British Thoracic Society

NICE National Institute for Health and Care Excellence

Healthcare Improvement Scotland | SIGN

Final

Asthma: diagnosis, monitoring and chronic asthma management (update)

[H] Evidence reviews for bronchial challenge with histamine and methacholine for the diagnosis of asthma

BTS/NICE/SIGN collaborative guideline NG245

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Final

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1.1 Review question

In people under investigation for asthma, what is the diagnostic test accuracy and clinical and cost (direct) effectiveness with histamine and methacholine?

1.1.1 Introduction

Asthma can be a difficult condition to diagnose, and it is not clear which tests are most useful in supporting a diagnosis. Histamine and methacholine both bind with receptors on airway smooth muscle and stimulate muscle contraction, and therefore airway narrowing. They are used in bronchial challenge tests whereby they are given in increasing doses via a nebuliser until a certain amount of narrowing has occurred (determined by spirometry) or the maximum dose has been given. Typically, people with asthma have an exaggerated responsive (bronchial hyperresponsiveness) to these agents compared to healthy people. Histamine and methacholine are therefore potentially useful in establishing a diagnosis of asthma and this evidence review was carried out to determine their clinical and cost-effectiveness as diagnostic tests.

1.1.2 Summary of the protocol

For full details see the review protocol in Appendix A.

No test-and-treat evidence was found so only the diagnostic accuracy evidence was reported.

Table 1: PICO characteristics of diagnostic accuracy review question

Population	People with suspected asthma (presenting with respiratory symptoms). **Ages stratified into the following 2 groups:** • Children/young people (5-16 years old) • Adults (≥17 years old) <u>Stratified on smoking status:</u> • Smokers • Non-smokers • Mixed/not reported **Exclusion**: • Children under 5 years old. • People on steroid medication (washout period minimum of 4 weeks for inclusion) • Occupational asthma /allergens
Target condition	Asthma
Index test	• Histamine PC20 and PD20 • Methacholine PC20 and PD20
Reference standard	Physician diagnosis of asthma based on symptoms plus an objective test from any one of the following: • Peak flow variability (cut-off value of more than 20% variability as indication of a positive test)

	• Bronchodilator reversibility (cut-off value of an improvement in FEV1 of more than or equal to 12%, and an increase in volume of more than or equal to 200mls as indication of a positive test). • FeNO Where no evidence is available using the cut-off values specified above, evidence will be included from studies using a reference standard of physician diagnosis with an objective test using an alternative threshold. Where no evidence is available from studies using physician diagnosis and an objective test, evidence will be included from studies using physician diagnosis based on symptoms alone, or patient report of a previous physician diagnosis. Maximum interval between initial diagnosis and confirmation of 'asthma' diagnosis: 12 months
Statistical measures	Asthma diagnosis • Sensitivity thresholds: upper 90%, lower 10% • Specificity thresholds: upper 80%, lower 50% • Raw data to calculate 2x2 tables to calculate sensitivity and specificity • Negative predictive value (NPV), Positive predictive value (PPV)
Study design	• Cross sectional studies • Cohort studies

1.1.3 Methods and process

This evidence review was developed using the methods and process described in Developing NICE guidelines: the manual. Methods specific to this review question are described in the review protocol in appendix A and the methods document.

Declarations of interest were recorded according to NICE's conflicts of interest policy.

1.1.4 Diagnostic evidence

1.1.4.1 Included studies

Eight cross-sectional diagnostic accuracy studies were included in the review (Alberts, et al., 1987, Anderson, et al., 2009, Hedman, et al., 1998, Kowal, et al., 2009, Louis, et al., 2020, Popovic-Grle, et al., 2002, Porpodis, et al., 2017, Zaczeniuk, et al., 2015) these are summarised in Table 2 below. Evidence from these studies is summarised in the clinical evidence summary below in Table 3and references in 1.3 References . The assessment of the evidence quality was conducted with emphasis on test sensitivity and specificity as this was identified by the committee as the primary measure in guiding decision-making. The committee set clinical decision thresholds as sensitivity: upper= 90% and lower= 10%, specificity: upper= 80% and lower= 50%. Values above the upper threshold indicated a test would be recommended and values below the lower threshold indicated a test is of no clinical use.

See also the study selection flow chart in Appendix C, sensitivity and specificity forest plots in Appendix E, and study evidence tables in Appendix D.

1.1.4.2 Excluded studies

Two studies included in the previous NICE guidance on this topic were excluded from this review. One study was excluded due to containing a population not relevant to the current review protocol, namely due to the inclusion of participants already diagnosed with asthma at

study entry. The other study was excluded due to including participants receiving inhaled corticosteroids and not including an adequate washout period prior to the study. See the excluded studies list in Appendix H.

1.1.5 Summary of studies included in the diagnostic evidence

Table 2: Summary of studies included in the evidence review

Study	Population	Target condition	Index test	Reference standard	Comments
Alberts 1987 (Alberts et al., 1987)	N=19 people referred to a bronchial challenge laboratory with symptoms suggestive of asthma in whom a diagnosis could be confirmed Age, mean (range): 38.2 (14-67) years USA	Asthma	Methacholine challenge test Cut-off: PC20 FEV_1 ≤16 mg/mL	Diagnosis of asthma, or an alternate diagnosis based on clinical information, by the referring physician	Retrospective cross-sectional study Age: Adults ICS use: Not reported Smoking status: Not reported Indirectness: Downgraded by two increments due to population (ICS use not reported, smoking status not reported and included both children/young people and adults) indirectness
Anderson 2009 (Anderson et al., 2009)	N=375 people with signs and symptoms suggestive of asthma according to NIH questionnaire and without a firm diagnosis of asthma Age, mean ± SD (range): 24.3 ± 10.2 (6-50) years USA	Asthma	Methacholine challenge test Cut-off: PC20 FEV_1 ≤16 mg/mL	Clinician diagnosis of asthma based on history, examination, skin prick tests, FEV_1 reversibility and exercise challenge results	Prospective cross-sectional study Age: Adults ICS use: 4-week washout Smoking status: Smokers excluded Indirectness: Downgraded by one increment due to population (included both children/young people and adults)

Study	Population	Target condition	Index test	Reference standard	Comments
Hedman 1998 (Hedman et al., 1998)	N= 230 people referred to a pulmonary department due to dyspnoea, wheezing or a cough of unknown cause Age, mean (SD): Asthma +ive; 44.0 (16.0), asthma -ive; 44.6 (16.2) Finland	Asthma	Methacholine challenge test Cut-offs: PD20 and PD15 FEV_1 ≤6900 µg	Asthma diagnosis by chest physician using ATS guidelines, including positivity according to bronchial reversibility, PEF variability, skin prick test or exercise test	Prospective cross-sectional study Age: Adults ICS use: 4-week washout Smoking status: Mixed – 23% and 16% current smokers in bronchial hyperresponsive +ive and -ive, respectively Indirectness: Downgraded by one increment due to population (mixed smoking and non-smoking participants) indirectness
Kowal 2009 (Kowal et al., 2009)	N=540 people referred by their family doctor to an asthma clinic for evaluation of chronic cough Age, mean (range): 26.5 (18-45) years Poland	Asthma	Histamine challenge test Cut-off: PC20 FEV_1 ≤8 mg/mL	Asthma was diagnosed over a 6-month period, based on demonstrated signification diurnal changes in PEF or significant bronchial reversibility upon administration of salbutamol	Prospective cross-sectional study Age: Adults ICS use: Therapy naïve Smoking status: Smokers excluded Indirectness: Downgraded by one increment due to reference standard (unclear if clinician diagnosis was involved) indirectness
Louis 2020 (Louis et al., 2020)	Patients with intermittent or chronic respiratory	Asthma	Methacholine challenge	Bronchodilator response to 400µg salbutamol	Retrospective cross-sectional study

Study	Population	Target condition	Index test	Reference standard	Comments
	symptoms referred by two asthma physicians for diagnosis (FEV_1 ≥70% predicted for inclusion) N=194; mean age (SD): 49 (16) years Belgium		Cut-off: PC20 ≤8 mg/mL		Age: Adults ICS use: Not previously treated for asthma Smoking status: Mixed (22% current smokers) Indirectness: Downgraded by two increments due to population (mixed smoking and non-smoking participants) and reference standard (unclear if clinician diagnosis was involved) indirectness
Popovic Grle 2002 (Popovic-Grle et al., 2002)	N=195 people treated for breathlessness, or referred by their GP because of suspected asthma, at an outpatient allergology department Age, mean (SD): 36.8 (6.2) Croatia	Asthma	Methacholine challenge test Cut-off: PC20 FEV_1 ≤8 mg/mL	Diagnosis of asthma based on questionnaire, history, symptoms and bronchial reversibility	Prospective cross-sectional study Age: Adults ICS use: Not reported Smoking status: 12% smokers Indirectness: Downgraded by one increment due to population (ICS use not reported and mixed smoking and non-smoking participants)
Porpodis 2017 (Porpodis et al., 2017)	N=88 people with asthma related symptoms in the past month visiting an asthma clinic for asthma diagnosis	Asthma	Methacholine challenge test Cut-off: PC20 FEV_1 ≤16 mg/mL	Asthma diagnosis according to GINA guidelines: combination of at least a ≥12% (and ≥200 mL) increase in	Prospective cross-sectional study Age: Adults ICS use: Treatment naïve

Study	Population	Target condition	Index test	Reference standard	Comments
	Age, mean (SD): 38.56 (16.73) years Greece			baseline FEV1 after albuterol, along with new symptoms of coughing, wheezing, or shortness of breath over the past month	Smoking status: 15% current smokers Indirectness: Downgraded by one increment due to population (mixed smoking and non-smoking participants) indirectness
Zaczeniuk 2015 (Zaczeniuk et al., 2015)	N=90 people attending an allergic outpatient clinic because of post-exercise symptoms such as cough and shortness of breath Age, range: 10-18 years Poland	Asthma	Methacholine challenge test Cut-off: PD20 FEV_1 ≤0.72 mg	Diagnosis of asthma established based on symptoms, physical examination and positive bronchial reversibility	Prospective cross-sectional study Age: Children/young people ICS use: ICS naïve Smoking status: Smokers excluded Indirectness: Downgraded by one increment due to population (inclusion of both children/young people and adults) indirectness

See Appendix D for full evidence tables.

1.1.6 Summary of the diagnostic evidence

The assessment of the evidence quality was conducted with emphasis on test sensitivity and specificity as this was identified by the committee as the primary measure in guiding decision-making. The committee set clinical decision thresholds as sensitivity: upper= 90% and lower= 10%, specificity: upper= 80% and lower= 50%. Values above the upper threshold indicated a test would be recommended and values below the lower threshold indicated a test is of no clinical use.

Table 3: Clinical evidence summary: histamine and methacholine challenge tests for the diagnosis of asthma in children/young people

Studies	N	Risk of bias	Inconsistency	Indirectness	Imprecision	Effect size (95%CI)	Quality
Methacholine challenge (PD20 FEV_1 ≤0.72 mg) vs clinician diagnosis and bronchodilator reversibility							
1 cross-sectional study	90	Very serious[1]	Not serious	Serious[2]	Serious[3]	Sensitivity= 0.90 (0.76-0.97)	VERY LOW
		Very serious[1]	Not serious	Serious[2]	Serious[4]	Specificity= 0.82 (0.69-0.92)	VERY LOW

1. *Downgraded by two increments due to concerns arising from the method of participant selection (recruitment method unclear), the interpretation of the index test and reference standard (unclear if blinded) and the flow and timing of patients through the study (11 patients excluded due to missing test data)*
2. *Downgraded by one increment due to population (inclusion of both children/young people and adults) indirectness*
3. *Downgraded by one increment due to the 95%CI overlapping the threshold corresponding to 'high sensitivity' (90%)*
4. *Downgraded by one increment due to the 95%CI overlapping the threshold corresponding to 'high specificity' (80%)*

Table 4: Clinical evidence summary: histamine and methacholine challenge tests for the diagnosis of asthma in non-smoking adults

Studies	N	Risk of bias	Inconsistency	Indirectness	Imprecision	Effect size (95%CI)	Quality
Methacholine challenge (PC20 FEV_1 ≤16 mg/mL) vs clinician diagnosis with exercise challenge, skin prick and bronchodilator reversibility tests							
1 cross-sectional study	375	Very serious[1]	Not serious	Serious[2]	Not serious	Sensitivity= 0.51 (0.44-0.57)	VERY LOW
		Very serious[1]	Not serious	Serious[2]	Very serious[3]	Specificity= 0.75 (0.67-0.82)	VERY LOW
Histamine challenge (PC20 FEV_1 ≤8 mg/mL) vs diagnosis with PEF variability or bronchodilator reversibility tests							
1 cross-sectional study	540	Very serious[4]	Not serious	Serious[5]	Serious[6]	Sensitivity= 0.93 (0.89-0.96)	VERY LOW
		Very serious[4]	Not serious	Serious[5]	Not serious	Specificity= 1.00 (0.99-1.00)	VERY LOW

1. *Downgraded by two increments due to concerns arising from the method of participant selection (method not reported) and flow and timing of participants through the study (16 participants excluded from analysis)*
2. *Downgraded by one increment due to population (mixed children/young people and adults) indirectness*
3. *Downgraded by two increments due to the 95%CI overlapping the threshold corresponding to both 'low and high specificity' (50% and 80%)*
4. *Downgraded by two increments due to concerns arising from the method of participant selection (method not reported) and interpretation of the index test and reference standard (unclear if blinded)*
5. *Downgraded by one increment due to reference standard (unclear if clinician decision was involved in diagnosis) indirectness*
6. *Downgraded by one increment due to the 95%CI overlapping the threshold corresponding to 'high sensitivity' (90%)*

Table 5: Clinical evidence summary: histamine and methacholine challenge tests for the diagnosis of asthma in adults with mixed/not reported smoking status

Studies	N	Risk of bias	Inconsistency	Indirectness	Imprecision	Effect size (95%CI)	Quality
Methacholine challenge (PC20 FEV_1 ≤8 mg/mL) vs clinician diagnosis with bronchodilator reversibility							
1 cross-sectional study	195	Serious[1]	Not serious	Very serious[2]	Not serious	Sensitivity= 0.97 (0.93-0.99)	VERY LOW
		Serious[1]	Very serious[3]	Very serious[2]	Serious[4]	Specificity= 0.83 (0.71-0.92)	VERY LOW
Methacholine challenge (PC20 FEV_1 ≤8 mg/mL) vs diagnosis with bronchodilator reversibility							
1 cross-sectional study	194	Serious[5]	Not serious	Very serious[6]	Serious[7]	Sensitivity= 0.82 (0.66-0.92)	VERY LOW
		Serious[5]	Very serious[3]	Very serious[6]	Not serious	Specificity= 0.30 (0.23-0.38)	VERY LOW
Methacholine challenge (PC20 FEV_1 ≤16 mg/mL) vs bronchodilator reversibility with/without clinician diagnosis or clinician diagnosis without an objective test							
3 cross-sectional studies	301	Very serious[8]	Very serious[3]	Very serious[9]	Serious[7]	Sensitivity= 0.72 (0.33-0.95	VERY LOW
		Very serious[8]	Very serious[3]	Very serious[9]	Very serious[10]	Specificity= 0.58 (0.23-0.98)	VERY LOW
Methacholine challenge (PD20 FEV_1 ≤6900 µg) vs clinician diagnosis and skin prick testing, PEF variability, bronchodilator reversibility or exercise challenge tests							
1 cross-sectional study	230	Not serious	Not serious	Serious[11]	Not serious	Sensitivity= 0.77 (0.65-0.87)	MODERATE
		Not serious	Not serious	Serious[11]	Serious[4]	Specificity= 0.82 (0.75-0.87)	LOW

1. *Downgraded by one increment due to concerns arising from the interpretation of the index test and reference standard (unclear if blinded in both studies)*
2. *Downgraded by two increments due to population (mixed smoking status and ICS use not reported) indirectness*
3. *Downgraded by two increments due to substantial differences between the point estimate and 95%CI reported in studies reporting the same threshold*
4. *Downgraded by one increment due to the 95%CI overlapping the threshold corresponding to 'high specificity' (80%)*
5. *Downgraded by one increment due to concerns arising from the method of participant selection (method not reported)*
6. *Downgraded by two increments due to population (mixed smoking status) and reference standard (unclear clinician decision involved in diagnosis) indirectness*
7. *Downgraded by one increment due to the 95%CI overlapping the threshold corresponding to 'high sensitivity' (90%)*
8. *Downgraded by two increments due to concerns arising from the method of participant selection (method not reported), interpretation of the index test and reference standard (unclear if blinded) and the flow and timing of participants through studies (participants missing from final analysis in two of three studies)*
9. *Downgraded by two increments due to population (mixed smoking status and ICS use not reported) and reference standard (unclear clinician decision in diagnosis) indirectness*
10. *Downgraded by two increments due to the 95%CI overlapping the threshold corresponding to both 'low and high specificity' (50% and 80%)*
11. *Downgraded by one increment due to population (mixed smoking status) indirectness*

1.1.7 Economic evidence

1.1.7.1 Included studies

No health economic studies were included.

1.1.7.2 Excluded studies

One economic study relating to this review question was identified but was excluded due to limited applicability(Kennedy, et al., 2007). This is listed in Appendix H, with reasons for exclusion given.

See also the health economic study selection flow chart in Appendix F.

1.1.8 Summary of included economic evidence

None.

1.1.9 Economic model

A health economic model was conducted focusing on sequences and combinations of diagnostic tests. This is reported in Evidence review 1.11.

1.1.10 Unit costs

Relevant unit costs are provided below to aid consideration of cost effectiveness.

Table 6: Cost of a bronchial challenge test

Resource	Unit costs	Source
Bronchial challenge test with methacholine	£179.49	National Cost Collection 2021-22 – DZ36Z(NHS England, 2022)

1.1.11 Evidence statements

Economic

- No relevant economic evaluations were identified.

1.2 The committee's discussion and interpretation of the evidence

1.2.1. The outcomes that matter most

Test and treat studies

The outcomes considered for this review were: severe asthma exacerbations, mortality, quality of life, asthma control, hospital admissions, reliever/rescue medication use, lung function (change in FEV1 or morning PEF – average over at least 7 days for morning PEF), adverse events (linear growth, pneumonia frequency, adrenal insufficiency, bone mineral density), inflammatory markers; exhaled nitric oxide (continuous outcome at ≥8 weeks). For the purpose of decision making, all outcomes were considered equally important and were therefore rated as critical by the committee. No relevant evidence was identified for any of the outcomes.

Diagnostic accuracy

The committee considered the diagnostic measures of sensitivity and specificity of the index test for diagnosing asthma as well as the positive and negative predictive values where these were reported by the studies. Clinical decision thresholds were set by the committee as sensitivity/specificity 0.9 and 0.8 above which a test would be recommended and 0.1 and 0.5 below which a test is of no clinical use. The committee were interested in establishing whether there was an optimal cut-off value of bronchial challenge testing with histamine or methacholine with sufficiently high sensitivity and specificity to be useful in making a diagnosis of asthma, but also in whether there are separate cut-off values which could usefully help either rule in or rule out an asthma diagnosis.

1.2.2 The quality of the evidence

Test and treat studies

No relevant clinical studies were identified comparing the clinical effectiveness of diagnosis of asthma based on bronchial challenge with histamine or methacholine, in terms of the clinical outcomes examined.

Diagnostic accuracy

Eight cross-sectional studies were included in this review, seven in adults and one in children/young people. In the adolescent/adult studies, two studies included only non-

smoking participants whilst the other five included a mixture of smoking and non-smoking participants or did not report smoking status. Seven studies examined the diagnostic accuracy of bronchial challenge testing with methacholine and a single study examined the diagnostic accuracy of bronchial challenge testing with histamine. A variety of cut-off values were applied in the included studies. Six studies expressed cut-offs as provocative concentration, with two studies applying a cut-off of PC20 FEV_1 ≤8 mg/mL (one with histamine and one with methacholine) and four studies using PC20 FEV_1 ≤16 mg/mL methacholine. Two studies expressed cut-offs as provocative dose of methacholine, with one study using a PD20 FEV_1 of ≤0.72 mg and another study using a PD20 FEV_1 ≤6900 µg.

Evidence ranged from moderate to very low quality. All evidence was downgraded by at least one increment due to risk of bias, typically due to unclear participants recruitment methods, unclear blinding on index test and reference standard results and less frequently due to missing participants in the reported data. Half of the evidence was downgraded due to indirectness, typically due to not reporting the ICS use of participants, mixing smoking and non-smoking participants or due to reference standard indirectness where it was unclear if asthma was diagnosed using protocol-specified criteria.

1.2.3 Benefits and harms

Children and young people

Very low-quality evidence from one study reported the accuracy of methacholine challenge testing using a cut-off of PD20 FEV_1 ≤0.72 mg compared to a clinician diagnosis with bronchodilator reversibility testing as an objective test. This evidence showed both a high sensitivity and specificity of 0.90 and 0.82, respectively, meeting the decision-making threshold for both outcomes. However, this evidence was at risk of bias due to an unclear recruitment method, limited detail over blinding of test results and missing outcome data from 10% of participants. Furthermore, this evidence was downgraded due to indirectness resulting from the inclusion of both children/young people and adolescent/adults, with an age range of 10-18 years being reported. Finally, imprecision was seen in both estimates, partially reflecting the small number of participants contributing to this evidence (n=90). Despite these concerns, the committee agreed that the evidence showed that bronchial challenge tests could be carried out by children and are useful in supporting a diagnosis of asthma.

Non-smoking adults

Very low-quality evidence from one study reported the accuracy of methacholine bronchial challenge testing using a cut-off of PC20 FEV_1 ≤16 mg/mL compared to a clinician diagnosis with exercise challenge, skin prick and bronchodilator reversibility tests. This evidence reported a sensitivity of 0.51 and a specificity of 0.75, neither of which met the threshold for decision-making.

Very low-quality evidence from one study reported the accuracy of histamine bronchial challenge testing using a cut-off of PC20 FEV_1 ≤8 mg/mL compared to diagnosis with peak flow variability or bronchodilator reversibility tests. This evidence reported a sensitivity of 0.93 and specificity of 1.00, meeting both thresholds for decision-making. However, the committee acknowledged the limitations of the evidence, namely due to risk of bias arising from an unclear recruitment method and unclear blinding of test results, and indirectness due to a lack of clarity over a clinician decision in the reference standard asthma diagnosis.

Adults with mixed or unreported smoking status

Very low-quality evidence from two studies reported the accuracy of methacholine bronchial challenge testing using a cut-off of PC20 FEV_1 ≤8 mg/mL. The first used a clinician diagnosis with bronchodilator reversibility testing as the reference standard, reporting both a high sensitivity of 0.97 and specificity of 0.83, both of which met the decision-making threshold. This evidence was at risk of bias due to unclear blinding of test results and was downgraded

due to indirectness as a result of mixing smoking and non-smoking participants, and not reporting pre-study ICS use. The second study used a reference diagnosis with bronchodilator reversibility alone, reporting a high sensitivity of 0.82 and a low specificity 0.30, neither of which met the decision-making threshold. This evidence was at risk of bias due to an unclear recruitment method and was downgraded due to indirectness resulting from mixing smoking and non-smoking participants and using a reference standard that didn't include a clinician decision in the final diagnosis. Given the differences between the estimates of specificity reported by the individual studies, both pieces of evidence were further downgraded due to inconsistency.

Very low-quality evidence from three studies reported the accuracy of methacholine bronchial challenge testing using a cut-off of PC20 FEV_1 ≤16 mg/mL. After meta-analysis, the pooled estimate of sensitivity from this evidence was 0.72, and specificity was 0.58, neither of which met the decision-making threshold. This evidence was at risk of bias arising from unclear recruitment methods, unclear blinding procedures and missing data. Additionally, indirectness was present due to the evidence containing a mixture of smoking and non-smoking participants, pre-study ICS status not being reported, and the absence of a clinician decision in the final asthma diagnosis. Furthermore, there was significant inconsistency seen in the estimates of both sensitivity and specificity across the included studies, leading to further downgrading.

Moderate-low-quality evidence from one study reported the diagnostic accuracy of methacholine bronchial challenge testing using a cut-off of PD20 FEV_1 ≤6900 µg, showing a moderate sensitivity of 0.77 and high specificity of 0.82. This evidence was downgraded for indirectness due to containing a mixture of smoking and non-smoking participants, with the specificity estimate being further downgraded due to the 95%CI overlapping the upper decision-making threshold.

The results overall are inconsistent, probably because of a combination of differences in study populations, reference standards and challenge test protocols. The cut-offs chosen to define asthma also varied which will affect the sensitivity and specificity results. Nonetheless, challenge tests generally showed better accuracy than other tests, and the committee noted that with a sensible cut-off value they can produce good sensitivity without sacrificing specificity too much, suggesting that they could be useful as a rule-out test for asthma.

1.2.4 Cost effectiveness and resource use

No relevant published health economic analyses were identified for this review question. The cost of a histamine or methacholine challenge test was presented to aid committee consideration of cost effectiveness. The cost was estimated to be £179.49 as the test is provided only in secondary care. The committee agreed that bronchial challenge stands out as the most accurate test for diagnosing asthma but acknowledged that its high cost, the low availability of methacholine and extended waiting list in secondary care made this test unsuitable for widespread use.

The committee considered bronchial challenge test with methacholine alongside or in combination with a variety of tests for asthma within a diagnostic algorithm in adults and children (see evidence review 1.11). The economic analysis found bronchial challenge test cost-effective when included in the final step of both adults and children diagnostic pathway. The committee noticed that this test may not be widely available across the country and, therefore, should be reserved for fewer people with complex clinical history who arrived at the end of the pathway with an uncertain diagnosis. Therefore, the committee recommended to include bronchial challenge test in the final stage of both diagnostic algorithms.

1.2.5 Other factors the committee took into account

One factor that was considered when discussing the evidence was the risk of adverse side effects occurring during bronchial challenge tests. This was initially raised as a point of concern by the lay members of the committee due to theoretical danger of deliberately restricting the airways. However, this concern was alleviated by the clinicians on the committee. It was explained that bronchial challenge tests only aim to achieve a reduction in FEV_1 of 20%, which in most individuals is not an uncomfortable level of bronchoconstriction. In very few cases a fall in FEV_1 of up to 40% may occur, but bronchial challenge tests are always followed by a rapid-acting bronchodilator to restore the functionality of the airway, thus limiting any discomfort experienced. Furthermore, bronchial challenge tests are conducted in secondary care where specialist attention and facilities are available should any adverse events occur. Given the low likelihood of adverse events, combined with the safe testing environment, the committee agreed that the benefits of bronchial challenge testing with methacholine strongly outweighed the risks. However, histamine is known to have other side effects, such as flushing and headaches. With these side effects in mind, the committee agreed that methacholine challenge testing was a more suitable bronchial challenge test for the diagnosis of asthma.

A second consideration was the limited availability of methacholine. Clinicians on the committee highlighted that issues with acquiring methacholine had been experienced in the past due to supplier issues. Historically, methacholine has been supplied in a concentrated form that requires specific preparation into a solution before it able to be used. This adds a layer of complexity to the testing procedure for the centre conducting tests. Furthermore, the amount of time that prepared methacholine solution can be stored for is limited to a number of days, resulting in wastage if a sufficient volume of tests are not conducted in a set time period. However, it was noted that with the introduction of specialised diagnostic hubs this wastage is less likely to occur as more tests per day will be conducted. Despite these considerations the committee still agreed that methacholine challenge testing demonstrated very good diagnostic accuracy and should be included in the diagnostic pathway.

The committee also noted that challenge tests require repeated spirometry measurements and people who find spirometry difficult may not produce reliable results. This is a minority of people but will limit the application of the test to some degree.

1.2.6 Recommendations supported by this evidence review

Recommendations 1.2.4 and 1.2.9.

1.3 References

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Zaczeniuk M, Woicka-Kolejwa K, Stelmach W, et al. (2015) Methacholine challenge testing is superior to the exercise challenge for detecting asthma in children *Annals of allergy, asthma & immunology : official publication of the American College of Allergy, Asthma, & Immunology* 115 (6): 481-484.

Appendices

Appendix A – Review protocols

Review protocol for diagnostic test accuracy and clinical and cost-effectiveness of histamine and methacholine challenge tests

Field	Content
PROSPERO registration number	CRD42023438302
Review title	Accuracy and clinical and cost-effectiveness of bronchial challenge testing with histamine and methacholine in the diagnosis of asthma
Review question	In people under investigation for asthma, what is the diagnostic test accuracy and clinical and cost-effectiveness of bronchial challenge testing with histamine and methacholine?
Objective	To evaluate the diagnostic test value of histamine and methacholine bronchial challenge in diagnosing asthma This evidence review will have two stages: (1) Identify the clinical and cost effectiveness of diagnosis with the test (test plus treatment) (2) If evidence on clinical effectiveness is limited, the diagnostic accuracy will instead be determined
Searches	The following databases (from inception) will be searched: • Cochrane Central Register of Controlled Trials (CENTRAL) • Cochrane Database of Systematic Reviews (CDSR)

	• Embase • MEDLINE • Epistemonikos Searches will be restricted by: • English language studies • Human studies Other searches: • Inclusion lists of systematic reviews The searches may be re-run 6 weeks before the final committee meeting and further studies retrieved for inclusion if relevant. The full search strategies will be published in the final review. Medline search strategy to be quality assured using the PRESS evidence-based checklist (see methods chapter for full details).
Condition or domain being studied	Asthma
Population	Inclusion: People with suspected asthma (presenting with respiratory symptoms). Ages stratified into the following 2 groups:

	• Children/young people (5-16 years old) • Adults (≥17 years old) Exclusion: • Children under 5 years old • People on steroid medication (washout period minimum of 4 weeks for inclusion)
Test	• Histamine PC20 and PD20 • Methacholine PC20 and PD20
Reference standard	Effectiveness (test-and-treat) • Compared to each other Diagnostic accuracy • Reference standard Reference standard: Physician diagnosis of asthma based on symptoms plus an objective test from any one of the following: • peak flow variability (cut-off value of more than 20% variability as indication of a positive test); • bronchodilator reversibility (cut-off value of an improvement in FEV1 of more than or equal to 12%, and an increase in volume of more than or equal to 200mls as indication of a positive test). • FeNO Where no evidence is available using the cut-off values specified above, evidence will be included from studies using a reference standard of physician diagnosis with an objective test using an alternative threshold.

	Where no evidence is available from studies using physician diagnosis and an objective test, evidence will be included from studies using physician diagnosis based on symptoms alone, or patient report of a previous physician diagnosis.
Types of study to be included	Clinical effectiveness (test and treat): • Systematic reviews of RCTs • Parallel RCTs Published NMAs and IPDs will be considered for inclusion. Diagnostic test accuracy: • Cross sectional studies • Cohort studies will be included
Other exclusion criteria	• Non-English language studies. • Non comparative cohort studies • Before and after studies • Conference abstracts will be excluded as it is expected there will be sufficient full text published studies available. • Not occupational asthma /allergens • Not looking at validation studies, or studies comparing different methods of measuring the same test • Not looking at factors which influence measurements
Context	Primary, secondary and community care settings
Primary outcomes (critical outcomes)	All outcomes are considered equally important for decision making a therefore have all been rated as critical:

	Clinical effectiveness (test and treat) outcomes:
	• Severe asthma exacerbations (defined as asthma exacerbations requiring oral corticosteroid use (dichotomous outcome at ≥6 months)
	• Mortality (dichotomous outcome at ≥6 months)
	• Quality of life (QOL; validated scale, including asthma specific questionnaires AQLQ; health-related) (continuous outcome at ≥3 months)
	• Asthma control assessed by a validated questionnaire (ACQ, ACT, St George's respiratory) (continuous outcome at ≥3 months)
	• Hospital admissions (dichotomous outcome at ≥6 months)
	• Reliever/rescue medication use (continuous outcome at ≥3 months)
	• Lung function (change in FEV1 or morning PEF – average over at least 7 days for morning PEF) (continuous outcome at ≥3 months). *Note: Extract FEV1 %pred over litres if both are reported. If only litres is reported, extract and analyse separately (do not extract both). For children, only use FEV1 %pred.*
	• Adverse events
	  ○ Linear growth (continuous outcome at ≥1 year),
	  ○ Pneumonia frequency (dichotomous outcome at ≥3 months)
	  ○ Adrenal insufficiency as defined by study, including short synacthen test and morning cortisol (dichotomous outcome at ≥3 months)
	  ○ Bone mineral density (continuous outcome at ≥6 months)
	  ○ Acute symptoms (dichotomous outcome reported immediately post-test (≤10 mins))
	• Inflammatory markers; exhaled nitric oxide (continuous outcome at ≥8 weeks)

	Diagnostic accuracy outcomes: • Sensitivity (thresholds: upper 90, lower 10) • Specificity (thresholds: upper 80, lower 50) • Raw data to calculate 2x2 tables to calculate sensitivity and specificity • Negative predictive value (NPV), Positive predictive value (PPV)
Data extraction (selection and coding)	All references identified by the searches and from other sources will be uploaded into EPPI reviewer and de-duplicated. 10% of the abstracts will be reviewed by two reviewers, with any disagreements resolved by discussion or, if necessary, a third independent reviewer. The full text of potentially eligible studies will be retrieved and will be assessed in line with the criteria outlined above. A standardised form will be used to extract data from studies (see Developing NICE guidelines: the manual section 6.4). 10% of all evidence reviews are quality assured by a senior research fellow. This includes checking: • papers were included /excluded appropriately • a sample of the data extractions • correct methods are used to synthesise data • a sample of the risk of bias assessments Disagreements between the review authors over the risk of bias in particular studies will be resolved by discussion, with involvement of a third review author where necessary.

	Study investigators may be contacted for missing data where time and resources allow.
Risk of bias (quality) assessment	Risk of bias will be assessed using the appropriate checklist as described in Developing NICE guidelines: the manual. • Systematic reviews: Risk of Bias in Systematic Reviews (ROBIS) • Randomised Controlled Trial: Cochrane RoB (2.0) • QUADAS-2 checklist
Strategy for data synthesis	<u>Diagnostic intervention (test and treat):</u> Pairwise meta-analyses will be performed using Cochrane Review Manager (RevMan5). Fixed-effects (Mantel-Haenszel) techniques will be used to calculate risk ratios for the binary outcomes where possible. Continuous outcomes will be analysed using an inverse variance method for pooling weighted mean differences. Heterogeneity between the studies in effect measures will be assessed using the I^2 statistic and visually inspected. An I^2 value greater than 50% will be considered indicative of substantial heterogeneity. Sensitivity analyses will be conducted based on pre-specified subgroups using stratified meta-analysis to explore the heterogeneity in effect estimates. If this does not explain the heterogeneity, the results will be presented pooled using random-effects. GRADEpro will be used to assess the quality of evidence for each outcome, taking into account individual study quality and the meta-analysis results. The 4 main quality elements (risk of bias, indirectness, inconsistency and imprecision) will be appraised for each outcome. Publication bias will be considered with the guideline committee, and if suspected will be tested for when there are more than 5 studies for that outcome. The risk of bias across all available evidence was evaluated for each outcome using an adaptation of the 'Grading of Recommendations Assessment, Development and

	Evaluation (GRADE) toolbox' developed by the international GRADE working group http://www.gradeworkinggroup.org/ Where meta-analysis is not possible, data will be presented and quality assessed individually per outcome. WinBUGS will be used for network meta-analysis, if possible given the data identified. Diagnostic accuracy: Where possible data will be meta-analysed where appropriate (if at least 3 studies reporting data at the same diagnostic threshold) in WinBUGS. Summary diagnostic outcomes will be reported from the meta-analyses with their 95% confidence intervals in adapted GRADE tables. Heterogeneity will be assessed by visual inspection of the sensitivity and specificity plots and summary area under the curve (AUC) plots. Particular attention will be placed on specificity determined by the committee to be the primary outcome for decision making. If meta-analysis is not possible, data will be presented as individual values in adapted GRADE profile tables and plots of un-pooled sensitivity and specificity from RevMan software.	
Analysis of sub-groups	Subgroups that will be investigated if heterogeneity is present: Strata: • Cut off used by the study • Reference standards (top block of physician diagnoses plus 1 of 3 objective measures vs physician diagnosis alone)	
Type and method of review	☒	Intervention
	☒	Diagnostic
	☐	Prognostic
	☐	Qualitative

	☐	Epidemiologic	
	☐	Service Delivery	
	☐	Other (please specify)	
Language	English		
Country	England		
Anticipated or actual start date			
Anticipated completion date	31 July 2024		
Stage of review at time of this submission	Review stage	Started	Completed
	Preliminary searches	☑	☑
	Piloting of the study selection process	☐	☐
	Formal screening of search results against eligibility criteria	☐	☐
	Data extraction	☐	☐
	Risk of bias (quality) assessment	☐	☐
	Data analysis	☐	☐
Named contact	5a. Named contact National Guideline Centre 5b Named contact e-mail asthmachronicmanagement@nice.org.uk		

	5e Organisational affiliation of the review National Institute for Health and Care Excellence (NICE) National Guideline Centre
Review team members	From the National Guideline Centre: Bernard Higgins (Guideline lead) Sharon Swain (Guideline lead) Melina Vasileiou (Senior systematic reviewer) Qudsia Malik (Systematic reviewer) Toby Sands (Systematic reviewer) Alfredo Mariani (Senior health economist) Lina Gulhane (Head of information specialists) Stephen Deed (Information specialist) Amy Crisp (Senior project manager)
Funding sources/sponsor	This systematic review is being completed by the National Guideline Centre which receives funding from NICE.
Conflicts of interest	All guideline committee members and anyone who has direct input into NICE guidelines (including the evidence review team and expert witnesses) must declare any potential conflicts of interest in line with NICE's code of practice for declaring and dealing with conflicts of interest. Any relevant interests, or changes to interests, will also be declared publicly at the start of each guideline committee meeting. Before each meeting, any potential conflicts of interest will be considered by the guideline committee Chair and a senior member of the development team. Any decisions to exclude a person from all or part of a meeting will be documented. Any changes to a member's declaration of interests will be recorded in the minutes of the meeting. Declarations of interests will be published with the final guideline.
Collaborators	Development of this systematic review will be overseen by an advisory committee who will use the review to inform the development of evidence-based recommendations in

	line with section 3 of Developing NICE guidelines: the manual. Members of the guideline committee are available on the NICE website: https://www.nice.org.uk/guidance/indevelopment/gid-ng10186	
Other registration details	N/A	
Reference/URL for published protocol	N/A	
Dissemination plans	NICE may use a range of different methods to raise awareness of the guideline. These include standard approaches such as: • notifying registered stakeholders of publication • publicising the guideline through NICE's newsletter and alerts • issuing a press release or briefing as appropriate, posting news articles on the NICE website, using social media channels, and publicising the guideline within NICE.	
Keywords	N/A	
Details of existing review of same topic by same authors	N/A	
	N/A	
Current review status	☒	Ongoing
	☐	Completed but not published
	☐	Completed and published
	☐	Completed, published and being updated
	☐	Discontinued
Additional information	N/A	
Details of final publication	N/A	

Health economic review protocol

Table 7: Health economic review protocol

Review question	All questions – health economic evidence
Objectives	To identify health economic studies relevant to any of the review questions.
Search criteria	• Populations, interventions and comparators must be as specified in the clinical review protocol above. • Studies must be of a relevant health economic study design (cost–utility analysis, cost-effectiveness analysis, cost–benefit analysis, cost–consequences analysis, comparative cost analysis). • Studies must not be a letter, editorial or commentary, or a review of health economic evaluations. (Recent reviews will be ordered although not reviewed. The bibliographies will be checked for relevant studies, which will then be ordered.) • Unpublished reports will not be considered unless submitted as part of a call for evidence. • Studies must be in English.
Search strategy	A health economic study search will be undertaken using population-specific terms and a health economic study filter – see appendix B below.
Review strategy	Studies not meeting any of the search criteria above will be excluded. Studies published before 2006, abstract-only studies and studies from non-OECD countries or the USA will also be excluded. Each remaining study will be assessed for applicability and methodological limitations using the NICE economic evaluation checklist which can be found in appendix H of Developing NICE guidelines: the manual (2014).(National Institute for Health and Care Excellence) **Inclusion and exclusion criteria** • If a study is rated as both 'Directly applicable' and with 'Minor limitations' then it will be included in the guideline. A health economic evidence table will be completed and it will be included in the health economic evidence profile. • If a study is rated as either 'Not applicable' or with 'Very serious limitations' then it will usually be excluded from the guideline. If it is excluded then a health economic evidence table will not be completed and it will not be included in the health economic evidence profile. • If a study is rated as 'Partially applicable', with 'Potentially serious limitations' or both then there is discretion over whether it should be included.

Where there is discretion

The health economist will make a decision based on the relative applicability and quality of the available evidence for that question, in discussion with the guideline committee if required. The ultimate aim is to include health economic studies that are helpful for decision-making in the context of the guideline and the current NHS setting. If several studies are considered of sufficiently high applicability and methodological quality that they could all be included, then the health economist, in discussion with the committee if required, may decide to include only the most applicable studies and to selectively exclude the remaining studies. All studies excluded on the basis of applicability or methodological limitations will be listed with explanation in the excluded health economic studies appendix below.

The health economist will be guided by the following hierarchies.

Setting:

- UK NHS (most applicable).
- OECD countries with predominantly public health insurance systems (for example, France, Germany, Sweden).
- OECD countries with predominantly private health insurance systems (for example, Switzerland).
- Studies set in non-OECD countries or in the USA will be excluded before being assessed for applicability and methodological limitations.

Health economic study type:

- Cost–utility analysis (most applicable).
- Other type of full economic evaluation (cost–benefit analysis, cost-effectiveness analysis, cost–consequences analysis).
- Comparative cost analysis.
- Non-comparative cost analyses including cost-of-illness studies will be excluded before being assessed for applicability and methodological limitations.

Year of analysis:

- The more recent the study, the more applicable it will be.
- Studies published in 2006 or later but that depend on unit costs and resource data entirely or predominantly from before 2006 will be rated as ‘Not applicable’.
- Studies published before 2006 be excluded before being assessed for applicability and methodological limitations.

Quality and relevance of effectiveness data used in the health economic analysis:

- The more closely the clinical effectiveness data used in the health economic analysis match with the outcomes of the studies included in the clinical review the more useful the analysis will be for decision-making in the guideline.

Appendix B – Literature search strategies

In people under investigation for asthma, what is the diagnostic test accuracy and clinical and cost-effectiveness of bronchial challenge testing with histamine and methacholine?

Clinical search literature search strategy

Searches were constructed using a PICO framework where population (P) terms were combined with Intervention (I) and in some cases Comparison (C) terms. Outcomes (O) are rarely used in search strategies as these concepts may not be indexed or described in the title or abstract and are therefore difficult to retrieve. Search filters were applied to the search where appropriate.

Table 8: Database parameters, filters and limits applied

Database	Dates searched	Search filter used
Medline (OVID)	1946 – 20 Dec 2023	Randomised controlled trials Systematic review studies Observational studies Diagnostic tests studies Exclusions (animal studies, letters, comments, editorials, case studies/reports) English language
Embase (OVID)	1974 – 20 Dec 2023	Randomised controlled trials Systematic review studies Observational studies Diagnostic tests studies Exclusions (conference abstracts, animal studies, letters, comments, editorials, case studies/reports) English language
The Cochrane Library (Wiley)	Cochrane Reviews to 2023 Issue 12 of 12 CENTRAL to 2023 Issue 12 of 12	Exclusions (clinical trials, conference abstracts)
Epistemonikos (The Epistemonikos Foundation)	Inception to 20 Dec 2023	Exclusions (Cochrane reviews) English language

Medline (Ovid) search terms

1.	exp Asthma/
2.	asthma*.ti,ab.
3.	1 or 2
4.	letter/
5.	editorial/
6.	news/
7.	exp historical article/
8.	Anecdotes as Topic/
9.	comment/
10.	case reports/

11.	(letter or comment*).ti.
12.	or/4-11
13.	randomized controlled trial/ or random*.ti,ab.
14.	12 not 13
15.	animals/ not humans/
16.	exp Animals, Laboratory/
17.	exp Animal Experimentation/
18.	exp Models, Animal/
19.	exp Rodentia/
20.	(rat or rats or mouse or mice or rodent*).ti.
21.	or/14-20
22.	3 not 21
23.	limit 22 to English language
24.	Bronchial Provocation Tests/
25.	(bronchial constrict* or bronchoconstrict* or broncho constrict* or bronchoprovocation or broncho provocation).ti,ab,kf.
26.	((bronchial or airway*) adj3 (provocat* or provok* or challeng* or test* or respons* or breath*)).ti,ab,kf.
27.	((challeng* or provocat* or inhalation or inhaling) adj2 test*).ti,ab,kf.
28.	BCT.ti,ab,kf.
29.	Bronchial Hyperreactivity/
30.	((bronchial or bronchus or airway) adj2 (hyperresponsiv* or hyperreactiv* or hyper-responsiv* or hyper-reactiv*)).ti,ab,kf.
31.	or/24-30
32.	exp Histamine/
33.	Methacholine Chloride/
34.	(histamin* or methacholine*).ti,ab,kf.
35.	provocholine*.ti,ab,kf.
36.	(HCT or MCT).ti,ab,kf.
37.	or/32-36
38.	exp Mannitol/
39.	mannit*.ti,ab,kf.
40.	or/38-39
41.	exp exercise tests/
42.	(exercise adj3 (provocat* or provok* or challeng* or test* or induced or inducing or brochosospasm* or stress or tolerance* or tolerating)).ti,ab,kf.
43.	((treadmill* or step* or bike* or bicycl* or cycl* or walk*) adj2 (test* or exert*)).ti,ab,kf.
44.	ergomet*.ti,ab,kf.
45.	or/41-44
46.	31 or 37 or 40 or 45
47.	23 and 46
48.	exp "sensitivity and specificity"/
49.	(sensitivity or specificity).ti,ab.
50.	((pre test or pretest or post test) adj probability).ti,ab.
51.	(predictive value* or PPV or NPV).ti,ab.
52.	likelihood ratio*.ti,ab.

53.	likelihood function/
54.	((area under adj4 curve) or AUC).ti,ab.
55.	(receive* operat* characteristic* or receive* operat* curve* or ROC curve*).ti,ab.
56.	gold standard.ab.
57.	exp Diagnostic errors/
58.	(false positiv* or false negativ*).ti,ab.
59.	Diagnosis, Differential/
60.	(diagnos* adj3 (performance* or accurac* or utilit* or value* or efficien* or effectiveness or precision or validat* or validity or differential or error*)).ti,ab.
61.	or/48-60
62.	Epidemiologic studies/
63.	Observational study/
64.	exp Cohort studies/
65.	(cohort adj (study or studies or analys* or data)).ti,ab.
66.	((follow up or observational or uncontrolled or non randomi#ed or epidemiologic*) adj (study or studies or data)).ti,ab.
67.	((longitudinal or retrospective or prospective) and (study or studies or review or analys* or cohort* or data)).ti,ab.
68.	Controlled Before-After Studies/
69.	Historically Controlled Study/
70.	Interrupted Time Series Analysis/
71.	(before adj2 after adj2 (study or studies or data)).ti,ab.
72.	exp case control study/
73.	case control*.ti,ab.
74.	Cross-sectional studies/
75.	(cross sectional and (study or studies or review or analys* or cohort* or data)).ti,ab.
76.	or/62-75
77.	randomized controlled trial.pt.
78.	controlled clinical trial.pt.
79.	randomi#ed.ab.
80.	placebo.ab.
81.	randomly.ab.
82.	clinical trials as topic.sh.
83.	trial.ti.
84.	or/77-83
85.	Meta-Analysis/
86.	Meta-Analysis as Topic/
87.	(meta analy* or metanaly* or metaanaly* or meta regression).ti,ab.
88.	((systematic* or evidence*) adj3 (review* or overview*)).ti,ab.
89.	(reference list* or bibliograph* or hand search* or manual search* or relevant journals).ab.
90.	(search strategy or search criteria or systematic search or study selection or data extraction).ab.
91.	(search* adj4 literature).ab.
92.	(medline or pubmed or cochrane or embase or psychlit or psyclit or psychinfo or psycinfo or cinahl or science citation index or bids or cancerlit).ab.
93.	cochrane.jw.

94.	((multiple treatment* or indirect or mixed) adj2 comparison*).ti,ab.
95.	or/85-94
96.	47 and (61 or 76 or 84 or 95)

Embase (Ovid) search terms

1.	exp Asthma/
2.	asthma*.ti,ab.
3.	1 or 2
4.	letter.pt. or letter/
5.	note.pt.
6.	editorial.pt.
7.	case report/ or case study/
8.	(letter or comment*).ti.
9.	(conference abstract* or conference review or conference paper or conference proceeding).db,pt,su.
10.	or/4-9
11.	randomized controlled trial/ or random*.ti,ab.
12.	10 not 11
13.	animal/ not human/
14.	nonhuman/
15.	exp Animal Experiment/
16.	exp Experimental Animal/
17.	animal model/
18.	exp Rodent/
19.	(rat or rats or mouse or mice or rodent*).ti.
20.	or/12-19
21.	3 not 20
22.	limit 21 to English language
23.	Inhalation Test/
24.	(bronchial constrict* or bronchoconstrict* or broncho constrict* or bronchoprovocation or broncho provocation).ti,ab,kf.
25.	((bronchial or airway*) adj3 (provocat* or provok* or challeng* or test* or respons* or breath*)).ti,ab,kf.
26.	((challeng* or provocat* or inhalation or inhaling) adj2 test*).ti,ab,kf.
27.	BCT.ti,ab,kf.
28.	Bronchus hyperreactivity/
29.	((bronchial or bronchus or airway) adj2 (hyperresponsiv* or hyperreactiv* or hyper-responsiv* or hyper-reactiv*)).ti,ab,kf.
30.	or/23-29
31.	exp Histamine/
32.	Methacholine Chloride/
33.	(histamin* or methacholine*).ti,ab,kf.
34.	provocholine*.ti,ab,kf.
35.	(HCT or MCT).ti,ab,kf.
36.	or/31-35
37.	exp Mannitol/
38.	mannit*.ti,ab,kf.

39.	or/37-38
40.	exp Exercise test/
41.	(exercise adj3 (provocat* or provok* or challeng* or test* or induced or inducing or brochosospasm* or stress or tolerance* or tolerating)).ti,ab,kf.
42.	((treadmill* or step* or bike* or bicycl* or cycl* or walk*) adj2 (test* or exert*)).ti,ab,kf.
43.	ergomet*.ti,ab,kf.
44.	or/40-43
45.	30 or 36 or 39 or 44
46.	22 and 45
47.	exp "sensitivity and specificity"/
48.	(sensitivity or specificity).ti,ab.
49.	((pre test or pretest or post test) adj probability).ti,ab.
50.	(predictive value* or PPV or NPV).ti,ab.
51.	likelihood ratio*.ti,ab.
52.	((area under adj4 curve) or AUC).ti,ab.
53.	(receive* operat* characteristic* or receive* operat* curve* or ROC curve*).ti,ab.
54.	diagnostic accuracy/
55.	diagnostic test accuracy study/
56.	gold standard.ab.
57.	exp diagnostic error/
58.	(false positiv* or false negativ*).ti,ab.
59.	differential diagnosis/
60.	(diagnos* adj3 (performance* or accurac* or utilit* or value* or efficien* or effectiveness or precision or validat* or validity or differential or error*)).ti,ab.
61.	or/47-60
62.	Clinical study/
63.	Observational study/
64.	Family study/
65.	Longitudinal study/
66.	Retrospective study/
67.	Prospective study/
68.	Cohort analysis/
69.	Follow-up/
70.	cohort*.ti,ab.
71.	69 and 70
72.	(cohort adj (study or studies or analys* or data)).ti,ab.
73.	((follow up or observational or uncontrolled or non randomi#ed or epidemiologic*) adj (study or studies or data)).ti,ab.
74.	((longitudinal or retrospective or prospective) and (study or studies or review or analys* or cohort* or data)).ti,ab.
75.	(before adj2 after adj2 (study or studies or data)).ti,ab.
76.	exp case control study/
77.	case control*.ti,ab.
78.	cross-sectional study/
79.	(cross sectional and (study or studies or review or analys* or cohort* or data)).ti,ab.
80.	or/62-68,71-79

81.	random*.ti,ab.
82.	factorial*.ti,ab.
83.	(crossover* or cross over*).ti,ab.
84.	((doubl* or singl*) adj blind*).ti,ab.
85.	(assign* or allocat* or volunteer* or placebo*).ti,ab.
86.	crossover procedure/
87.	single blind procedure/
88.	randomized controlled trial/
89.	double blind procedure/
90.	or/81-89
91.	Systematic Review/
92.	Meta-Analysis/
93.	(meta analy* or metanaly* or metaanaly* or meta regression).ti,ab.
94.	((systematic* or evidence*) adj3 (review* or overview*)).ti,ab.
95.	(reference list* or bibliograph* or hand search* or manual search* or relevant journals).ab.
96.	(search strategy or search criteria or systematic search or study selection or data extraction).ab.
97.	(search* adj4 literature).ab.
98.	(medline or pubmed or cochrane or embase or psychlit or psyclit or psychinfo or psycinfo or cinahl or science citation index or bids or cancerlit).ab.
99.	cochrane.jw.
100.	((multiple treatment* or indirect or mixed) adj2 comparison*).ti,ab.
101.	or/91-100
102.	46 and (61 or 80 or 90 or 101)

Cochrane Library (Wiley) search terms

#1.	MeSH descriptor: [Asthma] explode all trees
#2.	asthma*:ti,ab
#3.	#1 or #2
#4.	conference:pt or (clinicaltrials or trialsearch):so
#5.	#3 not #4
#6.	MeSH descriptor: [Bronchial Provocation Tests] this term only
#7.	(bronchial constrict* or bronchoconstrict* or "broncho constrict*" or bronchoprovocat* or "broncho provocat*"):ti,ab
#8.	((bronchial or airway*) near/3 (provocat* or provok* or challeng* or test* or respons* or breath*)):ti,ab
#9.	((challeng* or provocat* or inhalation or inhaling) near/2 test*):ti,ab
#10.	BCT:ti,ab
#11.	MeSH descriptor: [Bronchial Hyperreactivity] this term only
#12.	((bronchial or bronchus or airway) near/2 (hyperresponsiv* or hyperreactiv* or "hyper responsiv*" or "hyper reactiv*")):ti,ab
#13.	(or #6-#12)
#14.	MeSH descriptor: [Histamine] explode all trees
#15.	MeSH descriptor: [Methacholine Chloride] explode all trees
#16.	(histamin* or methacholine*):ti,ab

#17.	provocholine*:ti,ab
#18.	(HCT or MCT):ti,ab
#19.	(or #14-#18)
#20.	MeSH descriptor: [Mannitol] explode all trees
#21.	mannit*:ti,ab
#22.	(or #20-#21)
#23.	MeSH descriptor: [Exercise Test] explode all trees
#24.	(exercise near/3 (provocat* or provok* or challeng* or test* or induced or inducing or brochosospasm* or stress or tolerance* or tolerating)):ti,ab
#25.	((treadmill* or step* or bike* or bicycl* or cycl* or walk*) near/2 (test* or exert*)):ti,ab
#26.	ergomet*:ti,ab
#27.	(or #23-#26)
#28.	#13 or #19 or #22 or #27
#29.	#5 and #28

Epistemonikos search terms

1.	(title:((bronchial constrict* OR bronchoconstrict* OR "broncho constrict*" OR bronchoprovocat* OR "broncho provocat*")) OR abstract:((bronchial constrict* OR bronchoconstrict* OR "broncho constrict*" OR bronchoprovocat* OR "broncho provocat*"))) OR (title:((bronchial OR airway*) AND (provocat* OR provok* OR challeng* OR test* OR respons* OR breath*)) OR abstract:((bronchial OR airway*) AND (provocat* OR provok* OR challeng* OR test* OR respons* OR breath*))) OR (title:((challeng* OR provocat* OR inhalation OR inhaling) AND test*) OR abstract:((challeng* OR provocat* OR inhalation OR inhaling) AND test*)) OR (title:(bronchial OR bronchus OR airway) AND (hyperresponsiv* OR hyperreactiv* OR hyper-responsiv* OR hyper-reactiv*) OR abstract:(bronchial OR bronchus OR airway) AND (hyperresponsiv* OR hyperreactiv* OR hyper-responsiv* OR hyper-reactiv*)) OR (title:((histamin* OR methacholine*)) OR abstract:((histamin* OR methacholine*))) OR (title:(provocholine*) OR abstract:(provocholine*)) OR (title:(mannit*) OR abstract:(mannit*)) OR (title:(exercise AND (provocat* OR provok* OR challeng* OR test* OR induced OR inducing OR brochosospasm* OR stress OR tolerance* OR tolerating)) OR abstract:(exercise AND (provocat* OR provok* OR challeng* OR test* OR induced OR inducing OR brochosospasm* OR stress OR tolerance* OR tolerating))) OR (title:((treadmill* OR step* OR bike* OR bicycl* OR cycl* OR walk*) AND (test* OR exert*)) OR abstract:((treadmill* OR step* OR bike* OR bicycl* OR cycl* OR walk*) AND (test* OR exert*))) OR (title:(ergomet*) OR abstract:(ergomet*)) AND (title:(asthma*) OR abstract:(asthma*))

Health economic literature search strategy

Health economic evidence was identified by conducting searches using terms for a broad Asthma population. The following databases were searched: NHS Economic Evaluation Database (NHS EED - this ceased to be updated after 31st March 2015), Health Technology Assessment database (HTA - this ceased to be updated from 31st March 2018) and The International Network of Agencies for Health Technology Assessment (INAHTA). Searches for recent evidence were run on Medline and Embase from 2014 onwards for health economics, and all years for quality-of-life studies and modelling.

Table 9: Database parameters, filters and limits applied

Database	Dates searched	Search filters and limits applied
Medline (OVID)	Health Economics 1 January 2014 – 29 Dec 2023	Health economics studies Quality of life studies Modelling

Database	Dates searched	Search filters and limits applied
	Quality of Life 1946 – 29 Dec 2023	Exclusions (animal studies, letters, comments, editorials, case studies/reports) English language
	Modelling 1946 – 29 Dec 2023	
Embase (OVID)	Health Economics 1 January 2014 – 29 Dec 2023	Health economics studies Quality of life studies Modelling Exclusions (animal studies, letters, comments, editorials, case studies/reports, conference abstracts) English language
	Quality of Life 1974 – 29 Dec 2023	
	Modelling 1974 – 29 Dec 2023	
NHS Economic Evaluation Database (NHS EED) (Centre for Research and Dissemination - CRD)	Inception –31st March 2015	
Health Technology Assessment Database (HTA) (Centre for Research and Dissemination – CRD)	Inception – 31st March 2018	
The International Network of Agencies for Health Technology Assessment (INAHTA)	Inception - 29 Dec 2023	English language

Medline (Ovid) search terms

1.	exp Asthma/
2.	asthma*.ti,ab.
3.	1 or 2
4.	letter/
5.	editorial/
6.	news/
7.	exp historical article/
8.	Anecdotes as Topic/
9.	comment/
10.	case reports/
11.	(letter or comment*).ti.

12.	or/4-11
13.	randomized controlled trial/ or random*.ti,ab.
14.	12 not 13
15.	animals/ not humans/
16.	exp Animals, Laboratory/
17.	exp Animal Experimentation/
18.	exp Models, Animal/
19.	exp Rodentia/
20.	(rat or rats or mouse or mice or rodent*).ti.
21.	or/14-20
22.	3 not 21
23.	limit 22 to English language
24.	quality-adjusted life years/
25.	sickness impact profile/
26.	(quality adj2 (wellbeing or well being)).ti,ab.
27.	sickness impact profile.ti,ab.
28.	disability adjusted life.ti,ab.
29.	(qal* or qtime* or qwb* or daly*).ti,ab.
30.	(euroqol* or eq5d* or eq 5*).ti,ab.
31.	(qol* or hql* or hqol* or h qol* or hrqol* or hr qol*).ti,ab.
32.	(health utility* or utility score* or disutilit* or utility value*).ti,ab.
33.	(hui or hui1 or hui2 or hui3).ti,ab.
34.	(health* year* equivalent* or hye or hyes).ti,ab.
35.	discrete choice*.ti,ab.
36.	rosser.ti,ab.
37.	(willingness to pay or time tradeoff or time trade off or tto or standard gamble*).ti,ab.
38.	(sf36* or sf 36* or short form 36* or shortform 36* or shortform36*).ti,ab.
39.	(sf20 or sf 20 or short form 20 or shortform 20 or shortform20).ti,ab.
40.	(sf12* or sf 12* or short form 12* or shortform 12* or shortform12*).ti,ab.
41.	(sf8* or sf 8* or short form 8* or shortform 8* or shortform8*).ti,ab.
42.	(sf6* or sf 6* or short form 6* or shortform 6* or shortform6*).ti,ab.
43.	or/24-42
44.	exp models, economic/
45.	*Models, Theoretical/
46.	*Models, Organizational/
47.	markov chains/
48.	monte carlo method/
49.	exp Decision Theory/
50.	(markov* or monte carlo).ti,ab.
51.	econom* model*.ti,ab.

52.	(decision* adj2 (tree* or analy* or model*)).ti,ab.
53.	or/44-52
54.	Economics/
55.	Value of life/
56.	exp "Costs and Cost Analysis"/
57.	exp Economics, Hospital/
58.	exp Economics, Medical/
59.	Economics, Nursing/
60.	Economics, Pharmaceutical/
61.	exp "Fees and Charges"/
62.	exp Budgets/
63.	budget*.ti,ab.
64.	cost*.ti.
65.	(economic* or pharmaco?economic*).ti.
66.	(price* or pricing*).ti,ab.
67.	(cost* adj2 (effective* or utilit* or benefit* or minimi* or unit* or estimat* or variable*)).ab.
68.	(financ* or fee or fees).ti,ab.
69.	(value adj2 (money or monetary)).ti,ab.
70.	or/54-69
71.	23 and 43
72.	23 and 53
73.	23 and 70

Embase (Ovid) search terms

1.	exp Asthma/
2.	asthma*.ti,ab.
3.	1 or 2
4.	letter.pt. or letter/
5.	note.pt.
6.	editorial.pt.
7.	case report/ or case study/
8.	(letter or comment*).ti.
9.	(conference abstract or conference paper).pt.
10.	or/4-9
11.	randomized controlled trial/ or random*.ti,ab.
12.	10 not 11
13.	animal/ not human/
14.	nonhuman/
15.	exp Animal Experiment/
16.	exp Experimental Animal/

17.	animal model/
18.	exp Rodent/
19.	(rat or rats or mouse or mice or rodent*).ti.
20.	or/12-19
21.	3 not 20
22.	limit 21 to English language
23.	quality adjusted life year/
24.	"quality of life index"/
25.	short form 12/ or short form 20/ or short form 36/ or short form 8/
26.	sickness impact profile/
27.	(quality adj2 (wellbeing or well being)).ti,ab.
28.	sickness impact profile.ti,ab.
29.	disability adjusted life.ti,ab.
30.	(qal* or qtime* or qwb* or daly*).ti,ab.
31.	(euroqol* or eq5d* or eq 5*).ti,ab.
32.	(qol* or hql* or hqol* or h qol* or hrqol* or hr qol*).ti,ab.
33.	(health utility* or utility score* or disutilit* or utility value*).ti,ab.
34.	(hui or hui1 or hui2 or hui3).ti,ab.
35.	(health* year* equivalent* or hye or hyes).ti,ab.
36.	discrete choice*.ti,ab.
37.	rosser.ti,ab.
38.	(willingness to pay or time tradeoff or time trade off or tto or standard gamble*).ti,ab.
39.	(sf36* or sf 36* or short form 36* or shortform 36* or shortform36*).ti,ab.
40.	(sf20 or sf 20 or short form 20 or shortform 20 or shortform20).ti,ab.
41.	(sf12* or sf 12* or short form 12* or shortform 12* or shortform12*).ti,ab.
42.	(sf8* or sf 8* or short form 8* or shortform 8* or shortform8*).ti,ab.
43.	(sf6* or sf 6* or short form 6* or shortform 6* or shortform6*).ti,ab.
44.	or/23-43
45.	statistical model/
46.	exp economic aspect/
47.	45 and 46
48.	*theoretical model/
49.	*nonbiological model/
50.	stochastic model/
51.	decision theory/
52.	decision tree/
53.	monte carlo method/
54.	(markov* or monte carlo).ti,ab.
55.	econom* model*.ti,ab.
56.	(decision* adj2 (tree* or analy* or model*)).ti,ab.

57.	or/47-56
58.	health economics/
59.	exp economic evaluation/
60.	exp health care cost/
61.	exp fee/
62.	budget/
63.	funding/
64.	budget*.ti,ab.
65.	cost*.ti.
66.	(economic* or pharmaco?economic*).ti.
67.	(price* or pricing*).ti,ab.
68.	(cost* adj2 (effective* or utilit* or benefit* or minimi* or unit* or estimat* or variable*)).ab.
69.	(financ* or fee or fees).ti,ab.
70.	(value adj2 (money or monetary)).ti,ab.
71.	or/58-70
72.	22 and 44
73.	22 and 57
74.	22 and 71

NHS EED and HTA (CRD) search terms

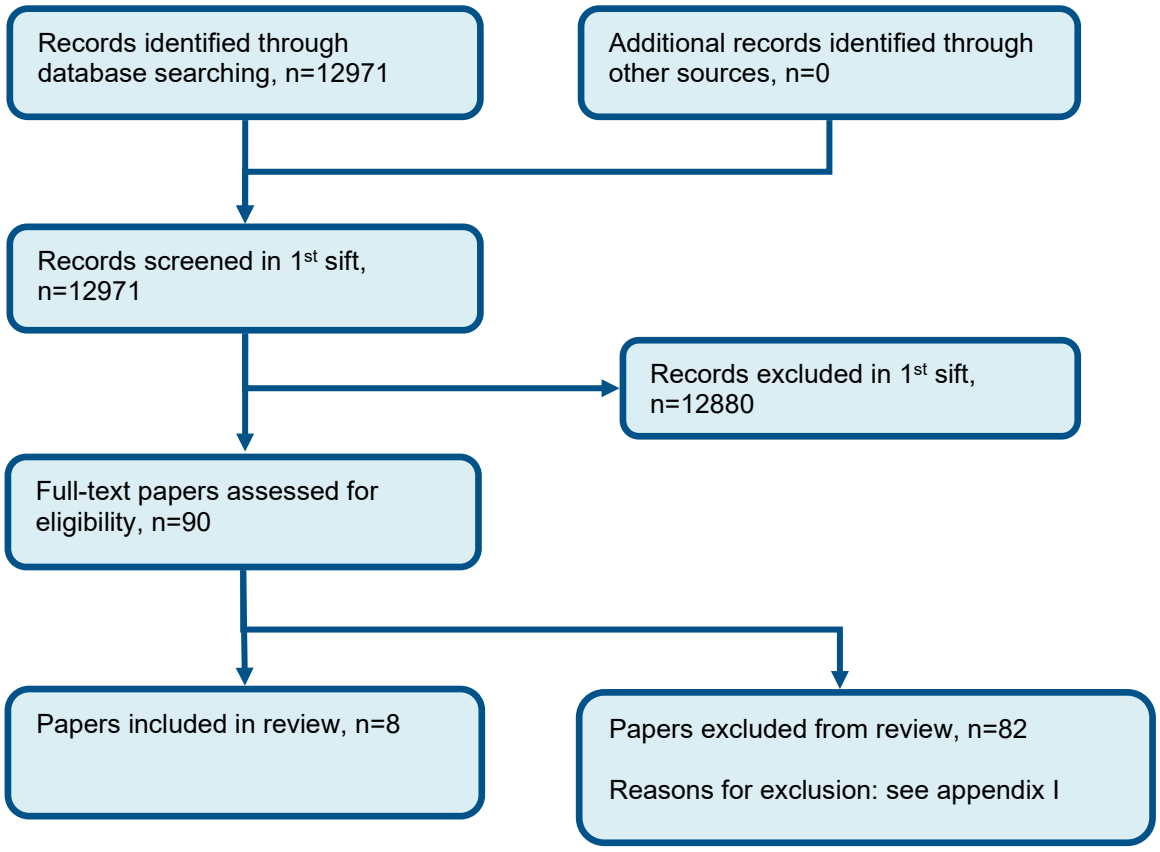
#1.	MeSH DESCRIPTOR Asthma EXPLODE ALL TREES
#2.	(asthma*)
#3.	#1 OR #2

INAHTA search terms

1.	(Asthma)[mh] OR (asthma*)[Title] OR (asthma*)[abs]

Appendix C –Diagnostic evidence study selection

Figure 1: Flow chart of clinical study selection for the review of diagnostic test accuracy of histamine and methacholine challenge tests



Appendix D –Diagnostic evidence

Reference	**Alberts 1987 (Alberts et al., 1987)**
Study type	Prospective cross-sectional study
Study methodology	Data source: Patients referred to the bronchial challenge laboratory for testing with symptoms suggestive of asthma Recruitment: Consecutive
Number of patients	n = 22 recruited and tested, 19 included in analyses as 3 had indeterminate test results (PC20 FEV_1 between 4 and 16 mg/mL)
Patient characteristics	Age, mean (range): 38.2 (14-67) years Gender (male to female ratio): 7:15 Ethnicity: Not reported Setting: Secondary care Country: USA Smoking status: Not reported ICS use: Not reported Inclusion criteria: Normal baseline spirometry (FEV_1 >80% predicted) Exclusion criteria: None reported
Target condition(s)	Asthma
Index test(s) and reference standard	Index test Test solutions were continuously aerosolised by a nebuliser to keep the output constant at 0.12-0.16 mL/minute. Solution was administered in two-minute periods, with spirometry repeated after an additional three minutes. The test began with inhalation of a saline solution control. Assuming no significant fall in FEV_1, progressively increasing concentrations of methacholine were administered, increasing two-

<table>
<tr><td>Reference</td><td colspan="5">Alberts 1987 (Alberts et al., 1987)</td></tr>
<tr><td></td><td colspan="5">fold from 0.06 mg/mL up to 16 mg/mL. The test was stopped when FEV_1 fell below 80% of the post-saline inhalation value, when the concentration reached 16 mg/mL or when the participant asked to stop.

Cut-off: PC20 FEV_1 ≤16 mg/mL

<u>Reference standard</u>
Participants were placed into two categories based on the suspicion of asthma by the referring physician. Group one contained participants with a clinical diagnosis of asthma who were referred for confirmation of this suspicion. Group two contained participants in whom asthma was a possibility, but based on clinical information were considered to have an alternate diagnosis.

Time between measurement of index test and reference standard: Not reported</td></tr>
<tr><td>2×2 table</td><td></td><td>Reference standard +</td><td>Reference standard −</td><td>Total</td><td rowspan="4">Prevalence= 68.4%</td></tr>
<tr><td></td><td>Index test +</td><td>8</td><td>1</td><td>9</td></tr>
<tr><td></td><td>Index test −</td><td>5</td><td>5</td><td>10</td></tr>
<tr><td></td><td>Total</td><td>13</td><td>6</td><td>19</td></tr>
<tr><td>Statistical measures</td><td colspan="5"><u>Index text</u>
Sensitivity: 0.62 (95%CI 0.32-0.86)
Specificity: 0.83 (95%CI 0.36-1.00)
PPV: 89%
NPV: 50%</td></tr>
<tr><td>Source of funding</td><td colspan="5">Supported by a grant from the Biomedical Research Support Grant Program at the National Institutes of Health</td></tr>
<tr><td>Limitations</td><td colspan="5">Risk of bias: Downgraded by two increments due to concerns arising from the interpretation of the index test and reference standard (unclear if blinded) and the flow and timing of the study (unclear interval between index test and reference standard and missing data from 3 participants who could not be diagnosed)
Indirectness: Downgraded by two increments due to population (smoking status not reported, ICS status not reported and inclusion of both children/young people and adolescents/adults) indirectness</td></tr>
<tr><td>Comments</td><td colspan="5">Sensitivity and specificity calculated from 2x2 data reported in paper</td></tr>
</table>

Reference	**Anderson 2009 (Anderson et al., 2009)**
Study type	Prospective cross-sectional diagnostic study
Study methodology	Data source: People with signs and symptoms suggestive of asthma without a firm diagnosis of asthma or non-asthma Recruitment: Not reported

Reference	Anderson 2009 (Anderson et al., 2009)
Number of patients	n = 375
Patient characteristics	Age, mean ± SD (range): 24.3 ± 10.2 (6-50) years Gender (male to female ratio): 51.5% female Ethnicity: 76.3% Caucasian, 8.3% Hispanic, 8.5% Black Setting: Secondary care Country: USA Smoking status: Smokers excluded ICS use: Four-week washout period applied Inclusion criteria: aged 6–50 years (BMI < 35) with signs and symptoms suggestive of asthma according to the National Institute of Health (NIH) Questionnaire but without a firm diagnosis of asthma or an exclusion of the diagnosis of asthma (e.g. had an equivocal diagnosis of asthma or had been referred for further investigation of asthma-type symptoms) were included. Subjects had at least Step 1 symptoms according to the NAEPPII asthma severity grading (symptoms ≤ 2 times per week; asymptomatic between exacerbations; exacerbations of only a few hours to a few days; and night-time symptoms of ≤ 2 times per month). They were required to have an FEV1 ≥ 70% of the predicted value at the Screening Visit Exclusion criteria: Any known other pulmonary disease; had smoked more than 1 cigarette per week within the past year or had a ≥ 10 pack year smoking history; had a respiratory tract infection within the previous 4 weeks; had been skin test positive to aeroallergens that were present in the environment during the time of enrolment and reported worsening of symptoms when exposed to these aeroallergens during the study; had been diagnosed at Screening Visit as definitively having asthma (95 to 100% likelihood) or not having asthma (0 to < 5% likelihood); had clinically significantly abnormal chest x-ray or ECG; or had failed to observe washout of medications that would interfere with BPT (including, but not limited to, no use of corticosteroids within 4 weeks of the Screening Visit)
Target condition(s)	Asthma
Index test(s) and reference standard	Methacholine Test Methacholine was delivered from a nebulizer by the dosimeter method. The concentrations were: 0.0312, 0.0625, 0.125, 0.25, 0.5, 1, 2, 4, 8, 16 mg/mL. Each concentration required five inhalations from functional residual capacity to total lung capacity. Spirometry was performed within 3 minutes. The response to methacholine was expressed as the concentration required to provoke a 20% fall in FEV_1 from the pre-challenge value.

Reference	**Anderson 2009 (Anderson et al., 2009)**
	Cut-off: positive test defined as PC20 FEV_1 ≤ 16 mg/ml Reference standard A respiratory physician was to make the Clinician diagnosis with access to the data on the exercise challenges (exercise induced asthma defined as ≥ 10%, 15% or 20% fall in FEV_1 after a standardized treadmill run), history, examination, skin tests, and FEV_1 reversibility but not the mannitol and methacholine challenge test result. Time between measurement of index test and reference standard: Unclear

2×2 table

	Reference standard +	Reference standard −	Total	Prevalence= 64%
Index test +	122	34	156	
Index test −	118	101	219	
Total	240	135	375	

Statistical measures	Index text: Sensitivity: 0.51 (95%CI 0.44-0.57) Specificity: 0.75 (95%CI 0.67-0.82) PPV: 78% NPV: 46%
Source of funding	Funded by Pharmaxis Ltd Who were involved in the design and statistical analysis of the study
Limitations	Risk of bias: Downgraded by two increments due to concerns arising from the method of participant selection (unclear method) and due to concerns arising from the flow and timing of the study (16 participants excluded from data analysis and unclear time between the index test and reference standard) Indirectness: Downgraded by one increment due to population indirectness (inclusion of both children/young people and adults)
Comments	2x2 data calculated from sensitivity, specificity and prevalence (64%) reported in paper

Reference	**Hedman 1998 (Hedman et al., 1998)**
Study type	Prospective cross-sectional diagnostic study
Study methodology	Data source: Adult patients tested with the rapid methacholine challenge at the Pulmonary Department of Lahti Central Hospital Recruitment: Consecutive patients, May – September 1994

Reference	Hedman 1998 (Hedman et al., 1998)
Number of patients	n = 230
Patient characteristics	Age, mean (SD): Asthma +ive; 44.0 (16.0), asthma -ive; 44.6 (16.2) Gender (male to female ratio): 90:140 Ethnicity: Not reported Setting: Secondary care Country: Finland Smoking status: Mixed – 23% and 16% current smokers in bronchial hyperresponsive +ive and -ive, respectively ICS use: Four-week washout applied Inclusion criteria: Patients referred to the clinic due to dyspnoea, wheezing or a cough of unknown cause, a FEV_1, of at least 65% of predicted before the challenge, and were not allowed to have any respiratory infection during the previous 4 weeks Exclusion criteria: Previous asthma diagnoses as well as those who had used inhaled steroids during the preceding 4 weeks
Target condition(s)	Asthma
Index test(s) and reference standard	Index test An automatic, inhalation synchronized dosimeter jet nebulizer was used for methacholine delivery. Prior to the challenges, patients were not allowed to use sympathomimetic drugs for 12 h, nor any other asthma or antihistaminic drugs for 48 h. Moreover, patients were not allowed to drink tea, coffee or cola drinks for 4 h before the test. After nebulization of 33 g isotonic saline, methacholine chloride was delivered in four cumulative doses of 80, 400, 1700 and 6900 µg. The concentrations of methacholine were 2.5, 10, 40 and 160 mg/mL. For the baseline, FEV_1, was recorded from at least three successive determinations after the inhalation of 33 g saline. Ninety seconds after each methacholine dose, three successive determinations of FEV_1, were made. If FEV_1, decreased from the baseline by 20% or more after any dose, further administration of methacholine was discontinued. The fall in FEV_1, was plotted against methacholine dose on a log scale, and the provocative dose causing a 20% fall in FEV_1, $PD_{20}FEV_1$ was estimated by interpolation from the dose-response curve. Cut-off: PD20 FEV_1 ≤6900 µg Reference standard

Reference	**Hedman 1998 (Hedman et al., 1998)**
	Asthma diagnosis was based on a clinical evaluation by the attending chest physicians. The guidelines of the American Thoracic Society for the diagnosis of bronchial asthma were used. The clinician who classified the patients as asthmatics or non-asthmatics was blinded to $PD20FEV_1$. Patients had to have a documented variation in FEV_1 or PEF of 15% or greater after medication, or repeatedly a 20% or greater spontaneous daily variation in PEF monitoring during a period of 2 weeks. In addition, a 15% or greater decrease in FEV_1 after a specific allergen provocation or during an exercise test was a criterion for diagnosing bronchial asthma. Time between measurement of index test and reference standard: Unclear

2×2 table

	Reference standard +	Reference standard −	Total	Prevalence= 26.5%
Index test +	47	31	78	
Index test −	14	138	152	
Total	61	169	230	

Statistical measures	Index text Sensitivity: 0.84 (95%CI 0.72-0.93) Specificity: 0.69 (95%CI 0.62-0.76) PPV: 50% (95%CI 49-71) NPV: 92% (95%CI 86-95)
Source of funding	None reported
Limitations	Risk of bias: None Indirectness: Downgraded by one increment due to population (mixed smoking and non-smoking participants) indirectness
Comments	Sensitivity and specificity calculated from 2x2 data reported in paper – 95%CI different to values reported in study

Reference	**Kowal 2009 (Kowal et al., 2009)**
Study type	Prospective cross-sectional study
Study methodology	Data source: Patients referred by family doctors to an asthma clinic for evaluation of chronic cough Recruitment: September 2000 – November 2006, method not stated
Number of patients	n = 540
Patient characteristics	Age, mean (range): 26.5 (18-45) years Gender (male to female ratio): Not reported

Reference	Kowal 2009 (Kowal et al., 2009)
	Ethnicity: Not reported Setting: Secondary care Country: Poland Smoking status: Smokers excluded ICS use: ICS naïve Inclusion criteria: Non-smokers, non-productive cough lasting at least 8 weeks, baseline lung function within normal limits Exclusion criteria: Use of any anti-asthma medication before the study, treatment with angiotensin converting enzyme inhibitors, use of codeine or other cough suppressant, upper respiratory tract infection within 4 weeks, presence of any systemic diseases and standard contradictions to a bronchial histamine test
Target condition(s)	Asthma
Index test(s) and reference standard	Index test All patients inhaled doubling concentrations of histamine starting with a concentration of 0.62 mg/mL. Aerosol was generated using a nebulizer attached to a dosimeter. All subjects performed five inspiratory-capacity breaths of each given histamine concentration. Forced expiratory manoeuvres were performed 90 seconds after each fifth inhalation. The procedure was continued until either at least a 20% decrease of FEV1 or a histamine concentration of 32 mg/mL was reached. Cut-off: PC20 FEV_1 ≤8 mg/mL Reference standard Participants were observed for at least 6 months. Asthma was diagnosed based on demonstrated signification diurnal changes in PEF or significant bronchial reversibility upon administration of salbutamol as per GINA guidelines Time between measurement of index test and reference standard: At least 6 months

2×2 table

	Reference standard +	Reference standard −	Total	Prevalence= 33%
Index test +	166	0	166	
Index test −	12	362	374	
Total	178	362	540	

Reference	Kowal 2009 (Kowal et al., 2009)
Statistical measures	Index text Sensitivity: 0.93 (95%CI 0.89-0.96) Specificity: 1.00 (95%CI 0.99-1.00) PPV: 100% NPV: 97%
Source of funding	Supported by a grant from the Medical university of Bialystok
Limitations	Risk of bias: Downgraded by two increments due to concerns arising from the method of patient selection (method of recruitment not reported) and unclear interpretation of the index test and reference standard (unclear if blinded) Indirectness: Downgraded by one increment due to reference standard (unclear if clinician diagnosis is included, or just an objective test) indirectness

Reference	Louis 2020 (Louis et al., 2020)
Study type	Retrospective cross-sectional diagnostic study
Study methodology	Data source: Asthma clinic database of patients referred by two asthma-dedicated respiratory physicians for a diagnosis of asthma Recruitment: Method not specified; people investigated from June 2006 – November 2018
Number of patients	n = Subset of 194 untreated patients from the patient database of 1610
Patient characteristics	Age, mean (SD): 49 (16) Gender (male to female ratio): 70:124 Smoking status:56% non-smokers, 21% ex-smokers, 22% current smokers Atopy: 85 atopic, 100 non-atopic (no data for 9 participants) Ethnicity: Not reported Setting: Secondary care Country: Belgium

Reference	**Louis 2020 (Louis et al., 2020)**				
	Inclusion criteria: Intermittent or chronic respiratory symptoms, referred to an asthma clinic by two asthma physicians for diagnosis who had not yet received treatment, FEV_1 ≥70% of predicted Exclusion criteria: None reported				
Target condition(s)	Asthma				
Index test(s) and reference standard	Index test The methacholine challenge was performed by using a jet nebulizer activated by an airflow rate of 6 L/minute and delivering 0.3 mL/ minute. Each patient successively inhaled for 1 minute quadrupling methacholine concentration starting from 0.06 mg/mL until a maximal concentration of 16 mg/mL. FEV1 was measured 30 and 90 seconds after each inhaled concentration and the best value was retained. The test was stopped if FEV1 had dropped by at least 20% from the baseline value. The PC20M was calculated by linear interpolation from the last 2 points of the curve. Cut-off: PC20 ≤8 mg/mL and ≤16 mg/mL (pre-specified) Reference standard Patients received 400 µg inhaled salbutamol administered by a metered-dose inhaler with a spacer one puff at a time into the spacer and spirometry was performed again 15 minutes later. Positive results were determined as >12% and 200 mL reversibility. Time between measurement of index test and reference standard: 7-14 days				
2×2 table PC20 ≤8 mg/mL		Reference standard +	Reference standard −	Total	Prevalence= 20.1%
	Index test +	32	109	141	
	Index test −	7	46	53	
	Total	39	155	194	
2×2 table PC20 ≤16 mg/mL		Reference standard +	Reference standard −	Total	
	Index test +	33	137	170	
	Index test −	6	18	24	
	Total	39	155	194	

Reference	Louis 2020 (Louis et al., 2020)
Statistical measures	Index text: PC20 ≤8 mg/mL Sensitivity: 0.82 (95%CI 0.66-0.92) Specificity: 0.30 (95%CI 0.23-0.38) PPV: 23% NPV: 87% Index text: PC20 ≤16 mg/mL Sensitivity: 0.85 (95%CI 0.69-0.94) Specificity: 0.12 (95%CI 0.07-0.18) PPV: 19% NPV: 75%
Source of funding	Federal grant from the Belgian Government
Limitations	Risk of bias: Downgraded by two increments due to high risk of bias arising from concerns due to the patient selection process (unclear how the 194 were selected from the 1610 available), and concerns due to the interpretation of the index test and reference standard (unclear if the results were interpreted by a blinded assessor) Indirectness: Downgraded by two increments due to population (mixed smoking and non-smoking participants) and reference standard (unclear if clinician diagnosis is involved or just an objective test) indirectness
Comments	Sensitivity and specificity calculated from 2x2 tables reported in study

Reference	Popovic-Grle 2002 (Popovic-Grle et al., 2002)
Study type	Prospective cross-sectional study
Study methodology	Data source: Patients treated for breathlessness at an outpatient allergology department. Patients were referred by their GPs because of suspected asthma Recruitment: Not reported
Number of patients	n = 195
Patient characteristics	Age, mean (SD): 36.8 (6.2) Gender (male to female ratio): 80:79 Ethnicity: Not reported

<table>
<tr><td>Reference</td><td colspan="5">Popovic-Grle 2002 (Popovic-Grle et al., 2002)</td></tr>
<tr><td></td><td colspan="5">Setting: Secondary care

Country: Croatia

Smoking status: 12% smokers

ICS use: Not reported

Inclusion criteria: None reported

Exclusion criteria: None reported</td></tr>
<tr><td>Target condition(s)</td><td colspan="5">Asthma</td></tr>
<tr><td>Index test(s) and reference standard</td><td colspan="5"><u>Index test</u>
Tidal volume was measured, using methacholine chloride as the provocative agent, dispersed by means of compressed air from a nebuliser. The initial concentration of methacholine was 0.03 mg/mL for two minutes, which was increased during the test by double concentration up to 8 mg/mL.

Cut-off: PC20 FEV_1 ≤8 mg/mL

<u>Reference standard</u>
Diagnosis of asthma was made on the basis of a questionnaire, with typical medical history data of occasional asthmatic attacks with wheezing and nocturnal awakening because of dyspnoea, as well as on the basis of reversible bronchial obstruction after salbutamol test.

Time between measurement of index test and reference standard: Not reported</td></tr>
<tr><td>2×2 table</td><td></td><td>Reference standard +</td><td>Reference standard −</td><td>Total</td><td rowspan="4">Prevalence= 72.3%</td></tr>
<tr><td></td><td>Index test +</td><td>137</td><td>45</td><td>182</td></tr>
<tr><td></td><td>Index test −</td><td>4</td><td>9</td><td>13</td></tr>
<tr><td></td><td>Total</td><td>141</td><td>54</td><td>195</td></tr>
<tr><td>Statistical measures</td><td colspan="5"><u>Index text</u>
Sensitivity: 0.97 (95%CI 0.93-0.99)
Specificity: 0.83 (95%CI 0.71-0.92)
PPV: 94%
NPV: 92%</td></tr>
</table>

Reference	**Popovic-Grle 2002 (Popovic-Grle et al., 2002)**
Source of funding	None reported
Limitations	Risk of bias: Downgraded by one increment due to concerns arising from unclear interpretation of the index test and reference standard (unclear if blinded) Indirectness: Downgraded by two increments due to population (smoking status not reported and ICS use not reported) indirectness
Comments	2x2 data calculated from sensitivity, specificity and prevalence (72.3%) reported in paper. Narrative reporting of number of positive methacholine tests does not match other results

Reference	**Porpodis 2017 (Porpodis et al., 2017)**
Study type	Prospective cross-sectional study
Study methodology	Data source: Conducted in the Outpatient Clinic for Asthma, Pulmonary Department, within the Aristotle University of Thessaloniki Recruitment: Subjects were recruited in the study when they visited the Asthma Clinic either for a formal examination of asthma diagnosis or after the referral of another specialist for work-up of respiratory symptoms
Number of patients	n = 88
Patient characteristics (per protocol)	Age, mean (SD): 38.56 (16.73) years Gender (male to female ratio): 41:47 Ethnicity: Not reported Smoking status: 55 non-smokers, 16 ex-smokers, 17 current smokers ICS use: Treatment naïve Setting: Secondary care Country: Greece Inclusion criteria: Asthma related symptoms in the previous month but without previous diagnosis of asthma and without initiation of treatment. Exclusion criteria: Any other known cardiopulmonary or systematic disease
Target condition(s)	Asthma

<table>
<tr><td>Reference</td><td colspan="5">Porpodis 2017 (Porpodis et al., 2017)</td></tr>
<tr><td>Index test(s) and reference standard</td><td colspan="5">Index test
Participants inhaled incremental doses of methacholine in wet aerosol form, until a drop of 20% of the FEV_1 baseline was obtained, where the test was considered positive. Initially baseline FEV_1 was measured and the test was performed if FEV_1 was >60% of predicted. Then normal saline was administered and if a decrease of FEV_1 > 10% was not observed sequential inhalations of increasing concentrations of methacholine ranging from 0.5 to 32 mg/ml were delivered. FEV_1 was measured after each inhalation step. The exact methacholine provocative concentration causing a 20% decrease in FEV_1 was estimated from a dose-response curve and was considered as a threshold value. The test was terminated when either the threshold value had been reached or the highest concentration of methacholine had been given. Patients were considered hyperresponsive if they reacted by bronchoconstriction to PC20 ≤16 mg/ml.

Cut-off: PC20 FEV_1 ≤16 mg/ml (pre-specified)

Reference standard
According to GINA guidelines, the clinician diagnosis of asthma was established by the combination of at least a ≥12% (and at least 200 mL) increase in baseline FEV_1 after albuterol, along with new symptoms of coughing, wheezing, or shortness of breath over the past month, and no previous diagnosis of asthma

Time between measurement of index test and reference standard: Unclear</td></tr>
<tr><td>2×2 table</td><td></td><td>Reference standard +</td><td>Reference standard −</td><td>Total</td><td>Prevalence= 76.1%</td></tr>
<tr><td></td><td>Index test +</td><td>42</td><td>3</td><td>45</td><td></td></tr>
<tr><td></td><td>Index test −</td><td>25</td><td>18</td><td>43</td><td></td></tr>
<tr><td></td><td>Total</td><td>67</td><td>21</td><td>88</td><td></td></tr>
<tr><td>Statistical measures</td><td colspan="5">Index text
Sensitivity: 0.63 (95%CI 0.50-0.74)
Specificity: 0.86 (95%CI 0.64-0.97)
PPV: 93%
NPV: 42%</td></tr>
<tr><td>Source of funding</td><td colspan="5">None reported</td></tr>
<tr><td>Limitations</td><td colspan="5">Risk of bias: Downgraded by two increments due to unclear method of patient selection (method not reported) and unclear interpretation of the index test and reference standard (unclear if clinician diagnosing asthma was blinded to methacholine challenge result)
Indirectness: Downgraded by one increment due to population (mixed smoking and non-smoking participants) indirectness</td></tr>
<tr><td>Comments</td><td colspan="5">Sensitivity and specificity calculated from 2x2 data reported in paper</td></tr>
</table>

Reference	Zaczeniuk 2015 (Zaczeniuk et al., 2015)
Study type	Prospective cross-sectional study
Study methodology	Data source: Middle-school children attending an allergic outpatient clinic because of post-exercise symptoms such as cough and shortness of breath during after physical education classes Recruitment: January 2013-December 2014, method not reported
Number of patients	n = 90
Patient characteristics (per protocol)	Age, range: 10-18 years Gender: 37.6% male, 62.4% female Smoking status: Active smokers excluded Ethnicity: Not reported ICS use: Therapy naïve Setting: Secondary care Country: Poland Inclusion criteria: Aged 10-18 years, post-exercise asthma symptoms Exclusion criteria: Acute or chronic lung diseases, active smoking
Target condition(s)	Asthma
Index test(s) and reference standard	Index test Methacholine challenge testing of the provocation was performed using the dosimeter technique. After pulverization of the physiologic diluent, methacholine was delivered in 4 cumulative doses: 0.015, 0.045, 0.18, and 0.72 mg. A negative result was defined as a provocative dose causing a 20% decrease higher than 0.72 mg. The test was continued at 2-minute intervals between inhalations until a decrease in FEV_1 of at least 20% was obtained. Cut-off: PD20 FEV_1 ≤0.72 mg

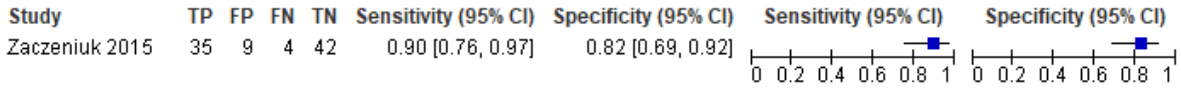
<table>
<tr><td>Reference</td><td colspan="5">Zaczeniuk 2015 (Zaczeniuk et al., 2015)</td></tr>
<tr><td></td><td colspan="5">Reference standard
Diagnosis of asthma was established by symptoms of asthma, physical examination findings of the respiratory system, and positive reversibility test findings. Positive reversibility test result was defined as improvement of at least 12% of pre-bronchodilator FEV_1 after administration of salbutamol.

Time between measurement of index test and reference standard: One-week</td></tr>
<tr><td>2×2 table</td><td></td><td>Reference standard +</td><td>Reference standard −</td><td>Total</td><td rowspan="4">Prevalence= 43.3%</td></tr>
<tr><td></td><td>Index test +</td><td>35</td><td>9</td><td>44</td></tr>
<tr><td></td><td>Index test −</td><td>4</td><td>42</td><td>46</td></tr>
<tr><td></td><td>Total</td><td>39</td><td>51</td><td>90</td></tr>
<tr><td>Statistical measures</td><td colspan="5">Index text
Sensitivity: 0.90 (95%CI 0.76-0.97)
Specificity: 0.82 (95%CI 0.69-0.92)
PPV: 80%
NPV: 91%</td></tr>
<tr><td>Source of funding</td><td colspan="5">This study was supported by the National Science Centre and the Medical University of Lodz.</td></tr>
<tr><td>Limitations</td><td colspan="5">Risk of bias: Downgraded by two increments due to concerns arising from patient selection (recruitment method unclear), the interpretation of the index test and reference standard (unclear if blinded) and the flow and timing of patients through the study (11 patients excluded due to missing test data)
Indirectness: Downgraded by one increment due to population indirectness (inclusion of both children/young people and adults)</td></tr>
<tr><td>Comments</td><td colspan="5">2x2 data calculated from sensitivity, specificity and prevalence (43.3%) reported in paper</td></tr>
</table>

Appendix E – Forest plots

Children/young people

Figure 2: Methacholine challenge (PD20 FEV_1 ≤0.72 mg) vs clinician diagnosis and bronchodilator reversibility



Non-smoking adults

Figure 3: Methacholine challenge (PC20 FEV_1 ≤16 mg/mL) vs clinician diagnosis with exercise challenge, skin prick and bronchodilator reversibility tests

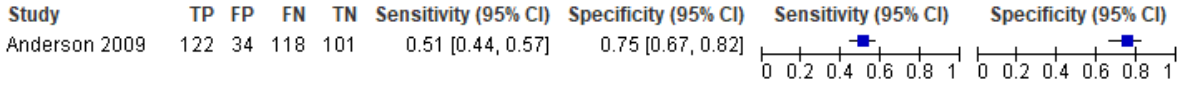
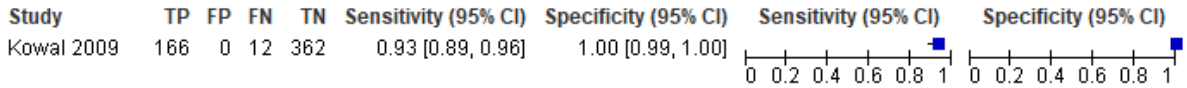


Figure 4: Histamine challenge (PC20 FEV1 ≤8 mg/mL) vs diagnosis with PEF variability or bronchodilator reversibility tests



Adults with mixed/not reported smoking status

Figure 5: Methacholine challenge (PC20 FEV_1 ≤8 mg/mL) vs bronchodilator reversibility with/without clinician decision

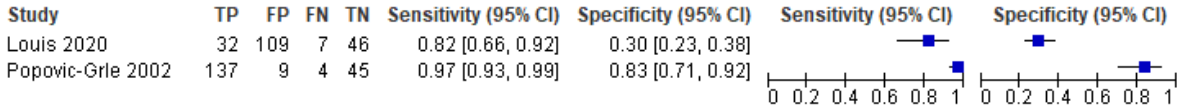


Figure 6: Methacholine challenge (PC20 FEV_1 ≤16 mg/mL) vs bronchodilator reversibility with/without clinician diagnosis or clinician diagnosis without an objective test

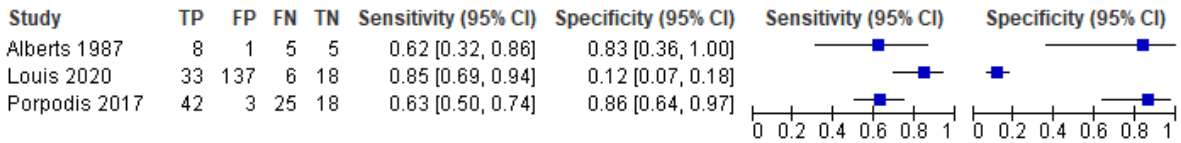
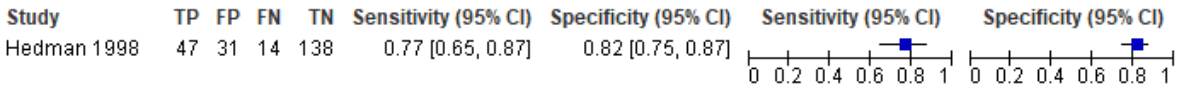
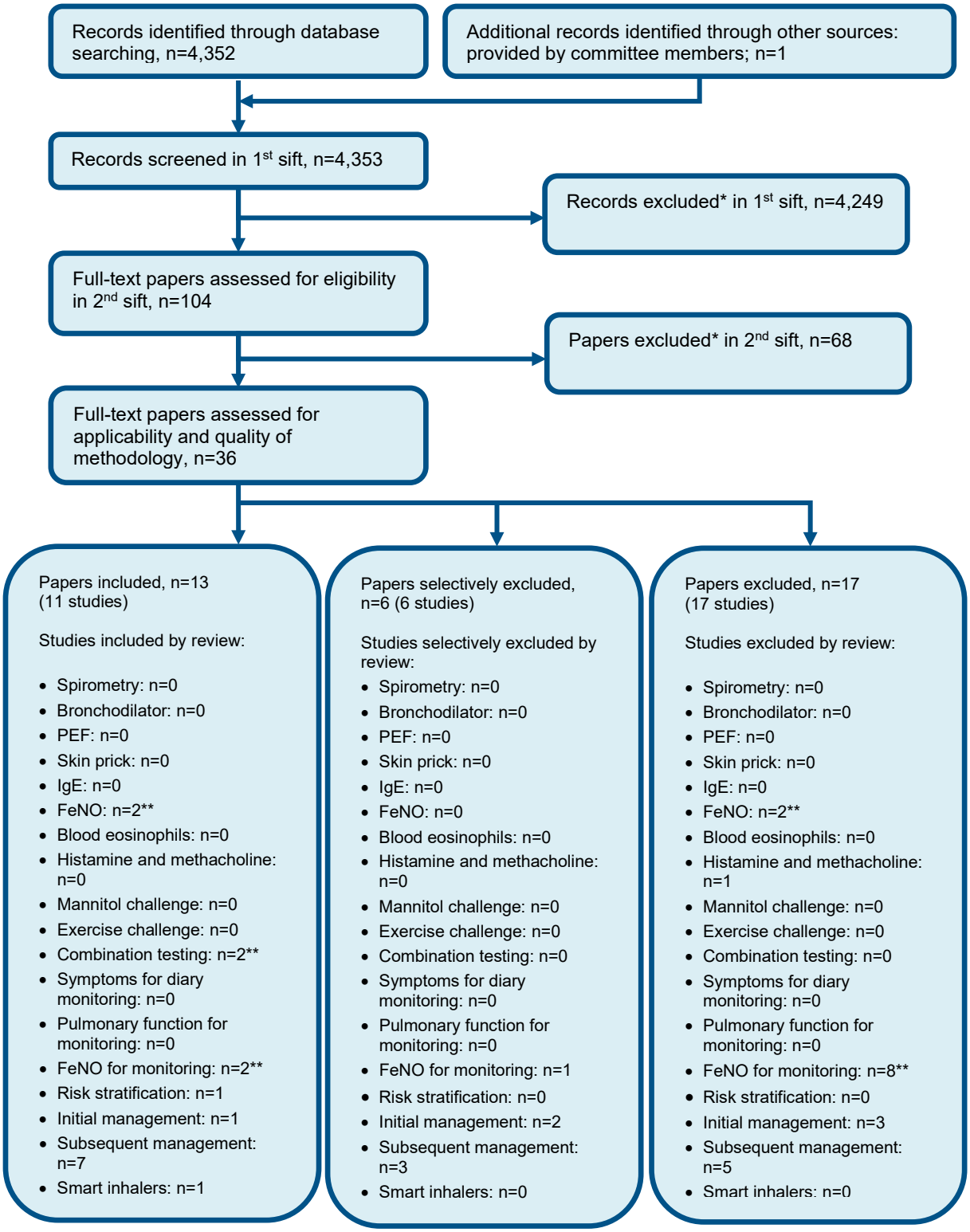


Figure 7: Methacholine challenge (PD20 FEV_1 ≤6900 µg) vs clinician diagnosis and skin prick testing, PEF variability, bronchodilator reversibility or exercise challenge tests



Appendix F – Economic evidence study selection

Figure 8: Flow chart of health economic study selection for the guideline



* Non-relevant population, intervention, comparison, design or setting; non-English language
** Includes studies that are in multiple reviews

Appendix G – Economic evidence tables

None.

Appendix H – Excluded studies

Clinical studies

Table 10: Studies excluded from the clinical review

Study	Code [Reason]
Abramson, Michael J; Puy, Robert M; Weiner, John M (2010) Injection allergen immunotherapy for asthma. The Cochrane database of systematic reviews: cd001186	- Study design not relevant to this review protocol *Systematic review including intervention studies*
Alberts, W M; Goldman, A L; Leaverton, P E (1992) Bronchodilator testing "confidence intervals" based on the level of bronchial responsiveness. Chest 102(3): 737-41	- Full text paper not available
Albornoz, C; Calvo, M; Marin, F (1995) Correlation between the clinical classification of bronchial asthma severity and the methacholine test in children. Journal of investigational allergology & clinical immunology 5(6): 322-4	- Population not relevant to this review protocol *Participants diagnosed with asthma prior to study entry*
Amakye, Daniel O, Davis, Beth E, Martin, Alexandra L et al. (2013) Refractoriness to inhaled mannitol 3 hours after allergen challenge. Annals of allergy, asthma & immunology : official publication of the American College of Allergy, Asthma, & Immunology 111(3): 182-4	- Population not relevant to this review protocol *Participants diagnosed with asthma prior to study entry*
Amayasu, H, Yoshida, S, Ebana, S et al. (2000) Clarithromycin suppresses bronchial hyperresponsiveness associated with eosinophilic inflammation in patients with asthma. Annals of allergy, asthma & immunology : official publication of the American College of Allergy, Asthma, & Immunology 84(6): 594-8	- Study design not relevant to this review protocol *Intervention study, not a diagnostic accuracy study*
Avital, A, Springer, C, Bar-Yishay, E et al. (1995) Adenosine, methacholine, and exercise challenges in children with asthma or paediatric chronic obstructive pulmonary disease. Thorax 50(5): 511-6	- Population not relevant to this review protocol *Participants diagnosed with asthma at study entry*
Ayala, L E and Ahmed, T (1989) Is there loss of protective muscarinic receptor mechanism in asthma?. Chest 96(6): 1285-91	- Index test does not match review protocol *Methacholine using PD50 not PD20; population not matching protocol: part was not suspected of asthma*

Study	Code [Reason]
Bacci, E, Di Franco, A, Cianchetti, S et al. (1995) Bronchial hyperresponsiveness to hypertonic saline, but not to methacholine, predicts the presence of eosinophils in induced sputum from asthmatic patients. European respiratory journal - supplement 8(suppl19): 472s	- Conference abstract
Backer, V and Ulrik, C S (1992) Bronchial responsiveness to exercise in a random sample of 494 children and adolescents from Copenhagen. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology 22(8): 741-7	- Population not relevant to this review protocol *Participants not presenting with respiratory symptoms*
Bakirtas, Arzu and Turktas, Ipek (2007) Methacholine and adenosine 5'-monophosphate challenges in preschool children with cough-variant and classic asthma. Pediatric pulmonology 42(10): 973-9	- Population not relevant to this review protocol *children under the age of 5 with known asthma*
Basir, R, Lehrman, S G, De Lorenzo, L J et al. (1995) Lack of significant bronchial reactivity to inhaled normal saline in subjects with a positive methacholine challenge test. The Journal of asthma : official journal of the Association for the Care of Asthma 32(1): 63-7	- Incorrect reference standard *No reference standard involved*
Bruschi, C, Cerveri, I, Zoia, M C et al. (1989) Bronchial responsiveness to inhaled methacholine in epidemiological studies: comparison of different indices. The European respiratory journal 2(7): 630-6	- Population not relevant to this review protocol *Participants not presenting with respiratory symptoms*
Caussade, S, Castro-Rodriguez, J A, Contreras, S et al. (2015) Methacholine challenge test by wheezing and oxygen saturation in preschool children with asthma. Allergologia et immunopathologia 43(2): 174-9	- Incorrect reference standard *Reference standard does not include an objective test - symptoms and response to treatment were used as pre-study diagnostic criteria*
Chenuel, B. (2020) A negative methacholine test rules out the diagnosis of asthma. Cons. Revue Francaise d'Allergologie 60(4): 290-291	- Study not reported in English
Choi, I.S., Lee, S., Kim, D.-H. et al. (2007) Airways are more reactive to histamine than to methacholine in patients with mild airway hyperresponsiveness, regardless of atopy. Korean Journal of Internal Medicine 22(3): 164-170	- Population not relevant to this review protocol *Participants diagnosed with asthma at study entry*

Study	Code [Reason]
Choi, Inseon S, Hong, Seo-Na, Lee, Yeon-Kyung et al. (2003) Asthmatic airway inflammation is more closely related to airway hyperresponsiveness to hypertonic saline than to methacholine. The Korean journal of internal medicine 18(2): 83-8	- Incorrect reference standard *Response to treatment used as reference standard*
Choi, Sun Hee, Kim, Do Kyun, Yoo, Young et al. (2007) Comparison of deltaFVC between patients with allergic rhinitis with airway hypersensitivity and patients with mild asthma. Annals of allergy, asthma & immunology : official publication of the American College of Allergy, Asthma, & Immunology 98(2): 128-33	- Population not relevant to this review protocol *Participants diagnosed with asthma prior to study entry*
Cockcroft, D W, Marciniuk, D D, Hurst, T S et al. (2001) Methacholine challenge: test-shortening procedures. Chest 120(6): 1857-60	- Study design not relevant to this review protocol *Not a diagnostic accuracy study*
Cockcroft, D W, Murdock, K Y, Berscheid, B A et al. (1992) Sensitivity and specificity of histamine PC20 determination in a random selection of young college students. The Journal of allergy and clinical immunology 89(1pt1): 23-30	- Population not relevant to this review protocol *Participants not presenting with respiratory symptoms*
Cockcroft, Donald W and Davis, Beth E (2006) The bronchoprotective effect of inhaling methacholine by using total lung capacity inspirations has a marked influence on the interpretation of the test result. The Journal of allergy and clinical immunology 117(6): 1244-8	- Review article but not a systematic review
Cockcroft, Donald W; Davis, Beth E; Blais, Christianne M (2020) Comparison of methacholine and mannitol challenges: importance of method of methacholine inhalation. Allergy, asthma, and clinical immunology : official journal of the Canadian Society of Allergy and Clinical Immunology 16: 14	- Population not relevant to this review protocol *Participants diagnosed with asthma at study entry*
de Jong, Carmen C M, Pedersen, Eva S L, Mozun, Rebeca et al. (2019) Diagnosis of asthma in children: the contribution of a detailed history and test results. The European respiratory journal 54(6)	- ICS washout period not appropriate *24-hour washout applied*
De Meer, Gea; Heederik, Dick; Postma, Dirkje S (2002) Bronchial responsiveness to adenosine 5'-monophosphate (AMP) and methacholine	- Population not relevant to this review protocol

Study	Code [Reason]
differ in their relationship with airway allergy and baseline FEV(1). American journal of respiratory and critical care medicine 165(3): 327-31	*Participants not presenting with respiratory symptoms*
De Souza, A.C.T.G. and Pereira, C.A.C. (2005) Bronchial provocation tests using methacholine, cycle ergometer exercise and free running in children with intermittent asthma. Jornal de Pediatria 81(1): 65-72	- Population not relevant to this review protocol *Participants diagnosed with asthma at study entry*
Deilami, G.D., Khandashpour, M., Paknejad, O. et al. (2009) Evaluation of methacholine challenge test results in chronic cough patients referring to clinic of pulmonary disease. Acta Medica Iranica 47(3): 175-179	- Incorrect reference standard *Methacholine challenge used as reference standard to assess diagnostic accuracy of chronic cough of varying durations*
den Otter, J J, Reijnen, G M, van den Bosch, W J et al. (1997) Testing bronchial hyper-responsiveness: provocation or peak expiratory flow variability?. The British journal of general practice : the journal of the Royal College of General Practitioners 47(421): 487-92	- Incorrect reference standard *Histamine challenge used as reference standard*
Di Lorenzo, Gabriele, Mansueto, Pasquale, Esposito-Pellitteri, Maria et al. (2007) The characteristics of different diagnostic tests in adult mild asthmatic patients: comparison with patients with asthma-like symptoms by gastro-oesophageal reflux. Respiratory medicine 101(7): 1455-61	- Population not relevant to this review protocol *Participants already diagnosed with asthma at study entry*
Drake, Sarah, Wang, Ran, Healy, Laura et al. (2021) Diagnosing Asthma with and without Aerosol-Generating Procedures. The journal of allergy and clinical immunology. In practice 9(12): 4243-4251e7	- Population not relevant to this review protocol *ICS washout period was 2 weeks - protocol specified a minimum of 4 weeks*
Fitzgerald, J.M. (1996) Relation of airway responsiveness to methacholine to parent and child reporting of symptoms suggesting asthma. Canadian Respiratory Journal 3(2): 115-123	- Population not relevant to this review protocol *Participants not presenting with respiratory symptoms*
Fruchter, Oren and Yigla, Mordechai (2008) Response to bronchodilators after exercise challenge predicts bronchial hyperreactivity. The Journal of asthma : official journal of the Association for the Care of Asthma 45(5): 353-6	- Incorrect outcome *Bronchial hyperresponsiveness*
Garcia-Rio, F, Mediano, O, Ramirez, M et al. (2004) Usefulness of bronchial reactivity analysis in the diagnosis of bronchial asthma in	- Inappropriate interval between index test and reference standard

Study	Code [Reason]
patients with bronchial hyperresponsiveness. Respiratory medicine 98(3): 199-204	*Two years between histamine challenge and clinician diagnosis*
Ghanei, M, Aliannejad, R, Mazloumi, M et al. (2015) Exertional-induced bronchoconstriction: comparison between cardiopulmonary exercise test and methacholine challenging test. Annals of cardiac anaesthesia 18(4): 479-485	- Incorrect reference standard *Methacholine challenge used as reference standard*
Ghodrati, S., Hormati, A., Mousavi, N.N. et al. (2011) Comparison of FEV1 and PEF values in cough variant asthma during methacholine challenge test. Journal of Zanjan University of Medical Sciences and Health Services 19(77): 3	- Study not reported in English
Giannini, D, Paggiaro, P L, Moscato, G et al. (1997) Comparison between peak expiratory flow and forced expiratory volume in one second (FEV1) during bronchoconstriction induced by different stimuli. The Journal of asthma : official journal of the Association for the Care of Asthma 34(2): 105-11	- Incorrect outcome *The sensitivity and specificity of different cut-off values of PEF change to detect a 15% decrease in FEV1 from baseline value, not detecting asthma; unclear reference standard*
Giovannini, M, Valli, M, Ribuffo, V et al. (2014) Relationship between Methacholine Challenge Testing and exhaled nitric oxide in adult patients with suspected bronchial asthma. European annals of allergy and clinical immunology 46(3): 109-13	- Study design not relevant to this review protocol *Not a diagnostic accuracy study*
Godfrey, Simon, Uwyyed, Kamal, Springer, Chaim et al. (2004) Is clinical wheezing reliable as the endpoint for bronchial challenges in preschool children?. Pediatric pulmonology 37(3): 193-200	- Population not relevant to this review protocol *Average population age <5 years*
Goldstein, M F, Veza, B A, Dunsky, E H et al. (2001) Comparisons of peak diurnal expiratory flow variation, postbronchodilator FEV(1) responses, and methacholine inhalation challenges in the evaluation of suspected asthma. Chest 119(4): 1001-10	- Incorrect reference standard *Methacholine challenge test used as part of clinician diagnosis*
Goldstein, Marc F, Veza, Bernadette A, Lauf-Goldstein, Arlene et al. (2002) Forced expiratory time and bronchial hyperresponsiveness to methacholine. The Journal of asthma : official journal of the Association for the Care of Asthma 39(2): 143-50	- Incorrect reference standard *Diagnostic accuracy result of methacholine inhalation challenge based on correlation with findings from shortened forced expiratory time (FET100%)*

Study	Code [Reason]
Higgins, B G, Britton, J R, Chinn, S et al. (1992) Comparison of bronchial reactivity and peak expiratory flow variability measurements for epidemiologic studies. The American review of respiratory disease 145(3): 588-93	- Population not relevant to this review protocol *Participants not presenting with respiratory symptoms*
James, A and Ryan, G (1997) Testing airway responsiveness using inhaled methacholine or histamine. Respirology (Carlton, Vic.) 2(2): 97-105	- Review article but not a systematic review
Johnson, K T and Funahashi, A (1987) Clinical characteristics and methacholine sensitivity in patients with suspected bronchial asthma. Wisconsin medical journal 86(4): 17-9	- Study design not relevant to this review protocol *No reference standard included, investigating correlation with symptoms*
Joyce, D P; Chapman, K R; Kesten, S (1996) Prior diagnosis and treatment of patients with normal results of methacholine challenge and unexplained respiratory symptoms. Chest 109(3): 697-701	- ICS washout period not appropriate *ICS not withheld prior to testing*
King, D K; Thompson, B T; Johnson, D C (1989) Wheezing on maximal forced exhalation in the diagnosis of atypical asthma. Lack of sensitivity and specificity. Annals of internal medicine 110(6): 451-5	- Incorrect reference standard *Methacholine challenge test used as reference standard with wheezing as the index test*
Koskela, Heikki O, Hyvarinen, Liisa, Brannan, John D et al. (2003) Responsiveness to three bronchial provocation tests in patients with asthma. Chest 124(6): 2171-7	- Population not relevant to this review protocol *Participants diagnosed with asthma at study entry*
Lee, Eun, Kim, Young-Ho, Han, Seungbong et al. (2017) Different cutoff values of methacholine bronchial provocation test depending on age in children with asthma. World journal of pediatrics : WJP 13(5): 439-445	- Population not relevant to this review protocol *Participants diagnosed with asthma prior to study entry*
Li, Bao-Hong, Guan, Wei-Jie, Zhu, Zheng et al. (2019) Methacholine bronchial provocation test for assessment of bronchial hyperresponsiveness in preschool children. Journal of thoracic disease 11(10): 4328-4336	- Population not relevant to this review protocol *Participants not presenting with respiratory symptoms*
Mazi, Ahlam and Lands, Larry C (2014) Effect of lowering methacholine challenge test cutoff in children. Annals of allergy, asthma & immunology : official publication of the American College of Allergy, Asthma, & Immunology 113(4): 393-7	- Incorrect reference standard *Unclear what the reference standard is*

Study	Code [Reason]
McGarvey, L P, Heaney, L G, Lawson, J T et al. (1998) Evaluation and outcome of patients with chronic non-productive cough using a comprehensive diagnostic protocol. Thorax 53(9): 738-43	- Incorrect reference standard *Histamine challenge test used as part of diagnostic decision*
McGeady, S.J., Schraeder, B.D., Waxenhiser, Z. et al. (1997) Methacholine challenge in detecting bronchial hyperreactivity in children. Pediatric Asthma, Allergy and Immunology 11(1): 31-38	- Index test does not match review protocol *Using breath units to determine positivity rather than PC20 or PD20*
Nazemiyah, Masoud, Ansarin, Khalil, Nouri-Vaskeh, Masoud et al. (2021) Comparison of spirometry and impulse oscillometry in methacholine challenge test for the detection of airway hyperresponsiveness in adults. Tuberkuloz ve toraks 69(1): 1-8	- Incorrect reference standard *No objective test or clinician diagnosis - study used response to therapy as reference standard*
Nieminen, M M (1992) Unimodal distribution of bronchial hyperresponsiveness to methacholine in asthmatic patients. Chest 102(5): 1537-43	- ICS washout period not appropriate *No washout applied*
Paknejad, O.; Hojjati, S.A.; Pazoki, M. (2011) The association between methacholine challenge test and respiratory symptoms: A study on 146 patients. Tehran University Medical Journal 68(11): 662-667	- Study not reported in English
Parameswaran, K; Belda, J; Sears, M R (1999) Use of peak flow variability and methacholine responsiveness in predicting changes from pre-test diagnosis of asthma. The European respiratory journal 14(6): 1358-62	- Index test does not match review protocol *Combination of methacholine and PEF reported, not in isolation (included in 1.11)*
Pedrosa, Maria, Barranco, Pilar, Caminoa, Magdalena et al. (2009) Comparison of methacholine and adenosine inhalation challenge in patients with suspected asthma. The Journal of asthma : official journal of the Association for the Care of Asthma 46(8): 773-6	- Index test does not match review protocol *diagnostic accuracy of adenosine monophosphate (AMP) with methacholine used as the reference standard*
Perez, M.P., Falcon, A.R., Galvan, M.F. et al. (2015) Comparative study of bronchial provocation tests using methacholine or mannitol in bronchial asthma. European Respiratory Journal 46(suppl59)	- Conference abstract
Pratter, M R; Hingston, D M; Irwin, R S (1983) Diagnosis of bronchial asthma by clinical	- Index test does not match review protocol *Methacholine but PC20/PD20 not used*

Study	Code [Reason]
evaluation. An unreliable method. Chest 84(1): 42-7	
Purokivi, Minna, Koskela, Heikki O, Koistinen, Tiina et al. (2007) Utility of hypertonic histamine challenge in distinguishing difficult-to-diagnose asthma. The clinical respiratory journal 1(2): 91-8	- Index test does not match review protocol *Hypertonic histamine challenge*
Raji, Hanieh, Haddadzadeh Shoushtari, Maryam, Idani, Esmaeil et al. (2018) Forced Expiratory Flow at 25-75% as a Marker for Airway Hyper Responsiveness in Adult Patients with Asthma-like Symptoms. Tanaffos 17(2): 90-95	- Incorrect reference standard *Objective test (spirometry) used without clinician diagnosis*
Remes, S T, Pekkanen, J, Remes, K et al. (2002) In search of childhood asthma: questionnaire, tests of bronchial hyperresponsiveness, and clinical evaluation. Thorax 57(2): 120-6	- Population not relevant to this review protocol *Study included participants already diagnosed with asthma and participants without respiratory symptoms*
Romero-Falcon, Maria Auxiliadora, Medina-Gallardo, Juan Francisco, Lopez-Campos, Jose Luis et al. (2022) Evaluation of the Diagnostic Accuracy of Non-Specific Bronchial Provocation Tests in the Diagnosis of Asthma: A Randomized Cross-Over Study. Archivos de bronconeumologia	- Incorrect reference standard *Response to treatment used as reference standard*
Salvador, R.O., Dijkers, E., Sterk, P. et al. (2017) Forced oscillation technique: An alternative outcome measure for methacholine provocation test. European Respiratory Journal 50(supplement61)	- Conference abstract
Shapiro, G G, Furukawa, C T, Pierson, W E et al. (1982) Methacholine bronchial challenge in children. The Journal of allergy and clinical immunology 69(4): 365-9	- Study design not relevant to this review protocol *No reference standard*
Siersted, H C, Mostgaard, G, Hyldebrandt, N et al. (1996) Interrelationships between diagnosed asthma, asthma-like symptoms, and abnormal airway behaviour in adolescence: the Odense Schoolchild Study. Thorax 51(5): 503-509	- Population not relevant to this review protocol *Participants not presenting with respiratory symptoms*
Son, K.M., Jang, S.H., Kang, H.R. et al. (2009) Role of methacholine PC20 in FEF25-75% for the diagnosis of bronchial asthma. Tuberculosis and Respiratory Diseases 67(4): 311-317	- Incorrect reference standard *Methacholine challenge involved in reference standard*

Study	Code [Reason]
Spiropoulos, K, Stevens, J, Eigen, H et al. (1986) Specificity and sensitivity of methacholine challenge test in children with normal and hyperreactive airways. Acta paediatrica Scandinavica 75(5): 737-43	- Incorrect reference standard *Unclear if reference standard (questionnaire) is diagnosing chronic cough or asthma*
Stocks, J.; Tripp, M.; Lin, T. (2014) Methacholine challenge is insufficient to exclude bronchial hyper-responsiveness in a symptomatic military population. Journal of Asthma 51(8): 886-890	- Incorrect reference standard *No reference standard included*
Suh, D.I.; Lee, J.K.; Koh, Y.Y. (2011) Mannitol challenge test to identify exercise-induced bronchoconstriction and refractoriness. Journal of Allergy and Clinical Immunology 127(2suppl1): ab6	- Conference abstract
Swartz, Edina and Lang, David (2008) When should a methacholine challenge be ordered for a patient with suspected asthma?. Cleveland Clinic journal of medicine 75(1): 37-40	- Not a peer-reviewed publication
Tan, Jessica H Y, Chew, Wui Mei, Lapperre, Therese S et al. (2017) Role of bronchoprovocation tests in identifying exercise-induced bronchoconstriction in a non-athletic population: a pilot study. Journal of thoracic disease 9(3): 537-542	- Index test does not match review protocol *Exercise challenge was index test, methacholine used as reference standard with no clinician diagnosis*
Ternesten-Hasseus, E, Farbrot, A, Lowhagen, O et al. (2002) Sensitivity to methacholine and capsaicin in patients with unclear respiratory symptoms. Allergy 57(6): 501-7	- Incorrect reference standard *Methacholine challenge test used as part of reference standard*
Urbankowski, Tomasz and Przybylowski, Tadeusz (2021) Methacholine Challenge Testing: Comparison of FEV1 and Airway Resistance Parameters. Respiratory care 66(3): 449-459	- Incorrect reference standard *Methacholine challenge test used as the reference standard*
van Noord, J A, Clement, J, van de Woestijne, K P et al. (1989) Total respiratory resistance and reactance as a measurement of response to bronchial challenge with histamine. The American review of respiratory disease 139(4): 921-6	- Study design not relevant to this review protocol *Study assessing reproducibility of IOS method*
Visser, F.J., van der Vegt, M.J.M.M., van der Wilt, G.J. et al. (2010) The optimization of the diagnostic work-up in patients with suspected	- Study design not relevant to this review protocol

Study	Code [Reason]
obstructive lung disease. BMC Pulmonary Medicine 10: 60	*Intervention trial, not a diagnostic accuracy study*
Waked, Myrna, Salameh, Pascale, Attoue, Rim et al. (2003) Methacholine challenge test: correlation with symptoms and atopy. Le Journal medical libanais. The Lebanese medical journal 51(2): 74-9	- Incorrect reference standard *No reference standard, instead examines correlations between symptoms and MCT positivity*
Wongtim, S, Mogmeud, S, Limthongkul, S et al. (1997) The role of the methacholine inhalation challenge in adult patients presenting with chronic cough. Asian Pacific journal of allergy and immunology 15(1): 9-14	- Incorrect reference standard *Methacholine challenge test included in clinician diagnosis*
Woo, Hyeonjin, Samra, Mona Salem, Lim, Dae Hyun et al. (2021) Current Asthma Prevalence Using Methacholine Challenge Test in Korean Children from 2010 to 2014. Journal of Korean medical science 36(19): e130	- Study design not relevant to this review protocol *Epidemiological study, not a diagnostic accuracy study*
Woolcock, A J; Green, W; Alpers, M P (1981) Asthma in a rural highland area of Papua New Guinea. The American review of respiratory disease 123(5): 565-7	- Study design not relevant to this review protocol *No reference standard included*
Yang, C L, Simons, E, Foty, R G et al. (2017) Misdiagnosis of asthma in schoolchildren. Pediatric pulmonology 52(3): 293-302	- Population not relevant to this review protocol *Participants diagnosed with asthma prior to study entry*
Yavuz, Suleyman Tolga, Civelek, Ersoy, Tuncer, Ayfer et al. (2011) Predictive factors for airway hyperresponsiveness in children with respiratory symptoms. Annals of allergy, asthma & immunology : official publication of the American College of Allergy, Asthma, & Immunology 106(5): 365-70	- Incorrect outcome *Investigating correlations between symptoms and positive methacholine challenge*
Zainudin, N M, Aziz, B A, Haifa, A L et al. (2001) Exercise-induced bronchoconstriction among Malay schoolchildren. Respirology (Carlton, Vic.) 6(2): 151-5	- Incorrect reference standard *No reference standard included*
Zhou, Ming-Juan, Chen, Yuan-Bin, Ouyang, Wen-Wei et al. (2021) Diagnostic value of the hypertonic saline bronchial provocation test in children with asthma using the high-power aerosol provocation system. The Journal of asthma : official journal of the Association for the Care of Asthma 58(5): 625-632	- Index test does not match review protocol *Hypertonic saline used as bronchial provocative agent*

Study	Code [Reason]
Zhu, Wenjing, Liu, Chuanhe, Sha, Li et al. (2022) Atypical asthma in children who present with isolated chest tightness: risk factors and clinical features. The Journal of asthma : official journal of the Association for the Care of Asthma 59(10): 1952-1960	- Incorrect reference standard *Methacholine challenge test included as part of clinician diagnosis*

Health Economic studies

Published health economic studies that met the inclusion criteria (relevant population, comparators, economic study design, published 2006 or later and not from non-OECD country or USA) but that were excluded following appraisal of applicability and methodological quality are listed below. See the health economic protocol for more details.

Table 11: Studies excluded from the health economic review

Reference	Reason for exclusion
Kennedy 2007(Kennedy et al., 2007)	Excluded as rated not applicable. Canadian resource use and costs from before 2002/2003 judged unlikely to be applicable to current UK NHS context.