	24	23	47	
	Index test −	16	180	196	
	Total	40	203	243	
2×2 table [ELISA (IgG) – serum], acute EM		Reference standard +	Reference standard −	Total	
	Index test +	20	4	24	
	Index test −	20	199	219	
	Total	40	203	242	
2×2 table [ELISA (IgM) – serum], conval EM		Reference standard +	Reference standard −	Total	
	Index test +	30	23	53	
	Index test −	8	180	188	
	Total	38	203	241	
2×2 table [ELISA (IgG) – serum], conval		Reference standard +	Reference standard −	Total	
	Index test +	28	4	32	

Reference	Molins 2017[73]				
EM	Index test −	10	199	209	
	Total	38	203	241	
2×2 table [ELISA (IgM) – serum], Lyme neuroborreliosis		Reference standard +	Reference standard −	Total	
	Index test +	10	23	33	
	Index test −	0	180	180	
	Total	10	203	213	
2×2 table [ELISA (IgG) – serum], Lyme neuroborreliosis		Reference standard +	Reference standard −	Total	
	Index test +	9	4	13	
	Index test −	1	199	200	
	Total	10	203	213	
2×2 table [ELISA (IgM) – serum], LC		Reference standard +	Reference standard −	Total	
	Index test +	5	23	28	
	Index test −	2	180	182	
	Total	7	203	210	
2×2 table [ELISA (IgG) – serum], LC		Reference standard +	Reference standard −	Total	
	Index test +	6	4	10	
	Index test −	1	199	200	
	Total	7	203	210	
2×2 table [ELISA (IgM) – serum], LA		Reference standard +	Reference standard −	Total	
	Index test +	19	23	42	

Reference	Molins 2017[73]				
	Index test −	10	180	190	
	Total	29	203	232	
2×2 table [ELISA (IgG) – serum], LA		Reference standard +	Reference standard −	Total	
	Index test +	29	4	33	
	Index test −	0	199	199	
	Total	29	203	232	
Statistical measures	ELISA (IgM) – serum (acute EM) Sensitivity 0.60 Specificity 0.89 ELISA (IgG) – serum (acute EM) Sensitivity 0.50 Specificity 0.98 ELISA (IgM) – serum (convalescent EM) Sensitivity 0.79 Specificity 0.89 ELISA (IgG) – serum (convalescent EM) Sensitivity 0.74 Specificity 0.98 ELISA (IgM) – serum (Lyme neuroborreliosis) Sensitivity 1.00 Specificity 0.89 ELISA (IgG) – serum (Lyme neuroborreliosis) Sensitivity 0.90				

Reference	Molins 2017[73]
	Specificity 0.98 ELISA (IgM) – serum (Lyme carditis) Sensitivity 0.71 Specificity 0.89 ELISA (IgG) – serum (Lyme carditis) Sensitivity 0.86 Specificity 0.98 ELISA (IgM) – serum (Lyme arthritis) Sensitivity 0.66 Specificity 0.89 ELISA (IgG) – serum (Lyme arthritis) Sensitivity 1.00 Specificity 0.98
Source of funding	Not reported
Limitations	Risk of bias: patient selection, index test, reference standard Indirectness: none

Reference	**Moter 1994[76]**				
Study type	Case-control study				
Study methodology	Data source: skin biopsies Recruitment: patients with skin manifestatiosn reporting to the clinic				
Number of patients	n = 22				
Patient characteristics	Age, mean: not reported Gender (male to female ratio): not reported Family origin: not reported Setting: university hospital Country: Germany Cases: EM (n=10), ACA (n=12) Controls: normal skin (n=4)				
Target condition(s)	EM, ACA				
Index test(s) and reference standard	Index tests PCR - skin Reference standard Clinical diagnosis Time between measurement of index test and reference standard: not reported				
2×2 table		Reference standard	Reference standard −	Total	

Reference	Moter 1994[76]				
[PCR - skin], EM		+			
	Index test +	8	0	8	
	Index test −	2	4	6	
	Total	10	4	14	
2×2 table [PCR – skin], ACA		Reference standard +	Reference standard −	Total	
	Index test +	11	0	11	
	Index test −	1	4	5	
	Total	12	4	16	
Statistical measures	PCR – skin (EM) Sensitivity 0.80 Specificity 1.00 PCR – skin (EM) Sensitivity 0.92 Specificity 1.00				
Source of funding	Government grant				
Limitations	Risk of bias: patient selection, index test, reference standard Indirectness: none				

Reference	Nocton 1994[78]
Study type	Case-control study
Study methodology	Data source: synovial fluid Recruitment: patients with Lyme arthritis seen at the clinic
Number of patients	n = 127
Patient characteristics	Age, mean (range): Test positive: 29 (8-67); Test negative: 38 (3-62) Gender (male to female ratio): 59:29 Family origin: not reported Setting: Lyme disease clinic Country: USA Cases: Lyme arthritis Controls: other forms of arthritis (n=69)
Target condition(s)	Lyme arthritis
Index test(s) and reference standard	Index tests PCR – synovial fluid Reference standard Clinical diagnosis (criteria: brief intermittent attacks of oligoarticular arthritis, exposure in an area of endemic disease, elevated antibody response to B.b on ELISA and exclusion of other known forms of arthritis) Time between measurement of index test and reference standard: not reported

Reference	Nocton 1994[78]				
2×2 table [PCR – synovial fluid]		Reference standard +	Reference standard −	Total	
	Index test +	75	0	75	
	Index test −	13	64	77	
	Total	88	64	152	
Statistical measures	PCR – synovial fluid Sensitivity 0.85 Specificity 1.00				
Source of funding	Supported by government grants				
Limitations	Risk of bias: patient selection, reference standard Indirectness: serious				

Reference	Nocton 1996[77]
Study type	Case-control study
Study methodology	Data source: CSF samples Recruitment: patients with neurological symptoms seen at the clinic
Number of patients	n = 60
Patient characteristics	Age, mean (range): Test positive: 41 (10-76); Test negative: 40 (8-81) Gender (male to female ratio): 34:26 Family origin: not reported Setting: Lyme disease clinic

Reference	Nocton 1996[77]				
	Country: USA Cases: Neuroborreliosis Controls: Sero-negative with no history of Lyme disease (n=22); evaluated for possible herpes simplex virus encephalitis (n=20)				
Target condition(s)	Neuroborreliosis				
Index test(s) and reference standard	Index tests PCR - CSF Reference standard Clinical diagnosis Time between measurement of index test and reference standard: not reported				
2×2 table [PCR – CSF]		Reference standard +	Reference standard −	Total	
	Index test +	17	0	17	
	Index test −	43	42	85	
	Total	60	42	102	
Statistical measures	PCR - CSF Sensitivity 0.28 Specificity 1.00				
Source of funding	Supported by government grants				
Limitations	Risk of bias: patient selection, reference standard Indirectness: none				

Reference	Nohlmans 1994[79]
Study type	Case-control study
Study methodology	Data source: serum samples Recruitment: not reported
Number of patients	n = 44
Patient characteristics	Age, mean: not reported Gender (male to female ratio): not reported Family origin: not reported Setting: not reported Country: Netherlands Cases: Early LB (EM, n=13), Late LB (arthralgia, arthritis, ACA, n=21) Controls: healthy controls (n=84)
Target condition(s)	EM, unspecified Lyme disease
Index test(s) and reference standard	Index tests ELISA (IgM/IgG) – serum ELISA (IgG) - serum Reference standard Clinical diagnosis Time between measurement of index test and reference standard: not reported

Reference	Nohlmans 1994[79]				
2×2 table [ELISA Dako (IgG) – serum], EM		Reference standard +	Reference standard −	Total	
	Index test +	8	1	9	
	Index test −	5	83	88	
	Total	13	84	97	
2×2 table [ELISA Diagast (IgG) – serum], EM		Reference standard +	Reference standard −	Total	
	Index test +	6	0	6	
	Index test −	7	84	91	
	Total	13	84	97	
2×2 table [ELISA Diamedix (IgM/IgG) – serum], EM		Reference standard +	Reference standard −	Total	
	Index test +	7	0	7	
	Index test −	6	84	90	
	Total	13	84	97	
2×2 table [ELISA Dako (IgG) – serum], unspec Lyme disease		Reference standard +	Reference standard −	Total	
	Index test +	18	1	19	
	Index test −	3	83	86	
	Total	21	84	105	
2×2 table [ELISA Diagast (IgG) – serum], unspec Lyme disease		Reference standard +	Reference standard −	Total	
	Index test +	18	0	18	
	Index test −	3	84	87	
	Total	21	84	105	

Reference	Nohlmans 1994[79]				
2×2 table [ELISA Diamedix (IgM/IgG) – serum], unspec Lyme disease		Reference standard +	Reference standard −	Total	
	Index test +	18	0	18	
	Index test −	3	84	87	
	Total	21	84	105	
2×2 table [ELISA Whittaker (IgM/IgG) – serum], unspec Lyme disease		Reference standard +	Reference standard −	Total	
	Index test +	15	0	15	
	Index test −	6	84	90	
	Total	21	84	105	
2×2 table [ELISA Whittaker (IgM/IgG) – serum], EM		Reference standard +	Reference standard −	Total	
	Index test +	4	0	4	
	Index test −	9	84	93	
	Total	13	84	97	
Statistical measures	ELISA Dako (IgG) – serum (EM) Sensitivity 0.62 Specificity 0.99 ELISA Diagast (IgG) – serum (EM) Sensitivity 0.46 Specificity 1.00 ELISA Diamedix (IgM/IgG) – serum (EM) Sensitivity 0.54 Specificity 1.00				

Reference	Nohlmans 1994[79]
	ELISA Dako (IgG) – serum (unspec Lyme disease) Sensitivity 0.86 Specificity 0.99 ELISA Diagast (IgG) – serum (unspec Lyme disease) Sensitivity 0.86 Specificity 1.00 ELISA Diamedix (IgM/IgG) – serum (unspec Lyme disease) Sensitivity 0.86 Specificity 1.00 ELISA Whittaker (IgM/IgG) – serum (unspec Lyme disease) Sensitivity 0.71 Specificity 1.00 ELISA Whittaker (IgM/IgG) – serum (EM) Sensitivity 0.31 Specificity 1.00
Source of funding	Not reported
Limitations	Risk of bias: patient selection, index test, reference standard Indirectness: none

Reference	Oksi 1995[81]
Study type	Case-control study
Study methodology	Data source: serum samples from patients before treatment Recruitment: endemic area, patients seen at hospital
Number of patients	n = 41
Patient characteristics	Age, mean (range): 37.6 years (4-76) Gender (male to female ratio): 19:22 Family origin: not reported Setting: university hospital Country: Finland Cases: late Lyme disease Controls: healthy controls (n=37)
Target condition(s)	Late Lyme disease
Index test(s) and reference standard	Index tests ELISA (IgM/IgG; flagella) – serum ELISA (IgM/IgG; recombinant) – serum ELISA (IgM/IgG; sonicated) – serum Reference standard Diagnosis based on clinical symptoms and positive culture and/or PCR Time between measurement of index test and reference standard: not reported

Reference	Oksi 1995[81]				
2×2 table [ELISA (IgM/IgG; flagella) – serum]		Reference standard +	Reference standard −	Total	
	Index test +	17	5	22	
	Index test −	24	32	56	
	Total	41	37	78	
2×2 table [ELISA (IgM/IgG; recombinant) – serum]		Reference standard +	Reference standard −	Total	
	Index test +	6	2	8	
	Index test −	35	35	70	
	Total	41	37	78	
2×2 table [ELISA (IgM/IgG; sonicated) – serum]		Reference standard +	Reference standard −	Total	
	Index test +	32	4	36	
	Index test −	9	33	42	
	Total	41	37	78	
Statistical measures	ELISA (IgM/IgG; flagella) - serum Sensitivity 0.41 Specificity 0.86 ELISA (IgM/IgG; recombinant) - serum Sensitivity 0.15 Specificity 0.95 ELISA (IgM/IgG; sonicated) - serum Sensitivity 0.78 Specificity 0.89				

Reference	Oksi 1995[81]
Source of funding	Supported by academic and private research grants
Limitations	Risk of bias: patient selection, index test, reference standard Indirectness: none

Reference	Padula 1994[82]
Study type	Case-control study
Study methodology	Data source: serum samples from patients after beginning of treatment Recruitment: not reported
Number of patients	n = 74
Patient characteristics	Age, mean: not reported Gender (male to female ratio): not reported Family origin: not reported Setting: not reported Country: USA Cases: EM Controls: Healthy individuals (n=70); Severe periodontitis (n=6)
Target condition(s)	EM
Index test(s)	Index tests

Reference	Padula 1994[82]				
and reference standard	ELISA (IgM) – serum WB/IB (IgM) – serum Reference standard Culture Time between measurement of index test and reference standard: not reported				
2×2 table [ELISA (IgM; recombinant) – serum], <6 weeks		Reference standard +	Reference standard −	Total	
	Index test +	29	0	29	
	Index test −	10	76	86	
	Total	39	76	115	
2×2 table 2×2 table [ELISA (IgM; recombinant) – serum], 6 weeks to 6 months		Reference standard +	Reference standard −	Total	
	Index test +	10	0	10	
	Index test −	5	76	81	
	Total	15	76	91	
2×2 table [ELISA (IgM; whole-cell) – serum], <6 weeks		Reference standard +	Reference standard −	Total	
	Index test +	25	0	25	
	Index test −	14	76	90	
	Total	39	76	115	
2×2 table [ELISA (IgM; whole-cell) – serum], 6		Reference standard +	Reference standard −	Total	
	Index test +	12	0	12	
	Index test −	3	76	79	

Reference	Padula 1994[82]				
weeks to 6 months	Total	15	76	91	
2×2 table [WB/IB (IgM) – serum], <6 weeks		Reference standard +	Reference standard −	Total	
	Index test +	28	2	30	
	Index test −	11	74	85	
	Total	39	76	115	
2×2 table [WB/IB (IgM) – serum], 6 weeks to 6 months		Reference standard +	Reference standard −	Total	
	Index test +	13	2	15	
	Index test −	2	75	76	
	Total	15	77	91	
Statistical measures	ELISA (IgM; recombinant) – serum (<6 weeks) Sensitivity 0.74 Specificity 1.00 ELISA (IgM; recombinant) – serum (6 weeks to 6 months) Sensitivity 0.67 Specificity 1.00 ELISA (IgM; whole-cell) – serum (<6 weeks) Sensitivity 0.64 Specificity 1.00 ELISA (IgM; whole-cell) – serum (6 weeks to 6 months) Sensitivity 0.80 Specificity 1.00				

Reference	Padula 1994[82]
	WB/IB (IgM) – serum (<6 weeks) Sensitivity 0.72 Specificity0.97 WB/IB (IgM) – serum (6 weeks to 6 months) Sensitivity 0.87 Specificity 0.97
Source of funding	Supported by government and private grants
Limitations	Risk of bias: patient selection, reference standard Indirectness: none

Reference	Panelius 2001[83]
Study type	Case-control study
Study methodology	Data source: not reported Recruitment: not reported
Number of patients	n = 28
Patient characteristics	Age, mean: not reported Gender (male to female ratio): not reported Family origin: not reported Setting: not reported Country: Finland Cases: Neuroborreliosis (n=14), Lyme arthritis (n=4) Controls: Syphilis (n=10), healthy donors (n=13)
Target condition(s)	Neuroborreliosis, Lyme arthritis
Index test(s) and reference standard	Index tests ELISA (IgM) – serum ELISA (IgG) - serum WB/IB (IgG) – serum Reference standard Clinical diagnosis (based on CDC guidelines) Time between measurement of index test and reference standard: not reported

Reference	Panelius 2001[83]				
2×2 table [ELISA (IgM) – serum], Lyme neuroborreliosis		Reference standard +	Reference standard −	Total	
	Index test +	11	0	11	
	Index test −	8	0	8	
	Total	19	0	19	
2×2 table [ELISA (IgG) – serum], Lyme neuroborreliosis		Reference standard +	Reference standard −	Total	
	Index test +	14	0	14	
	Index test −	5	0	5	
	Total	19	0	19	
2×2 table [WB/IB (IgG) – serum], Lyme neuroborreliosis		Reference standard +	Reference standard −	Total	
	Index test +	10	0	10	
	Index test −	4	0	4	
	Total	14	0	14	
2×2 table [ELISA (IgM) – serum], LA		Reference standard +	Reference standard −	Total	
	Index test +	7	0	7	
	Index test −	12	0	12	
	Total	19	0	19	
2×2 table [ELISA (IgG) – serum], LA		Reference standard +	Reference standard −	Total	
	Index test +	15	0	15	
	Index test −	4	0	4	
	Total	19	0	19	

Reference	Panelius 2001[83]				
2×2 table [WB/IB (IgG) – serum], LA		Reference standard +	Reference standard −	Total	
	Index test +	12	0	12	
	Index test −	2	0	2	
	Total	14	0	14	
Statistical measures	ELISA (IgM) – serum (Lyme neuroborreliosis) Sensitivity 0.58 Specificity N/A ELISA (IgG) – serum (Lyme neuroborreliosis) Sensitivity 0.74 Specificity N/A WB/IB (IgG) – serum (Lyme neuroborreliosis) Sensitivity 0.71 Specificity N/A ELISA (IgM) – serum (LA) Sensitivity 0.37 Specificity N/A ELISA (IgG) – serum (LA) Sensitivity 0.79 Specificity N/A WB/IB (IgG) – serum (LA) Sensitivity 0.86 Specificity N/A				

Reference	Panelius 2001[83]
Source of funding	Supported by government grants
Limitations	Risk of bias: patient selection, index test, reference standard Indirectness: none

Reference	Panelius 2008[84]
Study type	Case-control study
Study methodology	Data source: serum samples from patients before treatment Recruitment: not reported
Number of patients	n = 102
Patient characteristics	Age, mean: not reported Gender (male to female ratio): not reported Family origin: not reported Setting: not reported Country: Finland Cases: EM (n=25), Neuroborreliosis (n=67), ACA (n=10) Controls: blood donors (n=20), CSF samples from healthy individuals (n=20)
Target condition(s)	EM, Neuroborreliosis, ACA
Index test(s) and reference	Index tests ELISA (IgG) – serum

Reference	Panelius 2008[84]				
standard	ELISA (IgG) - CSF Reference standard Clinical diagnosis Time between measurement of index test and reference standard: not reported				
2×2 table [ELISA (IgG) – serum], acute Lyme disease (EM)		Reference standard +	Reference standard −	Total	
	Index test +	15	0	15	
	Index test −	10	20	30	
	Total	25	20	45	
2×2 table [ELISA (IgG) – serum], convalescent Lyme disease (EM)		Reference standard +	Reference standard −	Total	
	Index test +	16	0	16	
	Index test −	9	20	29	
	Total	25	20	42	
2×2 table [ELISA (IgG) – CSF], Lyme neuroborreliosis		Reference standard +	Reference standard −	Total	
	Index test +	19	0	19	
	Index test −	33	20	53	
	Total	52	20	72	
2×2 table [ELISA (IgG) – serum], ACA		Reference standard +	Reference standard −	Total	
	Index test +	8	0	8	
	Index test −	2	20	22	
	Total	10	20	30	

Reference	Panelius 2008[84]				
2×2 table [ELISA (IgG) – serum], Lyme neuroborreliosis		Reference standard +	Reference standard −	Total	
	Index test +	56	0	56	
	Index test −	11	20	31	
	Total	67	20	87	
Statistical measures	ELISA (IgG) – serum (EM, acute) Sensitivity 0.60 Specificity 1.00 ELISA (IgG) – serum (EM, convalescent) Sensitivity 0.64 Specificity 1.00 ELISA (IgG) – CSF (Lyme neuroborreliosis) Sensitivity 0.37 Specificity 1.00 ELISA (IgG) – serum (ACA) Sensitivity 0.80 Specificity 1.00 ELISA (IgG) – serum (Lyme neuroborreliosis) Sensitivity 0.84 Specificity 1.00				
Source of funding	Supported by government and industry grants				
Limitations	Risk of bias: patient selection, index test, reference standard Indirectness: none				

Reference	Peltomaa 2004[85]
Study type	Case-control study
Study methodology	Data source: serum samples from patients before treatment Recruitment: patients treated by study author at clinic
Number of patients	n = 47
Patient characteristics	Age, median: 35 years (4-74) Gender (male to female ratio): 25/22 Family origin: not reported Setting: clinic Country: USA Cases: facial palsy Controls: healthy persons (n=86)
Target condition(s)	Lyme disease associated facial palsy
Index test(s) and reference standard	Index tests WB/IB (IgG) – serum Reference standard Clinical diagnosis based on CDC criteria Time between measurement of index test and reference standard: not reported

Reference	**Peltomaa 2004[85]**				
2×2 table [WB/IB (IgG) – serum]		Reference standard +	Reference standard −	Total	
	Index test +	47	4	51	
	Index test −	0	82	82	
	Total	47	86	133	
Statistical measures	WB/IB (IgG) - serum Sensitivity 1.00 Specificity 0.95				
Source of funding	Supported by industry grants				
Limitations	Risk of bias: patient selection, index test, reference standard Indirectness: serious				

Reference	**Phillips 1998[86]**				
Study type	Case-control study				
Study methodology	Data source: serum samples from patients Recruitment: patients selected from private practice in hyper-endemic and non-endemic areas				
Number of patients	n = 47				
Patient characteristics	Age, median: 35 years (4-74) Gender (male to female ratio): not reported Family origin: not reported Setting: multi-centre Country: USA Cases: Lyme disease had failed or relapsed after extended oral and intravenous antibiotic therapy Controls: chronic illness other than Lyme disease (n=23)				
Target condition(s)	Lyme disease				
Index test(s) and reference standard	Index tests Culture - blood Reference standard Clinical diagnosis Time between measurement of index test and reference standard: not reported				
2×2 table		Reference standard	Reference standard −	Total	

Reference	Phillips 1998[86]				
[Culture – blood]		+			
	Index test +	43	0	43	
	Index test −	4	23	27	
	Total	47	23	70	
Statistical measures	Culture - blood Sensitivity 0.91 Specificity 1.00				
Source of funding	Not reported				
Limitations	Risk of bias: patient selection, index test, reference standard Indirectness: none				

Reference	**Pomelova 2015[87]**				
Study type	Case-control study				
Study methodology	Data source: serum samples from patients taken during summer 2010 Recruitment: not reported				
Number of patients	n = 146				
Patient characteristics	Age, mean: not reported Gender (male to female ratio): not reported Family origin: not reported Setting: not reported Country: Russia Cases: EM Controls: blood donors (n=197)				
Target condition(s)	EM				
Index test(s) and reference standard	Index tests ELISA (IgM/IgG) – serum Reference standard Clinical diagnosis Time between measurement of index test and reference standard: not reported				
2×2 table		Reference standard	Reference standard −	Total	

Reference	Pomelova 2015[87]				
[ELISA (IgM/IgG) – serum]		+			
	Index test +	40	96	136	
	Index test −	87	101	188	
	Total	127	197	224	
Statistical measures	ELISA (IgM/IgG) - serum Sensitivity 0.31 Specificity 0.51				
Source of funding	Supported by government grants				
Limitations	Risk of bias: patient selection, index test, reference standard Indirectness: none				

Reference	Porwancher 2011[88]
Study type	Case-control study
Study methodology	Data source: serum samples from patients Recruitment: not reported
Number of patients	n = 242
Patient characteristics	Age, mean: not reported Gender (male to female ratio): not reported Family origin: not reported Setting: not reported Country: USA Cases: Culture-proven early acute Lyme (n=79), early convalescent-phase (n=78), culture-proven EM (n=4), Stage-II and III Lyme (n=47), sera from patients receiving treatment (PTLDS, n=34) Controls: Healthy blood donors from New Mexico (n=300), healthy blood donors from New England (n=300), patients undergoing routine screening (n=99)
Target condition(s)	EM, Lyme arthritis, post-treatment Lyme disease syndrome
Index test(s) and reference standard	Index tests WB/IB (IgM) – serum WB/IB (IgG) – serum WB/IB (IgM/IgG) - serum Reference standard Culture only (n=83), clinical diagnosis

Reference	Porwancher 2011[88]				
	Time between measurement of index test and reference standard: not reported				
2×2 table [WB/IB (IgM) – serum], EM (acute)		Reference standard +	Reference standard −	Total	
	Index test +	29	0	29	
	Index test −	50	0	50	
	Total	79	0	79	
2×2 table [WB/IB (IgG) – serum], EM (acute)		Reference standard +	Reference standard −	Total	
	Index test +	6	0	6	
	Index test −	73	0	73	
	Total	79	0	79	
2×2 table [WB/IB (IgM/IgG) – serum], EM (acute)		Reference standard +	Reference standard −	Total	
	Index test +	31	22	53	
	Index test −	48	428	476	
	Total	79	450	529	
2×2 table [WB/IB (IgM) – serum], EM (convalescent)		Reference standard +	Reference standard −	Total	
	Index test +	60	0	60	
	Index test −	22	0	22	
	Total	82	0	82	
2×2 table [WB/IB (IgG) – serum], EM		Reference standard +	Reference standard −	Total	
	Index test +	17	0	17	

Reference	Porwancher 2011[88]				
(convalescent)	Index test −	65	0	65	
	Total	82	0	82	
2×2 table [WB/IB (IgM/IgG) – serum], EM (convalescent)		Reference standard +	Reference standard −	Total	
	Index test +	63	22	85	
	Index test −	19	428	447	
	Total	82	450	532	
2×2 table [WB/IB (IgM) – serum], LA		Reference standard +	Reference standard −	Total	
	Index test +	19	0	19	
	Index test −	10	0	10	
	Total	29	0	29	
2×2 table [WB/IB (IgG) – serum], LA		Reference standard +	Reference standard −	Total	
	Index test +	28	0	28	
	Index test −	54	0	54	
	Total	82	0	82	
2×2 table [WB/IB (IgM/IgG) – serum], LA		Reference standard +	Reference standard −	Total	
	Index test +	29	21	50	
	Index test −	0	429	429	
	Total	29	450	479	
2×2 table [WB/IB (IgM) – serum], PTLDS		Reference standard +	Reference standard −	Total	
	Index test +	13	0	13	

Reference	Porwancher 2011[88]				
	Index test −	21	0	21	
	Total	34	0	34	
2×2 table [WB/IB (IgG) – serum], PTLDS		Reference standard +	Reference standard −	Total	
	Index test +	17	0	17	
	Index test −	17	0	17	
	Total	34	0	34	
2×2 table [WB/IB (IgM/IgG) – serum], PTLDS		Reference standard +	Reference standard −	Total	
	Index test +	23	21	44	
	Index test −	11	429	440	
	Total	34	450	484	
Statistical measures	WB/IB (IgM) – serum (EM, acute) Sensitivity 0.37 Specificity N/A WB/IB (IgG) – serum (EM, acute) Sensitivity 0.08 Specificity N/A WB/IB (IgM/IgG) – serum (EM, acute) Sensitivity 0.39 Specificity N/A WB/IB (IgM) – serum (EM, convalescent) Sensitivity 0.73 Specificity N/A				

Reference	Porwancher 2011[88]
	WB/IB (IgG) – serum (EM, convalescent) Sensitivity 0.21 Specificity N/A WB/IB (IgM/IgG) – serum (EM, convalescent) Sensitivity 0.77 Specificity 0.95 WB/IB (IgM) – serum (LA) Sensitivity 0.66 Specificity N/A WB/IB (IgG) – serum (LA) Sensitivity 0.34 Specificity N/A WB/IB (IgM/IgG) – serum (LA) Sensitivity 1.00 Specificity 0.95 WB/IB (IgM) – serum (PTLDS) Sensitivity 0.38 Specificity N/A WB/IB (IgG) – serum (PTLDS) Sensitivity 0.50 Specificity N/A WB/IB (IgM/IgG) – serum (PTLDS) Sensitivity 0.68

Reference	Porwancher 2011[88]
	Specificity 0.95
Source of funding	Supported by government and academia grants
Limitations	Risk of bias: patient selection, index test, reference standard Indirectness: none

Reference	Priem 1997[89]
Study type	Case-control study
Study methodology	Data source: CSF samples Recruitment: not reported
Number of patients	n = 22
Patient characteristics	Age, mean: 44 years (7-82) Gender (male to female ratio): not reported Family origin: not reported Setting: not reported Country: Germany Cases: Neuroborreliosis Controls: rheumatic diseases (n=37), CNS diseases (n=21)
Target condition(s)	Neuroborreliosis

Reference	**Priem 1997[89]**				
Index test(s) and reference standard	Index tests PCR - CSF Reference standard Clinical diagnosis Time between measurement of index test and reference standard: not reported				
2×2 table [PCR – CSF]		Reference standard +	Reference standard −	Total	
	Index test +	15	0	15	
	Index test −	4	33	37	
	Total	19	33	52	
Statistical measures	PCR - CSF Sensitivity 0.79 Specificity 1.00				
Source of funding	Supported by government grants				
Limitations	Risk of bias: patient selection, index test, reference standard Indirectness: none				

Reference	Rauer 1995[90]
Study type	Case-control study
Study methodology	Data source: serum samples from patients Recruitment: not reported
Number of patients	n = 210
Patient characteristics	Age, range: not reported Gender (male to female ratio): not reported Family origin: not reported Setting: university clinic Country: Germany Cases: EM (n=118), Neuroborreliosis (n=33), Lyme arthritis (n=17), ACA (n=42) Controls: no current symptoms or history of Lyme (n=82)
Target condition(s)	EM, ACA, Neuroborreliosis, Lyme arthritis
Index test(s) and reference standard	Index tests ELISA (IgM/IgG) – serum ELISA (IgM) – serum ELISA (IgG) – serum Reference standard Clinical diagnosis Time between measurement of index test and reference standard: not reported

Reference	Rauer 1995[90]				
2×2 table [ELISA (IgM/IgG) – serum], EM		Reference standard +	Reference standard −	Total	
	Index test +	24	3	27	
	Index test −	94	79	173	
	Total	118	82	200	
2×2 table [ELISA (IgM) – serum], EM		Reference standard +	Reference standard −	Total	
	Index test +	7	3	10	
	Index test −	111	79	190	
	Total	118	82	200	
2×2 table [ELISA (IgG) – serum], EM		Reference standard +	Reference standard −	Total	
	Index test +	16	0	16	
	Index test −	102	82	184	
	Total	118	82	200	
2×2 table [ELISA (IgM/IgG) – serum], ACA		Reference standard +	Reference standard −	Total	
	Index test +	36	3	39	
	Index test −	6	79	85	
	Total	42	82	144	
2×2 table [ELISA (IgM) – serum], ACA		Reference standard +	Reference standard −	Total	
	Index test +	0	3	3	
	Index test −	42	79	121	
	Total	42	82	124	

Reference	Rauer 1995[90]				
2×2 table [ELISA (IgG) – serum], ACA		Reference standard +	Reference standard −	Total	
	Index test +	14	0	14	
	Index test −	28	82	110	
	Total	42	82	124	
2×2 table [ELISA (IgM/IgG) – serum], Lyme neuroborreliosis		Reference standard +	Reference standard −	Total	
	Index test +	14	3	17	
	Index test −	19	79	98	
	Total	33	82	115	
2×2 table [ELISA (IgM) – serum], Lyme neuroborreliosis		Reference standard +	Reference standard −	Total	
	Index test +	0	3	3	
	Index test −	33	79	112	
	Total	33	82	115	
2×2 table [ELISA (IgG) – serum], Lyme neuroborreliosis		Reference standard +	Reference standard −	Total	
	Index test +	16	3	19	
	Index test −	1	79	80	
	Total	17	82	99	
2×2 table [ELISA (IgM/IgG) – serum], LA		Reference standard +	Reference standard −	Total	
	Index test +	16	3	19	
	Index test −	1	79	80	
	Total	17	82	99	

Reference	Rauer 1995[90]				
2×2 table [ELISA (IgM) – serum], LA		Reference standard +	Reference standard −	Total	
	Index test +	0	3	3	
	Index test −	17	79	96	
	Total	17	82	99	
2×2 table [ELISA (IgG) – serum], LA		Reference standard +	Reference standard −	Total	
	Index test +	14	0	14	
	Index test −	3	82	85	
	Total	17	82	99	
Statistical measures	ELISA (IgM) – serum (EM) Sensitivity 0.06 Specificity 0.96 ELISA (IgG) – serum (EM) Sensitivity 0.14 Specificity 1.00 ELISA (IgM/IgG) – serum (EM) Sensitivity 0.20 Specificity 0.96 ELISA (IgM) – serum (ACA) Sensitivity 0.00 Specificity 0.96 ELISA (IgG) – serum (ACA) Sensitivity 0.33				

Reference	Rauer 1995[90]
	Specificity 1.00 ELISA (IgM/IgG) – serum (ACA) Sensitivity 0.86 Specificity0.96 ELISA (IgM) – serum (Lyme neuroborreliosis) Sensitivity 0.00 Specificity0.96 ELISA (IgG) – serum (Lyme neuroborreliosis) Sensitivity 0.33 Specificity 1.00 ELISA (IgM/IgG) – serum (Lyme neuroborreliosis) Sensitivity 0.42 Specificity 0.96 ELISA (IgM) – serum (LA) Sensitivity 0.00 Specificity 0.96 ELISA (IgG) – serum (LA) Sensitivity 0.82 Specificity 1.00 ELISA (IgM/IgG) – serum (LA) Sensitivity 0.94 Specificity 0.96

Reference	Rauer 1995[90]
Source of funding	Supported by government grants
Limitations	Risk of bias: patient selection, index test, reference standard Indirectness: none

Reference	Rauer 1998[91]
Study type	Case-control study
Study methodology	Data source: serum samples from patients Recruitment: not reported
Number of patients	n = 104
Patient characteristics	Age, range: not reported Gender (male to female ratio): not reported Family origin: not reported Setting: university clinic Country: Germany Cases: EM Controls: healthy controls (n=154)
Target condition(s)	EM
Index test(s) and reference	Index tests ELISA (IgM, recombinant) – serum

Reference	Rauer 1998[91]				
standard	ELISA (IgM, whole cell) – serum Reference standard Clinical diagnosis Time between measurement of index test and reference standard: not reported				
2×2 table [ELISA (IgM, recombinant) – serum]		Reference standard +	Reference standard −	Total	
	Index test +	48	8	56	
	Index test −	56	146	202	
	Total	104	154	258	
2×2 table [ELISA (IgM, whole cell) – serum]		Reference standard +	Reference standard −	Total	
	Index test +	47	8	55	
	Index test −	57	146	203	
	Total	104	154	258	
Statistical measures	ELISA (IgM, recombinant) - serum Sensitivity 0.46 Specificity 0.95 ELISA (IgM, whole cell) - serum Sensitivity 0.45 Specificity 0.95				
Source of funding	Supported by government grants				
Limitations	Risk of bias: patient selection, index test, reference standard				

Reference	Rauer 1998[91]
	Indirectness: none

Reference	Roux 2007[92]
Study type	Case-control study
Study methodology	Data source: serum samples from patients Recruitment: all patients presenting to clinic within 18 months
Number of patients	n = 11
Patient characteristics	Age, mean: 62 years (SD 15) Gender (male to female ratio): 2/9 Family origin: not reported Setting: tertiary clinic Country: France Cases: Lyme meningoradiculitis Controls: Consecutive patients referred for suspected Lyme meningoradiculitis (n=16)
Target condition(s)	Lyme meningoradiculitis
Index test(s) and reference standard	Index tests ELISA (IgM/IgG) – serum ELISA (IgM) – serum ELISA (IgG) – serum WB/IB (IgG) - serum

Reference	Roux 2007[92]				
	ELISA (IgM/IgG) – CSF ELISA (IgM) – CSF ELISA (IgG) – CSF WB/IB (IgG) - CSF Reference standard Clinical diagnosis Time between measurement of index test and reference standard: not reported				
2×2 table [ELISA (IgM/IgG) – CSF]		Reference standard +	Reference standard −	Total	
	Index test +	10	4	14	
	Index test −	1	12	13	
	Total	11	16	27	
2×2 table [WB/IB (IgG) – CSF]		Reference standard +	Reference standard −	Total	
	Index test +	9	1	10	
	Index test −	2	15	17	
	Total	11	16	27	
2×2 table [ELISA (IgG) – CSF]		Reference standard +	Reference standard −	Total	
	Index test +	10	4	14	
	Index test −	1	12	13	
	Total	11	16	27	
2×2 table		Reference standard +	Reference standard −	Total	

Reference	Roux 2007[92]				
[ELISA (IgM) – serum]	Index test +	7	1	8	
	Index test −	4	15	19	
	Total	11	16	27	
2×2 table [ELISA (IgG) – serum]		Reference standard +	Reference standard −	Total	
	Index test +	7	6	13	
	Index test −	4	10	14	
	Total	11	16	27	
2×2 table [ELISA (IgM/IgG) – serum]		Reference standard +	Reference standard −	Total	
	Index test +	10	6	16	
	Index test −	1	10	11	
	Total	11	16	27	
2×2 table [WB/IB (IgG) – serum]		Reference standard +	Reference standard −	Total	
	Index test +	7	6	13	
	Index test −	4	10	14	
	Total	11	16	27	
Statistical measures	ELISA (IgG) - CSF Sensitivity 0.91 Specificity 0.75 ELISA (IgM/IgG) - CSF Sensitivity 0.91 Specificity 0.75				

Reference	Roux 2007[92]
	WB/IB (IgG) - CSF Sensitivity 0.82 Specificity 0.94 ELISA (IgM) – serum Sensitivity 0.64 Specificity 0.94 ELISA (IgG) - serum Sensitivity 0.64 Specificity 0.63 ELISA (IgM/IgG) – serum Sensitivity 0.91 Specificity 0.63 WB/IB (IgG) - serum Sensitivity 0.64 Specificity 0.63
Source of funding	Not reported
Limitations	Risk of bias: patient selection, index test, reference standard Indirectness: none

Reference	Russell 1984[93]
Study type	Case-control study
Study methodology	Data source: serum samples from patients Recruitment: not reported
Number of patients	n = 45
Patient characteristics	Age, mean: not reported Gender (male to female ratio): not reported Family origin: not reported Setting: clinic Country: USA Cases: Lyme disease Controls: healthy persons (n=100)
Target condition(s)	Lyme disease
Index test(s) and reference standard	Index tests ELISA (IgM/IgG) – serum IFA (IgM/IgG) – serum WB/IB (IgG) – serum WB/IB (IgM/IgG) - serum CLIA (IgM/IgG) - serum Reference standard Clinical diagnosis

Reference	Russell 1984[93]				
	Time between measurement of index test and reference standard: not reported				
2×2 table [ELISA (IgM/IgG) – serum], EM		Reference standard +	Reference standard −	Total	
	Index test +	17	4	21	
	Index test −	17	96	113	
	Total	34	100	134	
2×2 table [IFA (IgM/IgG) – serum], EM		Reference standard +	Reference standard −	Total	
	Index test +	17	0	17	
	Index test −	17	100	117	
	Total	34	100	134	
2×2 table [ELISA (IgM/IgG) – serum], Lyme neuroborreliosis		Reference standard +	Reference standard −	Total	
	Index test +	26	4	30	
	Index test −	0	96	96	
	Total	26	100	126	
2×2 table [IFA (IgM/IgG) – serum], Lyme neuroborreliosis		Reference standard +	Reference standard −	Total	
	Index test +	24	0	24	
	Index test −	2	100	102	
	Total	26	100	126	
2×2 table [ELISA (IgM/IgG) –		Reference standard +	Reference standard −	Total	
	Index test +	38	4	42	

Reference	Russell 1984[93]				
serum], LA	Index test −	0	96	96	
	Total	38	100	138	
2×2 table [IFA (IgM/IgG) – serum], LA		Reference standard +	Reference standard −	Total	
	Index test +	38	0	38	
	Index test −	0	100	100	
	Total	38	100	138	
2×2 table [ELISA (IgM/IgG) – serum], carditis		Reference standard +	Reference standard −	Total	
	Index test +	6	4	10	
	Index test −	0	96	96	
	Total	6	100	106	
2×2 table [IFA (IgM/IgG) – serum], carditis		Reference standard +	Reference standard −	Total	
	Index test +	6	0	6	
	Index test −	0	100	100	
	Total	6	100	106	
Statistical measures	ELISA (IgM/IgG) – serum (EM) Sensitivity 0.50 Specificity 0.96 IFA (IgM/IgG) – serum (EM) Sensitivity 0.50 Specificity 1.00 ELISA (IgM/IgG) – serum (Lyme neuroborreliosis)				

Reference	Russell 1984[93]
	Sensitivity 1.00 Specificity 0.96 IFA (IgM/IgG) – serum (Lyme neuroborreliosis) Sensitivity 0.92 Specificity 1.00 ELISA (IgM/IgG) – serum (LA) Sensitivity 1.00 Specificity 0.96 IFA (IgM/IgG) – serum (LA) Sensitivity 1.00 Specificity 1.00 ELISA (IgM/IgG) – serum (LC) Sensitivity 1.00 Specificity 0.96 IFA (IgM/IgG) – serum (LC) Sensitivity 1.00 Specificity 1.00
Source of funding	Not reported
Limitations	Risk of bias: patient selection, index test, reference standard Indirectness: none

Reference	Ruzic-Sabljic 2002[94]
Study type	Case-control study
Study methodology	Data source: serum samples from patients Recruitment: not reported
Number of patients	n = 117
Patient characteristics	Age, mean: not reported Gender (male to female ratio): not reported Family origin: not reported Setting: university clinic Country: Slovenia Cases: EM Controls: healthy persons (n=96)
Target condition(s)	EM
Index test(s) and reference standard	Index tests WB/IB (IgM) – serum WB/IB (IgG) – serum IFA (IgM) - serum IFA (IgG) - serum Reference standard Clinical diagnosis Time between measurement of index test and reference standard: not reported

Reference	Ruzic-Sabljic 2002[94]				
2×2 table [WB/IB (IgM) – serum]		Reference standard +	Reference standard −	Total	
	Index test +	33	23	56	
	Index test −	33	28	61	
	Total	66	51	117	
2×2 table [WB/IB (IgG) – serum]		Reference standard +	Reference standard −	Total	
	Index test +	20	16	36	
	Index test −	46	35	81	
	Total	66	51	117	
2×2 table [IFA (IgM) – serum],		Reference standard +	Reference standard −	Total	
	Index test +	0	2	2	
	Index test −	66	49	115	
	Total	66	51	117	
2×2 table [IFA (IgG) – serum]		Reference standard +	Reference standard −	Total	
	Index test +	1	2	3	
	Index test −	65	49	114	
	Total	66	51	117	
Statistical measures	WB/IB (IgM) - serum Sensitivity 0.50 Specificity 0.55 WB/IB (IgG) - serum Sensitivity 0.30				

Reference	Ruzic-Sabljic 2002[94]
	Specificity 0.69 IFA (IgM) - serum Sensitivity 0.00 Specificity 0.96 IFA (IgG) – serum Sensitivity 0.02 Specificity 0.96
Source of funding	Not reported
Limitations	Risk of bias: patient selection, index test, reference standard Indirectness: none

Reference	Sapi 2013[95]
Study type	Case-control study
Study methodology	Data source: blood samples Recruitment: not reported
Number of patients	n = 72
Patient characteristics	Age, mean (range): 42 years (3-80) Gender (male to female ratio): 26/46 Family origin: not reported

Reference	Sapi 2013[95]				
	Setting: not reported Country: USA Cases: Lyme disease (n=72) Controls: healthy persons (n=48)				
Target condition(s)	Lyme disease				
Index test(s) and reference standard	Index test Culture Reference standard Clinical diagnosis Time between measurement of index test and reference standard: not reported				
2×2 table [Culture], @ 6 days		Reference standard +	Reference standard −	Total	
	Index test +	34	0	34	
	Index test −	38	48	86	
	Total	72	48	120	
2×2 table [Culture], @ 8 weeks		Reference standard +	Reference standard −	Total	
	Index test +	60	0	60	
	Index test −	12	48	60	
	Total	72	48	120	

Reference	**Sapi 2013[95]**				
2×2 table [Culture], @ 16 weeks		Reference standard +	Reference standard −	Total	
	Index test +	68	0	68	
	Index test −	4	48	52	
	Total	72	48	120	
Statistical measures	Culture @ 6 days Sensitivity 0.47 Specificity 1.00 Culture @ 8 weeks Sensitivity 0.83 Specificity 1.00 Culture @ 16 weeks Sensitivity 0.94 Specificity 1.00				
Source of funding	Supported by industry grants				
Limitations	Risk of bias: patient selection, index test, reference standard Indirectness: serious				

Reference	Schnarr 2001[96]				
Study type	Case-control study				
Study methodology	Data source: synovial fluid samples from patients Recruitment: not reported				
Number of patients	n = 16				
Patient characteristics	Age, mean: not reported Gender (male to female ratio): not reported Family origin: not reported Setting: clinic Country: Germany Cases: Lyme arthritis Controls: rheumatoid arthritis (n=31)				
Target condition(s)	Lyme arthritis				
Index test(s) and reference standard	Index tests PCR – synovial fluid Reference standard Clinical diagnosis Time between measurement of index test and reference standard: not reported				
2×2 table [PCR – SF]		Reference standard +	Reference standard −	Total	
	Index test +	11	0	11	

Reference	**Schnarr 2001[96]**				
	Index test −	5	31	36	
	Total	16	31	47	
Statistical measures	PCR – synovial fluid Sensitivity 0.69 Specificity 1.00				
Source of funding	Supported by academic grants				
Limitations	Risk of bias: patient selection, index test, reference standard Indirectness: none				

Reference	Schulte-Spechtel 2004[97] (Schulte-Spechtel 2003[98])
Study type	Case-control study
Study methodology	Data source: serum samples from patients Recruitment: not reported
Number of patients	n = 36
Patient characteristics	Age, mean: not reported Gender (male to female ratio): not reported Family origin: not reported Setting: clinic Country: Germany Cases: Neuroborreliosis Controls: blood donors (n=49), syphilis (n=10), rheumatoid factor (n=8)
Target condition(s)	Neuroborreliosis
Index test(s) and reference standard	Index tests WB/IB (IgG, recombinant) – serum WB/IB (IgG, whole cell) – serum Reference standard Clinical diagnosis Time between measurement of index test and reference standard: not reported

Reference	Schulte-Spechtel 2004[97] (Schulte-Spechtel 2003[98])				
2×2 table [WB/IB (IgG, recombinant) – serum]		Reference standard +	Reference standard −	Total	
	Index test +	31	0	31	
	Index test −	5	67	72	
	Total	36	67	103	
2×2 table [WB/IB (IgG, whole cell) – serum]		Reference standard +	Reference standard −	Total	
	Index test +	23	2	25	
	Index test −	13	65	78	
	Total	36	67	103	
Statistical measures	WB/IB (IgG, recombinant) - serum Sensitivity 0.86 Specificity 1.00 WB/IB (IgG, whole cell) - serum Sensitivity 0.64 Specificity 0.97				
Source of funding	Not reported				
Limitations	Risk of bias: patient selection, index test, reference standard Indirectness: none				

Reference	**Schwartz 1992[99]**				
Study type	Case-control study				
Study methodology	Data source: skin biopsy samples from patients Recruitment: recruitment at clinics				
Number of patients	n = 35				
Patient characteristics	Age, range: not reported Gender (male to female ratio): not reported Family origin: not reported Setting: clinic Country: USA Cases: EM (untreated) Controls: people undergoing plastic surgery (n=10)				
Target condition(s)	EM				
Index test(s) and reference standard	Index tests PCR - skin Reference standard Clinical diagnosis Time between measurement of index test and reference standard: not reported				
2×2 table		Reference standard	Reference standard −	Total	

Reference	Schwartz 1992[99]				
[PCR – skin]		+			
	Index test +	20	1	21	
	Index test −	15	9	24	
	Total	35	10	45	
Statistical measures	PCR - skin Sensitivity 0.57 Specificity 0.90				
Source of funding	Supported by government grants				
Limitations	Risk of bias: patient selection, index test, reference standard Indirectness: none				

Reference	Senel 2010[100]
Study type	Case-control study
Study methodology	Data source: CSF samples from patients Recruitment: not reported
Number of patients	n = 37 (28 with definite neuroborreliosis, 9 with systemic borreliosis)
Patient characteristics	Age, median: 58 years (32-70) Gender (male to female ratio): 19/9 Family origin: not reported Setting: university clinic Country: Germany Cases: definite Neuroborreliosis (n=28) Controls: CNS bacterial infections (n=16), Viral CNS diseases (n=18), Guillain-Barre syndrome (n=11), Bell's palsy (n=19), Other cranial nerve palsies (n=5), Cephalgia (n=20)
Target condition(s)	Neuroborreliosis
Index test(s) and reference standard	Index tests CXCL13 – CSF Reference standard Clinical diagnosis Time between measurement of index test and reference standard: not reported

Reference	**Senel 2010[100]**				
2×2 table [CXCL13 – CSF]		Reference standard +	Reference standard −	Total	
	Index test +	27	2	29	
	Index test −	1	67	68	
	Total	28	69	97	
Statistical measures	CXCL13 - CSF Sensitivity 0.96 Specificity 0.97				
Source of funding	Not reported				
Limitations	Risk of bias: patient selection, index test, reference standard Indirectness: none				

Reference	Sillanpaa 2007[101]
Study type	Case-control study
Study methodology	Data source: serum samples from patients Recruitment: not reported
Number of patients	n = 70
Patient characteristics	Age, mean: not reported Gender (male to female ratio): not reported Family origin: not reported Setting: clinic Country: USA, Finland Cases: EM (n=42), Neuroborreliosis (n=14), Lyme arthritis (n=14) Controls: Syphilis (n=10), Rheumatoid factor (n=8), Anti-streptolysin antibodies (n=13), EBV (n=11), Anti-nuclear antibodies (n=12), Salmonella (n=5), Yersinia enterocolitica (n=4), Healthy blood donors (n=20)
Target condition(s)	EM, Neuroborreliosis, Lyme arthritis
Index test(s) and reference standard	Index tests ELISA (IgG) – serum Reference standard Clinical diagnosis Time between measurement of index test and reference standard: not reported

Reference	Sillanpaa 2007[101]				
2×2 table [ELISA (IgG) – serum], EM (acute)		Reference standard +	Reference standard −	Total	
	Index test +	25	0	25	
	Index test −	17	83	100	
	Total	42	83	125	
2×2 table [ELISA (IgG) – serum], EM (convalescent)		Reference standard +	Reference standard −	Total	
	Index test +	9	0	9	
	Index test −	13	83	96	
	Total	22	83	105	
2×2 table [ELISA (IgG) – serum], Lyme neuroborreliosis		Reference standard +	Reference standard −	Total	
	Index test +	14	0	14	
	Index test −	0	83	83	
	Total	14	83	97	
2×2 table [ELISA (IgG) – serum], LA		Reference standard +	Reference standard −	Total	
	Index test +	13	0	13	
	Index test −	1	83	84	
	Total	14	83	97	
Statistical measures	ELISA (IgG) – serum (EM, acute) Sensitivity 0.60 Specificity 1.00 ELISA (IgG) – serum (EM, convalescent) Sensitivity 0.41				

Reference	Sillanpaa 2007[101]
	Specificity 1.00 ELISA (IgG) – serum (Lyme neuroborreliosis) Sensitivity 1.00 Specificity 1.00 ELISA (IgG) – serum (LA) Sensitivity 0.93 Specificity 1.00
Source of funding	Supported by government, academic and industry grants
Limitations	Risk of bias: patient selection, index test, reference standard Indirectness: none

Reference	**Sivak 1996[102]**				
Study type	Case-control study				
Study methodology	Data source: serum samples from patients Recruitment: not reported				
Number of patients	n = 44				
Patient characteristics	Age, mean: not reported Gender (male to female ratio): not reported Family origin: not reported Setting: clinic Country: USA Cases: EM Controls: asymptomatic healthy controls (n=272)				
Target condition(s)	EM				
Index test(s) and reference standard	Index tests WB/IB (IgM) – serum Reference standard Positive culture Time between measurement of index test and reference standard: not reported				
2×2 table		Reference standard	Reference standard −	Total	

Reference	Sivak 1996[102]				
[WB/IB (IgM) – serum], acute EM		+			
	Index test +	11	8	19	
	Index test −	33	264	297	
	Total	44	272	316	
2×2 table [WB/IB (IgM) – serum], convalescent EM		Reference standard +	Reference standard −	Total	
	Index test +	31	8	39	
	Index test −	13	264	277	
	Total	44	272	316	
2×2 table [WB/IB (IgM) – serum], EM over 7 days		Reference standard +	Reference standard −	Total	
	Index test +	36	8	44	
	Index test −	8	264	272	
	Total	44	272	316	
Statistical measures	WB/IB (IgM) – serum (acute EM) Sensitivity 0.25 Specificity 0.97 WB/IB (IgM) – serum (convalescent EM) Sensitivity 0.70 Specificity 0.97 WB/IB (IgM) – serum (EM over 7 days) Sensitivity 0.82 Specificity 0.97				
Source of	Supported by government grants				

Reference	Sivak 1996[102]
funding	
Limitations	Risk of bias: patient selection, index test, reference standard Indirectness: none

Reference	Smismans 2006[104]
Study type	Case-control study
Study methodology	Data source: serum samples from patients before treatment Recruitment: not reported
Number of patients	n = 45
Patient characteristics	Age, mean: not reported Gender (male to female ratio): not reported Family origin: not reported Setting: clinic Country: USA Cases: Early localised cutaneous (n=23); Early disseminated (n=22): arthritis (n=2), cranial neuritis (n=9), radiculoneuropathy (n=3), EM with dissemination (n=7), polyneuropathy (n=1) Controls: Epstein-Barr virus (n=10), Acute cytomegalovirus (n=10), Syphilis (n=10), Rheumatoid factor positivity (n=10)
Target condition(s)	Early Lyme disease
Index test(s) and reference standard	Index tests ELISA (IgM/IgG, purified) – serum

Reference	Smismans 2006[104]				
	ELISA (IgM, purified) – serum ELISA (IgG, purified) – serum ELISA (IgM/IgG, synthetic C6) – serum ELISA (IgM, synthetic C6) – serum ELISA (IgG, synthetic C6) – serum ELISA (IgM/IgG, whole cell) – serum ELISA (IgM, whole cell) – serum ELISA (IgG, whole cell) – serum Reference standard Clinical diagnosis Time between measurement of index test and reference standard: not reported				
2×2 table [ELISA (IgM, purified) – serum], EM		Reference standard +	Reference standard −	Total	
	Index test +	14	9	25	
	Index test −	9	31	40	
	Total	23	40	63	
2×2 table [ELISA (IgG, purified) – serum], EM		Reference standard +	Reference standard −	Total	
	Index test +	5	0	5	
	Index test −	7	40	47	
	Total	13	40	53	
2×2 table [ELISA (IgM/IgG,		Reference standard +	Reference standard −	Total	
	Index test +	18	9	27	

Reference	Smismans 2006[104]				
purified) – serum], EM	Index test −	5	31	36	
	Total	23	40	63	
2×2 table [ELISA (IgM, purified) – serum], unspec Lyme disease		Reference standard +	Reference standard −	Total	
	Index test +	9	9	18	
	Index test −	4	31	35	
	Total	13	40	53	
2×2 table [ELISA (IgG, purified) – serum], unspec Lyme disease		Reference standard +	Reference standard −	Total	
	Index test +	13	0	13	
	Index test −	9	40	49	
	Total	22	40	62	
2×2 table [ELISA (IgM/IgG, purified) – serum], unspec Lyme disease		Reference standard +	Reference standard −	Total	
	Index test +	20	9	29	
	Index test −	2	31	33	
	Total	22	40	62	
2×2 table [ELISA (IgM, synthetic C6) – serum], EM		Reference standard +	Reference standard −	Total	
	Index test +	21	3	24	
	Index test −	2	37	39	
	Total	23	40	63	
2×2 table [ELISA (IgG, synthetic C6) –		Reference standard +	Reference standard −	Total	
	Index test +	11	3	14	

Reference	Smismans 2006[104]				
serum], EM	Index test −	1	37	38	
	Total	22	40	62	
2×2 table [ELISA (IgM/IgG, synthetic C6) – serum], EM		Reference standard +	Reference standard −	Total	
	Index test +	21	3	24	
	Index test −	2	37	39	
	Total	23	40	63	
2×2 table [ELISA (IgM, synthetic C6) – serum], unspec Lyme disease		Reference standard +	Reference standard −	Total	
	Index test +	11	3	14	
	Index test −	2	37	39	
	Total	13	40	53	
2×2 table [ELISA (IgG, synthetic C6) – serum], unspec Lyme disease		Reference standard +	Reference standard −	Total	
	Index test +	20	3	23	
	Index test −	2	37	39	
	Total	22	40	62	
2×2 table [ELISA (IgM/IgG, synthetic C6) – serum], unspec Lyme disease		Reference standard +	Reference standard −	Total	
	Index test +	20	3	23	
	Index test −	2	37	39	
	Total	22	40	62	
2×2 table [ELISA (IgM, whole cell) –		Reference standard +	Reference standard −	Total	
	Index test +	21	19	40	

Reference	Smismans 2006[104]				
serum], EM	Index test −	2	21	23	
	Total	23	40	63	
2×2 table [ELISA (IgG, whole cell) – serum], EM		Reference standard +	Reference standard −	Total	
	Index test +	10	3	13	
	Index test −	2	37	39	
	Total	12	40	52	
2×2 table [ELISA (IgM/IgG, whole cell) – serum], EM		Reference standard +	Reference standard −	Total	
	Index test +	23	20	43	
	Index test −	0	20	20	
	Total	23	40	63	
2×2 table [ELISA (IgM, whole cell) – serum], unspec Lyme disease		Reference standard +	Reference standard −	Total	
	Index test +	11	19	30	
	Index test −	2	21	23	
	Total	13	40	53	
2×2 table [ELISA (IgG, whole cell) – serum], unspec Lyme disease		Reference standard +	Reference standard −	Total	
	Index test +	20	3	23	
	Index test −	2	37	39	
	Total	22	40	62	
2×2 table [ELISA (IgM/IgG,		Reference standard +	Reference standard −	Total	
	Index test +	22	20	42	

Reference	Smismans 2006[104]				
whole cell) – serum], unspec Lyme disease	Index test −	0	20	20	
	Total	22	40	62	
Statistical measures	ELISA (IgM, purified) – serum (EM) Sensitivity 0.61 Specificity 0.78 ELISA (IgG, purified) – serum (EM) Sensitivity 0.42 Specificity 1.00 ELISA (IgM/IgG, purified) – serum (EM) Sensitivity 0.78 Specificity 0.78 ELISA (IgM, purified) – serum (unspec Lyme disease) Sensitivity 0.69 Specificity 0.78 ELISA (IgG, purified) – serum (unspec Lyme disease) Sensitivity 0.59 Specificity 1.00 ELISA (IgM/IgG, purified) – serum (unspec Lyme disease) Sensitivity 0.91 Specificity 0.78 ELISA (IgM, synthetic C6) – serum (EM) Sensitivity 0.91 Specificity 0.93				

Reference	Smismans 2006[104]
	ELISA (IgG, synthetic C6) – serum (EM) Sensitivity 0.92 Specificity 0.93 ELISA (IgM/IgG, synthetic C6) – serum (EM) Sensitivity 0.91 Specificity 0.93 ELISA (IgM, synthetic C6) – serum (unspec Lyme disease) Sensitivity 0.85 Specificity 0.93 ELISA (IgG, synthetic C6) – serum (unspec Lyme disease) Sensitivity 0.91 Specificity 0.93 ELISA (IgM/IgG, synthetic C6) – serum (unspec Lyme disease) Sensitivity 0.91 Specificity 0.93 ELISA (IgM, whole cell) – serum (EM) Sensitivity 0.91 Specificity 0.53 ELISA (IgG, whole cell) – serum (EM) Sensitivity 0.83 Specificity 0.93 ELISA (IgM/IgG, whole cell) – serum (EM) Sensitivity 1.00 Specificity 0.50

Reference	Smismans 2006[104]
	ELISA (IgM, whole cell) – serum (unspec Lyme disease) Sensitivity 0.85 Specificity 0.53 ELISA (IgG, whole cell) – serum (unspec Lyme disease) Sensitivity 0.91 Specificity 0.93 ELISA (IgM/IgG, whole cell) – serum (unspec Lyme disease) Sensitivity 1.00 Specificity 0.50
Source of funding	None reported
Limitations	Risk of bias: patient selection, index test, reference standard Indirectness: none

Reference	Stanek 1999[105]
Study type	Case-control study
Study methodology	Data source: serum samples from patients before treatment Recruitment: consecutive patients with tick bite
Number of patients	n = 99
Patient characteristics	Age, median: Female: (n=55): 49 years (10-80) Male (n=44): 51 (18-77)

Reference	Stanek 1999[105]				
	Gender (male to female ratio): 44/55 Family origin: not reported Setting: university clinic Country: Austria Cases: EM Controls: blood donors (n=100)				
Target condition(s)	Early Lyme disease				
Index test(s) and reference standard	Index tests ELISA (IgM) – serum ELISA (IgG) – serum Reference standard Clinical diagnosis Time between measurement of index test and reference standard: not reported				
2×2 table [ELISA (IgM) – serum]		Reference standard +	Reference standard −	Total	
	Index test +	5	1	6	
	Index test −	94	99	193	
	Total	99	100	199	
2×2 table [ELISA (IgG) – serum]		Reference standard +	Reference standard −	Total	
	Index test +	24	5	29	

Reference	Stanek 1999[105]				
	Index test −	75	95	170	
	Total	99	100	199	
Statistical measures	ELISA (IgM) - serum Sensitivity 0.05 Specificity 0.99 ELISA (IgG) - serum Sensitivity 0.24 Specificity 0.95				
Source of funding	Supported by government grants				
Limitations	Risk of bias: patient selection, index test, reference standard Indirectness: none				

Reference	Steere 2008[106]
Study type	Case-control study
Study methodology	Data source: serum samples from patients Recruitment: by 2 primary care physicians
Number of patients	n = 134
Patient characteristics	Age, mean: not reported Gender (male to female ratio): not reported Family origin: not reported

Reference	Steere 2008[106]				
	Setting: primary care Country: USA Cases: EM (n=76), Acute neurologic or cardiac involvement (n=13), Arthritis or chronic neurologic involvement (n=31), Post-Lyme disease symptoms (n=14) Controls: healthy persons (n=136)				
Target condition(s)	EM (single or multiple, acute or convalescent), acute/chronic disseminated Lyme disease				
Index test(s) and reference standard	Index tests ELISA (IgM/IgG) – serum Reference standard EM: CDC criteria and culture-positive Time between measurement of index test and reference standard: not reported				
2×2 table [ELISA (IgM/IgG) – serum], acute disseminated Lyme disease		Reference standard +	Reference standard −	Total	
	Index test +	13	5	18	
	Index test −	0	131	131	
	Total	13	136	149	
2×2 table [ELISA (IgM/IgG) – serum], acute multiple EM		Reference standard +	Reference standard −	Total	
	Index test +	15	5	20	
	Index test −	25	131	156	
	Total	40	136	176	
2×2 table		Reference standard	Reference standard −	Total	

Reference	Steere 2008[106]				
[ELISA (IgM/IgG) – serum], acute single EM		+			
	Index test +	7	5	13	
	Index test −	29	131	160	
	Total	36	136	172	
2×2 table [ELISA (IgM/IgG) – serum], chronic disseminated Lyme disease		Reference standard +	Reference standard −	Total	
	Index test +	31	5	36	
	Index test −	0	131	131	
	Total	31	136	167	
2×2 table [ELISA (IgM/IgG) – serum], convalescent multiple EM		Reference standard +	Reference standard −	Total	
	Index test +	25	5	30	
	Index test −	15	131	146	
	Total	40	136	176	
2×2 table [ELISA (IgM/IgG) – serum], convalescent single EM		Reference standard +	Reference standard −	Total	
	Index test +	17	5	22	
	Index test −	19	131	150	
	Total	36	136	172	
Statistical measures	ELISA (IgM/IgG) – serum (acute disseminated) Sensitivity 1.00 Specificity 0.96 ELISA (IgM/IgG) – serum (acute multiple EM) Sensitivity 0.38 Specificity 0.96				

Reference	Steere 2008[106]
	ELISA (IgM/IgG) – serum (acute single EM) Sensitivity 0.19 Specificity 0.96 ELISA (IgM/IgG) – serum (chronic disseminated) Sensitivity 1.00 Specificity 0.96 ELISA (IgM/IgG) – serum (convalescent multiple EM) Sensitivity 0.63 Specificity 0.96 ELISA (IgM/IgG) – serum (convalescent single EM) Sensitivity 0.47 Specificity 0.96
Source of funding	Supported by government and charity grants
Limitations	Risk of bias: patient selection, index test, reference standard Indirectness: none

Reference	Stiernstedt 1986[108] (Stiernstedt 1985[107])
Study type	Case-control study
Study methodology	Data source: serum samples from patients Recruitment: not reported
Number of	n = 26

Reference	Stiernstedt 1986[108] (Stiernstedt 1985[107])				
patients					
Patient characteristics	Age, median: 38 years (18-66) Gender (male to female ratio): 10/16 Family origin: not reported Setting: clinics, multicentre Country: Sweden Cases: EM Controls: healthy persons (63 for IFA, 120 for ELISA)				
Target condition(s)	EM				
Index test(s) and reference standard	Index tests ELISA (IgM/IgG) – serum ELISA (IgM) – serum ELISA (IgG) – serum IFA - serum Reference standard Clinical diagnosis Time between measurement of index test and reference standard: not reported				
2×2 table [ELISA (IgM/IgG) – serum]		Reference standard +	Reference standard −	Total	
	Index test +	7	11	18	
	Index test −	18	109	127	

Reference	Stiernstedt 1986[108] (Stiernstedt 1985[107])				
	Total	25	120	145	
2×2 table [ELISA (IgM) – serum]		Reference standard +	Reference standard −	Total	
	Index test +	2	0	2	
	Index test −	23	0	23	
	Total	25	0	25	
2×2 table [ELISA (IgG) – serum]		Reference standard +	Reference standard −	Total	
	Index test +	5	0	5	
	Index test −	20	0	20	
	Total	25	0	25	
2×2 table [IFA – serum]		Reference standard +	Reference standard −	Total	
	Index test +	3	3	6	
	Index test −	22	60	82	
	Total	25	63	88	
Statistical measures	ELISA (IgM/IgG) - serum Sensitivity 0.28 Specificity 0.91 ELISA (IgM) - serum Sensitivity 0.08 Specificity N/A ELISA (IgG) - serum Sensitivity 0.20				

Reference	Stiernstedt 1986[108] (Stiernstedt 1985[107])
	Specificity N/A IFA – serum Sensitivity 0.12 Specificity 0.95
Source of funding	None reported
Limitations	Risk of bias: patient selection, index test, reference standard Indirectness: none

Reference	Stricker 2001[109]
Study type	Case-control study
Study methodology	Data source: serum samples from patients Recruitment: not reported
Number of patients	n = 83
Patient characteristics	Age, mean: Acute Lyme disease: Male: 36 (13-52) Female: 43 (37-50) Chronic Lyme disease: Male: 45 (14-71) Female: 43 (15-76) Gender (male to female ratio): 33/50

Reference	Stricker 2001[109]				
	Family origin: not reported Setting: referral practice Country: USA Cases: acute Lyme disease (n=10), chronic Lyme disease (n=73) Controls: people with AIDS (n=22)				
Target condition(s)	Acute/chronic Lyme disease				
Index test(s) and reference standard	Index tests CD57 Reference standard Clinical diagnosis based on CDC criteria Time between measurement of index test and reference standard: not reported				
2×2 table (CD57], acute Lyme disease		Reference standard +	Reference standard −	Total	
	Index test +	0	4	4	
	Index test −	10	18	28	
	Total	10	22	32	
2×2 table [CD57], chronic Lyme disease		Reference standard +	Reference standard −	Total	
	Index test +	31	4	35	
	Index test −	0	18	18	
	Total	31	22	53	

Reference	Stricker 2001[109]
Statistical measures	CD57 (acute Lyme disease) Sensitivity 0.00 Specificity 0.82 CD57 (chronic Lyme disease) Sensitivity 1.00 Specificity 0.82
Source of funding	Not reported
Limitations	Risk of bias: patient selection, reference standard Indirectness: none

Reference	Tjernberg 2007[111]
Study type	Case-control study
Study methodology	Data source: serum samples from patients Recruitment: not reported
Number of patients	n = 273
Patient characteristics	Age, median: 54.5 years (4-85) Gender (male to female ratio): 79/121 Family origin: not reported Setting: clinic

Reference	Tjernberg 2007[111]				
	Country: Sweden Cases: EM (n=158), Neuroborreliosis (n=26), Acrodermatitis (n=9), Lyme arthritis (n=3), Possible LB (n=31) Controls: blood donors (n=200)				
Target condition(s)	EM, Lyme arthritis, Neuroborreliosis, ACA				
Index test(s) and reference standard	Index tests ELISA C6 (IgM/IgG) – serum ELISA Virotech (IgM) – serum CLIA (IgM/IgG) - serum Reference standard Clinical diagnosis Time between measurement of index test and reference standard: not reported				
2×2 table [ELISA C6 (IgM/IgG) – serum], EM		Reference standard +	Reference standard −	Total	
	Index test +	58	184	242	
	Index test −	100	16	116	
	Total	158	200	358	
2×2 table [ELISA C6 (IgM/IgG) – serum], ACA		Reference standard +	Reference standard −	Total	
	Index test +	8	184	192	
	Index test −	1	16	17	
	Total	9	200	209	
2×2 table		Reference standard +	Reference standard −	Total	

Reference	Tjernberg 2007[111]				
[ELISA C6 (IgM/IgG) – serum], Lyme neuroborreliosis	Index test +	23	184	207	
	Index test −	3	16	19	
	Total	26	200	226	
2×2 table [ELISA C6 (IgM/IgG) – serum], LA		Reference standard +	Reference standard −	Total	
	Index test +	2	184	186	
	Index test −	1	16	17	
	Total	3	200	203	
2×2 table [ELISA (IgM/IgG) – serum], EM		Reference standard +	Reference standard −	Total	
	Index test +	73	152	225	
	Index test −	85	48	133	
	Total	158	200	358	
2×2 table [ELISA (IgM/IgG) – serum], ACA		Reference standard +	Reference standard −	Total	
	Index test +	9	152	161	
	Index test −	0	48	48	
	Total	9	200	209	
2×2 table [ELISA (IgM/IgG) – serum], Lyme neuroborreliosis		Reference standard +	Reference standard −	Total	
	Index test +	25	152	177	
	Index test −	1	48	49	
	Total	26	200	226	
2×2 table		Reference standard +	Reference standard −	Total	

Reference	Tjernberg 2007[111]				
[ELISA (IgM/IgG) – serum], LA	Index test +	2	152	154	
	Index test −	1	48	49	
	Total	3	200	203	
2×2 table [CLIA (IgM/IgG) – serum], EM		Reference standard +	Reference standard −	Total	
	Index test +	66	162	228	
	Index test −	92	38	130	
	Total	158	200	358	
2×2 table [CLIA (IgM/IgG) – serum], ACA		Reference standard +	Reference standard −	Total	
	Index test +	6	162	168	
	Index test −	3	38	41	
	Total	9	200	209	
2×2 table [CLIA (IgM/IgG) – serum], Lyme neuroborreliosis		Reference standard +	Reference standard −	Total	
	Index test +	22	162	184	
	Index test −	4	38	42	
	Total	26	200	226	
2×2 table [CLIA (IgM/IgG) – serum], LA		Reference standard +	Reference standard −	Total	
	Index test +	2	162	164	
	Index test −	1	38	39	
	Total	3	200	203	
Statistical measures	ELISA C6 (IgM/IgG) – serum (EM)				

Reference	Tjernberg 2007[111]
	Sensitivity 0.37 Specificity 0.08 ELISA C6 (IgM/IgG) – serum (ACA) Sensitivity 0.89 Specificity 0.08 ELISA C6 (IgM/IgG) – serum (Lyme neuroborreliosis) Sensitivity 0.88 Specificity 0.08 ELISA C6 (IgM/IgG) – serum (LA) Sensitivity 0.67 Specificity 0.08 ELISA (IgM/IgG) – serum (EM) Sensitivity 0.46 Specificity 0.24 ELISA (IgM/IgG) – serum (ACA) Sensitivity 1.00 Specificity 0.24 ELISA (IgM/IgG) – serum (Lyme neuroborreliosis) Sensitivity 0.96 Specificity 0.24 ELISA (IgM/IgG) – serum (LA) Sensitivity 0.67 Specificity 0.24

Reference	Tjernberg 2007[111]
	CLIA (IgM/IgG) – serum (EM) Sensitivity 0.42 Specificity 0.19 CLIA (IgM/IgG) – serum (ACA) Sensitivity 0.67 Specificity 0.19 CLIA (IgM/IgG) – serum (Lyme neuroborreliosis) Sensitivity 0.85 Specificity 0.19 CLIA (IgM/IgG) – serum (LA) Sensitivity 0.67 Specificity 0.19
Source of funding	Supported by government grants
Limitations	Risk of bias: patient selection, index test, reference standard Indirectness: none

Reference	**Tjernberg 2009[112]**				
Study type	Case-control study				
Study methodology	Data source: serum samples from patients Recruitment: not reported				
Number of patients	n = 148				
Patient characteristics	Age, median: 58 years (7-84) Gender (male to female ratio): 58/90 Family origin: not reported Setting: clinic Country: Sweden Cases: EM Controls: blood donors (n=200)				
Target condition(s)	EM				
Index test(s) and reference standard	Index tests ELISA (IgM/IgG) – serum Reference standard Clinical diagnosis Time between measurement of index test and reference standard: not reported				
2×2 table		Reference standard	Reference standard −	Total	

Reference	Tjernberg 2009[112]				
[ELISA (IgM/IgG) – serum], cut-off 0.0689		+			
	Index test +	97	5	102	
	Index test −	51	166	217	
	Total	148	171	319	
2×2 table [ELISA (IgM/IgG) – serum], cut-off 0.15		Reference standard +	Reference standard −	Total	
	Index test +	76	0	76	
	Index test −	72	171	243	
	Total	148	171	319	
Statistical measures	ELISA (IgM/IgG) – serum (cut-off 0.0689) Sensitivity 0.66 Specificity 0.97 ELISA (IgM/IgG) – serum (cut-off 0.15) Sensitivity 0.51 Specificity 1.00				
Source of funding	Supported by government grants				
Limitations	Risk of bias: patient selection, index test, reference standard Indirectness: none				

Reference	Trevejo 2001[113]
Study type	Case-control study
Study methodology	Data source: serum samples from patients taken a median of 4 days after illness onset (acute phase) or 36 days after illness onset (convalescent phase) Recruitment: not reported
Number of patients	n = 74
Patient characteristics	Age, median: 41 years (3-83) Gender (male to female ratio): 41/33 Family origin: not reported Setting: primary care Country: USA Cases: EM (66 acute phase, 55 convalescent phase) Controls: healthy controls (n=38)
Target condition(s)	EM
Index test(s) and reference standard	Index tests ELISA (IgM/IgG) – serum Reference standard Clinical diagnosis Time between measurement of index test and reference standard: not reported

Reference	Trevejo 2001[113]				
2×2 table [ELISA (IgM/IgG) – serum], EM (acute phase)		Reference standard +	Reference standard −	Total	
	Index test +	28	1	29	
	Index test −	38	36	74	
	Total	66	37	103	
2×2 table [WB/IB (IgM/IgG) – serum], EM (acute phase)		Reference standard +	Reference standard −	Total	
	Index test +	25	1	26	
	Index test −	41	37	78	
	Total	66	38	104	
2×2 table [ELISA (IgM/IgG) – serum], EM (convalescent phase)		Reference standard +	Reference standard −	Total	
	Index test +	43	1	44	
	Index test −	12	36	48	
	Total	55	37	92	
2×2 table [WB/IB (IgM/IgG) – serum], EM (convalescent phase)		Reference standard +	Reference standard −	Total	
	Index test +	17	1	18	
	Index test −	39	37	76	
	Total	56	38	94	
Statistical measures	ELISA (IgM/IgG) – serum (EM, acute phase) Sensitivity 0.42 Specificity 0.97 WB/IB (IgM/IgG) – serum (EM, acute phase) Sensitivity 0.38				

Reference	Trevejo 2001[113]
	Specificity 0.97 ELISA (IgM/IgG) – serum (EM, convalescent phase) Sensitivity 0.78 Specificity 0.97 WB/IB (IgM/IgG) – serum (EM, convalescent phase) Sensitivity 0.30 Specificity 0.97
Source of funding	Not reported
Limitations	Risk of bias: patient selection, reference standard Indirectness: none

Reference	Tumani 1995[114]
Study type	Case-control study
Study methodology	Data source: serum samples from patients Recruitment: not reported
Number of patients	n = 24
Patient characteristics	Age, mean: not reported Gender (male to female ratio): not reported Family origin: not reported Setting: university clinic Country: Germany Cases: acute neuroborrleiosis Controls: disease controls (n=45), healthy controls (n=28)
Target condition(s)	Acute neuroborreliosis
Index test(s) and reference standard	Index tests Bb-IgM-AI (antibody index: CSF, serum) Bb-IgG-AI Reference standard No reference standard Time between measurement of index test and reference standard: not applicable

Reference	Tumani 1995[114]
Statistical measures	Bb-IgM-AI Sensitivity 0.79 Specificity 0.96 Bb-IgG-AI Sensitivity 0.63 Specificity 0.89 All CSF values Sensitivity 0.70 Specificity 0.98 3 out of 4 CSF values Sensitivity 0.80 Specificity 0.98
Source of funding	None reported
Limitations	Risk of bias: patient selection, index test, reference standard Indirectness: none

Reference	van Burgel 2011[115]
Study type	Case-control study
Study methodology	Data source: serum samples from patients Recruitment: laboratory information system
Number of patients	n = 95
Patient characteristics	Age, mean: Lyme neuroborreliosis: 39 years (SD 24) LB: 51 years (SD 17) Gender (male to female ratio): 53/42 Family origin: not reported Setting: urban medical centres and hospitals Country: Netherlands Cases: Lyme neuroborreliosis (n=59), Lyme borreliosis (n=36) Controls: Infectious meningitis/encephalitis (n=69), Neurological controls (n=74)
Target condition(s)	Neuroborreliosis
Index test(s) and reference standard	Index tests ELISA (IgM/IgG) – AI Reference standard Lyme neuroborreliosis: 4 of the following 5 criteria: detection of B. burgdorferi antibodies in serum, CSF pleocytosis, absence of other evident cause of meningitis, evidence of intrathecal production of specific B. burgdorferi antibodies, objective neurological complaints with favourable outcome after treatment

Reference	van Burgel 2011[115]				
	Time between measurement of index test and reference standard: not reported				
2×2 table [ELISA (IgM/IgG) – AI]		Reference standard +	Reference standard −	Total	
	Index test +	56	5	61	
	Index test −	3	138	141	
	Total	59	143	202	
Statistical measures	ELISA (IgM/IgG) - AI Sensitivity 0.95 Specificity 0.97				
Source of funding	None declared				
Limitations	Risk of bias: patient selection, index test, reference standard Indirectness: serious				

Reference	**van der Heijden 1999[116]**				
Study type	Case-control study				
Study methodology	Data source: synovial fluid samples from patients Recruitment: not reported				
Number of patients	n = 4				
Patient characteristics	Age, median: 28 years (17-38) Gender (male to female ratio): 3/1 Family origin: not reported Setting: university clinic Country: Netherlands Cases: Lyme arthritis Controls: other arthritis forms (n=9)				
Target condition(s)	Lyme arthritis				
Index test(s) and reference standard	Index tests PCR – synovial fluid Reference standard Clinical diagnosis Time between measurement of index test and reference standard: not reported				
2×2 table		Reference standard	Reference standard −	Total	

Reference	van der Heijden 1999[116]			
[PCR – synovial fluid]		+		
	Index test +	3	0	3
	Index test −	1	9	10
	Total	4	9	13
Statistical measures	PCR – synovial fluid Sensitivity 0.75 Specificity 1.00			
Source of funding	Not reported			
Limitations	Risk of bias: patient selection, reference standard Indirectness: none			

Reference	Vasiliu 1998[117]
Study type	Case-control study
Study methodology	Data source: synovial fluid samples from patients Recruitment: not reported
Number of patients	n = 20
Patient characteristics	Age, mean: 39.2 years (SD 13.2) Gender (male to female ratio): 10/10 Family origin: not reported Setting: not reported

Reference	Vasiliu 1998[117]				
	Country: Germany Cases: Lyme arthritis Controls: rheumatic disease (n=10)				
Target condition(s)	Lyme arthritis				
Index test(s) and reference standard	Index tests PCR – synovial fluid Reference standard Clinical diagnosis Time between measurement of index test and reference standard: not reported				
2×2 table [PCR – synovial fluid]		Reference standard +	Reference standard −	Total	
	Index test +	13	0	13	
	Index test −	7	10	17	
	Total	20	10	30	
Statistical measures	PCR – synovial fluid Sensitivity 0.65 Specificity 1.00				
Source of funding	Not reported				
Limitations	Risk of bias: patient selection, index test, reference standard Indirectness: none				

Reference	von Baehr 2012[118]
Study type	Case-control study
Study methodology	Data source: venous blood samples from patients Recruitment: not reported
Number of patients	n = 94
Patient characteristics	Age, mean: not reported Gender (male to female ratio): not reported Family origin: not reported Setting: not reported Country: Germany Cases: EM (n=28), Acute mono-arthritis (n=14), Bannwarth's syndrome (n=6), Migrating arthromyalgias (n=34), Facial palsy (n=5), Acute Lyme neuroborreliosis (n=7) Controls: Blood donors (n=120), Autoimmune diseases (n=40), Seropositive clinically healthy outdoor workers (n=48)
Target condition(s)	Lyme disease
Index test(s) and reference standard	Index tests Lymphocyte transformation test – venous blood Reference standard Clinical diagnosis Time between measurement of index test and reference standard: not reported

Reference	von Baehr 2012[118]				
2×2 table [LTT – venous blood]		Reference standard +	Reference standard −	Total	
	Index test +	84	2	86	
	Index test −	10	158	168	
	Total	94	160	254	
Statistical measures	LTT – venous blood Sensitivity 0.89 Specificity 0.99				
Source of funding	None declared				
Limitations	Risk of bias: patient selection, index test, reference standard Indirectness: none				

Reference	von Stedingk 1995[119]				
Study type	Case-control study				
Study methodology	Data source: skin samples from patients Recruitment: not reported				
Number of patients	n = 62				
Patient characteristics	Age, mean: not reported Gender (male to female ratio): not reported Family origin: not reported Setting: department of dermatology Country: Sweden Cases: EM (n=26), ACA (n=36) Controls: Skin removed during plastic surgery (n=67), Volunteers among medical staff (n=5), Non-borrelial disorders (n=4)				
Target condition(s)	EM, ACA				
Index test(s) and reference standard	Index tests PCR - skin Reference standard Clinical diagnosis Time between measurement of index test and reference standard: not reported				
2×2 table		Reference standard	Reference standard −	Total	

Reference	von Stedingk 1995[119]				
[PCR - skin], EM		+			
	Index test +	18	0	18	
	Index test −	8	76	8	
	Total	26	76	102	
2×2 table [PCR - skin], ACA		Reference standard +	Reference standard −	Total	
	Index test +	22	0	22	
	Index test −	14	76	90	
	Total	36	76	112	
Statistical measures	PCR – skin (EM) Sensitivity 0.69 Specificity 1.00 PCR – skin (ACA) Sensitivity 0.61 Specificity 1.00				
Source of funding	Supported by industry grants				
Limitations	Risk of bias: patient selection, reference standard Indirectness: none				

Reference	Widhe 2004[121]
Study type	Case-control study
Study methodology	Data source: serum samples from patients Recruitment: not reported
Number of patients	n = 56
Patient characteristics	Age, median: Lyme neuroborreliosis: 51 years (22-80) EM: 54 years (30-78) ACA: 65 years (32-73) Gender (male to female ratio): 31/25 Family origin: not reported Setting: not reported Country: Sweden Cases: Lyme neuroborreliosis (n=39), EM (n=12), ACA (n=5) Controls: healthy blood donors (n=23)
Target condition(s)	EM, ACA, Neuroborreliosis
Index test(s) and reference standard	Index tests ELISA (IgM) – serum ELISA (IgG) – serum Reference standard Clinical diagnosis

Reference	Widhe 2004[121]				
	Time between measurement of index test and reference standard: not reported				
2×2 table [ELISA (IgM) – serum], EM		Reference standard +	Reference standard −	Total	
	Index test +	4	0	4	
	Index test −	1	23	24	
	Total	5	23	28	
2×2 table [ELISA (IgG) – serum], EM		Reference standard +	Reference standard −	Total	
	Index test +	1	0	1	
	Index test −	4	23	27	
	Total	5	23	28	
2×2 table [ELISA (IgM) – serum], ACA		Reference standard +	Reference standard −	Total	
	Index test +	3	0	3	
	Index test −	2	23	25	
	Total	5	23	28	
2×2 table [ELISA (IgG) – serum], ACA		Reference standard +	Reference standard −	Total	
	Index test +	5	0	5	
	Index test −	0	23	23	
	Total	5	23	28	
2×2 table [ELISA (IgM) – serum], Lyme		Reference standard +	Reference standard −	Total	
	Index test +	11	0	11	

Reference	**Widhe 2004[121]**				
neuroborreliosis	Index test −	17	23	40	
	Total	28	23	51	
2×2 table [ELISA (IgG) – serum], Lyme neuroborreliosis		Reference standard +	Reference standard −	Total	
	Index test +	23	0	23	
	Index test −	6	23	29	
	Total	29	23	52	
Statistical measures	ELISA (IgM) – serum (EM) Sensitivity 0.80 Specificity 1.00 ELISA (IgM) – serum (EM) Sensitivity 0.20 Specificity 1.00 ELISA (IgM) – serum (ACA) Sensitivity 0.60 Specificity 1.00 ELISA (IgM) – serum (ACA) Sensitivity 1.00 Specificity 1.00 ELISA (IgM) – serum (Lyme neuroborreliosis) Sensitivity 0.39 Specificity 1.00 ELISA (IgM) – serum (Lyme neuroborreliosis) Sensitivity 0.79				

Reference	Widhe 2004[121]
	Specificity 1.00
Source of funding	Not reported
Limitations	Risk of bias: patient selection, index test, reference standard Indirectness: none

Reference	Wilske 1993[122]
Study type	Case-control study
Study methodology	Data source: serum samples from patients Recruitment: not reported
Number of patients	n = 134
Patient characteristics	Age, range: not reported Gender (male to female ratio): not reported Family origin: not reported Setting: not reported Country: Germany Cases: EM (n=31), Lyme neuroborreliosis (n=60), Late Lyme disease (LA (n=24), ACA (n=19)) Controls: Blood donors (n=100), Antibodies against T. pallidum (n=20), Antibodies against Epstein-Barr virus (n=12), Rheumatoid factor (n=10)

Reference	Wilske 1993[122]				
Target condition(s)	Lyme disease, EM, Neuroborreliosis				
Index test(s) and reference standard	Index tests ELISA (IgM, flagellin) – serum ELISA (IgG, flagellin) – serum ELISA (IgM, OGP-ELISA) – serum ELISA (IgG, OGP-ELISA) – serum WB/IB (IgM, OspC-blot) – serum WB/IB (IgG, OspC-blot) – serum WB/IB (IgM, p41/i-blot) – serum WB/IB (IgG, p41/i-blot) – serum WB/IB (IgM, p100-blot) – serum WB/IB (IgG, p100-blot) – serum IFA (IgM) – serum IFA (IgG) – serum Reference standard Clinical diagnosis Time between measurement of index test and reference standard: not reported				
2×2 table [ELISA (IgM, flagellin) – serum], all Lyme disease		Reference standard +	Reference standard −	Total	
	Index test +	48	6	54	
	Index test −	86	136	222	
	Total	134	142	276	
2×2 table [ELISA (IgG, flagellin) – serum], all		Reference standard +	Reference standard −	Total	
	Index test +	95	9	104	
	Index test −	39	133	172	

Reference	Wilske 1993[122]				
Lyme disease	Total	134	142	276	
2×2 table [IFA (IgM) – serum], all Lyme disease		Reference standard +	Reference standard −	Total	
	Index test +	31	4	35	
	Index test −	103	138	241	
	Total				
2×2 table [IFA (IgG) – serum], all Lyme disease		Reference standard +	Reference standard −	Total	
	Index test +	102	4	106	
	Index test −	32	138	170	
	Total	134	142	276	
2×2 table [ELISA (IgM, OGP) – serum], all Lyme disease		Reference standard +	Reference standard −	Total	
	Index test +	62	4	66	
	Index test −	72	138	210	
	Total	134	142	276	
2×2 table [ELISA (IgG, OGP) – serum], all Lyme disease		Reference standard +	Reference standard −	Total	
	Index test +	80	4	84	
	Index test −	54	138	192	
	Total	134	142	276	
2×2 table [WB/IB (IgM, OspC-blot) – serum], all		Reference standard +	Reference standard −	Total	
	Index test +	58	4	62	
	Index test −	76	138	214	

Reference	Wilske 1993[122]				
Lyme disease	Total	134	142	276	
2×2 table [WB/IB (IgG, OspC-blot) – serum], all Lyme disease		Reference standard +	Reference standard −	Total	
	Index test +	19	1	20	
	Index test −	115	141	256	
	Total	134	142	276	
2×2 table [WB/IB (IgM, p100) – serum], all Lyme disease		Reference standard +	Reference standard −	Total	
	Index test +	17	1	18	
	Index test −	117	141	258	
	Total	134	142	276	
2×2 table [WB/IB (IgG, p100) – serum], all Lyme disease		Reference standard +	Reference standard −	Total	
	Index test +	68	9	77	
	Index test −	66	133	199	
	Total	134	142	276	
2×2 table [WB/IB (IgM, p41/i) – serum], all Lyme disease		Reference standard +	Reference standard −	Total	
	Index test +	20	1	21	
	Index test −	114	141	255	
	Total	134	142	276	
2×2 table [WB/IB (IgG, p41/i) – serum], all		Reference standard +	Reference standard −	Total	
	Index test +	43	6	49	
	Index test −	91	136	227	

Reference	Wilske 1993[122]				
Lyme disease	Total	134	142	276	
2×2 table [ELISA (IgM, flagellin) – serum], EM		Reference standard +	Reference standard −	Total	
	Index test +	12	6	18	
	Index test −	19	136	155	
	Total	31	142	173	
2×2 table [ELISA (IgG, flagellin) – serum], EM		Reference standard +	Reference standard −	Total	
	Index test +	12	9	21	
	Index test −	19	133	152	
	Total	31	142	173	
2×2 table [ELISA (IgM, flagellin) – serum], Lyme neuroborreliosis		Reference standard +	Reference standard −	Total	
	Index test +	30	6	36	
	Index test −	30	136	166	
	Total	60	142	202	
2×2 table [ELISA (IgG, flagellin) – serum], Lyme neuroborreliosis		Reference standard +	Reference standard −	Total	
	Index test +	45	9	54	
	Index test −	15	133	148	
	Total	60	142	202	
2×2 table [ELISA (IgG, flagellin) – serum], all		Reference standard +	Reference standard −	Total	
	Index test +	38	9	47	
	Index test −	5	133	138	

Reference	Wilske 1993[122]				
Lyme disease	Total	43	142	185	
2×2 table [IFA (IgM) – serum], EM		Reference standard +	Reference standard −	Total	
	Index test +	10	4	14	
	Index test −	21	138	159	
	Total	31	142	173	
2×2 table [ELISA (IgG, flagellin) – serum], EM		Reference standard +	Reference standard −	Total	
	Index test +	14	4	18	
	Index test −	17	138	155	
	Total	31	142	173	
2×2 table [IFA (IgM) – serum], Lyme neuroborreliosis		Reference standard +	Reference standard −	Total	
	Index test +	18	4	22	
	Index test −	42	138	180	
	Total	60	142	202	
2×2 table [IFA (IgG) – serum], Lyme neuroborreliosis		Reference standard +	Reference standard −	Total	
	Index test +	45	4	49	
	Index test −	15	138	153	
	Total	60	142	202	
2×2 table [ELISA (IgM, flagellin) – serum], late		Reference standard +	Reference standard −	Total	
	Index test +	6	6	12	
	Index test −	37	136	173	

Reference	Wilske 1993[122]				
Lyme disease	Total	43	142	185	
2×2 table [IFA (IgM) – serum], late Lyme disease		Reference standard +	Reference standard −	Total	
	Index test +	2	4	6	
	Index test −	41	138	179	
	Total	43	142	185	
2×2 table [IFA (IgG) – serum], late Lyme disease		Reference standard +	Reference standard −	Total	
	Index test +	43	4	47	
	Index test −	0	138	138	
	Total	43	142	185	
2×2 table [ELISA (IgM, OGB) – serum], late Lyme disease		Reference standard +	Reference standard −	Total	
	Index test +	10	4	14	
	Index test −	33	138	171	
	Total	43	142	185	
2×2 table [WB/IB (IgM, OspC) – serum], late Lyme disease		Reference standard +	Reference standard −	Total	
	Index test +	17	4	21	
	Index test −	26	138	164	
	Total	43	142	185	
2×2 table [WB/IB (IgG, OspC) – serum], late		Reference standard +	Reference standard −	Total	
	Index test +	7	1	8	
	Index test −	36	141	177	

Reference	Wilske 1993[122]				
Lyme disease	Total	43	142	185	
2×2 table [WB/IB (IgM, p100) – serum], late Lyme disease		Reference standard +	Reference standard −	Total	
	Index test +	8	1	9	
	Index test −	35	141	176	
	Total	43	142	185	
2×2 table [WB/IB (IgG, p100) – serum], late Lyme disease		Reference standard +	Reference standard −	Total	
	Index test +	43	9	52	
	Index test −	0	133	133	
	Total	43	142	185	
2×2 table [WB/IB (IgM, p41/i) – serum], late Lyme disease		Reference standard +	Reference standard −	Total	
	Index test +	5	1	6	
	Index test −	38	141	179	
	Total	43	142	185	
2×2 table [WB/IB (IgG, p41/i) – serum], late Lyme disease		Reference standard +	Reference standard −	Total	
	Index test +	26	6	32	
	Index test −	17	136	153	
	Total	43	142	185	
2×2 table [ELISA (IgM, OGP) – serum], EM		Reference standard +	Reference standard −	Total	
	Index test +	14	4	18	
	Index test −	17	138	155	

Reference	Wilske 1993[122]				
	Total	31	142	173	
2×2 table [ELISA (IgG, OGP) – serum], EM		Reference standard +	Reference standard −	Total	
	Index test +	11	4	15	
	Index test −	20	138	158	
	Total	31	142	173	
2×2 table [ELISA (IgM, OGP) – serum], Lyme neuroborreliosis		Reference standard +	Reference standard −	Total	
	Index test +	37	4	41	
	Index test −	23	138	161	
	Total	60	142	202	
2×2 table [ELISA (IgG, OGP) – serum], Lyme neuroborreliosis		Reference standard +	Reference standard −	Total	
	Index test +	27	4	31	
	Index test −	33	138	171	
	Total	60	142	202	
2×2 table [ELISA (IgG, OGP) – serum], unspec Lyme disease		Reference standard +	Reference standard −	Total	
	Index test +	43	4	47	
	Index test −	0	138	138	
	Total	43	142	185	
2×2 table [WB/IB (IgM, OspC) – serum], EM		Reference standard +	Reference standard −	Total	
	Index test +	14	4	18	
	Index test −	17	138	155	

Reference	Wilske 1993[122]				
	Total	31	142	173	
2×2 table [WB/IB (IgG, OspC) – serum], EM		Reference standard +	Reference standard −	Total	
	Index test +	3	1	4	
	Index test −	28	141	169	
	Total	31	142	173	
2×2 table [WB/IB (IgM, OspC) – serum], Lyme neuroborreliosis		Reference standard +	Reference standard −	Total	
	Index test +	26	4	30	
	Index test −	34	138	172	
	Total	60	142	202	
2×2 table [WB/IB (IgG, OspC) – serum], Lyme neuroborreliosis		Reference standard +	Reference standard −	Total	
	Index test +	9	1	10	
	Index test −	51	141	192	
	Total	60	142	202	
2×2 table [WB/IB (IgM, p100) – serum], EM		Reference standard +	Reference standard −	Total	
	Index test +	3	1	4	
	Index test −	28	141	169	
	Total	31	142	173	
2×2 table [WB/IB (IgG, p100) – serum], EM		Reference standard +	Reference standard −	Total	
	Index test +	7	9	16	
	Index test −	24	133	157	

Reference	Wilske 1993[122]				
	Total	31	142	173	
2×2 table [WB/IB (IgM, p100) – serum], EM		Reference standard +	Reference standard −	Total	
	Index test +	7	1	8	
	Index test −	53	141	194	
	Total	60	142	202	
2×2 table [WB/IB (IgG, p100) – serum], EM		Reference standard +	Reference standard −	Total	
	Index test +	26	9	35	
	Index test −	34	133	167	
	Total	60	142	202	
2×2 table [WB/IB (IgM, p41/i) – serum], EM		Reference standard +	Reference standard −	Total	
	Index test +	3	1	4	
	Index test −	28	141	169	
	Total	31	142	173	
2×2 table [WB/IB (IgG, p41/i) – serum], EM		Reference standard +	Reference standard −	Total	
	Index test +	2	6	8	
	Index test −	29	136	165	
	Total	31	142	173	
2×2 table [WB/IB (IgM, p41/i) – serum], EM		Reference standard +	Reference standard −	Total	
	Index test +	12	1	13	
	Index test −	48	141	189	

Reference	Wilske 1993[122]				
	Total	60	142	202	
2×2 table [WB/IB (IgG, p41/i) – serum], EM		Reference standard +	Reference standard −	Total	
	Index test +	15	6	21	
	Index test −	45	136	181	
	Total	60	142	202	
Statistical measures	ELISA (IgM, flagellin) – serum (all Lyme) Sensitivity 0.36 Specificity 0.96 ELISA (IgG, flagellin) – serum (all Lyme) Sensitivity 0.71 Specificity 0.94 IFA (IgM) – serum (all Lyme) Sensitivity 0.23 Specificity 0.97 IFA (IgG) – serum (all Lyme) Sensitivity 0.76 Specificity 0.97 ELISA (IgM, OGP) – serum (all Lyme) Sensitivity 0.46 Specificity 0.97 ELISA (IgG, OGP) – serum (all Lyme) Sensitivity 0.60 Specificity 0.97				

Reference	Wilske 1993[122]
	WB/IB (IgM, OspC-blot) – serum (all Lyme) Sensitivity 0.43 Specificity 0.97 WB/IB (IgG, OspC-blot) – serum (all Lyme) Sensitivity 0.14 Specificity 0.99 WB/IB (IgM, p100-blot) – serum (all Lyme) Sensitivity 0.13 Specificity 0.99 WB/IB (IgG, p100-blot) – serum (all Lyme) Sensitivity 0.51 Specificity 0.94 WB/IB (IgM, p41/i-blot) – serum (all Lyme) Sensitivity 0.15 Specificity 0.99 WB/IB (IgG, p41/i-blot) – serum (all Lyme) Sensitivity 0.32 Specificity 0.96 ELISA (IgM, flagellin) – serum (EM) Sensitivity 0.39 Specificity 0.96 ELISA (IgG, flagellin) – serum (EM) Sensitivity 0.39

Reference	Wilske 1993[122]
	Specificity 0.94 ELISA (IgM, flagellin) – serum (Lyme neuroborreliosis) Sensitivity 0.50 Specificity 0.96 ELISA (IgG, flagellin) – serum (Lyme neuroborreliosis) Sensitivity 0.75 Specificity 0.94 ELISA (IgG, flagellin) – serum (unspecified Lyme disease) Sensitivity 0.88 Specificity 0.94 IFA (IgM) – serum (EM) Sensitivity 0.32 Specificity 0.97 IFA (IgG) – serum (EM) Sensitivity 0.45 Specificity 0.97 IFA (IgM) – serum (Lyme neuroborreliosis) Sensitivity 0.30 Specificity 0.97 IFA (IgG) – serum (Lyme neuroborreliosis) Sensitivity 0.75 Specificity 0.97 ELISA (IgM, flagellin) – serum (late Lyme)

Reference	Wilske 1993[122]
	Sensitivity 0.14 Specificity 0.96 IFA (IgM) – serum (late Lyme) Sensitivity 0.05 Specificity 0.97 IFA (IgG) – serum (late Lyme) Sensitivity 1.00 Specificity 0.97 ELISA (IgM, OGP) – serum (late Lyme) Sensitivity 0.23 Specificity 0.97 WB/IB (IgM, OspC) – serum (late Lyme) Sensitivity 0.40 Specificity 0.97 WB/IB (IgG, OspC) – serum (late Lyme) Sensitivity 0.16 Specificity 0.99 WB/IB (IgM, p100) – serum (late Lyme) Sensitivity 0.19 Specificity 0.99 WB/IB (IgG, p100) – serum (late Lyme) Sensitivity 1.00 Specificity 0.94

Reference	Wilske 1993[122]
	WB/IB (IgM,p41/i) – serum (late Lyme) Sensitivity 0.12 Specificity 0.99 WB/IB (IgG, p41/i) – serum (late Lyme) Sensitivity 0.60 Specificity 0.96 ELISA (IgM, OGP) – serum (EM) Sensitivity 0.45 Specificity 0.97 ELISA (IgG, OGP) – serum (EM) Sensitivity 0.35 Specificity 0.97 ELISA (IgM, OGP) – serum (Lyme neuroborreliosis) Sensitivity 0.62 Specificity 0.97 ELISA (IgG, OGP) – serum (Lyme neuroborreliosis) Sensitivity 0.45 Specificity 0.97 ELISA (IgG, OGP) – serum (unspecified Lyme disease) Sensitivity 1.00 Specificity 0.97 WB/IB (IgM, OspC) – serum (EM) Sensitivity 0.45 Specificity 0.97

Reference	Wilske 1993[122]
	WB/IB (IgG, OspC) – serum (EM) Sensitivity 0.10 Specificity 0.99 WB/IB (IgM, OspC) – serum (Lyme neuroborreliosis) Sensitivity 0.43 Specificity 0.97 WB/IB (IgG, OspC) – serum (Lyme neuroborreliosis) Sensitivity 0.15 Specificity 0.99 WB/IB (IgM, p100) – serum (EM) Sensitivity 0.10 Specificity 0.99 WB/IB (IgG, p100) – serum (EM) Sensitivity 0.23 Specificity 0.94 WB/IB (IgM, p100) – serum (Lyme neuroborreliosis) Sensitivity 0.12 Specificity 0.99 WB/IB (IgG, p100) – serum (Lyme neuroborreliosis) Sensitivity 0.43 Specificity 0.94 WB/IB (IgM, p41/i) – serum (EM) Sensitivity 0.10

Reference	Wilske 1993[122]
	Specificity 0.99 WB/IB (IgM, p41/i) – serum (EM) Sensitivity 0.06 Specificity 0.96 WB/IB (IgM, p41/i) – serum (Lyme neuroborreliosis) Sensitivity 0.20 Specificity 0.99 WB/IB (IgM, p41/i) – serum (Lyme neuroborreliosis) Sensitivity 0.25 Specificity 0.96
Source of funding	Not reported
Limitations	Risk of bias: patient selection, index test, reference standard Indirectness: none

Reference	Wilske 1999[123]
Study type	Case-control study
Study methodology	Data source: serum samples from patients Recruitment: not reported
Number of patients	n = 147
Patient characteristics	Age, mean: not reported Gender (male to female ratio): not reported Family origin: not reported Setting: not reported Country: Germany Cases: EM (n=66), Lyme neuroborreliosis (n=42), Acrodermatitis (n=29), Lyme arthritis (n=10) Controls: Blood donors (n=118), Syphilis (n=11), Rheumatoid factor (n=10)
Target condition(s)	Early Lyme disease
Index test(s) and reference standard	Index tests ELISA (IgM/IgG) – serum WB/IB (IgM) – serum WB/IB (IgG) – serum WB/IB (IgM/IgG) - serum CLIA (IgM/IgG) - serum Reference standard Clinical diagnosis

Reference	Wilske 1999[123]				
	Time between measurement of index test and reference standard: not reported				
2×2 table [WB/IB (IgG, recombinant (new)) – serum], late Lyme disease		Reference standard +	Reference standard −	Total	
	Index test +	38	3	41	
	Index test −	1	136	137	
	Total	39	139	178	
2×2 table [WB/IB (IgG, recombinant (old)) – serum], late Lyme disease		Reference standard +	Reference standard −	Total	
	Index test +	29	3	32	
	Index test −	10	136	146	
	Total				
2×2 table [WB/IB (IgG, whole cell) – serum], late Lyme disease		Reference standard +	Reference standard −	Total	
	Index test +	39	3	42	
	Index test −	0	136	136	
	Total	39	139	178	
2×2 table [WB/IB (IgG, recombinant (new)) – serum], EM		Reference standard +	Reference standard −	Total	
	Index test +	7	3	10	
	Index test −	59	136	185	
	Total	66	139	205	
2×2 table [WB/IB (IgG, recombinant		Reference standard +	Reference standard −	Total	
	Index test +	19	3	22	

Reference	Wilske 1999[123]				
(new)) – serum], Lyme neuroborreliosis	Index test −	23	136	159	
	Total	42	139	181	
2×2 table [WB/IB (IgG, recombinant (old)) – serum], EM		Reference standard +	Reference standard −	Total	
	Index test +	3	3	6	
	Index test −	63	136	199	
	Total	66	139	205	
2×2 table [WB/IB (IgG, recombinant (old)) – serum], Lyme neuroborreliosis		Reference standard +	Reference standard −	Total	
	Index test +	12	3	15	
	Index test −	30	136	166	
	Total	42	139	181	
2×2 table [WB/IB (IgG, whole cell) – serum], EM		Reference standard +	Reference standard −	Total	
	Index test +	22	3	25	
	Index test −	44	136	180	
	Total	66	139	205	
2×2 table [WB/IB (IgG, whole cell) – serum], Lyme neuroborreliosis		Reference standard +	Reference standard −	Total	
	Index test +	24	3	27	
	Index test −	18	136	154	
	Total	42	139	181	
Statistical measures	WB/IB (IgG, recombinant (new)) – serum (late Lyme disease) Sensitivity 0.97				

Reference	Wilske 1999[123]
	Specificity 0.98 WB/IB (IgG, recombinant (old)) – serum (late Lyme disease) Sensitivity 0.74 Specificity 0.98 WB/IB (IgG, whole cell) – serum (late Lyme disease) Sensitivity 1.00 Specificity 0.98 WB/IB (IgG, recombinant (new)) – serum (EM) Sensitivity 0.11 Specificity 0.98 WB/IB (IgG, recombinant (new)) – serum (Lyme neuroborreliosis) Sensitivity 0.45 Specificity 0.98 WB/IB (IgG, recombinant (old)) – serum (EM) Sensitivity 0.05 Specificity 0.98 WB/IB (IgG, recombinant (old)) – serum (Lyme neuroborreliosis) Sensitivity 0.29 Specificity 0.98 WB/IB (IgG, whole cell) – serum (EM) Sensitivity 0.33 Specificity 0.98 WB/IB (IgG, whole cell) – serum (Lyme neuroborreliosis)

Reference	Wilske 1999[123]
	Sensitivity 0.57 Specificity 0.98
Source of funding	Not reported
Limitations	Risk of bias: patient selection, index test, reference standard Indirectness: none

D.1.3 Cohort studies (children)

Reference	Avery 2005[3]
Study type	Cohort study
Study methodology	Data source: CSF samples from children Recruitment: children presenting to the children's hospital
Number of patients	n = 108
Patient characteristics	Age, mean (SD): Lyme meningitis: 9.0 years (2.7-13.0) Aseptic meningitis: 9.6 years (3.1-17.8) Gender (male to female ratio): 75/33 Family origin: not reported Setting: children's hospital Country: USA Meningitis and suspected Lyme disease (defined as both Lyme serology and Lyme CSF-PCR ordered by physician)
Target condition(s)	Lyme meningitis
Index test(s) and reference standard	Index tests PCR – CSF Reference standard Clinical diagnosis (EM) plus positive serology

Reference	Avery 2005[3]				
	Time between measurement of index test and reference standard: not reported				
2×2 table		Reference standard +	Reference standard −	Total	Lyme meningitis PCR Serum
	Index test +	1	1	2	
	Index test −	19	87	106	
	Total	20	88	108	
Statistical measures	PCR – CSF Sensitivity 0.05 Specificity 0.99				
Source of funding	None declared				
Limitations	Risk of bias: patient selection, index test, reference standard Indirectness: none				

Reference	Barstad 2017[5]
Study type	Cohort study
Study methodology	Data source: CSF samples from children Recruitment: children aged 3 months to 18 years admitted to five different paediatric departments in the region with symptoms suggestive of Neuroborreliosis; eligible for inclusion if Neuroborreliosis or other possible causes of aseptic meningitis were suspected based on symptoms identified by attending physician and if a lumbar puncture and a Bb Ab in the CSF were ordered for clinical reasons
Number of patients	n = 210
Patient characteristics	Age, median (IQR): Confirmed Lyme neuroborreliosis (n=59): 6.5 (5, 8.5); probable Lyme neuroborreliosis (n=18): 5.5 (4, 9); possible Lyme neuroborreliosis (n=7): 6.5 (5.5, 13.5); non-Lyme aseptic meningitis (n=12): 7.5 (3.4, 10.3); possible peripheral Lyme neuroborreliosis (n=7): 11 (7, 13); non-meningitis (n=91): 10 (7.5, 14.5); negative controls (n=16): 12 (8.8, 14.5) Gender (male to female ratio): 93/117 Family origin: not reported Setting: paediatric departments, multi-centre Country: Norway Children who had antibiotics prior to admission were excluded
Target condition(s)	Neuroborreliosis
Index test(s) and reference standard	Index tests CXCL13 - CSF Reference standard Clinical diagnosis according to European guidelines

Reference	Barstad 2017[5]				
	Time between measurement of index test and reference standard: not reported				
2×2 table [CXCL13 – CSF], cut-off 18 pg/ml		Reference standard +	Reference standard −	Total	
	Index test +	57	3	60	
	Index test −	2	116	118	
	Total	59	119	178	
2×2 table [CXCL13 – CSF], cut-off 81 pg/ml		Reference standard +	Reference standard −	Total	
	Index test +	55	2	57	
	Index test −	4	117	121	
	Total	59	119	178	
2×2 table [CXCL13 – CSF], cut-off 213 pg/ml		Reference standard +	Reference standard −	Total	
	Index test +	54	0	54	
	Index test −	5	119	124	
	Total	59	119	178	
Statistical measures	CXCL13 – CSF (18 pg/ml) Sensitivity 0.97 Specificity 0.97 CXCL13 – CSF (81 pg/ml) Sensitivity 0.93 Specificity 0.98 CXCL13 – CSF (213 pg/ml)				

Reference	Barstad 2017[5]
	Sensitivity 0.92 Specificity 1.00
Source of funding	Supported by government and academic funding
Limitations	Risk of bias: reference standard Indirectness: none

Reference	Bennet 2008[6]				
Study type	Cohort study				
Study methodology	Data source: serum samples from children Recruitment: all children who had a Borrelia antibody analysis performed in both CSF and serum from May 2000 to April 2001 at the hospital				
Number of patients	n = 267				
Patient characteristics	Age, mean (SD): not reported Gender (male to female ratio): not reported Family origin: not reported Setting: children's hospital Country: Sweden				
Target condition(s)	Neuroborreliosis				
Index test(s) and reference standard	Index tests ELISA (IgM) – serum ELISA (IgG) – serum Reference standard Clinical assessment: Based on history, presenting symptoms, clinical examinations, CSF and serum analyses, response to antibiotic treatment Time between measurement of index test and reference standard: not reported				
2×2 table		Reference standard	Reference standard −	Total	Lyme neuroborreliosis

Reference	Bennet 2008[6]				
		+			ELISA (IgM) Serum
	Index test +	52	34	86	
	Index test −	18	142	160	
	Total	70	176	246	
2×2 table		Reference standard +	Reference standard −	Total	Lyme neuroborreliosis ELISA (IgG) Serum
	Index test +	33	3	36	
	Index test −	37	173	210	
	Total	70	176	246	
2×2 table		Reference standard +	Reference standard −	Total	EM ELISA (IgM) Serum
	Index test +	5	34	39	
	Index test −	1	142	143	
	Total	6	176	182	
2×2 table		Reference standard +	Reference standard −	Total	EM ELISA (IgG) Serum
	Index test +	0	3	3	
	Index test −	6	173	179	
	Total	6	176	182	
2×2 table		Reference standard +	Reference standard −	Total	Facial palsy ELISA (IgM) Serum
	Index test +	7	34	41	
	Index test −	8	142	150	
	Total	15	176	191	
2×2 table		Reference standard	Reference standard −	Total	Facial palsy

Reference	Bennet 2008[6]				
		+			ELISA (IgG) Serum
	Index test +	0	3	3	
	Index test −	15	173	188	
	Total	15	176	191	
Statistical measures	ELISA (IgM) – serum (EM) Sensitivity 0.83 Specificity 0.81 ELISA (IgG) – serum (EM) Sensitivity 0.00 Specificity 0.98 ELISA (IgM) – serum (Lyme neuroborreliosis) Sensitivity 0.74 Specificity 0.81 ELISA (IgG) – serum (Lyme neuroborreliosis) Sensitivity 0.47 Specificity 0.98 ELISA (IgM) – serum (FP) Sensitivity 0.47 Specificity 0.81 ELISA (IgG) – serum (FP) Sensitivity 0.00 Specificity 0.98				
Source of funding	Not reported				

Reference	Bennet 2008[6]
Limitations	Risk of bias: index test, reference standard Indirectness: none

Reference	Lipsett 2016[62]
Study type	Cohort study
Study methodology	Data source: serum samples from children Recruitment: children undergoing serological testing for Lyme disease
Number of patients	n = 944
Patient characteristics	Age, median (IQR): 10.9 years (6.4-15.2) Gender (male to female ratio): 421/524 Family origin: not reported Setting: hospital-based laboratory Country: USA Children and adolescents undergoing serologic evaluation for Lyme disease
Target condition(s)	Lyme disease
Index test(s) and reference standard	Index tests ELISA C6 - serum ELISA (WCS – serum

Reference	Lipsett 2016[62]				
	Reference standard Clinician-diagnosed EM or a positive 2-tiered serologic result in the presence of a Lyme disease-associated clinical syndrome Time between measurement of index test and reference standard: not reported				
2×2 table		Reference standard +	Reference standard −	Total	Unspecified Lyme disease ELISA C6 Serum
	Index test +	91	48	139	
	Index test −	23	782	805	
	Total	114	830	944	
2×2 table		Reference standard +	Reference standard −	Total	Unspecified Lyme disease ELISA WCS Serum
	Index test +	100	160	260	
	Index test −	14	670	674	
	Total	114	830	944	
Statistical measures	ELISA C6 – serum Sensitivity 0.80 Specificity 0.94 ELISA WCS – serum Sensitivity 0.88 Specificity 0.81				
Source of funding	Academic grants				
Limitations	Risk of bias: patient selection, index test, reference standard Indirectness: none				

D.1.4 Case-control studies (children)

Reference	Gerber 1995[26]
Study type	Case-control
Study methodology	Data source: children diagnosed with solitary EM at 5 paediatric offices and controls from an earlier investigation of streptococcal pharyngitis Recruitment: not reported
Number of patients	n = 82 cases, 50 controls
Patient characteristics	Age, median (range): cases 6 years (1-18), controls 9 years (3-17) Gender (male to female ratio): not reported Family origin: not reported Setting: 5 paediatric offices Country: USA Cases: children diagnosed with a solitary EM Controls: children from whom serum specimens had been collected as part of an earlier investigation of streptococcal pharyngitis, no history of Lyme disease and Lyme disease not endemic in the area
Target condition(s)	Lyme disease
Index test(s) and reference standard	Index test(s) ELISA (IgM) Immunoblot (IgM) Reference standard

Reference	Gerber 1995[26]				
	Clinical diagnosis Time between measurement of index test and reference standard: samples collected median 2 days (range 0-30) after EM was first detected				
2×2 table		Reference standard +	Reference standard −	Total	EM ELISA (IgM) rOspC Serum
	Index test +	38	1	39	
	Index test −	44	49	93	
	Total	82	50	132	
		Reference standard +	Reference standard −	Total	EM ELISA (IgM) whole-cell Serum
	Index test +	23	0	23	
	Index test −	59	50	109	
	Total	82	50	132	
		Reference standard +	Reference standard −	Total	EM Immunoblot (IgM) Serum
	Index test +	24	0	24	
	Index test −	58	50	108	
	Total	82	50	132	
Statistical measures	Index test: ELISA IgM rOspC (serum) - EM Sensitivity 0.46 Specificity 0.98 Index test: ELISA IgM whole-cell (serum) - EM Sensitivity 0.28 Specificity 1.00 Index test: Immunoblot IgM (serum) – EM Sensitivity 0.29				

Reference	Gerber 1995[26]
	Specificity 1.00
Source of funding	Public Health Service, Apollo Kingsley award from Connecticut Innovations Inc., Department of Economics Development, State of Connecticut and departmental funds
Limitations	Risk of bias: selection, index test, reference standard Indirectness: none

Reference	Heikkilä 2002[38]
Study type	Case-control
Study methodology	Data source: not reported Recruitment: not reported
Number of patients	n = 52 cases, 40 controls
Patient characteristics	Age, mean (SD): not reported Gender (male to female ratio): not reported Family origin: not reported Setting: not reported Country: Finland Cases: children with well-characterised Lyme arthritis from Germany Controls: children with other inflammatory joint diseases (20), healthy blood donors (20)
Target	Lyme disease

Reference	**Heikkilä 2002[38]**				
condition(s)					
Index test(s) and reference standard	Index test(s) ELISA (IgG) Reference standard Clinical diagnosis Time between measurement of index test and reference standard: not reported				
2×2 table		Reference standard + Index test +	Reference standard − 2	Total 42	Arthritis ELISA (IgG) Serum
	Index test +	40	2	42	
	Index test −	12	38	50	
	Total	52	40	92	
Statistical measures	Index test: ELISA IgG (serum) – arthritis Sensitivity 0.77 Specificity 0.95				
Source of funding	Foundation for Paediatric Research, Finland and the National Technology Agency (Tekes), Finland				
Limitations	Risk of bias: selection, index test, reference standard, flow and timing Indirectness: none				
Comments	Adult data not analysable as control numbers are not reported				

Reference	Krbková 2016[51]
Study type	Case-control
Study methodology	Data source: children admitted to the Department of Children's Infectious Diseases in a Lyme endemic area Recruitment: not reported
Number of patients	n = 116 cases, 66 controls
Patient characteristics	Age, mean (SD): not reported Gender (male to female ratio): not reported Family origin: not reported Setting: Department of Clinical Biochemistry and Department of Clinical Microbiology, University Hospital Country: Czech Republic Cases: children with proven neuroborreliosis, manifested by aseptic meningitis, facial/abducens palsy or both (86) and children with suspicion of neuroborreliosis (30) Controls: children with neuroinfections other than neuroborreliosis – tick-borne encephalitis (3), enteroviral meningitis (3), other viral meningitis (10), isolated facial palsy of non-borrelial aetiology (22), myelitis (1), or children with negative CSF finding in which neuroinfection had been excluded
Target condition(s)	Lyme disease
Index test(s) and reference standard	Index test(s) ELISA (IgM) ELISA (IgG) Immunoblot (IgM) Immunoblot (IgG)

Reference	Krbková 2016[51]				
	Reference standard Time between measurement of index test and reference standard:				
2×2 table		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA (IgM) recombinant CSF
	Index test +	22	0	22	
	Index test −	64	66	130	
	Total	86	66	152	
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA (IgM) recombinant Serum
	Index test +	43	4	47	
	Index test −	43	62	105	
	Total	86	66	152	
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA (IgM) whole-cell CSF
	Index test +	37	0	37	
	Index test −	49	66	115	
	Total	86	66	152	
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA (IgM) whole-cell Serum
	Index test +	47	11	58	
	Index test −	39	55	94	
	Total	86	66	152	
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA (IgG) recombinant CSF
	Index test +	69	2	71	
	Index test −	17	64	81	
	Total	86	66	152	

Reference	**Krbková 2016[51]**				
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA (IgG) recombinant Serum
	Index test +	75	12	87	
	Index test −	11	54	65	
	Total	86	66	152	
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA (IgG) whole-cell CSF
	Index test +	55	0	55	
	Index test −	31	66	97	
	Total	86	66	152	
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA (IgG) whole-cell Serum
	Index test +	63	13	76	
	Index test −	23	53	76	
	Total	86	66	152	
		Reference standard +	Reference standard −	Total	Neuroborreliosis Immunoblot (IgM) CSF
	Index test +	11	0	11	
	Index test −	75	66	141	
	Total	86	66	152	
		Reference standard +	Reference standard −	Total	Neuroborreliosis Immunoblot (IgM) Serum
	Index test +	31	2	33	
	Index test −	55	64	119	
	Total	86	66	152	
		Reference standard +	Reference standard −	Total	Neuroborreliosis Immunoblot (IgG) CSF
	Index test +	31	2	33	
	Index test −	55	64	119	

Reference	**Krbková 2016[51]**				
	Total	86	66	152	
		Reference standard +	Reference standard −	Total	Neuroborreliosis Immunoblot (IgG) Serum
	Index test +	47	6	53	
	Index test −	39	60	99	
	Total	86	66	152	
Statistical measures	Index test: ELISA IgM recombinant (CSF) - neuroborreliosis Sensitivity 0.26 Specificity 1.00 Index test: ELISA IgM recombinant (serum) - neuroborreliosis Sensitivity 0.50 Specificity 0.94 Index test: ELISA IgM whole-cell (CSF) - neuroborreliosis Sensitivity 0.43 Specificity 1.00 Index test: ELISA IgM whole-cell (serum) - neuroborreliosis Sensitivity 0.55 Specificity 0.83 Index test: ELISA IgG recombinant (CSF) - neuroborreliosis Sensitivity 0.80 Specificity 0.97 Index test: ELISA IgG recombinant (serum) - neuroborreliosis Sensitivity 0.87 Specificity 0.82 Index test: ELISA IgG whole-cell (CSF) - neuroborreliosis				

Reference	Krbková 2016[51]
	Sensitivity 0.64 Specificity 1.00 Index test: ELISA IgG whole-cell (serum) - neuroborreliosis Sensitivity 0.73 Specificity 0.80 Index test: Immunoblot IgM (CSF) - neuroborreliosis Sensitivity 0.13 Specificity 1.00 Index test: Immunoblot IgM (serum) - neuroborreliosis Sensitivity 0.36 Specificity 0.97 Index test: Immunoblot IgG (CSF) - neuroborreliosis Sensitivity 0.36 Specificity 0.97 Index test: Immunoblot IgG (serum) - neuroborreliosis Sensitivity 0.55 Specificity 0.91
Source of funding	Study was not sponsored
Limitations	Risk of bias: selection, index test, reference standard Indirectness: none
Comments	Suspicion of neuroborreliosis not included in analysis because there is no clear reference standard

Reference	Skogman 2008[103]
Study type	Case-control study

Reference	Skogman 2008[103]				
Study methodology	Data source: CSF samples from children before treatment Recruitment: not reported				
Number of patients	n = 24				
Patient characteristics	Age, mean: not reported Gender (male to female ratio): not reported Family origin: not reported Setting: clinic Country: Sweden Cases: Children with Neuroborreliosis Controls: Children with other neurological diseases (n=20), Adults with no proven infection (n=16)				
Target condition(s)	Neuroborreliosis				
Index test(s) and reference standard	Index tests ELISA (IgG) – CSF Reference standard Clinical diagnosis (based on clinical features and lab findings) Time between measurement of index test and reference standard: not reported				
2×2 table		Reference standard +	Reference standard −	Total	Lyme neuroborreliosis

Reference	Skogman 2008[103]				
	Index test +	32	0	32	ELISA (IgG) CSF
	Index test −	8	36	44	
	Total	40	36	76	
Statistical measures	ELISA (IgG) - CSF Sensitivity 0.80 Specificity 1.00				
Source of funding	Not reported				
Limitations	Risk of bias: patient selection, index test, reference standard Indirectness: none				

Reference	Wutte 2011[124]
Study type	Case-control study
Study methodology	Data source: serum samples from children Recruitment: not reported
Number of patients	n = 75 (33 children, 42 adults)
Patient characteristics	Age, mean (SD): 19 years (SD 3) Gender (male to female ratio): 17/5 Family origin: not reported Setting: clinic Country: Sweden

Reference	Wutte 2011[124]				
	Cases: People with neuroborreliosis (15 children, 7 adults) Controls: Healthy blood donors (n=300)				
Target condition(s)	Neuroborreliosis				
Index test(s) and reference standard	Index tests CXCL13 - serum Reference standard Clinical diagnosis (German Neurological Society guidelines) Time between measurement of index test and reference standard: not reported				
2×2 table		Reference standard + Reference standard −	Total	Lyme neuroborreliosis CXCL13 Serum	
	Index test +	16	39	55	
	Index test −	6	261	267	
	Total	22	300	322	
Statistical measures	CXCL13 - serum Sensitivity 0.73 Specificity 0.87				
Source of funding	Not reported				
Limitations	Risk of bias: patient selection, index test, reference standard Indirectness: serious				

D.2 Confirmatory tests

Reference	Blaauw 1999[8]				
Study type	Cohort study				
Study methodology	Data source: serum samples from patients Recruitment: Diagnosed or suspected chronic Lyme with musculoskeletal complaints				
Number of patients	n = 105				
Patient characteristics	Age, mean (range): 48.7 years (6-82) Gender (male to female ratio): 41/62 Family origin: not reported Setting: university hospital Country: Netherlands				
Target condition(s)	Lyme disease				
Index test(s) and reference standard	Index tests IB (IgG) – serum Reference standard Clinical diagnosis Time between measurement of index test and reference standard: not reported				
2×2 table		Reference standard	Reference standard −	Total	

Reference	Blaauw 1999[8]				
[IB (IgG) – serum], unspec Lyme disease		+			
	Index test +	10	7	17	
	Index test −	0	5	5	
	Total	10	12	22	
Statistical measures	IB (IgG) – serum (unspec Lyme disease) Sensitivity 1.00 Specificity 0.42				
Source of funding	None declared				
Limitations	Risk of bias: index test, reference standard Indirectness: none				

Reference	Christova 2003[16]
Study type	Case-control
Study methodology	Data source: not reported Recruitment: not reported
Number of patients	n = 105 cases, 90 controls
Patient characteristics	Age, mean (SD): not reported Gender (male to female ratio): not reported Family origin: not reported Setting: Microbiology department, National Centre of Infectious and Parasitic Diseases Country: Bulgaria

Reference	Christova 2003[16]				
	Cases: patients with EM lesions (105) Controls: healthy blood donors (90)				
Target condition(s)	Lyme disease				
Index test(s) and reference standard	Index test(s) Immunofluorescence assay (IgM) Immunofluorescence assay (IgG) Immunoblot (IgM) Immunoblot (IgG) Reference standard Clinical diagnosis Time between measurement of index test and reference standard: not reported				
2×2 table		Reference standard +	Reference standard −	Total	EM IFA (IgM) Serum
	Index test +	24	0	24	
	Index test −	27	90	117	
	Total	51	90	141	
		Reference standard +	Reference standard −	Total	EM IFA (IgG) Serum
	Index test +	15	0	15	
	Index test −	3	90	93	
	Total	18	90	108	
		Reference standard +	Reference standard −	Total	EM Immunoblot (IgM)
	Index test +	36	0	36	

Reference	**Christova 2003[16]**				
	Index test −	15	90	105	Serum
	Total	51	90	141	
		Reference standard +	Reference standard −	Total	EM Immunoblot (IgG)
	Index test +	12	0	12	
	Index test −	6	90	96	
	Total	18	90	108	
Statistical measures	Index test: IFA IgM (serum) - EM Sensitivity 0.47 Specificity 1.00 Index test: IFA IgG (serum) - EM Sensitivity 0.83 Specificity 1.00 Index test: Immunoblot IgM (serum) - EM Sensitivity 0.71 Specificity 1.00 Index test: Immunoblot IgG (serum) - EM Sensitivity 0.67 Specificity 1.00				
Source of funding	Not reported				
Limitations	Risk of bias: selection, index test, reference standard Indirectness: none				
Comments	-				

Reference	**Coyle 1993[18]**

Reference	Coyle 1993[18]
Study type	Case-control
Study methodology	Data source: patients from an endemic region presenting with clinical or laboratory evidence of B. burgdorferi infection and neurological complaints Recruitment: not reported
Number of patients	n = 77 cases, 34 controls
Patient characteristics	Age, mean (range): 34 years (3 – 84) Gender (male to female ratio): Lyme disease 33:44 Family origin: not reported Setting: Department of Neurology Country: USA Cases: clinical/laboratory evidence of B. burgdorferi infection and neurological complaints, only patients who underwent lumbar puncture as part of a work-up for neurologic Lyme disease and in whom sufficient CSF was collected, 24 had received prior antibiotic treatment but had persistent symptoms, 5 were currently receiving antibiotics Controls: other neurological diseases
Target condition(s)	Lyme disease
Index test(s) and reference standard	Index test(s) Immunoblot (IgG) Reference standard Clinical diagnosis

Reference	Coyle 1993[18]				
	Time between measurement of index test and reference standard: not reported				
2×2 table		Reference standard +	Reference standard −	Total	Neuroborreliosis Immunoblot (IgG) CSF
	Index test +	12	0	12	
	Index test −	10	11	21	
	Total	22	11	33	
Statistical measures	Index test: Immunoblot IgG (CSF) - neuroborreliosis Sensitivity 0.55 Specificity 1.00				
Source of funding	NIH grants, New York State grant for Lyme disease research, East End Lyme foundation				
Limitations	Risk of bias: selection, index test, reference standard Indirectness: serious (adults and children)				
Comments	-				

Reference	Magnarelli 1992[66]
Study type	Case-control study
Study methodology	Data source: serum samples previously used Recruitment: not reported
Number of patients	n = 53
Patient characteristics	Age, mean (SD): not reported Gender (male to female ratio): not reported

Reference	Magnarelli 1992[66]				
	Family origin: not reported Setting: tick-infested area Country: USA Cases: EM with antibodies (n=17), EM without antibodies (n=36) Controls: healthy persons (n=40)				
Target condition(s)	EM with or without antibodies				
Index test(s) and reference standard	Index tests ELISA (IgG, recombinant) – serum ELISA (IgG, whole-cell) - serum Reference standard Clinical diagnosis Time between measurement of index test and reference standard: not reported				
2×2 table [ELISA (IgG, recombinant) – serum]		Reference standard +	Reference standard −	Total	
	Index test +	16	0	16	
	Index test −	1	40	41	
	Total	17	40	57	
2×2 table [ELISA (IgG, whole-cell) – serum]		Reference standard +	Reference standard −	Total	
	Index test +	17	0	16	
	Index test −	0	40	41	
	Total	17	40	57	

Reference	Magnarelli 1992[66]
Statistical measures	ELISA (IgG, recombinant) – serum Sensitivity 0.94 Specificity 1.00 ELISA (IgG, whole-cell) – serum Sensitivity 1.00 Specificity 1.00
Source of funding	Supported by CDC grants and grants from the National Institutes of Health and the Mathers Foundation.
Limitations	Risk of bias: patient selection, index test, reference standard Indirectness: none

Reference	Trevejo 2001[113]
Study type	Case-control study
Study methodology	Data source: serum samples from patients taken a median of 4 days after illness onset (acute phase) or 36 days after illness onset (convalescent phase) Recruitment: not reported
Number of patients	n = 74
Patient characteristics	Age, median: 41 years (3-83) Gender (male to female ratio): 41/33 Family origin: not reported Setting: primary care

Reference	Trevejo 2001[113]				
	Country: USA Cases: EM (66 acute phase, 55 convalescent phase) Controls: healthy controls (n=38)				
Target condition(s)	EM				
Index test(s) and reference standard	Index tests WB/IB (IgM/IgG) - serum Reference standard Clinical diagnosis Time between measurement of index test and reference standard: not reported				
2×2 table [WB/IB (IgM/IgG) – serum], EM (acute phase)		Reference standard +	Reference standard −	Total	
	Index test +	21	1	22	
	Index test −	7	37	44	
	Total	28	38	66	
2×2 table [WB/IB (IgM/IgG) – serum], EM (convalescent phase)		Reference standard +	Reference standard −	Total	
	Index test +	16	1	17	
	Index test −	27	37	64	
	Total	43	38	81	
Statistical measures	WB/IB (IgM/IgG) – serum (EM, acute phase) Sensitivity 0.75				

Reference	Trevejo 2001[113]
	Specificity 0.97 WB/IB (IgM/IgG) – serum (EM, convalescent phase) Sensitivity 0.37 Specificity 0.97
Source of funding	Not reported
Limitations	Risk of bias: patient selection, reference standard Indirectness: none

D.3 Combination tests

Reference	Ang 2015[1]
Study type	Case-control
Study methodology	Data source: validation studies for B. burgdorferi antibody assays from 8 laboratories Recruitment: not reported
Number of patients	n = 369 cases, 228 healthy controls
Patient characteristics	Age, mean (SD): not reported Gender (male to female ratio): not reported Family origin: not reported Setting: 8 laboratories Country: the Netherlands

Reference	Ang 2015[1]				
	Cases: patients with typical manifestations of Lyme borreliosis (according to ESGBOR guidelines – EM clinical information or positive PCR on skin biopsy, ACA clinical information or positive PCR and matching histopathology, neuroborreliosis defined as meningoradiculitis and/or bilateral facial palsy, CSF pleocytosis and/or positive CSF PCR for Borrelia spp., arthritis defined as monoarthritis of the knee and/or positive PCR for Borrelia spp. In synovial fluid or synovial biopsy Controls : healthy controls from healthcare workers for a check on hepatitis B vaccination and from stem cell donors				
Target condition(s)	Lyme disease				
Index test(s) and reference standard	Index test(s) ELISA (IgM/IgG) + Immunoblot (IgM/IgG) – serum ELISA (C6) + Immunoblot (IgM/IgG) – serum Reference standard ESGBOR guidelines: EM clinical information or positive PCR on skin biopsy, ACA clinical information or positive PCR and matching histopathology, neuroborreliosis defined as meningoradiculitis and/or bilateral facial palsy, CSF pleocytosis and/or positive CSF PCR for Borrelia spp., arthritis defined as monoarthritis of the knee and/or positive PCR for Borrelia spp. In synovial fluid or synovial biopsy Time between measurement of index test and reference standard: not reported				
2×2 table		Reference standard +	Reference standard −	Total	EM ELISA (IgM/IgG) + Immunoblot (IgM/IgG) Serum
	Index test +	40	0	40	
	Index test −	18	90	108	
	Total	58	90	148	
		Reference standard +	Reference standard −	Total	EM ELISA (C6) + Immunoblot (IgM/IgG) Serum
	Index test +	42	0	42	
	Index test −	24	104	128	
	Total	66	104	170	
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA (IgM/IgG) + Immunoblot (IgM/IgG)
	Index test +	29	0	29	

Reference	**Ang 2015[1]**				
	Index test −	1	90	91	Serum
	Total	30	90	120	
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA (C6) + Immunoblot (IgM/IgG)
	Index test +	47	0	47	
	Index test −	4	104	108	
	Total	51	104	155	
Statistical measures	Index test: ELISA IgM/IgG + Immunoblot IgM/IgG (serum) – EM Sensitivity 0.69 Specificity 1.00 Index test: ELISA C6 + Immunoblot IgM/IgG (serum) – EM Sensitivity 0.64 Specificity 1.00 Index test: ELISA IgM/IgG + Immunoblot IgM/IgG (serum) – Neuroborreliosis Sensitivity 0.97 Specificity 1.00 Index test: ELISA C6 + Immunoblot IgM/IgG (serum) – Neuroborreliosis Sensitivity 0.92 Specificity 1.00				
Source of funding	Not reported				
Limitations	Risk of bias: selection, index test, reference standard Indirectness: none				
Comments	Borderline results excluded from the analysis as the study authors did not necessarily interpret them as positive evidence of infection				

Reference	**Bacon 2003[4]**

Reference	Bacon 2003[4]
Study type	Case-control
Study methodology	Data source: panel of 839 samples Recruitment: not reported
Number of patients	n = 280 cases, 559 controls (257 healthy controls used in the analysis)
Patient characteristics	Age, mean (SD): not reported Gender (male to female ratio): not reported Family origin: not reported Setting: not reported Country: USA Cases: patients with acute Lyme disease (80), early convalescent Lyme disease (106), early neurologic (15), early neurologic convalescent (11), arthritis (33), arthritis convalescent (24), late neurologic (11) Controls: healthy individuals (257), evidence of autoimmune disease or spirochaetal infection other than Lyme disease (302)
Target condition(s)	Lyme disease
Index test(s) and reference standard	Index test(s) ELISA (IgM/IgG) + Immunoblot (IgM/IgG) – serum 10 Reference standard Clinical diagnosis (CDC criteria) Time between measurement of index test and reference standard: not reported

Reference	Bacon 2003[4]				
2×2 table		Reference standard +	Reference standard −	Total	Acute disseminated EM ELISA (IgM/IgG) + Immunoblot (IgM/IgG) Serum
	Index test +	19	0	19	
	Index test −	19	257	276	
	Total	38	257	295	
		Reference standard +	Reference standard −	Total	Acute single EM ELISA (IgM/IgG) + Immunoblot (IgM/IgG) Serum
	Index test +	11	0	11	
	Index test −	31	257	288	
	Total	42	257	299	
		Reference standard +	Reference standard −	Total	Early convalescent disseminated EM ELISA (IgM/IgG) + Immunoblot (IgM/IgG) Serum
	Index test +	33	0	33	
	Index test −	13	257	270	
	Total	46	257	303	
		Reference standard +	Reference standard −	Total	Early convalescent single EM ELISA (IgM/IgG) + Immunoblot (IgM/IgG) Serum
	Index test +	38	0	38	
	Index test −	22	257	279	
	Total	60	257	317	
		Reference standard +	Reference standard −	Total	Early neurologic convalescent ELISA (IgM/IgG) + Immunoblot (IgM/IgG) Serum
	Index test +	9	0	9	
	Index test −	2	257	259	
	Total	11	257	268	
		Reference standard +	Reference standard −	Total	Early neurologic ELISA (IgM/IgG) + Immunoblot (IgM/IgG) Serum
	Index test +	11	0	11	
	Index test −	0	257	257	

Reference	**Bacon 2003[4]**				
	Total	11	257	268	
		Reference standard +	Reference standard −	Total	Late neurologic ELISA (IgM/IgG) + Immunoblot (IgM/IgG) Serum
	Index test +	13	1	14	
	Index test −	2	99	101	
	Total	15	100	115	
		Reference standard +	Reference standard −	Total	Arthritis convalescent ELISA (IgM/IgG) + Immunoblot (IgM/IgG) Serum
	Index test +	23	0	23	
	Index test −	1	257	258	
	Total	24	257	281	
		Reference standard +	Reference standard −	Total	Arthritis ELISA (IgM/IgG) + Immunoblot (IgM/IgG) Serum
	Index test +	32	0	32	
	Index test −	1	257	258	
	Total	33	257	290	
		Reference standard +	Reference standard −	Total	Unspecified Lyme diseaseLyme disease ELISA (IgM/IgG) + Immunoblot (IgM/IgG) Serum
	Index test +	189	0	189	
	Index test −	91	257	348	
	Total	280	257	537	
Statistical measures	Index test: ELISA IgM/IgG + Immunoblot IgM/IgG (serum) - acute disseminated EM Sensitivity 0.50 Specificity 1.00 Index test: ELISA IgM/IgG + Immunoblot IgM/IgG (serum) - acute single EM Sensitivity 0.26 Specificity 1.00 Index test: ELISA IgM/IgG + Immunoblot IgM/IgG (serum) - early convalescent disseminated EM				

Reference	Bacon 2003[4]
	Sensitivity 0.72 Specificity 1.00 Index test: ELISA IgM/IgG + Immunoblot IgM/IgG (serum) - early convalescent single EM Sensitivity 0.63 Specificity 1.00 Index test: ELISA IgM/IgG + Immunoblot IgM/IgG (serum) - early neurologic convalescent Sensitivity 0.82 Specificity 1.00 Index test: ELISA IgM/IgG + Immunoblot IgM/IgG (serum) - early neurologic Sensitivity 0.87 Specificity 1.00 Index test: ELISA IgM/IgG + Immunoblot IgM/IgG (serum) - late neurologic Sensitivity 1.00 Specificity 1.00 Index test: ELISA IgM/IgG + Immunoblot IgM/IgG (serum) - arthritis convalescent Sensitivity 0.96 Specificity 1.00 Index test: ELISA IgM/IgG + Immunoblot IgM/IgG (serum) - arthritis Sensitivity 0.97 Specificity 1.00 Index test: ELISA IgM/IgG + Immunoblot IgM/IgG (serum) - unspecified Lyme disease Sensitivity 0.68 Specificity 1.00

Reference	Bacon 2003[4]
Source of funding	National Center for Research Resources, NIH, CDC
Limitations	Risk of bias: selection, index test, reference standard Indirectness: none
Comments	Separate IgM and IgG immunoblots used

Reference	Branda 2010[9]
Study type	Case-control
Study methodology	Data source: patients meeting the CDC criteria for Lyme disease, healthy controls and disease controls Recruitment: not reported
Number of patients	n = 162 cases, 269 controls (166 healthy controls used in analysis)
Patient characteristics	Age, mean (SD): not reported Gender (male to female ratio): not reported Family origin: not reported Setting: 2 medical centres Country: USA Cases: patients meeting the CDC criteria for Lyme disease – culture confirmed EM (106), acute neuritis/carditis (27), arthritis/late neuritis (29) Controls: healthy controls (166), other illnesses (103)
Target condition(s)	Lyme disease

Reference	Branda 2010[9]				
Index test(s) and reference standard	Index test(s) ELISA (IgM/IgG) + Immunoblot (IgM/IgG) ELISA (IgM/IgG) + VlsE band ELISA (IgM/IgG) + Immunoblot (IgG with VlsE band) Reference standard Clinical diagnosis (CDC criteria) Time between measurement of index test and reference standard: not reported				
2×2 table		Reference standard +	Reference standard −	Total	Acute neuritis/carditis ELISA (IgM/IgG) + Immunoblot (IgM/IgG) Serum
	Index test +	17	0	17	
	Index test −	10	166	176	
	Total	27	166	193	
		Reference standard +	Reference standard −	Total	Acute neuritis/carditis ELISA (IgM/IgG) + VlsE band Serum
	Index test +	26	0	26	
	Index test −	1	166	167	
	Total	27	166	193	
		Reference standard +	Reference standard −	Total	Acute neuritis/carditis ELISA (IgM/IgG) + Immunoblot (IgG with VlsE band) Serum
	Index test +	26	0	26	
	Index test −	1	166	167	
	Total	27	166	193	
		Reference standard +	Reference standard −	Total	Arthritis/late neuritis ELISA (IgM/IgG) + Immunoblot (IgM/IgG) Serum
	Index test +	29	0	29	
	Index test −	0	166	166	
	Total	29	166	195	

Reference	Branda 2010[9]				
		Reference standard +	Reference standard −	Total	Arthritis/late neuritis ELISA (IgM/IgG) + VlsE band Serum
	Index test +	28	0	28	
	Index test −	1	166	167	
	Total	29	166	195	
		Reference standard +	Reference standard −	Total	Arthritis/late neuritis ELISA (IgM/IgG) + Immunoblot (IgG with VlsE band) Serum
	Index test +	29	0	29	
	Index test −	0	166	166	
	Total	29	166	195	
Statistical measures	Index test: ELISA IgM/IgG + Immunoblot IgM/IgG (serum) – acute neuritis/carditis Sensitivity 0.63 Specificity 1.00 Index test: ELISA IgM/IgG + VlsE band (serum) – acute neuritis/carditis Sensitivity 0.96 Specificity 1.00 Index test: ELISA IgM/IgG + Immunoblot IgG with VlsE band – acute neuritis/carditis Sensitivity 0.96 Specificity 1.00 Index test: ELISA IgM/IgG + Immunoblot IgM/IgG (serum) – arthritis/late neuritis Sensitivity 1.00 Specificity 1.00 Index test: ELISA IgM/IgG + VlsE band (serum) – arthritis/late neuritis Sensitivity 0.97 Specificity 1.00 Index test: ELISA IgM/IgG + Immunoblot IgG with VlsE band – arthritis/late neuritis				

Reference	Branda 2010[9]
	Sensitivity 1.00 Specificity 1.00
Source of funding	Research grant from Viramed Biotech
Limitations	Risk of bias: selection, reference standard Indirectness: none
Comments	Acute and convalescent phase EM not included in the analysis - more samples than patients

Reference	Branda 2011[10]
Study type	Case-control
Study methodology	Data source: Phase 1 - well-characterised Lyme disease patients and symptomatic controls who had been evaluated by Lyme disease experts Phase 2 – serum samples submitted for Lyme disease testing with review of medical records and healthy controls during routine visits Recruitment: not reported
Number of patients	n = 169 cases, 1300 controls
Patient characteristics	Age, mean (SD): not reported Gender (male to female ratio): not reported Family origin: not reported Setting: general hospital Country: USA

Reference	Branda 2011[10]				
	Cases: EM (114), acute neuritis or carditis (26), arthritis or late neuritis (29) Controls : symptomatic patients not meeting CDC criteria for Lyme disease (54), healthy controls during routine visits (1246)				
Target condition(s)	Lyme disease				
Index test(s) and reference standard	Index test(s) ELISA (IgM/IgG) + Immunoblot (IgM/IgG) ELISA (WCS) + ELISA (C6) Reference standard Clinical diagnosis (CDC surveillance criteria) Time between measurement of index test and reference standard: not reported				
2×2 table		Reference standard +	Reference standard −	Total	EM ELISA (IgM/IgG) + Immunoblot (IgM/IgG) Serum
	Index test +	48	7	55	
	Index test −	66	1293	1359	
	Total	114	1300	1414	
		Reference standard +	Reference standard −	Total	EM ELISA (WCS) + ELISA (C6) Serum
	Index test +	60	7	67	
	Index test −	54	1293	1347	
	Total	114	1300	1414	
		Reference standard +	Reference standard −	Total	Acute neuritis/carditis ELISA (IgM/IgG) + Immunoblot (IgM/IgG) Serum
	Index test +	19	7	26	
	Index test −	7	1293	1300	
	Total	26	1300	1326	
		Reference standard +	Reference standard −	Total	Acute neuritis/carditis

Reference	**Branda 2011[10]**				
	Index test +	26	7	33	ELISA (WCS) + ELISA (C6) Serum
	Index test −	0	1293	1293	
	Total	26	1300	1326	
		Reference standard +	Reference standard −	Total	Arthritis/late neuritis ELISA (IgM/IgG) + Immunoblot (IgM/IgG) Serum
	Index test +	29	7	36	
	Index test −	0	1293	1293	
	Total	29	1300	1329	
		Reference standard +	Reference standard −	Total	Arthritis/late neuritis ELISA (WCS) + ELISA (C6) Serum
	Index test +	29	7	36	
	Index test −	0	1293	1293	
	Total	29	1300	1329	
		Reference standard +	Reference standard −	Total	Unspecified Lyme disease ELISA (IgM/IgG) + Immunoblot (IgM/IgG) Serum
	Index test +	96	7	103	
	Index test −	73	1293	1366	
	Total	169	1300	1469	
		Reference standard +	Reference standard −	Total	Unspecified Lyme disease ELISA (WCS) + ELISA (C6) Serum
	Index test +	115	7	122	
	Index test −	54	1293	1347	
	Total	169	1300	1469	
Statistical measures	Index test: ELISA IgM/IgG + Immunoblot IgM/IgG (serum) – EM Sensitivity 0.42 Specificity 0.99 Index test: ELISA WCS + ELISA C6 (serum) - EM Sensitivity 0.53				

Reference	Branda 2011[10]
	Specificity 0.99 Index test: ELISA IgM/IgG + Immunoblot IgM/IgG (serum) – acute neuritis/carditis Sensitivity 0.73 Specificity 0.99 Index test: ELISA WCS + ELISA C6 (serum) - acute neuritis/carditis Sensitivity 1.00 Specificity 0.99 Index test: ELISA IgM/IgG + Immunoblot IgM/IgG (serum) – arthritis/late neuritis Sensitivity 1.00 Specificity 0.99 Index test: ELISA WCS + ELISA C6 (serum) - arthritis/late neuritis Sensitivity 1.00 Specificity 0.99 Index test: ELISA IgM/IgG + Immunoblot IgM/IgG (serum) – unspecified Lyme disease Sensitivity 0.57 Specificity 0.99 Index test: ELISA WCS + ELISA C6 (serum) - unspecified Lyme disease Sensitivity 0.68 Specificity 0.99
Source of funding	CDC, the English, Bonter, Mitchell Foundation, the Eshe Fund and the Lyme Disease and Arthritis Research Fund at Massachusetts General Hospital
Limitations	Risk of bias: selection, index test, reference standard Indirectness: none
Comments	

Reference	Branda 2013[11]
Study type	Case-control
Study methodology	Data source: patients evaluated at a Lyme borreliosis outpatient clinic meeting European criteria for Lyme disease and blood donors. Recruitment: not reported
Number of patients	n = 64 cases, 100 controls
Patient characteristics	Age, mean (SD): not reported Gender (male to female ratio): not reported Family origin: not reported Setting: Lyme borreliosis outpatient clinic Country: Slovenia Cases: patients meeting European criteria for Lyme disease: EM (20, 15 with positive cultures), neuroborreliosis with CSF pleocytosis, concomitant EM isolation of B. burgdorferi spirochetes from CSF or demonstration of intrathecal synthesis of specific antibodies (15), arthritis with swelling in 1 or more joints and specific antibodies in serum without alternative explanation (15), ACA with characteristic clinical picture, supportive histologic findings and high specific serum IgG antibody levels (14) Controls: 100 healthy blood donors in New Zealand, a non-endemic region
Target condition(s)	Lyme disease
Index test(s) and reference standard	Index test(s) ELISA (IgM/IgG) + Immunoblot (IgM/IgG) ELISA C6 + Immunoblot (IgM/IgG) ELISA WCS + ELISA C6

Reference	Branda 2013[11]				
	Reference standard Clinical diagnosis (European Lyme disease criteria) Time between measurement of index test and reference standard: not reported				
2×2 table		Reference standard +	Reference standard −	Total	EM ELISA (IgM/IgG) + Immunoblot (IgM/IgG) European tests Serum
	Index test +	11	1	12	
	Index test −	9	99	108	
	Total	20	100	120	
		Reference standard +	Reference standard −	Total	EM ELISA (IgM/IgG) + Immunoblot (IgM/IgG) USA tests Serum
	Index test +	4	0	4	
	Index test −	16	100	116	
	Total	20	100	120	
		Reference standard +	Reference standard −	Total	EM ELISA C6 (IgT) + Immunoblot (IgM/IgG) USA tests Serum
	Index test +	4	0	4	
	Index test −	16	100	116	
	Total	20	100	120	
		Reference standard +	Reference standard −	Total	EM ELISA WCS + ELISA C6 USA tests Serum
	Index test +	13	0	13	
	Index test −	7	100	107	
	Total	20	100	120	
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA (IgM/IgG) + Immunoblot (IgM/IgG) European tests Serum
	Index test +	13	1	14	
	Index test −	2	99	101	
	Total	15	100	115	

Reference	Branda 2013[11]				
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA (IgM/IgG) + Immunoblot (IgM/IgG) USA tests Serum
	Index test +	6	0	6	
	Index test −	9	100	109	
	Total	15	100	115	
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA C6 (IgT) + Immunoblot (IgM/IgG) USA tests Serum
	Index test +	6	0	6	
	Index test −	9	100	109	
	Total	15	100	115	
		Reference standard +	Reference standard −	Total	Neuroborreliosis ELISA WCS + ELISA C6 USA tests Serum
	Index test +	13	0	13	
	Index test −	2	100	102	
	Total	15	100	115	
		Reference standard +	Reference standard −	Total	Arthritis ELISA (IgM/IgG) + Immunoblot (IgM/IgG) European tests Serum
	Index test +	14	1	15	
	Index test −	1	99	100	
	Total	15	100	115	
		Reference standard +	Reference standard −	Total	Arthritis ELISA (IgM/IgG) + Immunoblot (IgM/IgG) USA tests Serum
	Index test +	9	0	9	
	Index test −	6	100	106	
	Total	15	100	115	
		Reference standard +	Reference standard −	Total	Arthritis ELISA C6 (IgT) + Immunoblot (IgM/IgG) USA tests Serum
	Index test +	10	0	10	
	Index test −	5	100	105	

Reference	**Branda 2013[11]**				
	Total	15	100	115	
		Reference standard +	Reference standard −	Total	Arthritis ELISA WCS + ELISA C6
	Index test +	14	0	14	USA tests Serum
	Index test −	1	100	101	
	Total	15	100	115	
		Reference standard +	Reference standard −	Total	ACA ELISA (IgM/IgG) + Immunoblot (IgM/IgG)
	Index test +	14	1	15	European tests Serum
	Index test −	0	99	99	
	Total	14	100	114	
		Reference standard +	Reference standard −	Total	ACA ELISA (IgM/IgG) + Immunoblot (IgM/IgG)
	Index test +	14	0	14	USA tests Serum
	Index test −	0	100	100	
	Total	14	100	114	
		Reference standard +	Reference standard −	Total	ACA ELISA C6 (IgT) + Immunoblot (IgM/IgG)
	Index test +	14	0	14	USA tests Serum
	Index test −	0	100	100	
	Total	14	100	114	
		Reference standard +	Reference standard −	Total	ACA ELISA WCS + ELISA C6
	Index test +	14	0	14	USA tests Serum
	Index test −	0	100	100	
	Total	14	100	114	
		Reference standard +	Reference standard −	Total	Unspecified Lyme disease ELISA (IgM/IgG) + Immunoblot (IgM/IgG)
	Index test +	52	1	53	

Reference	**Branda 2013[11]**				
	Index test −	12	99	111	European tests Serum
	Total	64	100	164	
		Reference standard +	Reference standard −	Total	Unspecified Lyme disease ELISA (IgM/IgG) + Immunoblot (IgM/IgG)
	Index test +	33	0	33	USA tests Serum
	Index test −	31	100	131	
	Total	64	100	164	
		Reference standard +	Reference standard −	Total	Unspecified Lyme disease ELISA C6 + Immunoblot (IgM/IgG)
	Index test +	34	0	34	USA tests Serum
	Index test −	30	100	130	
	Total	64	100	164	
		Reference standard +	Reference standard −	Total	Unspecified Lyme disease ELISA WCS + ELISA C6
	Index test +	54	0	54	USA tests Serum
	Index test −	10	100	110	
	Total	64	100	164	
Statistical measures	Index test: ELISA IgM/IgG + Immunoblot IgM/IgG European tests (serum) - EM Sensitivity 0.55 Specificity 0.99 Index test: ELISA IgM/IgG + Immunoblot IgM/IgG USA tests (serum) - EM Sensitivity 0.20 Specificity 1.00 Index test: ELISA C6 (IgT) + Immunoblot IgM/IgG USA tests (serum) - EM Sensitivity 0.20 Specificity 1.00 Index test: ELISA WCS + ELISA C6 USA tests (serum) - EM				

Reference	Branda 2013[11]
	Sensitivity 0.65 Specificity 1.00 Index test: ELISA IgM/IgG + Immunoblot IgM/IgG European tests (serum) - neuroborreliosis Sensitivity 0.87 Specificity 0.99 Index test: ELISA IgM/IgG + Immunoblot IgM/IgG USA tests (serum) - neuroborreliosis Sensitivity 0.40 Specificity 1.00 Index test: ELISA C6 (IgT) + Immunoblot IgM/IgG USA tests (serum) - neuroborreliosis Sensitivity 0.40 Specificity 1.00 Index test: ELISA WCS + ELISA C6 USA tests (serum) - neuroborreliosis Sensitivity 0.87 Specificity 1.00 Index test: ELISA IgM/IgG + Immunoblot IgM/IgG European tests (serum) - arthritis Sensitivity 0.93 Specificity 0.99 Index test: ELISA IgM/IgG + Immunoblot IgM/IgG USA tests (serum) - arthritis Sensitivity 0.60 Specificity 1.00 Index test: ELISA C6 (IgT) + Immunoblot IgM/IgG USA tests (serum) - arthritis Sensitivity 0.67 Specificity 1.00

Reference	Branda 2013[11]
	Index test: ELISA WCS + ELISA C6 USA tests (serum) - arthritis Sensitivity 0.93 Specificity 1.00 Index test: ELISA IgM/IgG + Immunoblot IgM/IgG European tests (serum) - ACA Sensitivity 1.00 Specificity 0.99 Index test: ELISA IgM/IgG + Immunoblot IgM/IgG USA tests (serum) - ACA Sensitivity 1.00 Specificity 1.00 Index test: ELISA C6 (IgT) + Immunoblot IgM/IgG USA tests (serum) - ACA Sensitivity 1.00 Specificity 1.00 Index test: ELISA WCS + ELISA C6 USA tests (serum) - ACA Sensitivity 1.00 Specificity 1.00 Index test: ELISA IgM/IgG + Immunoblot IgM/IgG European tests (serum) – unspecified Lyme disease Sensitivity 0.81 Specificity 0.99 Index test: ELISA IgM/IgG + Immunoblot IgM/IgG USA tests (serum) - unspecified Lyme disease Sensitivity 0.52 Specificity 1.00 Index test: ELISA C6 (IgT) + Immunoblot IgM/IgG USA tests (serum) - unspecified Lyme disease Sensitivity 0.53 Specificity 1.00

Reference	Branda 2013[11]
	Index test: ELISA WCS + ELISA C6 USA tests (serum) - unspecified Lyme disease Sensitivity 0.84 Specificity 1.00
Source of funding	Austin L. Vickery Jr award from the Department of Pathology, Massachusetts General Hospital, Boston
Limitations	Risk of bias: selection, index test, reference standard Indirectness: none
Comments	-

Reference	Fallon 2014
Study type	Case-control
Study methodology	Data source: specimens obtained during the conduct of 2 research protocols on individuals with post-treatment Lyme syndrome and controls Recruitment: not reported
Number of patients	n = 37 cases, 40 controls
Patient characteristics	Age, mean (SD): cases 46.5 years (10.5), controls 43.9 years (11.7) Gender (male to female ratio): cases 13:24, controls 16:24 Family origin: not reported Setting: 4 laboratories Country: USA

Reference	Fallon 2014				
	Cases: patients with post-treatment Lyme syndrome with historical evidence meeting CDC criteria for Lyme disease Controls: no history of prior diagnosis or treatment for Lyme disease, no history of Lyme-like symptoms or illness, no history of another major neurologic or medical disorder, residence in non-endemic area and no recent exposure to a highly Lyme endemic area				
Target condition(s)	Lyme disease				
Index test(s) and reference standard	Index test(s) ELISA + Immunoblot (IgG) ELISA C6 + Immunoblot (IgG) ELISA + ELISA C6 Reference standard Clinical diagnosis (CDC criteria) Time between measurement of index test and reference standard: not reported				
2×2 table		Reference standard +	Reference standard −	Total	PTLDS ELISA + Immunoblot (IgG) Serum Commercial lab
	Index test +	15	0	15	
	Index test −	22	40	62	
	Total	37	40	77	
		Reference standard +	Reference standard −	Total	PTLDS ELISA + Immunoblot (IgG) Serum Speciality lab A
	Index test +	14	0	14	
	Index test −	23	40	63	
	Total	37	40	77	
		Reference standard +	Reference standard −	Total	PTLDS ELISA + Immunoblot (IgG) Serum Speciality lab B
	Index test +	16	1	17	
	Index test −	21	39	60	

Reference	**Fallon 2014**				
	Total	37	40	77	
		Reference standard +	Reference standard −	Total	PTLDS ELISA + Immunoblot (IgG) Serum University reference lab
	Index test +	18	0	18	
	Index test −	19	40	59	
	Total	37	40	77	
		Reference standard +	Reference standard −	Total	PTLDS ELISA C6 + Immunoblot (IgG) Serum Speciality lab A
	Index test +	15	0	15	
	Index test −	22	40	62	
	Total	37	40	77	
		Reference standard +	Reference standard −	Total	PTLDS ELISA C6 + Immunoblot (IgG) Serum Speciality lab B
	Index test +	17	0	17	
	Index test −	20	40	60	
	Total	37	40	77	
		Reference standard +	Reference standard −	Total	PTLDS ELISA + ELISA C6 Serum Speciality lab A
	Index test +	22	0	22	
	Index test −	15	40	55	
	Total	37	40	77	
		Reference standard +	Reference standard −	Total	PTLDS ELISA + ELISA C6 Serum Speciality lab B
	Index test +	18	0	18	
	Index test −	19	40	59	
	Total	37	40	77	
Statistical measures	Index test: ELISA + Immunoblot IgG (serum) – PTLDS (commercial lab) Sensitivity 0.41 Specificity 1.00				

Reference	Fallon 2014
	Index test: ELISA + Immunoblot IgG (serum) – PTLDS (speciality lab A) Sensitivity 0.38 Specificity 1.00 Index test: ELISA + Immunoblot IgG (serum) – PTLDS (speciality lab B) Sensitivity 0.43 Specificity 0.97 Index test: ELISA + Immunoblot IgG (serum) – PTLDS (university reference lab) Sensitivity 0.49 Specificity 1.00
Source of funding	Lyme Research Alliance Inc, the Lyme Disease association Inc, the Lyme and Tick-borne Diseases Research Center
Limitations	Risk of bias: selection, reference standard Indirectness: none
Comments	Borderline results counted as positive

Reference	Goossens 1999[29]/Goossens 2000[30]
Study type	Case-control
Study methodology	Data source: not reported Recruitment: not reported
Number of patients	n = 39 cases, 190 controls
Patient characteristics	Age, mean (SD): not reported Gender (male to female ratio): not reported

Reference	Goossens 1999[29]/Goossens 2000[30]				
	Family origin: not reported Setting: not reported Country: The Netherlands Cases: early Lyme borreliosis presenting with EM and a history of tick bite and/isolation of B. burgdorferi from their skin lesion (26), late Lyme borreliosis presenting with ACA/neuroborreliosis (13) Controls: healthy controls with no history of Lyme borreliosis and tick exposure				
Target condition(s)	Lyme disease				
Index test(s) and reference standard	Index test(s) ELISA (IgM) + Immunoblot (IgM) ELISA (IgG) + Immunoblot (IgG) ELISA (IgM/IgG) + Immunoblot (IgM) ELISA (IgM/IgG) + Immunoblot (IgG) Reference standard Clinical diagnosis Time between measurement of index test and reference standard: not reported				
2×2 table		Reference standard +	Reference standard −	Total	Early Lyme disease ELISA (IgM) + Immunoblot (IgM) Serum Behring EIA + Genzyme Virotech IB
	Index test +	12	0	12	
	Index test −	14	62	76	
	Total	26	62	88	
		Reference standard +	Reference standard −	Total	Early Lyme disease

Reference	**Goossens 1999[29]/Goossens 2000[30]**				
	Index test +	12	0	12	ELISA (IgM) + Immunoblot (IgM) Serum Behring EIA + MRL IB
	Index test −	14	62	76	
	Total	26	62	88	
		Reference standard +	Reference standard −	Total	Early Lyme disease ELISA (IgM) + Immunoblot (IgM) Serum Boehringer EIA + Genzyme Virotech IB
	Index test +	8	0	8	
	Index test −	18	62	80	
	Total	26	62	88	
		Reference standard +	Reference standard −	Total	Early Lyme disease ELISA (IgM) + Immunoblot (IgM) Serum Boehringer EIA + MRL IB
	Index test +	9	0	9	
	Index test −	17	62	79	
	Total	26	62	88	
		Reference standard +	Reference standard −	Total	Early Lyme disease ELISA (IgM) + Immunoblot (IgM) Serum Dako EIA + Genzyme Virotech IB
	Index test +	9	0	9	
	Index test −	17	62	79	
	Total	26	62	88	
		Reference standard +	Reference standard −	Total	Early Lyme disease ELISA (IgM) + Immunoblot (IgM) Serum Dako EIA + MRL IB
	Index test +	11	0	11	
	Index test −	15	62	77	
	Total	26	62	88	
		Reference standard +	Reference standard −	Total	Early Lyme disease ELISA (IgM) + Immunoblot (IgM) Serum Genzyme Virotech EIA + Genzyme Virotech IB
	Index test +	13	0	13	
	Index test −	13	62	75	
	Total	26	62	88	
		Reference standard	Reference standard −	Total	Early Lyme disease

Reference	Goossens 1999[29]/Goossens 2000[30]				
		+			ELISA (IgM) + Immunoblot (IgM) Serum Genzyme Virotech EIA + MRL IB
	Index test +	12	0	12	
	Index test −	14	62		
	Total	26	62	88	
		Reference standard +	Reference standard −	Total	Early Lyme disease ELISA (IgM) + Immunoblot (IgM) Serum IBL EIA + Genzyme Virotech IB
	Index test +	9	2	11	
	Index test −	17	60	77	
	Total	26	62	88	
		Reference standard +	Reference standard −	Total	Early Lyme disease ELISA (IgM) + Immunoblot (IgM) Serum IBL EIA + MRL IB
	Index test +	12	0	12	
	Index test −	14	62	76	
	Total	26	62	88	
		Reference standard +	Reference standard −	Total	Early Lyme disease ELISA (IgG) + Immunoblot (IgG) Serum Behring EIA + Genzyme Virotech IB
	Index test +	6	4	10	
	Index test −	20	58	78	
	Total	26	62	88	
		Reference standard +	Reference standard −	Total	Early Lyme disease ELISA (IgG) + Immunoblot (IgG) Serum Behring EIA + MRL IB
	Index test +	1	2	3	
	Index test −	25	60	85	
	Total	26	62	88	
		Reference standard +	Reference standard −	Total	Early Lyme disease ELISA (IgG) + Immunoblot (IgG) Serum Boehringer EIA + Genzyme Virotech IB
	Index test +	4	4	8	
	Index test −	22	58	80	
	Total	26	62	88	

Reference	Goossens 1999[29]/Goossens 2000[30]				
		Reference standard +	Reference standard −	Total	Early Lyme disease ELISA (IgG) + Immunoblot (IgG) Serum Boehringer EIA + MRL IB
	Index test +	1	2	3	
	Index test −	25	60	85	
	Total	26	62	88	
		Reference standard +	Reference standard −	Total	Early Lyme disease ELISA (IgG) + Immunoblot (IgG) Serum Dako EIA + Genzyme Virotech IB
	Index test +	5	2	7	
	Index test −	21	60	81	
	Total	26	62	88	
		Reference standard +	Reference standard −	Total	Early Lyme disease ELISA (IgG) + Immunoblot (IgG) Serum Dako EIA + MRL IB
	Index test +	1	2	3	
	Index test −	25	60	85	
	Total	26	62	88	
		Reference standard +	Reference standard −	Total	Early Lyme disease ELISA (IgG) + Immunoblot (IgG) Serum Genzyme Virotech EIA + Genzyme Virotech IB
	Index test +	5	3	8	
	Index test −	21	59	80	
	Total	26	62	88	
		Reference standard +	Reference standard −	Total	Early Lyme disease ELISA (IgG) + Immunoblot (IgG) Serum Genzyme Virotech EIA + MRL IB
	Index test +	1	2	3	
	Index test −	25	60	85	
	Total	26	62	88	
		Reference standard +	Reference standard −	Total	Early Lyme disease ELISA (IgG) + Immunoblot (IgG) Serum IBL EIA + Genzyme Virotech IB
	Index test +	4	4	8	
	Index test −	22	58	80	

Reference	**Goossens 1999[29]/Goossens 2000[30]**				
	Total	26	62	88	
		Reference standard +	Reference standard −	Total	Early Lyme disease ELISA (IgG) + Immunoblot (IgG) Serum IBL EIA + MRL IB
	Index test +	1	2	3	
	Index test −	25	60	85	
	Total	26	62	88	
		Reference standard +	Reference standard −	Total	Early Lyme disease ELISA (IgM/IgG) + Immunoblot (IgM) Serum Milenia EIA + Genzyme Virotech IB
	Index test +	3	3	6	
	Index test −	23	59	82	
	Total	26	62	88	
		Reference standard +	Reference standard −	Total	Early Lyme disease ELISA (IgM/IgG) + Immunoblot (IgM) Serum Milenia EIA + MRL IB
	Index test +	1	2	3	
	Index test −	25	60	85	
	Total	26	62	88	
		Reference standard +	Reference standard −	Total	Early Lyme disease ELISA (IgM/IgG) + Immunoblot (IgG) Serum Milenia EIA + Genzyme Virotech IB
	Index test +	3	3	6	
	Index test −	23	59	82	
	Total	26	62	88	
		Reference standard +	Reference standard −	Total	Early Lyme disease ELISA (IgM/IgG) + Immunoblot (IgG) Serum Milenia EIA + MRL IB
	Index test +	1	2	3	
	Index test −	25	60	85	
	Total	26	62	88	
		Reference standard +	Reference standard −	Total	Late Lyme disease ELISA (IgM) + Immunoblot (IgM)
	Index test +	5	0	5	

Reference	**Goossens 1999[29]/Goossens 2000[30]**				
	Index test −	8	62	70	Serum Behring EIA + Genzyme Virotech IB
	Total	13	62	75	
		Reference standard +	Reference standard −	Total	Late Lyme disease ELISA (IgM) + Immunoblot (IgM)
	Index test +	6	0	6	Serum Behring EIA + MRL IB
	Index test −	7	62	69	
	Total	13	62	75	
		Reference standard +	Reference standard −	Total	Late Lyme disease ELISA (IgM) + Immunoblot (IgM)
	Index test +	4	0	4	Serum Boehringer EIA + Genzyme Virotech IB
	Index test −	9	62	71	
	Total	13	62	75	
		Reference standard +	Reference standard −	Total	Late Lyme disease ELISA (IgM) + Immunoblot (IgM)
	Index test +	6	0	6	Serum Boehringer EIA + MRL IB
	Index test −	7	62	69	
	Total	13	62	75	
		Reference standard +	Reference standard −	Total	Late Lyme disease ELISA (IgM) + Immunoblot (IgM)
	Index test +	5	0	5	Serum Dako EIA + Genzyme Virotech IB
	Index test −	8	62	70	
	Total	13	62	75	
		Reference standard +	Reference standard −	Total	Late Lyme disease ELISA (IgM) + Immunoblot (IgM)
	Index test +	6	0	6	Serum Dako EIA + MRL IB
	Index test −	7	62	69	
	Total	13	62	75	
		Reference standard +	Reference standard −	Total	Late Lyme disease

Reference	**Goossens 1999[29]/Goossens 2000[30]**				
	Index test +	5	0	5	ELISA (IgM) + Immunoblot (IgM) Serum Genzyme Virotech EIA + Genzyme Virotech IB
	Index test −	8	62	70	
	Total	13	62	75	
		Reference standard +	Reference standard −	Total	Late Lyme disease ELISA (IgM) + Immunoblot (IgM) Serum Genzyme Virotech EIA + MRL IB
	Index test +	6	0	6	
	Index test −	7	62	69	
	Total	13	62	75	
		Reference standard +	Reference standard −	Total	Late Lyme disease ELISA (IgM) + Immunoblot (IgM) Serum IBL EIA + Genzyme Virotech IB
	Index test +	4	2	6	
	Index test −	9	60	69	
	Total	13	62	75	
		Reference standard +	Reference standard −	Total	Late Lyme disease ELISA (IgM) + Immunoblot (IgM) Serum IBL EIA + MRL IB
	Index test +	5	0	5	
	Index test −	8	62	70	
	Total	13	62	75	
		Reference standard +	Reference standard −	Total	Late Lyme disease ELISA (IgG) + Immunoblot (IgG) Serum Behring EIA + Genzyme Virotech IB
	Index test +	6	4	10	
	Index test −	7	58	65	
	Total	13	62	75	
		Reference standard +	Reference standard −	Total	Late Lyme disease ELISA (IgG) + Immunoblot (IgG) Serum Behring EIA + MRL IB
	Index test +	5	2	7	
	Index test −	8	60	68	
	Total	13	62	75	
		Reference standard	Reference standard −	Total	Late Lyme disease

Reference	Goossens 1999[29]/Goossens 2000[30]				
		+			ELISA (IgG) + Immunoblot (IgG) Serum Boehringer EIA + Genzyme Virotech IB
	Index test +	6	4	10	
	Index test −	7	58	65	
	Total	13	62	75	
		Reference standard +	Reference standard −	Total	Late Lyme disease ELISA (IgG) + Immunoblot (IgG) Serum Boehringer EIA + MRL IB
	Index test +	5	2	7	
	Index test −	8	60	68	
	Total	13	62	75	
		Reference standard +	Reference standard −	Total	Late Lyme disease ELISA (IgG) + Immunoblot (IgG) Serum Dako EIA + Genzyme Virotech IB
	Index test +	5	2	7	
	Index test −	8	60	68	
	Total	13	62	75	
		Reference standard +	Reference standard −	Total	Late Lyme disease ELISA (IgG) + Immunoblot (IgG) Serum Dako EIA + MRL IB
	Index test +	5	2		
	Index test −	8	60		
	Total	13	62		
		Reference standard +	Reference standard −	Total	Late Lyme disease ELISA (IgG) + Immunoblot (IgG) Serum Genzyme Virotech EIA + Genzyme Virotech IB
	Index test +	5	3	8	
	Index test −	8	59	67	
	Total	13	62	75	
		Reference standard +	Reference standard −	Total	Late Lyme disease ELISA (IgG) + Immunoblot (IgG) Serum Genzyme Virotech EIA + MRL IB
	Index test +	5	2	7	
	Index test −	8	60	68	
	Total	13	62	75	

Reference	Goossens 1999[29]/Goossens 2000[30]				
		Reference standard +	Reference standard −	Total	Late Lyme disease ELISA (IgG) + Immunoblot (IgG) Serum IBL EIA + Genzyme Virotech IB
	Index test +	4	4	8	
	Index test −	9	58	67	
	Total	13	62	75	
		Reference standard +	Reference standard −	Total	Late Lyme disease ELISA (IgG) + Immunoblot (IgG) Serum IBL EIA + MRL IB
	Index test +	4	2	6	
	Index test −	9	60	69	
	Total	13	62	75	
		Reference standard +	Reference standard −	Total	Late Lyme disease ELISA (IgM/IgG) + Immunoblot (IgM) Serum Milenia EIA + Genzyme Virotech IB
	Index test +	2	3	5	
	Index test −	11	59	70	
	Total	13	62	75	
		Reference standard +	Reference standard −	Total	Late Lyme disease ELISA (IgM/IgG) + Immunoblot (IgM) Serum Milenia EIA + MRL IB
	Index test +	1	2	3	
	Index test −	12	60	72	
	Total	13	62	75	
		Reference standard +	Reference standard −	Total	Late Lyme disease ELISA (IgM/IgG) + Immunoblot (IgG) Serum Milenia EIA + Genzyme Virotech IB
	Index test +	6	3	9	
	Index test −	7	59	66	
	Total	13	62	75	
		Reference standard +	Reference standard −	Total	Late Lyme disease ELISA (IgM/IgG) + Immunoblot (IgG) Serum Milenia EIA + MRL IB
	Index test +	6	2	8	
	Index test −	7	60	67	

Reference	Goossens 1999[29]/Goossens 2000[30]				
	Total	13	62	75	
Statistical measures	Index test: ELISA IgM + Immunoblot IgM Behring + Genzyme Virotech (serum) – early Lyme disease Sensitivity 0.46 Specificity 1.00 Index test: ELISA IgM + Immunoblot IgM Behring + MRL (serum) – early Lyme disease Sensitivity 0.46 Specificity 1.00 Index test: ELISA IgM + Immunoblot IgM Boehringer + Genzyme Virotech (serum) – early Lyme disease Sensitivity 0.31 Specificity 1.00 Index test: ELISA IgM + Immunoblot IgM Boehringer + MRL (serum) – early Lyme disease Sensitivity 0.35 Specificity 1.00 Index test: ELISA IgM + Immunoblot IgM Dako + Genzyme Virotech (serum) – early Lyme disease Sensitivity 0.35 Specificity 1.00 Index test: ELISA IgM + Immunoblot IgM Dako + MRL (serum) – early Lyme disease Sensitivity 0.42 Specificity 1.00 Index test: ELISA IgM + Immunoblot IgM Genzyme Virotech + Genzyme Virotech (serum) – early Lyme disease Sensitivity 0.50 Specificity 1.00 Index test: ELISA IgM + Immunoblot IgM Genzyme Virotech + MRL (serum) – early Lyme disease Sensitivity 0.46				

Reference	Goossens 1999[29]/Goossens 2000[30]
	Specificity 1.00 Index test: ELISA IgM + Immunoblot IgM IBL + Genzyme Virotech (serum) – early Lyme disease Sensitivity 0.35 Specificity 0.97 Index test: ELISA IgM + Immunoblot IgM IBL + MRL (serum) – early Lyme disease Sensitivity 0.46 Specificity 1.00 Index test: ELISA IgG + Immunoblot IgM Behring + Genzyme Virotech (serum) – early Lyme disease Sensitivity 0.23 Specificity 0.94 Index test: ELISA IgM + Immunoblot IgG Behring + MRL (serum) – early Lyme disease Sensitivity 0.04 Specificity 0.97 Index test: ELISA IgG + Immunoblot IgG Boehringer + Genzyme Virotech (serum) – early Lyme disease Sensitivity 0.15 Specificity 0.94 Index test: ELISA IgG + Immunoblot IgG Boehringer + MRL (serum) – early Lyme disease Sensitivity 0.04 Specificity 0.97 Index test: ELISA IgG + Immunoblot IgG Dako + Genzyme Virotech (serum) – early Lyme disease Sensitivity 0.19 Specificity 0.97 Index test: ELISA IgG + Immunoblot IgG Dako + MRL (serum) – early Lyme disease

Reference	Goossens 1999[29]/Goossens 2000[30]
	Sensitivity 0.04 Specificity 0.97 Index test: ELISA IgG + Immunoblot IgG Genzyme Virotech + Genzyme Virotech (serum) – early Lyme disease Sensitivity 0.19 Specificity 0.95 Index test: ELISA IgG + Immunoblot IgG Genzyme Virotech + MRL (serum) – early Lyme disease Sensitivity 0.04 Specificity 0.97 Index test: ELISA IgG + Immunoblot IgG IBL + Genzyme Virotech (serum) – early Lyme disease Sensitivity 0.15 Specificity 0.94 Index test: ELISA IgG + Immunoblot IgG IBL + MRL (serum) – early Lyme disease Sensitivity 0.04 Specificity 0.97 Index test: ELISA IgM/IgG + Immunoblot IgM Milenia + Genzyme Virotech (serum) – early Lyme disease Sensitivity 0.12 Specificity 0.95 Index test: ELISA IgM/IgG + Immunoblot IgM Milenia + MRL (serum) – early Lyme disease Sensitivity 0.04 Specificity 0.97 Index test: ELISA IgM/IgG + Immunoblot IgG Milenia + Genzyme Virotech (serum) – early Lyme disease Sensitivity 0.12 Specificity 0.95

Reference	Goossens 1999[29]/Goossens 2000[30]
	Index test: ELISA IgM/IgG + Immunoblot IgG Milenia + MRL (serum) – early Lyme disease Sensitivity 0.04 Specificity 0.97 Index test: ELISA IgM + Immunoblot IgM Behring + Genzyme Virotech (serum) – late Lyme disease Sensitivity 0.38 Specificity 1.00 Index test: ELISA IgM + Immunoblot IgM Behring + MRL (serum) – late Lyme disease Sensitivity 0.46 Specificity 1.00 Index test: ELISA IgM + Immunoblot IgM Boehringer + Genzyme Virotech (serum) – late Lyme disease Sensitivity 0.31 Specificity 1.00 Index test: ELISA IgM + Immunoblot IgM Boehringer + MRL (serum) – late Lyme disease Sensitivity 0.46 Specificity 1.00 Index test: ELISA IgM + Immunoblot IgM Dako + Genzyme Virotech (serum) – late Lyme disease Sensitivity 0.38 Specificity 1.00 Index test: ELISA IgM + Immunoblot IgM Dako + MRL (serum) – late Lyme disease Sensitivity 0.46 Specificity 1.00 Index test: ELISA IgM + Immunoblot IgM Genzyme Virotech + Genzyme Virotech (serum) – late Lyme disease Sensitivity 0.38 Specificity 1.00

Reference	Goossens 1999[29]/Goossens 2000[30]
	Index test: ELISA IgM + Immunoblot IgM Genzyme Virotech + MRL (serum) – late Lyme disease Sensitivity 0.46 Specificity 1.00 Index test: ELISA IgM + Immunoblot IgM IBL + Genzyme Virotech (serum) – late Lyme disease Sensitivity 0.31 Specificity 0.97 Index test: ELISA IgM + Immunoblot IgM IBL + MRL (serum) – late Lyme disease Sensitivity 0.38 Specificity 1.00 Index test: ELISA IgG + Immunoblot IgG Behring + Genzyme Virotech (serum) – late Lyme disease Sensitivity 0.46 Specificity 0.94 Index test: ELISA IgG + Immunoblot IgG Behring + MRL (serum) – late Lyme disease Sensitivity 0.38 Specificity 0.97 Index test: ELISA IgG + Immunoblot IgG Boehringer + Genzyme Virotech (serum) – late Lyme disease Sensitivity 0.46 Specificity 0.94 Index test: ELISA IgG + Immunoblot IgG Boehringer + MRL (serum) – late Lyme disease Sensitivity 0.38 Specificity 0.97 Index test: ELISA IgG + Immunoblot IgG Dako + Genzyme Virotech (serum) – late Lyme disease Sensitivity 0.38

Reference	Goossens 1999[29]/Goossens 2000[30]
	Specificity 0.97 Index test: ELISA IgG + Immunoblot IgG Dako + MRL (serum) – late Lyme disease Sensitivity 0.38 Specificity 0.97 Index test: ELISA IgG + Immunoblot IgG Genzyme Virotech + Genzyme Virotech (serum) – late Lyme disease Sensitivity 0.38 Specificity 0.95 Index test: ELISA IgG + Immunoblot IgG Genzyme Virotech + MRL (serum) – late Lyme disease Sensitivity 0.38 Specificity 0.97 Index test: ELISA IgG + Immunoblot IgG IBL + Genzyme Virotech (serum) – late Lyme disease Sensitivity 0.31 Specificity 0.94 Index test: ELISA IgG + Immunoblot IgG IBL + MRL (serum) – late Lyme disease Sensitivity 0.31 Specificity 0.97 Index test: ELISA IgM/IgG + Immunoblot IgM Milenia + Genzyme Virotech (serum) – late Lyme disease Sensitivity 0.15 Specificity 0.95 Index test: ELISA IgM/IgG + Immunoblot IgM Milenia + MRL (serum) – late Lyme disease Sensitivity 0.08 Specificity 0.97 Index test: ELISA IgM/IgG + Immunoblot IgG Milenia + Genzyme Virotech (serum) – late Lyme disease

Reference	Goossens 1999[29]/Goossens 2000[30]
	Sensitivity 0.46 Specificity 0.95 Index test: ELISA IgM/IgG + Immunoblot IgG Milenia + MRL (serum) – late Lyme disease Sensitivity 0.46 Specificity 0.97
Source of funding	Not reported
Limitations	Risk of bias: selection, index test, reference standard Indirectness: none
Comments	Healthy subjects used as control group in analysis, borderline results considered positive

Reference	Johnson 1996[44]
Study type	Case-control
Study methodology	Data source: serum samples contributed by physicians with extensive experience in the clinical diagnosis of Lyme disease, healthy blood donors, patients with other illnesses Recruitment: not reported
Number of patients	n = 111 cases, 224 controls
Patient characteristics	Age, mean (SD): not reported Gender (male to female ratio): not reported Family origin: not reported Setting: not reported

Reference	Johnson 1996[44]				
	Country: USA Cases: EM (58), early neurologic (3), Lyme arthritis (36), late neurologic (14) Controls: healthy blood donors (113), autoimmune disorders, leptospirosis, periodontitis, relapsing fever, syphilis, tularemia and other illnesses (111)				
Target condition(s)	Lyme disease				
Index test(s) and reference standard	Index test(s) ELISA (IgM/IgG) + Immunoblot (IgM/IgG) Reference standard Clinical diagnosis Time between measurement of index test and reference standard: not reported				
2×2 table		Reference standard +	Reference standard −	Total	EM (disseminated) FLA-ELISA (IgM/IgG) + IB (IgM/IgG) Serum
	Index test +	8	0	8	
	Index test −	0	113	113	
	Total	8	113	121	
		Reference standard +	Reference standard −	Total	EM (localised) FLA-ELISA (IgM/IgG) + IB (IgM/IgG) Serum
	Index test +	29	0	29	
	Index test −	21	113	134	
	Total	50	113	163	
		Reference standard +	Reference standard −	Total	Neuroborreliosis (early) FLA-ELISA (IgM/IgG) + IB (IgM/IgG) Serum
	Index test +	3	0	3	
	Index test −	0	113	113	

Reference	**Johnson 1996[44]**				
	Total	3	113	116	
		Reference standard +	Reference standard −	Total	Neuroborreliosis (late) FLA-ELISA (IgM/IgG) + IB (IgM/IgG) Serum
	Index test +	14	0	14	
	Index test −	0	113	113	
	Total	14	113	127	
		Reference standard +	Reference standard −	Total	Arthritis FLA-ELISA (IgM/IgG) + IB (IgM/IgG) Serum
	Index test +	36	0	36	
	Index test −	0	113	113	
	Total	36	113	149	
		Reference standard +	Reference standard −	Total	Unspecified Lyme disease FLA-ELISA (IgM/IgG) + IB (IgM/IgG) Serum
	Index test +	90	0	90	
	Index test −	21	113	134	
	Total	111	113	224	
Statistical measures	Index test: ELISA IgM/IgG + IB IgM/IgG (serum) – EM (disseminated) Sensitivity 1.00 Specificity 1.00 Index test: ELISA IgM/IgG + IB IgM/IgG (serum) – EM (localised) Sensitivity 0.58 Specificity 1.00 Index test: ELISA IgM/IgG + IB IgM/IgG (serum) - neuroborreliosis (early) Sensitivity 1.00 Specificity 1.00 Index test: ELISA IgM/IgG + IB IgM/IgG (serum) – neuroborreliosis (late) Sensitivity 1.00				

Reference	Johnson 1996[44]
	Specificity 1.00 Index test: ELISA IgM/IgG + IB IgM/IgG (serum) – arthritis Sensitivity 1.00 Specificity 1.00 Index test: ELISA IgM/IgG + IB IgM/IgG (serum) – unspecified Lyme disease Sensitivity 0.81 Specificity 1.00
Source of funding	Not reported
Limitations	Risk of bias: selection, reference standard Indirectness: none
Comments	Healthy controls used in the analysis

Reference	Lipsett 2016[62]
Study type	Cohort study
Study methodology	Data source: serum samples from children Recruitment: children undergoing serological testing for Lyme disease
Number of patients	n = 944
Patient characteristics	Age, median (IQR): 10.9 years (6.4-15.2) Gender (male to female ratio): 421/524 Family origin: not reported

<table>
<tr><td>Reference</td><td colspan="5">Lipsett 2016[62]</td></tr>
<tr><td></td><td colspan="5">Setting: hospital-based laboratory

Country: USA

Children and adolescents undergoing serologic evaluation for Lyme disease</td></tr>
<tr><td>Target condition(s)</td><td colspan="5">Lyme disease</td></tr>
<tr><td>Index test(s) and reference standard</td><td colspan="5">Index tests
ELISA C6 + IB (IgM/IgG) – serum
ELISA WCS + ELISA C6 – serum
ELISA WCS + IB (IgM/IgG) – serum

Reference standard
Clinician-diagnosed EM or a positive 2-tiered serologic result in the presence of a Lyme disease-associated clinical syndrome

Time between measurement of index test and reference standard: not reported</td></tr>
<tr><td rowspan="4">2×2 table [ELISA C6 + IB (IgM/IgG) – serum], unspec Lyme disease</td><td></td><td>Reference standard +</td><td>Reference standard −</td><td>Total</td><td rowspan="4"></td></tr>
<tr><td>Index test +</td><td>89</td><td>12</td><td>101</td></tr>
<tr><td>Index test −</td><td>25</td><td>818</td><td>843</td></tr>
<tr><td>Total</td><td>114</td><td>830</td><td>944</td></tr>
<tr><td rowspan="4">2×2 table [ELISA WCS + ELISA C6 – serum], unspec Lyme disease</td><td></td><td>Reference standard +</td><td>Reference standard −</td><td>Total</td><td rowspan="4"></td></tr>
<tr><td>Index test +</td><td>91</td><td>29</td><td>120</td></tr>
<tr><td>Index test −</td><td>23</td><td>801</td><td>824</td></tr>
<tr><td>Total</td><td>114</td><td>830</td><td>944</td></tr>
</table>

Reference	Lipsett 2016[62]				
2×2 table [ELISA WCS + IB (IgM/IgG) – serum], unspec Lyme disease		Reference standard +	Reference standard −	Total	
	Index test +	93	10	103	
	Index test −	21	820	841	
	Total	114	830	944	
Statistical measures	ELISA C6 + IB (IgM/IgG) – serum Sensitivity 0.78 Specificity 0.99 ELISA WCS + ELISA C6 – serum Sensitivity 0.80 Specificity 0.97 ELISA WCS + IB (IgM/IgG) – serum Sensitivity 0.82 Specificity 0.99				
Source of funding	Academic grants				
Limitations	Risk of bias: patient selection, index test, reference standard Indirectness: none				

Reference	Molins 2014[75]
Study type	Case-control study
Study methodology	Data source: not reported Recruitment: not reported
Number of	n = 124

Reference	Molins 2014[75]
patients	
Patient characteristics	Age, mean (SD): not reported Gender (male to female ratio): not reported Family origin: not reported Setting: not reported Country: USA Cases: Early Lyme disease with EM acute phase (n=40) Early Lyme disease with EM convalescent phase (n=38) Early disseminated Lyme carditis (n=7) Early disseminated Lyme neuroborreliosis (n=10) Late Lyme disease, LA (29) Controls: healthy persons (n=203) Standard CDC algorithm used for ELISA (IgM and IgG) + IB (IgM and IgG) – IgG used only after 1 month
Target condition(s)	EM, Neuroborreliosis, Lyme carditis, Lyme arthritis, unspecified Lyme disease
Index test(s) and reference standard	Index tests ELISA (IgM/IgG) + Immunoblot (IgM/IgG) – serum ELISA (IgM/IgG) + Immunoblot (IgM) – serum ELISA (IgM/IgG) + Immunoblot (IgG) – serum WB/IB (IgG) – serum WB/IB (IgM/IgG) – serum PCR – blood and skin PCR – blood, skin and heart tissue

Reference	Molins 2014[75]				
	PCR – synovial fluid Culture – blood and skin Culture – blood, skin and heart tissue Reference standard Clinical diagnosis Time between measurement of index test and reference standard: not reported				
2×2 table [ELISA (IgM/IgG) + IB (IgM/IgG) – serum], acute EM		Reference standard +	Reference standard −	Total	
	Index test +	16	2	18	
	Index test −	24	201	225	
	Total	40	203	243	
2×2 table [ELISA (IgM/IgG) + IB (IgM/IgG) – serum], convalescent EM		Reference standard +	Reference standard −	Total	
	Index test +	23	2	25	
	Index test −	15	201	216	
	Total	38	203	241	
2×2 table [ELISA (IgM/IgG) + IB (IgM) – serum], acute EM		Reference standard +	Reference standard −	Total	
	Index test +	12	1	13	
	Index test −	28	202	230	
	Total	40	203	243	
2×2 table [ELISA (IgM/IgG) + IB (IgM) –		Reference standard +	Reference standard −	Total	
	Index test +	20	1	21	

Reference	Molins 2014[75]				
serum], convalescent EM	Index test −	18	202	220	
	Total	38	203	241	
2×2 table [ELISA (IgM/IgG) + IB (IgG) – serum], acute EM		Reference standard +	Reference standard −	Total	
	Index test +	8	2	10	
	Index test −	32	201	233	
	Total	40	203	243	
2×2 table [ELISA (IgM/IgG) + IB (IgG) – serum], convalescent EM		Reference standard +	Reference standard −	Total	
	Index test +	13	2	15	
	Index test −	25	201	226	
	Total	38	203	241	
2x2 table [ELISA (IgM/IgG) + IB (IgM/IgG) – serum], neuroborreliosis		Reference standard +	Reference standard −	Total	
	Index test +	9	2	11	
	Index test −	1	203	204	
	Total	10	205	215	
2x2 table [ELISA (IgM/IgG) + IB (IgM) – serum], neuroborreliosis		Reference standard +	Reference standard −	Total	
	Index test +	9	1	10	
	Index test −	1	202	203	
	Total	10	203	213	
2x2 table [ELISA (IgM/IgG) + IB (IgG) –		Reference standard +	Reference standard −	Total	
	Index test +	3	2	5	

Reference	Molins 2014[75]				
serum], neuroborreliosis	Index test −	7	201	208	
	Total	10	203	213	
2x2 table [ELISA (IgM/IgG) + IB (IgM/IgG) – serum], arthritis		Reference standard +	Reference standard −	Total	
	Index test +	29	2	31	
	Index test −	0	203	203	
	Total	29	205	234	
2x2 table [ELISA (IgM/IgG) + IB (IgM) – serum], arthritis		Reference standard +	Reference standard −	Total	
	Index test +	9	1	10	
	Index test −	20	202	222	
	Total	29	203	232	
2x2 table [ELISA (IgM/IgG) + IB (IgG) – serum], arthritis		Reference standard +	Reference standard −	Total	
	Index test +	29	4	33	
	Index test −	0	201	201	
	Total	29	205	234	
2x2 table [ELISA (IgM/IgG) + IB (IgM/IgG) – serum], carditis		Reference standard +	Reference standard −	Total	
	Index test +	6	2	8	
	Index test −	1	203	204	
	Total	7	205	212	
2x2 table [ELISA (IgM/IgG) + IB (IgM) –		Reference standard +	Reference standard −	Total	
	Index test +	4	1	5	

Reference	Molins 2014[75]				
serum], carditis	Index test −	3	202	205	
	Total	7	203	210	
2x2 table [ELISA (IgM/IgG) + IB (IgG) – serum], carditis		Reference standard +	Reference standard −	Total	
	Index test +	4	2	6	
	Index test −	3	201	204	
	Total	7	203	210	
2x2 table [ELISA (IgM/IgG) + IB (IgM/IgG) – serum], unspecified		Reference standard +	Reference standard −	Total	
	Index test +	83	2	85	
	Index test −	41	201	242	
	Total	124	203	327	
Statistical measures	ELISA IgM/IgG + Immunoblot IgM/IgG – serum (acute EM) Sensitivity 0.40 Specificity 0.99 ELISA IgM/IgG + Immunoblot IgM/IgG – serum (convalescent EM) Sensitivity 0.61 Specificity 0.99 ELISA IgM/IgG + Immunoblot IgM – serum (acute EM) Sensitivity 0.30 Specificity 1.00 ELISA IgM/IgG + Immunoblot IgM – serum (convalescent EM) Sensitivity 0.53 Specificity 1.00				

Reference	Molins 2014[75]
	ELISA IgM/IgG + Immunoblot IgG – serum (acute EM) Sensitivity 0.20 Specificity 0.99 ELISA IgM/IgG + Immunoblot IgG – serum (convalescent EM) Sensitivity 0.34 Specificity 0.99 ELISA IgM/IgG + Immunoblot IgM/IgG – serum (neuroborreliosis) Sensitivity 0.90 Specificity 0.99 ELISA IgM/IgG + Immunoblot IgM – serum (neuroborreliosis) Sensitivity 0.90 Specificity 1.00 ELISA IgM/IgG + Immunoblot IgG – serum (neuroborreliosis) Sensitivity 0.30 Specificity 0.99 ELISA IgM/IgG + Immunoblot IgM/IgG – serum (arthritis) Sensitivity 1.00 Specificity 0.99 ELISA IgM/IgG + Immunoblot IgM – serum (arthritis) Sensitivity 0.31 Specificity 1.00 ELISA IgM/IgG + Immunoblot IgG – serum (arthritis) Sensitivity 1.00

Reference	Molins 2014[75]
	Specificity 0.99 ELISA IgM/IgG + Immunoblot IgM/IgG – serum (carditis) Sensitivity 0.86 Specificity 0.99 ELISA IgM/IgG + Immunoblot IgM – serum (carditis) Sensitivity 0.57 Specificity 1.00 ELISA IgM/IgG + Immunoblot IgG – serum (carditis) Sensitivity 0.57 Specificity 0.99 ELISA IgM/IgG + Immunoblot IgM/IgG – serum (unspecified Lyme disease) Sensitivity 0.67 Specificity 0.99
Source of funding	Supported by a CDC grant
Limitations	Risk of bias: patient selection, reference standard Indirectness: none

Reference	Molins 2016[74]
Study type	Case-control study
Study methodology	Data source: serum samples from CDC Recruitment: not reported
Number of	n = 124

Reference	Molins 2016[74]				
patients					
Patient characteristics	Age, mean (SD): not reported Gender (male to female ratio): not reported Family origin: not reported Setting: not reported Country: USA Cases: Acute and convalescent stage (n=78), Lyme neuroborreliosis (n=10), Lyme carditis (n=7), LA (n=29) Controls: healthy donors (n=203)				
Target condition(s)	EM, Neuroborreliosis, Lyme carditis, Lyme arthritis, unspecified Lyme disease				
Index test(s) and reference standard	Index tests ELISA (IgM/IgG) + Immunoblot (IgM/IgG) ELISA (C6) + Immunoblot IgM/IgG) ELISA (WCS) + ELISA (C6) ELISA (WCS) + Immunoblot (VlsE) Reference standard Clinical diagnosis Time between measurement of index test and reference standard: not reported				
2×2 table [ELISA (IgM/IgG) + IB (IgM/IgG) –		Reference standard +	Reference standard –	Total	
	Index test +	19	4	23	

Reference	Molins 2016[74]				
serum], acute EM	Index test −	21	199	220	
	Total	40	203	243	
2×2 table [ELISA (IgM/IgG) + IB (IgM/IgG) – serum], convalescent EM		Reference standard +	Reference standard −	Total	
	Index test +	24	4	28	
	Index test −	14	199	213	
	Total	38	203	241	
2×2 table [ELISA (C6) + IB, Marblot (IgM/IgG) – serum], acute EM		Reference standard +	Reference standard −	Total	
	Index test +	16	2	18	
	Index test −	24	201	225	
	Total	40	203	243	
2×2 table [ELISA (C6) + IB, ViraStripe (IgM/IgG) – serum], acute EM		Reference standard +	Reference standard −	Total	
	Index test +	17	1	18	
	Index test −	23	202	225	
	Total	40	203	243	
2×2 table [ELISA (C6) + IB, Marblot (IgM/IgG) – serum], convalescent EM		Reference standard +	Reference standard −	Total	
	Index test +	24	2	26	
	Index test −	14	201	215	
	Total	38	203	241	
2×2 table [ELISA (C6) + IB, ViraStripe		Reference standard +	Reference standard −	Total	
	Index test +	24	1	25	

Reference	Molins 2016[74]				
(IgM/IgG) – serum], convalescent EM	Index test −	14	202	216	
	Total	38	203	241	
2x2 table [ELISA (WCS) + ELISA (C6) – serum], acute EM		Reference standard +	Reference standard −	Total	
	Index test +	20	1	21	
	Index test −	20	202	222	
	Total	40	203	243	
2x2 table [ELISA (WCS) + ELISA (C6) – serum], convalescent EM		Reference standard +	Reference standard −	Total	
	Index test +	30	1	31	
	Index test −	8	202	210	
	Total	38	203	241	
2x2 table [ELISA (WCS) + IB (VlsE) – serum], acute EM		Reference standard +	Reference standard −	Total	
	Index test +	19	1	20	
	Index test −	21	202	223	
	Total	40	203	243	
2x2 table [ELISA (WCS) + IB (VlsE) – serum], convalescent EM		Reference standard +	Reference standard −	Total	
	Index test +	28	1	29	
	Index test −	10	202	212	
	Total	38	203	241	
2x2 table [ELISA (IgM/IgG) + IB (IgM/IgG) –		Reference standard +	Reference standard −	Total	
	Index test +	8	4	12	

Reference	Molins 2016[74]				
serum], neuroborreliosis	Index test −	2	199	201	
	Total	10	203	213	
2x2 table [ELISA (C6) + IB Marblot (IgM/IgG) – serum], neuroborreliosis		Reference standard +	Reference standard −	Total	
	Index test +	9	2	11	
	Index test −	1	201	202	
	Total	10	203	213	
2x2 table [ELISA (C6) + IB ViraStripe (IgM/IgG) – serum], neuroborreliosis		Reference standard +	Reference standard −	Total	
	Index test +	9	1	10	
	Index test −	1	202	203	
	Total	10	203	213	
2x2 table [ELISA (WCS) + ELISA (C6) – serum], neuroborreliosis		Reference standard +	Reference standard −	Total	
	Index test +	9	1	10	
	Index test −	1	202	203	
	Total	10	203	213	
2x2 table [ELISA (WCS) + IB (VlsE) – serum], neuroborreliosis		Reference standard +	Reference standard −	Total	
	Index test +	9	1	10	
	Index test −	1	202	203	
	Total	10	203	213	
2x2 table [ELISA (IgM/IgG) + IB (IgM/IgG) –		Reference standard +	Reference standard −	Total	
	Index test +	28	4	32	

Reference	Molins 2016[74]				
serum], arthritis	Index test −	1	199	200	
	Total	29	203	232	
2x2 table [ELISA (C6) + IB Marblot (IgM/IgG) – serum], arthritis		Reference standard +	Reference standard −	Total	
	Index test +	29	2	31	
	Index test −	0	201	201	
	Total	29	203	232	
2x2 table [ELISA (C6) + IB ViraStripe (IgM/IgG) – serum], arthritis		Reference standard +	Reference standard −	Total	
	Index test +	28	1	29	
	Index test −	1	202	203	
	Total	29	203	232	
2x2 table [ELISA (WCS) + ELISA (C6) – serum], arthritis		Reference standard +	Reference standard −	Total	
	Index test +	29	1	29	
	Index test −	0	202	202	
	Total	29	203	232	
2x2 table [ELISA (WCS) + IB (VlsE) – serum], arthritis		Reference standard +	Reference standard −	Total	
	Index test +	29	1	30	
	Index test −	0	202	202	
	Total	29	203	232	
2x2 table [ELISA (IgM/IgG) + IB (IgM/IgG) –		Reference standard +	Reference standard −	Total	
	Index test +	7	4	11	

Reference	Molins 2016[74]				
serum], carditis	Index test −	0	199	199	
	Total	7	203	210	
2x2 table [ELISA (C6) + IB Marblot (IgM/IgG) – serum], carditis		Reference standard +	Reference standard −	Total	
	Index test +	6	2	8	
	Index test −	1	201	202	
	Total	7	203	210	
2x2 table [ELISA (C6) + IB ViraStripe (IgM/IgG) – serum], carditis		Reference standard +	Reference standard −	Total	
	Index test +	6	1	7	
	Index test −	1	202	203	
	Total	7	203	210	
2x2 table [ELISA (WCS) + ELISA (C6) – serum], carditis		Reference standard +	Reference standard −	Total	
	Index test +	6	1	7	
	Index test −	1	202	203	
	Total	7	203	210	
2x2 table [ELISA (WCS) + IB (VlsE) – serum], carditis		Reference standard +	Reference standard −	Total	
	Index test +	6	1	7	
	Index test −	1	202	203	
	Total	7	203	210	
2x2 table [ELISA (IgM/IgG) + IB (IgM/IgG) –		Reference standard +	Reference standard −	Total	
	Index test +	86	4	90	

Reference	Molins 2016[74]				
serum], unspecified	Index test −	38	199	237	
	Total	124	203	327	
2x2 table [ELISA (C6) + IB Marblot (IgM/IgG) – serum], unspecifed		Reference standard +	Reference standard −	Total	
	Index test +	84	2	86	
	Index test −	40	201	241	
	Total	124	203	327	
2x2 table [ELISA (C6) + IB ViraStripe (IgM/IgG) – serum], unspecified		Reference standard +	Reference standard −	Total	
	Index test +	84	1	85	
	Index test −	40	202	242	
	Total	124	203	327	
2x2 table [ELISA (WCS) + ELISA (C6) – serum], unspecified		Reference standard +	Reference standard −	Total	
	Index test +	94	1	95	
	Index test −	30	202	232	
	Total	124	203	327	
2x2 table [ELISA (WCS) + IB (VlsE) – serum], unspecified		Reference standard +	Reference standard −	Total	
	Index test +	91	1	92	
	Index test −	33	202	235	
	Total	124	203	327	
Statistical measures	ELISA IgM/IgG + Immunoblot IgM/IgG – serum (acute EM) Sensitivity 0.47 Specificity 0.98				

Reference	Molins 2016[74]
	ELISA IgM/IgG + Immunoblot IgM/IgG – serum (convalescent EM) Sensitivity 0.63 Specificity0.98 ELISA C6 + Immunoblot IgM/IgG Marblot – serum (acute EM) Sensitivity 0.40 Specificity 0.99 ELISA C6 + Immunoblot IgM/IgG ViraStripe – serum (acute EM) Sensitivity 0.42 Specificity 1.00 ELISA C6 + Immunoblot IgM/IgG Marblot – serum (convalescent EM) Sensitivity 0.63 Specificity 0.99 ELISA C6 + Immunoblot IgM/IgG ViraStripe – serum (convalescent EM) Sensitivity 0.63 Specificity 1.00 ELISA WCS + ELISA C6 – serum (acute EM) Sensitivity 0.50 Specificity 1.00 ELISA WCS + ELISA C6 – serum (convalescent EM) Sensitivity 0.79 Specificity 1.00 ELISA WCS + Immunoblot VlsE – serum (acute EM) Sensitivity 0.47

Reference	Molins 2016[74]
	Specificity 1.00 ELISA WCS + Immunoblot VlsE – serum (convalescent EM) Sensitivity 0.74 Specificity 1.00 ELISA IgM/IgG + Immunoblot IgM/IgG – serum (neuroborreliosis) Sensitivity 0.80 Specificity 0.98 ELISA C6 + Immunoblot IgM/IgG Marblot – serum (neuroborreliosis) Sensitivity 0.90 Specificity 0.99 ELISA C6 + Immunoblot IgM/IgG ViraStripe – serum (neuroborreliosis) Sensitivity 0.90 Specificity 1.00 ELISA WCS + ELISA C6 – serum (neuroborreliosis) Sensitivity 0.90 Specificity 1.00 ELISA WCS + Immunoblot VlsE – serum (neuroborreliosis) Sensitivity 0.90 Specificity 1.00 ELISA IgM/IgG + Immunoblot IgM/IgG – serum (arthritis) Sensitivity 0.97 Specificity 0.98 ELISA C6 + Immunoblot IgM/IgG Marblot – serum (arthritis)

Reference	Molins 2016[74]
	Sensitivity 1.00 Specificity 0.99 ELISA C6 + Immunoblot IgM/IgG ViraStripe – serum (arthritis) Sensitivity 0.97 Specificity 1.00 ELISA WCS + ELISA C6 – serum (arthritis) Sensitivity 1.00 Specificity 1.00 ELISA WCS + Immunoblot VlsE – serum (arthritis) Sensitivity 1.00 Specificity 1.00 ELISA IgM/IgG + Immunoblot IgM/IgG – serum (carditis) Sensitivity 1.00 Specificity 0.98 ELISA C6 + Immunoblot IgM/IgG Marblot – serum (carditis) Sensitivity 0.86 Specificity 0.99 ELISA C6 + Immunoblot IgM/IgG ViraStripe – serum (carditis) Sensitivity 0.86 Specificity 1.00 ELISA WCS + ELISA C6 – serum (carditis) Sensitivity 0.86 Specificity 1.00

Reference	Molins 2016[74]
	ELISA WCS + Immunoblot VlsE – serum (carditis) Sensitivity 0.86 Specificity 1.00 ELISA IgM/IgG + Immunoblot IgM/IgG – serum (unspecified Lyme disease) Sensitivity 0.69 Specificity 0.98 ELISA C6 + Immunoblot IgM/IgG Marblot – serum (unspecified Lyme disease) Sensitivity 0.68 Specificity 0.99 ELISA C6 + Immunoblot IgM/IgG ViraStripe – serum (unspecified Lyme disease) Sensitivity 0.68 Specificity 1.00 ELISA WCS + ELISA C6 – serum (unspecified Lyme disease) Sensitivity 0.76 Specificity 1.00 ELISA WCS + Immunoblot VlsE – serum (unspecified Lyme disease) Sensitivity 0.73 Specificity 1.00
Source of funding	None
Limitations	Risk of bias: patient selection, reference standard Indirectness: none

Reference	Peltomaa 2004[85]

Reference	Peltomaa 2004[85]				
Study type	Case-control study				
Study methodology	Data source: serum samples from patients before treatment Recruitment: patients treated by study author at clinic				
Number of patients	n = 47				
Patient characteristics	Age, median: 35 years (4-74) Gender (male to female ratio): 25/22 Family origin: not reported Setting: clinic Country: USA Cases: facial palsy Controls: healthy persons (n=86)				
Target condition(s)	Lyme disease associated facial palsy				
Index test(s) and reference standard	Index tests ELISA (IgM/IgG) + Immunoblot IgM/IgG) Reference standard Clinical diagnosis based on CDC criteria Time between measurement of index test and reference standard: not reported				
2×2 table		Reference standard	Reference standard −	Total	

Reference	Peltomaa 2004[85]				
[ELISA (IgM/IgG) + WB/IB (IgM/IgG) – serum]		+			
	Index test +	47	2	49	
	Index test −	0	86	86	
	Total	47	88	135	
Statistical measures	ELISA IgM/IgG + Immunoblot IgM/IgG (serum) – neuroborreliosis Sensitivity 1.00 Specificity 0.98				
Source of funding	Supported by industry grants				
Limitations	Risk of bias: patient selection, index test, reference standard Indirectness: serious				

Reference	Steere 2008[106]
Study type	Case-control study
Study methodology	Data source: serum samples from patients Recruitment: by 2 primary care physicians
Number of patients	n = 134
Patient characteristics	Age, mean: not reported Gender (male to female ratio): not reported Family origin: not reported Setting: primary care Country: USA

Reference	Steere 2008[106]				
	Cases: EM (n=76), Acute neurologic or cardiac involvement (n=13), Arthritis or chronic neurologic involvement (n=31), Post-Lyme disease symptoms (n=14) Controls: healthy persons (n=136)				
Target condition(s)	EM (single or multiple, acute or convalescent), acute/chronic disseminated Lyme disease				
Index test(s) and reference standard	Index tests ELISA (IgM/IgG) + Immunoblot (IgM/IgG) ELISA (IgM/IgG) + Immunoblot (IgM) ELISA (IgM/IgG) + Immunoblot (IgG) Reference standard EM: CDC criteria and culture-positive Time between measurement of index test and reference standard: not reported				
2×2 table [ELISA (IgM/IgG) + IB (IgM/IgG) – serum], acute disseminated EM		Reference standard +	Reference standard −	Total	
	Index test +	17	2	19	
	Index test −	23	136	159	
	Total	40	138	178	
2×2 table [ELISA (IgM/IgG) + IB (IgM/IgG) – serum], acute EM without dissemination		Reference standard +	Reference standard −	Total	
	Index test +	6	2	8	
	Index test −	30	136	166	
	Total	36	138	174	
2×2 table		Reference standard	Reference standard −	Total	

Reference	Steere 2008[106]				
[ELISA (IgM/IgG) + IB (IgM/IgG) – serum], convalescent EM without dissemination		+			
	Index test +	19	2	21	
	Index test −	17	136	153	
	Total	36	138	174	
2×2 table [ELISA (IgM/IgG) + IB (IgM/IgG) – serum], convalescent disseminated EM		Reference standard +	Reference standard −	Total	
	Index test +	30	2	32	
	Index test −	10	136	146	
	Total	40	138	178	
2×2 table [ELISA (IgM/IgG) + IB (IgM) – serum], acute disseminated EM		Reference standard +	Reference standard −	Total	
	Index test +	15	1	16	
	Index test −	25	136	161	
	Total	40	137	177	
2×2 table [ELISA (IgM/IgG) + IB (IgM) – serum], acute EM without dissemination		Reference standard +	Reference standard −	Total	
	Index test +	4	1	5	
	Index test −	32	136	168	
	Total	36	137	173	
2×2 table [ELISA (IgM/IgG) + IB (IgM) – serum],		Reference standard +	Reference standard −	Total	
	Index test +	14	1	15	
	Index test −	22	136	158	

Reference	Steere 2008[106]				
convalescent EM without dissemination	Total	36	137	173	
2×2 table [ELISA (IgM/IgG) + IB (IgM) – serum], convalescent disseminated EM		Reference standard +	Reference standard −	Total	
	Index test +	28	1	29	
	Index test −	12	136	148	
	Total	40	137	177	
2×2 table [ELISA (IgM/IgG) + IB (IgG) – serum], acute disseminated EM		Reference standard +	Reference standard −	Total	
	Index test +	6	1	7	
	Index test −	34	136	170	
	Total	40	137	177	
2×2 table [ELISA (IgM/IgG) + IB (IgG) – serum], acute EM without dissemination		Reference standard +	Reference standard −	Total	
	Index test +	2	1	3	
	Index test −	34	136	170	
	Total	36	137	173	
2×2 table [ELISA (IgM/IgG) + IB (IgG) – serum], convalescent EM without dissemination		Reference standard +	Reference standard −	Total	
	Index test +	6	1	7	
	Index test −	30	136	166	
	Total	36	137	173	
2×2 table		Reference standard +	Reference standard −	Total	

Reference	Steere 2008[106]				
[ELISA (IgM/IgG) + IB (IgG) – serum], convalescent disseminated EM	Index test +	8	1	9	
	Index test −	32	136	168	
	Total	40	137	177	
2×2 table [ELISA (IgM/IgG) + IB (IgM/IgG) – serum], acute neuritis/carditis		Reference standard +	Reference standard −	Total	
	Index test +	13	2	15	
	Index test −	0	136	136	
	Total	13	138	151	
2×2 table [ELISA (IgM/IgG) + IB (IgM) – serum], acute neuritis/carditis		Reference standard +	Reference standard −	Total	
	Index test +	11	1	12	
	Index test −	2	136	138	
	Total	13	137	150	
2×2 table [ELISA (IgM/IgG) + IB (IgG) – serum], acute neuritis/carditis		Reference standard +	Reference standard −	Total	
	Index test +	11	1	12	
	Index test −	2	136	138	
	Total	13	137	150	
2×2 table [ELISA (IgM/IgG) + IB (IgM/IgG) – serum], arthritis/late neuritis		Reference standard +	Reference standard −	Total	
	Index test +	31	2	33	
	Index test −	0	136	136	
	Total	31	138	169	
2×2 table		Reference standard	Reference standard −	Total	

Reference	Steere 2008[106]				
[ELISA (IgM/IgG) + IB (IgM) – serum], arthritis/late neuritis		+			
	Index test +	7	1	8	
	Index test −	24	136	160	
	Total	31	137	168	
2×2 table [ELISA (IgM/IgG) + IB (IgG) – serum], arthritis/late neuritis		Reference standard +	Reference standard −	Total	
	Index test +	31	1	32	
	Index test −	0	136	136	
	Total	31	137	168	
Statistical measures	ELISA IgM/IgG + Immunoblot IgM/IgG – serum (acute disseminated EM) Sensitivity 0.42 Specificity 0.99 ELISA IgM/IgG + Immunoblot IgM/IgG – serum (acute EM without dissemination) Sensitivity 0.17 Specificity 0.99 ELISA IgM/IgG + Immunoblot IgM/IgG – serum (convalescent EM without dissemination) Sensitivity 0.53 Specificity 0.99 ELISA IgM/IgG + Immunoblot IgM/IgG – serum (convalescent disseminated EM) Sensitivity 0.75 Specificity 0.99 ELISA IgM/IgG + Immunoblot IgM – serum (acute disseminated EM) Sensitivity 0.38 Specificity 0.99				

Reference	Steere 2008[106]
	ELISA IgM/IgG + Immunoblot IgM – serum (acute EM without dissemination) Sensitivity 0.11 Specificity 0.99 ELISA IgM/IgG + Immunoblot IgM – serum (convalescent EM without dissemination) Sensitivity 0.39 Specificity 0.99 ELISA IgM/IgG + Immunoblot IgM – serum (convalescent disseminated EM) Sensitivity 0.70 Specificity 0.99 ELISA IgM/IgG + Immunoblot IgG – serum (acute disseminated EM) Sensitivity 0.15 Specificity 0.99 ELISA IgM/IgG + Immunoblot IgG – serum (acute EM without dissemination) Sensitivity 0.06 Specificity 0.99 ELISA IgM/IgG + Immunoblot IgG – serum (convalescent EM without dissemination) Sensitivity 0.17 Specificity 0.99 ELISA IgM/IgG + Immunoblot IgG – serum (convalescent disseminated EM) Sensitivity 0.20 Specificity 0.99 ELISA IgM/IgG + Immunoblot IgM/IgG – serum (acute neuritis/carditis) Sensitivity 1.00 Specificity 0.99

Reference	Steere 2008[106]
	ELISA IgM/IgG + Immunoblot IgM – serum (acute neuritis/carditis) Sensitivity 0.85 Specificity 0.99 ELISA IgM/IgG + Immunoblot IgG – serum (acute neuritis/carditis) Sensitivity 0.85 Specificity 0.99 ELISA IgM/IgG + Immunoblot IgM/IgG – serum (arthritis/late neuritis) Sensitivity 1.00 Specificity 0.99 ELISA IgM/IgG + Immunoblot IgM – serum (arthritis/late neuritis) Sensitivity 0.23 Specificity 0.99 ELISA IgM/IgG + Immunoblot IgG – serum (arthritis/late neuritis) Sensitivity 1.00 Specificity 0.99
Source of funding	Supported by government and charity grants
Limitations	Risk of bias: patient selection, index test, reference standard Indirectness: none

Reference	Trevejo 2001[113]
Study type	Case-control study
Study methodology	Data source: serum samples from patients taken a median of 4 days after illness onset (acute phase) or 36 days after illness onset (convalescent phase)

Reference	Trevejo 2001[113]				
	Recruitment: not reported				
Number of patients	n = 74				
Patient characteristics	Age, median: 41 years (3-83) Gender (male to female ratio): 41/33 Family originFamily origin: not reported Setting: primary care Country: USA Cases: EM (66 acute phase, 55 convalescent phase) Controls: healthy controls (n=38)				
Target condition(s)	EM				
Index test(s) and reference standard	Index tests ELISA (IgM/IgG) + Immunoblot (IgM/IgG) Reference standard Clinical diagnosis Time between measurement of index test and reference standard: not reported				
2×2 table [ELISA (IgM/IgG) + IB (IgM/IgG) –		Reference standard +	Reference standard −	Total	
	Index test +	27	0	27	
	Index test −	39	37	76	

Reference	Trevejo 2001[113]				
serum], EM (acute phase) simplified approach	Total	66	37	103	
2×2 table [ELISA (IgM/IgG) + IB (IgM/IgG) – serum], EM (acute phase) CDC approach		Reference standard +	Reference standard −	Total	
	Index test +	21	0	21	
	Index test −	45	37	82	
	Total	66	37	103	
2×2 table [ELISA (IgM/IgG) + IB (IgM/IgG) – serum], EM (convalescent phase) CDC approach		Reference standard +	Reference standard −	Total	
	Index test +	16	0	16	
	Index test −	39	37	76	
	Total	55	37	92	
2×2 table [ELISA (IgM/IgG) + IB (IgM/IgG) – serum], EM (convalescent phase) simplified approach		Reference standard +	Reference standard −	Total	
	Index test +	39	0	39	
	Index test −	16	37	53	
	Total	55	37	92	
Statistical measures	ELISA IgM/IgG + Immunoblot IgM/IgG – serum (EM, acute phase) simplified approach Sensitivity 0.41 Specificity 1.00 ELISA IgM/IgG + Immunoblot IgM/IgG – serum (EM, acute phase) CDC approach Sensitivity 0.32				

Reference	Trevejo 2001[113]
	Specificity 1.00 ELISA IgM/IgG + Immunoblot IgM/IgG – serum (EM, convalescent phase) CDC approach Sensitivity 0.29 Specificity 1.00 ELISA IgM/IgG + Immunoblot IgM/IgG – serum (EM, convalescent phase) simplified approach Sensitivity 0.71 Specificity 1.00
Source of funding	Not reported
Limitations	Risk of bias: patient selection, reference standard Indirectness: none

Reference	Weiner 2015[120]
Study type	Case-control study
Study methodology	Data source: Lyme serum repository composed of serum obtained from well-characterised Lyme disease patients, control serum from healthy individuals and patients with other diseases Recruitment: not reported
Number of patients	n = 70 cases, 80 controls
Patient characteristics	Age, mean (SD): not reported Gender (male to female ratio): not reported Family originFamily origin: not reported

Reference	Weiner 2015[120]				
	Setting: Division of Vector Borne Diseases, Bacterial Diseases Branch, CDC Country: USA Cases: Lyme with EM - acute and convalescent Lyme (46), neuroborreliosis (10), Lyme carditis (6), Lyme arthritis (8) Controls: healthy patients (32), other diseases (48)				
Target condition(s)	Lyme disease				
Index test(s) and reference standard	Index tests ELISA (IgM/IgG) + Immunoblot (IgM/IgG) ELISA (IgM/IgG) + Immunoblot (IgM) ELISA (IgM/IgG) + Immunoblot (IgG) Reference standard Clinical diagnosis Time between measurement of index test and reference standard: not reported				
2x2 table [ELISA (IgM/IgG) + IB (IgM/IgG) – serum], acute EM		Reference standard +	Reference standard −	Total	
	Index test +	7	0	7	
	Index test −	16	32	48	
	Total	23	32	55	
2x2 table [ELISA (IgM/IgG) + IB (IgM/IgG) – serum], acute		Reference standard +	Reference standard −	Total	
	Index test +	18	0	18	
	Index test −	5	32	37	

Reference	Weiner 2015[120]				
EM	Total	23	32	55	
2x2 table [ELISA (IgM/IgG) + IB (IgM) – serum], acute EM		Reference standard +	Reference standard −	Total	
	Index test +	7	0	7	
	Index test −	16	0	16	
	Total	23	0	23	
2x2 table [ELISA (IgM/IgG) + IB (IgM) – serum], acute EM		Reference standard +	Reference standard −	Total	
	Index test +	16	0	16	
	Index test −	7	32	39	
	Total	23	32	55	
2x2 table [ELISA (IgM/IgG) + IB (IgG) – serum], acute EM		Reference standard +	Reference standard −	Total	
	Index test +	1	0	1	
	Index test −	22	32	54	
	Total	23	32	55	
2x2 table [ELISA (IgM/IgG) + IB (IgG) – serum], acute EM		Reference standard +	Reference standard −	Total	
	Index test +	7	0	7	
	Index test −	16	32	48	
	Total	23	32	55	
2x2 table [ELISA (IgM/IgG) + IB (IgM/G) – serum],		Reference standard +	Reference standard −	Total	
	Index test +	9	0	9	
	Index test −	1	32	33	

Reference	Weiner 2015[120]				
neuroborreliosis	Total	10	32	42	
2x2 table [ELISA (IgM/IgG) + IB (IgM) – serum], neuroborreliosis		Reference standard +	Reference standard −	Total	
	Index test +	9	0	9	
	Index test −	1	32	33	
	Total	10	32	42	
2x2 table [ELISA (IgM/IgG) + IB (IgG) – serum], neuroborreliosis		Reference standard +	Reference standard −	Total	
	Index test +	4	0	4	
	Index test −	6	32	38	
	Total	10	32	42	
2x2 table [ELISA (IgM/IgG) + IB (IgM/G) – serum], arthritis		Reference standard +	Reference standard −	Total	
	Index test +	8	0	8	
	Index test −	0	32	32	
	Total	8	32	40	
2x2 table [ELISA (IgM/IgG) + IB (IgM) – serum], arthritis		Reference standard +	Reference standard −	Total	
	Index test +	1	0	1	
	Index test −	7	32	39	
	Total	8	32	40	
2x2 table [ELISA (IgM/IgG) + IB (IgG) – serum], arthritis		Reference standard +	Reference standard −	Total	
	Index test +	8	0	8	
	Index test −	0	32	32	

Reference	Weiner 2015[120]				
	Total	8	32	40	
2x2 table [ELISA (IgM/IgG) + IB (IgM/G) – serum], carditis		Reference standard +	Reference standard −	Total	
	Index test +	5	0	5	
	Index test −	1	32	33	
	Total	6	32	38	
2x2 table [ELISA (IgM/IgG) + IB (IgM) – serum], carditis		Reference standard +	Reference standard −	Total	
	Index test +	4	0	4	
	Index test −	2	32	34	
	Total	6	32	38	
2x2 table [ELISA (IgM/IgG) + IB (IgG) – serum], carditis		Reference standard +	Reference standard −	Total	
	Index test +	3	0	3	
	Index test −	3	32	35	
	Total	6	32	38	
2x2 table [ELISA (IgM/IgG) + IB (IgM/G) – serum], unspecified		Reference standard +	Reference standard −	Total	
	Index test +	47	0	47	
	Index test −	23	32	55	
	Total	70	32	102	
2x2 table [ELISA (IgM/IgG) + IB (IgM) – serum], unspecified		Reference standard +	Reference standard −	Total	
	Index test +	37	0	37	
	Index test −	33	32	65	

Reference	Weiner 2015[120]				
	Total	70	32	102	
2x2 table [ELISA (IgM/IgG) + IB (IgG) – serum], unspecified		Reference standard +	Reference standard −	Total	
	Index test +	23	0	23	
	Index test −	47	32	79	
	Total	70	32	102	
Statistical measures	ELISA IgM/IgG + Immunoblot IgM/IgG – serum (EM, acute phase) Sensitivity 0.30 Specificity 1.00 ELISA IgM/IgG + Immunoblot IgM/IgG – serum (EM, convalescent phase) Sensitivity 0.78 Specificity 1.00 ELISA IgM/IgG + Immunoblot IgM – serum (EM, acute phase) Sensitivity 0.30 Specificity not estimable ELISA IgM/IgG + Immunoblot IgM – serum (EM, convalescent phase) Sensitivity 0.70 Specificity 1.00 ELISA IgM/IgG + Immunoblot IgG – serum (EM, acute phase) Sensitivity 0.04 Specificity 1.00 ELISA IgM/IgG + Immunoblot IgG – serum (EM, convalescent phase) Sensitivity 0.30 Specificity 1.00				

Reference	Weiner 2015[120]
	ELISA IgM/IgG + Immunoblot IgM/IgG – serum (neuroborreliosis) Sensitivity 0.90 Specificity 1.00 ELISA IgM/IgG + Immunoblot IgM – serum (neuroborreliosis) Sensitivity 0.90 Specificity 1.00 ELISA IgM/IgG + Immunoblot IgG – serum (neuroborreliosis) Sensitivity 0.40 Specificity 1.00 ELISA IgM/IgG + Immunoblot IgM/IgG – serum (arthritis) Sensitivity 1.00 Specificity 1.00 ELISA IgM/IgG + Immunoblot IgM – serum (arthritis) Sensitivity 0.13 Specificity 1.00 ELISA IgM/IgG + Immunoblot IgG – serum (arthritis) Sensitivity 1.00 Specificity 1.00 ELISA IgM/IgG + Immunoblot IgM/IgG – serum (carditis) Sensitivity 0.83 Specificity 1.00 ELISA IgM/IgG + Immunoblot IgM – serum (carditis) Sensitivity 0.67

Reference	Weiner 2015[120]
	Specificity 1.00 ELISA IgM/IgG + Immunoblot IgG – serum (carditis) Sensitivity 0.50 Specificity 1.00 ELISA IgM/IgG + Immunoblot IgM/IgG – serum (unspecified Lyme disease) Sensitivity 0.67 Specificity 1.00 ELISA IgM/IgG + Immunoblot IgM – serum (unspecified Lyme disease) Sensitivity 0.53 Specificity 1.00 ELISA IgM/IgG + Immunoblot IgG – serum (unspecified Lyme disease) Sensitivity 0.33 Specificity 1.00
Source of funding	American Society for Microbiology/CDC Program in Infectious Disease and Public Health Microbiology and the Association for Public Health Laboratories
Limitations	Risk of bias: patient selection, index test, reference standard Indirectness: none
Comments	Standard CDC algorithm used for IB (IgG only after 30 days)

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