A systematic review and economic evaluation of intraoperative tests (RD-100i OSNA system and Metasin test) for detecting sentinel lymph node metastases in breast cancer

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APPENDICES

Appendix 1: Accuracy of ultrasound and FNAC of the axilla

Extract from NICE clinical guideline on early and locally advanced breast cancer

Recommendation

• Pretreatment ultrasound evaluation of the axilla should be performed for all patients being investigated for early invasive breast cancer and, if morphologically abnormal lymph nodes are identified, ultrasound-guided needle sampling should be offered.

Qualifying statement: These recommendations are based on good evidence, including from a meta-analysis, of clinical effectiveness in reducing the number of patients who undergo SLNB and then need further axillary surgery, and reasonable evidence of cost effectiveness.

Clinical Evidence

The evidence for this topic comes from case series studies and one meta-analysis which pooled estimates.

Eight studies reported the proportion of cases in whom it was possible to visualise axillary lymph nodes on ultrasound. This proportion had a mean of 76% and median 81% but varied widely, with a range 35% to 99%. The remaining proportion represents patients for whom ultrasound does not add any information. (Altinyollar et al., 2005; Brancato et al., 2004; Damera et al., 2003; Deurloo et al., 2003; Dixon et al., 1992; Esen et al., 2005; Nori et al., 2005 and Podkrajsek et al., 2005).

The systematic review by Alvarez et al. (2006) performed a meta-analysis of staging outcomes for 'grey scale' axillary ultrasound based upon 16 case series studies. The metaanalysis provided pooled estimates of staging outcomes. When patients with palpable and non-palpable axillary lymph nodes were combined, lymph nodes that were suspicious on ultrasound based on their size (> 5mm); sensitivity was 69.2% and specificity was 75.2%.

If lymph nodes were suspicious on ultrasound based on their morphology the sensitivity was 71.0% and specificity was 86.2%. Considering only studies of patients with non-palpable lymph nodes, ultrasound had reduced sensitivity (using the morphologic criterion for nodal involvement) and there was little change in specificity. When a meta-analysis including only patients in whom it was possible to obtain biopsy material by ultrasound were considered, the pooled sensitivity was 75.0% and the pooled specificity was 98.3%. In a meta-analysis of patients in whom ultrasound-

guided biopsy was planned, and defining failure to find a lymph node on ultrasound or failure to collect biopsy material as a negative screen was conducted, the effect of these classifications was to reduce the sensitivity of ultrasound compared to earlier values, with little change in its specificity.

From case series studies the staging performance of 'grey scale' ultrasound alone showed a mean sensitivity of 62%, a mean specificity of 87%, a positive predictive value of 86% and a negative predictive value of 71%. (Altinyollar et al., 2005; Bartonkova et al., 2006; Brancato et al., 2004; Chandawarkar and Shinde, 1997; Esen et al., 2005; Heusinger et al., 2005; Lee et al., 1996; Hergan et al., 1996; Sato et al., 2004 and Van Rijk et al., 2006).

The staging performance of 'grey scale' ultrasound plus colour doppler ultrasound showed a mean sensitivity of 65%; a mean specificity of 89% a positive predictive value of 78% and a negative predictive value of 81%. (Couto et al., 2004; Dixon et al., 1992; Esen et al., 2005; Lee et al., 1996;, Nori et al., 2005; Perre et al., 1996; Podkrajsek et al., 2005 and Walsh et al., 1994).

The staging performance of ultrasound guided FNAC showed a mean sensitivity of 43%, a mean specificity of 100%, a positive predictive value of 99% and a negative predictive value of 72%. (Brancato et al., 2004; Damera et al., 2003; De Kanter et al., 2006; Deurloo et al., 2003; Lemos et al., 2005; Podkrajsek et al., 2005; Stewart et al., 2006 and Van Rijk et al., 2006). Ciatto et al. (2007) reported an overall sensitivity of 72.6% and specificity of 95.6% with a negative predictive value of 67.2% and a positive predictive value 96.6% when excluding inadequate results from analysis; including inadequate results as a negative gave a sensitivity of 64.6%, specificity of 95.7%, negative predictive value of 61.3% and a positive predictive value of 96.6%. Sahoo et al. (2007) reported an overall sensitvity of 96% and specificity of 93%. Somasunder et al. (2006) reported an increase in sensitivity from T1 (35%) to T3/4 (78%) and specificity from T1 (96%) to T3/4 (100%). The likelihood of lymph node FNAC being positive was linked with tumour stage (Ciatto et al., 2007 and Somasunder et al., 2006). Ciatto et al. (2007) also reported a significant association with histological grade and number of lymph nodes involved. Sahoo et al. (2007) reported that 40 (70%) patients with positive ultrasound FNAC were spared the additional step of SLNB while Somasunder et al. (2006) reported that 79 (47%) patients with positive ultrasound FNAC were spared SLNB.

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Appendix 2: Accuracy and side-effects of SLNB relative to ALND

Extract from NICE clinical guideline on early and locally advanced breast cancer

Recommendations

• Minimal surgery, rather than lymph node clearance, should be performed to stage the axilla for patients with early invasive breast cancer and no evidence of lymph node involvement on ultrasound or a negative ultrasound-guided needle biopsy. SLNB is the preferred technique.

• SLNB should only be performed by a team that is validated in the use of the technique, as identified in the New Start training programme1 .

• Perform SLNB using the dual technique with isotope and blue dye.

• Breast units should audit their axillary recurrence rates.

Qualifying statement: These recommendations are based on evidence from a meta-analysis of the results of observational studies and RCTs confirming the accuracy of SLNB in staging the axilla, RCT evidence of less morbidity with SLNB compared to axillary clearance and limited evidence that SLNB does not result in poorer overall or disease-free survival. Published health economic evidence is difficult to interpret in the UK context.

Clinical Evidence

Invasive breast cancer SLNB versus axillary clearance or axillary sampling

There is a large volume of evidence on SLNB both from RCTs and case series studies (Agarwal et al., 2005; Blanchard et al., 2003; BMJ Clinical Evidence 2005; Carlo et al., 2005; Clarke et al., 2004; Cody et al., 1999; Cox. et al., 2000; Cserni et al., 2002; Fleissig et al., 2006; Giuliano et al., 1997; Haid et al., 2002; Imoto et al., 2004; Julian et al., 2004; Katz et al., 2006; Kim et al., 2006; Kokke et al.,2005; Krag et al., 2001 and 2007; Langer et al., 2004; Langer et al., 2005; Leidenius 2004; Lucci et al., 2007; Mansel et al., 2006; Naik et al., 2004; Purushotham et al., 2005; Reitsamer et al., 2004; Rietman et al., 2003; Ung et al., 2004; Veronesi et al., 2003 and 2006 and Zavagno et al., 2005 a and b and 2008).

A well conducted systematic review and meta-analysis of 69 studies (of mixed study design) was undertaken by Kim, Giuliano and Lyman (2006) with data from over 8,000 patients. The overall sentinal lymph node localisation rate was 96.4%, the pooled estimate of false negative rate was

7.0%, the mean proportion of patients with positive sentinel lymph nodes was 42% and the post test probability negative was 4.6%. From other studies, the sentinel lymph node localisation rate ranged from 81.4% to 100% (mean 94.0% and median 94.9%) (Agarwal et al., 2005; Carlo et al., 2005; Clarke et al., 2004; Cody et al., 1999; Cox. et al., 2000; Cserni et al., 2002; Giuliano et al., 1997; Haid et al., 2002; Imoto et al., 2004; Julian et al., 2004; Krag et al., 2001; Langer et al., 2004; Langer et al., 2005; Naik et al., 2004; Reitsamer et al., 2004; Ung et al., 2004 and Veronesi et al., 2003).

The false negative rate of SLNB ranges from 0% to 10.7% (mean 5.8%, median 5.9%) (Agarwal et al., 2005; Clarke et al., 2004; Cody et al., 1999; Cox et al., 2000; Cserni et al., 2002; Giuliano et al., 1997; Julian et al., 2004; Krag et al., 2001; Langer et al., 2004; Ung et al., 2004 and Veronesi et al., 2003). The accuracy of SLNB ranges from 94.6% to 100% (mean 97.7% with a median of 98.3%) (Agarwal et al., 2005; Clarke et al., 2004; Cody et al., 1999; Cserni et al., 2002; Giuliano et al., 1997; Krag et al., 2001; Langer et al., 2004; Ung et al., 2004; Veronesi et al., 2003 and Cox et al., 2000.). The prevalence of axillary disease has a mean of 39.1%, median 35.4% and a range from 28.8% to 57.6% (Agarwal et al., 2005; Clarke et al., 2004; Cody et al., 1999; Cserni et al., 2002; Giuliano et al., 1997; Krag et al., 2001, Langer et al., 2004; Leidenius et al., 2004; Ung et al., 2004; Veronesi et al., 2003 and 2006 and Cox et al., 2000.).

The evidence on morbidity, including lymphoedema, favours SLNB over axillary clearance (Mansel et al., 2006; Fleissig et al., 2006; Purushotham et al., 2005; Lucci et al., 2007 and Zavagno et al., 2008). The ALMANAC RCT (reported by Mansel et al., 2006 and Fleissig et al., 2006) and the RCT by Purushotham et al. (2005) found little evidence, by intention to treat, that a difference exists in psychological morbidity between patients treated by SLNB compared to axillary clearance.

The follow-up periods in the studies ranged from a mean of 24 months from surgery (Blanchard et al., 2003) to a median of 60 months by Carlo et al. (2005) and up to 78 months as reported by Veronesi et al. (2006). The extent of follow-up is therefore immature and results should be interpreted with caution. However, findings showed that patients treated by SLNB do not appear to have poorer rates of disease-free survival or overall survival, or of axillary recurrence in the short term, compared to patients treated by axillary clearance.

The retrospective review conducted by Katz et al. (2006) of SLNB procedures in 1,133 patients, the majority of whom had invasive disease, identified the following factors as risk factors for involvement of the sentinel lymph node: younger age; mastectomy as definitive surgery; larger tumour size; invasive histology; and tumour lymphovascular invasion. In the same study in patients with involved sentinel lymph nodes, the following factors were found to be risk factors for

further axillary node involvement revealed by axillary clearance: tumour lymphovascular invasion; higher number of positive sentinel lymph nodes; larger sentinel lymph node deposits; and lower number of uninvolved sentinel lymph nodes.

A RCT by Lucci et al. (2007) reported that the use of SLNB plus ALND resulted in more wound infections, axillary seromas, and paresthesias than SLNB alone. Lymphoedema was more common after SLNB plus ALND but was significantly different only by subjective report. The use of SLNB alone resulted in fewer complications. Zavagno et al. (2008) reported that the analysis of the Psychological General Well Being Index questionnaire showed a statistically more positive outcome in the anxiety domain and in the general index for the sentinel lymph node group.

Axillary sampling as staging surgery

In addition to SLNB, a literature search was performed to identify studies which evaluated axillary sampling as staging surgery in early breast cancer. 15 studies were identified: two RCTs (Chetty et al., 2000 and Forrest et al., 1995) and 13 case series studies (Hadjiminas and Burke, 1994; Rampaul et al., 2004; Tanaka et al., 2006; Thompson et al., 1995; Mathew et al., 2006; Sato et al., 2001; Ishikawa et al., 2005; Narredy et al., 2006; Macmillan et al., 2001; Hoar and Stonelake, 2003; Gui et al., 2005; Cserni, 1999 and Kingsmore et al., 2003).

Staging performance: staging data for axillary sampling were identified in five case series studies, most of which were very small in size. From these limited data, axillary sampling appears to have a median false negative rate of 3.6% (range 0%-6.5%) and a median accuracy of 98.5% (range 98%-100%). Although these values appear favourable to those of SLNB2 they should be interpreted with caution due to the small volume of low-quality evidence. However the studies present no evidence that axillary sampling is inferior to SLNB in terms of detecting axillary disease.

Physical morbidity: evidence from one RCT is suggestive of reduced morbidity from axillary sample over axillary clearance or axillary sample plus radiotherapy, expressed as greater arm flexion at six months from surgery and smaller forearm circumference at three years from surgery. There were no other significant differences in morbidity outcomes, including upper arm circumference and other arm movements. Evidence from three observational studies comparing axillary sampling with axillary clearance favours axillary sample in terms of arm volume increase. Two of these studies suggest that radiotherapy, when used after axillary sampling in patients with disease-positive lymph nodes, has an adverse effect on shoulder mobility and arm volume.

A meta-analysis by Kim, Giuliano & Lyman (2006) provided a pooled estimate of FNR for SLNB as 7.0% [95% CI 5.2%-8.8%]. In studies of SLNB reviewed for this guideline, the accuracy of SLNB had median 98.3% (range 94.6% to 100%), based on 10 series of patients (three series were within RCTs). The FNR of SLNB had median 5.9% (range 0% to 10.7%) based upon 11 series of patients (four series were within RCTs).

Recurrence and survival: two RCTs comparing axillary sampling with axillary clearance found no significant difference in terms of survival or recurrence. One retrospective analysis of a large series of patients who were treated in the pre-SLNB era, concluded that survival is significantly improved if four or more lymph nodes are sampled, compared to sampling fewer than four lymph nodes. This effect was demonstrated for patients with metastatic axillary lymph nodes and for patients with no detectable nodal metastases. A second observational study was suggestive of an inverse relationship between survival and the number of positive lymph nodes, with the best survival in patients with no detectable nodal disease.

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Zavagno G, Carcoforo P, Franchini Z, Renier M, Barutta L, De Salvo GL, et al. (2005a) Axillary recurrence after negative sentinel lymph node biopsy without axillary dissection: a study on 479 breast cancer patients. Eur J Surg Oncol, 31 (7): 715–720.

Zavagno G, Carcoforo P, Marconato R, Franchini Z, Scalco G, Burelli P, et al. (2005b) Role of axillary sentinel lymph node biopsy in patients with pure ductal carcinoma in situ of the breast. BMC Cancer, 5: 28.

Zavagno G, De Salvo GL, Scalco G, Bozza F, Barutta L, Del Bianco P, et al. (2008) A Randomized clinical trial on sentinel lymph node biopsy versus axillary lymph node dissection in breast cancer: results of the Sentinella/GIVOM trial. Annals of Surgery, 247: 207–213.

Appendix 3: Literature search strategy

The search strategy focuses on the interventions under consideration for this review in context of the specific area in which the tests are applied: the lymph nodes. The search also, independently of the interventions, draws in literature on the biological markers CK19 and Mammaglobin (in context of the test area) which aims to help serve any modelling which may relate to this project. The search was not limited by language, methodology or to humans exclusively. The search was run from database inception.

Database search results

The following table details the databases search. The Web of Science searching included the Conference Proceedings Citation Index. Records were downloaded and managed in Endnote X5.

Table 1: Database search results

Database	Hits
Medline via OVID	197
Medline in Process via OVD	15
Embase via OVID	624
Web of Science via ISI	93
Cochrane via The Cochrane Library	18
HEED via The Cochrane Collaboration	4
Total	951
Endnote De-duplication	-286
Unique Records to Screen	665

Bibliographic Search Annex

1. Database: Ovid MEDLINE(R)

Host: Ovid

Data Parameters: 1946 to July Week 3 2012

Date Searched: Wednesday, August 1st 2012

Search Strategy: See Table 2 below

Hits: 197

Notes: N/A

Table 2: Search strategy for Ovid MEDLINE(R)

#	Searches	Results
1	Sysmex.mp.	464
2	(RD100i or RD-100i or (RD and 100i) or OSNA or One-step nucleic acid amplification).mp.	23
3	1 or 2	486
4	Metasin.mp.	0
5	"98/79/EC".tw.	16
6	3 or 4 or 5	502
7	Cytokeratin 19.mp.	1217
8	(CK19 adj5 (gene or lymph)).mp.	42
9	Mammaglobin B/ or Mammaglobin A/	179
10	mammaglobin.mp.	242
11	7 or 8 or 9 or 10	1441
12	6 or 11	1933
13	Sentinel Lymph Node Biopsy/	6859
14	exp Lymph Nodes/	65568
15	(lymph$ adj3 node$).mp.	169538
16	13 or 14 or 15	171356

17	12 and 16	197

2. Database: Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations

Host: Ovid

Data Parameters: July 31, 2012

Date Searched: Wednesday, August 1st 2012

Search Strategy: See Table 3 below

Hits: 15

Notes: N/A

Table 3: Search strategy for Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations

#	Searches	Results
1	Sysmex.mp.	26
2	(RD100i or RD-100i or (RD adj1 100i) or OSNA or One-step nucleic acid amplification).mp.	5
3	1 or 2	30
4	Metasin.mp.	0
5	"98/79/EC".tw.	0
6	3 or 4 or 5	30
7	Cytokeratin 19.mp.	61
8	(CK19 adj5 (gene or lymph)).mp.	5
9	Mammaglobin B/ or Mammaglobin A/	0
10	mammaglobin.mp.	5
11	7 or 8 or 9 or 10	69
12	6 or 11	97
13	Sentinel Lymph Node Biopsy/	0
14	exp Lymph Nodes/	0

15	(lymph$ adj3 node$).mp.	4941
16	13 or 14 or 15	4941
17	12 and 16	15

3. Database: Embase

Host: Ovid

Data Parameters: 1974 to 2012 Week 30

Date Searched: Wednesday, August 1st 2012

Search Strategy: See Table 4 below

Hits: 624

Notes: N/A

Table 4: Search strategy for Embase

#	Searches	Results
1	Sysmex.mp.	1135
2	(RD100i or RD-100i or (RD and 100i) or OSNA or "One-step nucleic acid amplification").mp.	98
3	1 or 2	1225
4	Metasin.mp.	11
5	"98/79/EC".tw.	32
6	3 or 4 or 5	1268
7	Cytokeratin 19.mp.	3691
8	(CK19 adj5 (gene or lymph)).mp.	79
9	Mammaglobin B/ or Mammaglobin A/	44
10	mammaglobin.mp.	425
11	7 or 8 or 9 or 10	4053

12	6 or 11	5266
13	Sentinel Lymph Node Biopsy/	7986
14	exp lymph node/	96163
15	(lymph$ adj3 node$).mp.	239855
16	13 or 14 or 15	241216
17	12 and 16	624

4. **Database: Web of Science (SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, BKCI-S, BKCI-SSH)**

Host: ISI

Data Parameters: 1899-2012

Date Searched: Wednesday, August 1st 2012

Search Strategy: See Table 5 below

Hits: 93

Notes: N/A

Table 5: Search strategy for Web of Science

#	Searches	Results
1	Topic=(("RD100i" or "RD-100i" or (RD NEAR/1 100i) or "OSNA" or "One-step nucleic acid amplification"))	--
2	Topic=("Metasin")	--
3	1 or 2	93

5. **Database: Cochrane Library**

Host: http://www.thecochranelibrary.com/view/0/index.html

Data Parameters: **Issue 7 of 12, July 2012**

Date Searched: Wednesday, August 1st 2012

Search Strategy: See Table 6 below

Hits: Reviews = 4; Central 13 and NHS EEDS 1. Total = 18

Notes: N/A

Table 6: Search strategy for Cochrane Library

#	Searches	Results
1	(Sysmex):ti,ab,kw	10
2	(RD100i or RD-100i or (RD and 100i) or OSNA or (One-step nucleic acid amplification))	8
3	Metasin	0
4	(#1 OR #2 OR #3)	18

6. **Database: Health Economic Evaluations Database (HEED)**

Host: via the Cochrane Collaboration

Data Parameters: **Issue 7 of 12, July 2012**

Date Searched: Wednesday, August 1st 2012

Search Strategy: See Table 7 below

Notes: N/A

Table 7: Search strategy for Health Economic Evaluations Database (HEED)

#	Searches	Results
1	All Data: Sysmex	--
2	All Data: (RD100i or RD-100i or (RD and 100i) or OSNA or (One-step nucleic acid amplification))	--
3	All Data: Metasin	4

Trials Registries

Trials registries were searched as follows:

Table 8: Trial registries searched

Registry	Hits
NIH ClinicalTrials.gov	3
Current Controlled Trials	0
WHO International Clinical Trials Registry Platform (ICTRP)	4
EU Clinical Trials Register	0
Total	7

1. NIH ClinicalTrials.gov

http://www.clinicaltrials.gov/

Searched: August 1st 2012

Results n=3 (see Table 9 below)

Table 9: NIH ClinicalTrials.gov searches

Search	Hits
OSNA	3
One-step nucleic acid amplification	0
Metasin	0

- Clinical Evaluation of OSNA Breast Cancer System to Extensive Frozen Section Histopathology via http://www.clinicaltrials.gov/ct2/show/NCT01368744?term=OSNA&rank=1

- Clinical Evaluation of OSNA Breast Cancer System in Breast Cancer Patients Receiving Neoadjuvant Therapy via http://www.clinicaltrials.gov/ct2/show/NCT01140776?term=OSNA&rank=2

- Clinical Evaluation of OSNA Breast Cancer System to Test Sentinel Lymph Nodes From Patients With Breast Cancer via http://www.clinicaltrials.gov/ct2/show/NCT01136369?term=OSNA&rank=3

2. Current Controlled Trials

http://www.controlled-trials.com/

Searched: August 1st 2012

Results n=0 (see Table 10 below)

Table 10: Current Controlled Trials searches

Search	Hits
OSNA	0
One-step nucleic acid amplification	0
Metasin	0

3. WHO International Clinical Trials Registry Platform (ICTRP)

http://www.who.int/ictrp/en/

Searched: August 1st 2012

Results n=4 (see Table 11 below)

Table 11: ICTRP searches

Search	Hits
OSNA	4
One-step nucleic acid amplification	1
Metasin	0

- Clinical Evaluation of OSNA Breast Cancer System to Extensive Frozen Section Histopathology via http://apps.who.int/trialsearch/Trial.aspx?TrialID=NCT01368744

- Clinical evaluation of molecular detection for sentinel lymph node examination in breast cancer patients via http://apps.who.int/trialsearch/Trial.aspx?TrialID=JPRN-UMIN000005321

- Clinical Evaluation of OSNA Breast Cancer System in Breast Cancer Patients Receiving Neoadjuvant Therapy via http://apps.who.int/trialsearch/Trial.aspx?TrialID=NCT01140776

- Clinical Evaluation of OSNA Breast Cancer System to Test Sentinel Lymph Nodes From Patients With Breast Cancer via http://apps.who.int/trialsearch/Trial.aspx?TrialID=NCT01136369

- A clinical study of intraoperative diagnosis of sentinel lymph node metastasis in head and neck cancer patients using bimolecular methods via http://apps.who.int/trialsearch/Trial.aspx?TrialID=JPRN-UMIN000006508

4. EU Clinical Trials Register

https://www.clinicaltrialsregister.eu/

Searched: August 1st 2012

Results: n=0 (see Table 12 below)

Table 12: EU Clinical Trials Register searches

Search	Hits
OSNA	0
One-step nucleic acid amplification	0
Metasin	0

GOOGLE Searches

Searched: August 1st 2012

All the searches below were conducted using the advanced search function with a limit to PDF.

Search Term: OSNA

- http://www.sysmex-lifescience.com/files/lifescience_patients_en.pdf
- http://www.sysmex-lifescience.com/files/lifescience_en.pdf
- http://www.sysmex-lifescience.com/files/OSNA%20Produktflyer_EN_150.pdf
- http://www.sysmex-lifescience.com/files/English%20OSNA%20study%20-%20poster%20-%20Pathological%20society%20London_08-01-2009%20-%20English.pdf
- http://www.sysmex-lifescience.com/files/poster_san_antonio_breast_cancer_meeting_2007_german_osna_study.pdf
- http://www.osnaelectronics.net/safety_light/interfaces-process-automation.pdf
- http://www.translational-medicine.com/content/pdf/1479-5876-8-83.pdf
- http://www.sysmex-lifescience.com/files/sysmex_OSNA_breastcancer_en.pdf
- http://pannonia-pathology.com/sites/default/files/presentations/anna_sapino.pdf

All the searches below were conducted using the advanced search function without limit or filter

Search Term: OSNA

- http://www.translational-medicine.com/content/8/1/83
- http://www.asco.org/ascov2/Meetings/Abstracts?&vmview=abst_detail_view&confID=58&abstractID=40334

All the searches below were conducted using the advanced search function with a limit to PDF.

Search Term: METASIN

- https://docs.google.com/viewer?a=v&q=cache:InSL07ar5KgJ:web.me.com/pathologist/SENTINELNODEPCR/Update_of_Metasin_files/metasin%2520for%2520aprton.pdf+metasin+filetype:pdf&hl=en&gl=ca&pid=bl&srcid=ADGEESgSmQ9pWNx71jXzkiy3h8cx63faCeVSXSUFHb--5TwWuD998C-O5NnjXn3B-Hach6ViPCIcLcHJlxqeh_-wwmh5jVkCCiFX7GMUEnxr1fwA7doRdlVO9nthcRyDhpF7hWfn4Q3i&sig=AHIEtbR54NhNqzX3z8M70BiSxseJskXr9A
- http://www.pathsoc.org/files/meetings/winter2010/05.01.106552ProgMAINv10(web).pdf

All the searches below were conducted using the advanced search function

Search Term: METASIN

No hits

Forward Citation Chasing

Review of Effectiveness

Database: Web of Science

Host: Thomson Reuters

Date Searched: 15th October 2012

Search by: Jenny Lowe

Results: See Table 13 below

Table 13: Forward Citation Chasing for the effectiveness review

#	Citation	Hits	Notes
51	Intra-operative sentinel lymph node metastasis detection in breast cancer by "One-step Nucleic Acid Amplification (OSNA)" - results of the	0	

	French multicentre prospective study		
105	Reliability of Whole Sentinel Lymph Node Analysis by One-Step Nucleic Acid Amplification for Intraoperative Diagnosis of Breast Cancer Metastases	2	
121	One-step Nucleic Acid Amplification (OSNA): Intraoperative Rapid Molecular Diagnostic Method for the Detection of Sentinel Lymph Node Metastases in Breast Cancer Patients in Korean Cohort	0	
188	A Novel Automated Assay for the Rapid Identification of Metastatic Breast Carcinoma in Sentinel Lymph Nodes	9	
242	Sentinel lymph node analysis in breast cancer: contribution of one-step nucleic acid amplification (OSNA)	0	
260	One-step nucleic acid amplification (OSNA) assay for sentinel lymph node metastases as an alternative to conventional postoperative histology in breast cancer: A cost-benefit analysis	2	
355	Implementation of molecular intra-operative assessment of sentinel lymph node in breast cancer	4	
556	Accurate staging of axillary lymph nodes from breast cancer patients using a novel molecular	1	

	method		
653	One-step nucleic acid amplification-a molecular method for the detection of lymph node metastases in breast cancer patients; results of the German study group	25	
697	Multicentre evaluation of intraoperative molecular analysis of sentinel lymph nodes in breast carcinoma	11	
744	Molecular Detection of Lymph Node Metastases in Breast Cancer Patients: Results of a Multicenter Trial Using the One-Step Nucleic Acid Amplification Assay	44	
746	Routine clinical use of the one-step nucleic acid amplification assay for detection of sentinel lymph node metastases in breast cancer patients Results of a Multicenter Study in Japan	0	
775	One-step nucleic acid amplification for intraoperative detection of lymph node metastasis in breast cancer patients	71	
804	Comparison of molecular analysis and histopathology for axillary lymph node staging in primary breast cancer: Results of the B-CLOSER-I study	0	
820	Importance of assessing CK19 immunostaining in core biopsies in patients subjected to sentinel node study by OSNA	0	
Total		169	169

De-dupe		-76	
Unique items to screen		93	
De-dupe against the master search		-35	
Unique Items to Screen		58	

Review of Cost Effectiveness

Database: Web of Science

Host: Thomson Reuters

Date Searched: 9th October 2012

Search by: Chris Cooper

Results: See Table 14 below

Table 14: Forward Citation Chasing for the effectiveness review

Citation	N	Notes
ID 136. Cutress 2010: Observational and cost analysis of the implementation of breast cancer sentinel node intraoperative molecular diagnosis	9	N/A
ID 260. Guillen Paredes 2011: One-step nucleic acid amplification (OSNA) assay for sentinel lymph node metastases as an alternative to conventional postoperative histology in breast cancer: A cost-benefit analysis	2	N/A
ID 314. Iqbal et al., 2012 : Implementation of one step nucleic acid amplification	0	N/A

(OSNA) for Intra-operative assessment of sentinel lymph nodes in a DGH		
Total	11	

Appendix 4: Clinical effectiveness: quality appraisal and data extraction forms

Design	Participants	Tests	OUTCOMES
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]			
[redacted]			
[redacted]			
[redacted]			
[redacted]			
[redacted]			

[redacted]	
[redacted]	U
[redacted]	Y
[redacted]	U
[redacted]	U
[redacted]	L
[redacted]	U
[redacted]	Y
[redacted]	U
[redacted]	L
[redacted]	Y
[redacted]	U
[redacted]	U
[redacted]	L
[redacted]	Y
[redacted]	Y
[redacted]	Y
[redacted]	U
[redacted]	N
[redacted]	L

Design	Participants	Tests	OUTCOMES
Sundaresan (unpub)	**Number of participants:**	**Index (technical details):** The initial	**Accuracy outcomes:**

Objective: **Study design:** Description of the describe the validation of Metasin, a novel real time PCR assay for the detection of metastatic cancer in sentinel lymph nodes from breast cancer patients. **Country:** UK **No. of centres:** 6 centres **Funding:** NR **Notes**	1265 **Number of SLNs or ALNs:** 2279 SLNs **Recruitment procedure:** NR **Inclusion criteria:** NR **Exclusion criteria:** NR **Sample attrition / dropout:** NR	early beta study of internal validation of the Metasin-BLNA (M-BLNA) assay for preliminary use was carried out on a series of 245 cases. This high level of determination of the cut off values was carried out against the Veridex data set and morphology, enabling the verification of the thresholds for macro-metastasis (>2mm) and micro-metastasis (<2mm & more than 0.2mm) determination. The Cp values were determined for Ck19 (Cp values <25) and for Mammaglobin (<25.9). Thresholds for micro-metastasis were similarly determined (CK19>25 and <32) and for MGB the micro-metastasis were identified (Cp>25.9 and <32). The detailed methodology for the assay is presented in a companion manuscript (Ramadhani et al, manuscript in preparation) detailing PCR primers and PCR machine assay conditions. For RNA extraction and quantification, the protocol was adopted from the Genesearch assay. BMS staff were trained over a 3 day period. **Reference standard (technical details):** Sentinel lymph nodes were sectioned at 3 levels/steps of 150um. Nodal micro-metastasis (<2 mm and >0.2 mm) and macro-metastatic disease (>2 mm) were interpreted as positive for histologically confirmed positive disease	Sensitivity, specificity and concordance **Process outcomes:** **Clinical outcomes:** NR **Other:**NR **Unit of analysis:** Patient **Discordant case analysis:** Yes **Test failures:**

		Details of SLN detection: Sentinel nodes were identified by a combination of the use of blue dye and radiation: as per established conventional protocol following NEW START. **Extraction and division of SLN:** Six centres contributed tissue homogenates and RNA from patients treated for breast cancer. Two centres were only able to provide frozen RNA. The remaining institutions contributed lymph node homogenates stored at -80C. Lymph nodes were serially sliced in the longitudinal plane into an even number of approximately 2 mm slices. Alternate slices were submitted for conventional histopathological analysis and for homogenization and RNA preparation. **Discordance analysis:** Cases with discrepancy were further followed up by examination of the block by extra levels and selectively examined with MNF116 immunostaining. Cases deemed discordant if molecular assay was positive but histology negative were subject to a further round of analysis, subject to availability of homogenates for analysis. RNA was re-extracted where possible and was examined by an independent panel of markers. Retrospective discordant case analysis could not be uniformly followed in view of the lack of a formal process for informing patients of the different outcome if deeper levels were positive for tumour on the histological sections **Outcome assessor:** NR **Blinding:** NR	
Participant characteristics			
NR			
Results			
	n = 1265 patients		

	Three level histopathology	
Metasin	Positive	Negative
Positive	249	26
Negative	20	940

	Sensitivity (%)	Specificity (%)	Discordance (%)
n=1265 patients	92	97	4.4

Nodes (n)	**Median time to analysis (min)**
1	36
2	42
3	46

Test failure – 1.2% due to insufficient mRNA in sample

Methodological issues

See STARD table

Quality appraisal

Was a consecutive or random sample of patients enrolled? (Y/N/U)	U
Was a cohort study design avoided?[a](Y/N/U)	Y
Did the study avoid inappropriate exclusions? (Y/N/U)[g]	U
Could the selection of patients have introduced bias? (H/L/U)	U
Concerns that the included patients do not match the review question? (H/L/U)	L
Were the index test results interpreted without knowledge of the results of the reference standard? (Y/N/U)	U
If a threshold was used, was it pre-specified? (Y/N/U)	Y

Could the conduct or interpretation of the index test have introduced bias? (H/L/U)[e]	U
Are there concerns that the index test, its conduct, or interpretation differ from the review question? (H/L/U)	L
Is the reference standard likely to correctly classify the target condition? (Y/N/U)	Y
Were the reference standard results interpreted without knowledge of the results of the index test? (Y/N/U)	U
Could the reference standard, its conduct, or its interpretation have introduced bias?[f] (H/L/U)	U
Are there concerns that the target condition as defined by the reference standard does not match the review question?	L
Did all patients receive a reference standard? (Y/N/U)	Y
Did all patients receive the same reference standard? (Y/N/U)	Y
Were all samples (that should have been[b]) included in the analysis? (Y/N/U)	Y
Could the patient flow have introduced bias? (H/L/U)	U
Were samples suspected of TAB excluded from the analysis? (H/L/U)[c]	N
Are there concerns about selective reporting of outcomes? (H/L/U)	L

Design	Participants	Tests	OUTCOMES
Castellano et al. (2012) Objective: To assess the reliability of OSNA as a single test on whole SLNs as a method of intraoperative diagnosis and staging of SLNs in breast cancer. Study design: Cohort Country: Italy No. of centres: 1 Funding: Unknown **Notes** OSNA SLNs were also analysed using imprint cytology and the two results compared (almost like a single-gate study embedded within the parallel group study), but that comparison is not relevant for this review.	Number of participants: 110 OSNA, 169 histology Number of SLNs or ALNs: Unclear Recruitment procedure: Unclear Inclusion criteria: Patients who did not have suspicious ALNs after US, nor positive cytological smears. For the OSNA cohort, the primary tumour had to express CK19 in >80% of tumour cells. Exclusion criteria:NR Sample attrition / dropout: 13 patients transferred to histology due to lack of CK19 expression	Index (technical details):Min. weight for 1 OSNA reaction 50-600mg. SLNs homogenised using lysis buffer for 90s on ice. Homogenate centrifuged at 10,000g at room temperature for 1 min. 20 ul aliquots used as a template for RT-LAMP reaction. CK19 mRNA determined on RD100i system. According to standard curve, (+) corresponded to 250 to 5000 CK19 mRNA copies/ul, defined as micrometastases, (++) corresponded to >5000 CK19 mRNA copies/ul, defined as macrometastases. <250 CK19 copies/ul corresponded to a negative result. Reference standard (technical details): Histopathology; 4 slices placed in bioboxes , formalin fixed and paraffin-embedded. Slices step sectioned at 100 um intervals until extinction. First two consecutive sections for each step used for H&E staining and IHC. Metastatic deposits were measured in 2 dimensions and categorised according to AJCC. The categories were: pN0(i+), malignant cells <0.2mm, single tumour cells or a cluster of <200 cells; pN1mi, micrometastases >0.2mm and or >200 cells, pN1a, metastases in 1 to 3 ALNs or at least 1 metastasis >2.0mm. Details of SLN detection: SLNs were identified using a combination of blue dye and radioactive isotopes. Blue stained nodes and nodes with high radioactive counts were considered to be SLNs Extraction and division of SLN: SLNs were excised and sent to path lab before primary tumour surgery was conducted (to avoid tissue contamination). SLNs were cleared from fat tissue, weighed and cut along short axis. Four slices step sectioned at 100 um intervals until extinction. Discordance analysis: N/A Outcome assessor:NR Blinding:N/A	Accuracy outcomes: Positive and negative rates Process outcomes:NR Clinical outcomes:NR Other:NR Unit of analysis:Patient Discordant case analysis:N/A Test failures:NR

Participant characteristics

Intervention	OSNA	Histology
Patient No.	110	169
Median age, yrs (range)	66.7 (38-82)	61.2 (23-86)
Tumour size (%)		
<10 mm	33 (30)	41 (24)
1.1-1.5 cm	19 (17)	45 (27)
>1.5 cm	58 (53)	83 (49)
Histopathologic type (%)		
IDC	81 (74)	109 (64)
ILC	16 (14)	29 (17)
DCIS		
Others	13 (12)	31 (18)
HER2 (%)		
Negative	108 (98)	144 (85)
Positive	2 (2)	25 (15)

Results

	Total Cases	Negative (%)	ITC (%)	Micrometastases (%)	Macrometastases (%)
OSNA	110	78(71)	-	20(18)	12(11)
Histology	169	112 (66)	11(7)	13(8)	33(20)

Methodological issues	
Recruitment: Unclear **Replicates: Unclear whether replicate samples were analysed** **Outcome assessment: Unclear whether the histology was checked by more than one independent pathologist**	
Quality appraisal	
Was a consecutive or random sample of patients enrolled? (Y/N/U)	**U**
Was a cohort study design avoided?(Y/N/U)	**N**
Did the study avoid inappropriate exclusions? (Y/N/U)	**Y**
Could the selection of patients have introduced bias? (H/L/U)	**U**
Concerns that the included patients do not match the review question? (H/L/U)	**L**
Were the index test results interpreted without knowledge of the results of the reference standard? (Y/N/U)	**NA**
If a threshold was used, was it pre-specified? (Y/N/U)	**Y**
Could the conduct or interpretation of the index test have introduced bias? (H/L/U)	**L**
Are there concerns that the index test, its conduct, or interpretation differ from the review question? (H/L/U)	**L**
Is the reference standard likely to correctly classify the target condition? (Y/N/U)	**Y**
Were the reference standard results interpreted without knowledge of the results of the index test? (Y/N/U)	**NA**
Could the reference standard, its conduct, or its interpretation have introduced bias?(H/L/U)	**U**
Are there concerns that the target condition as defined by the reference standard does not match the review question?	**L**
Did all patients receive a reference standard? (Y/N/U)	**N**
Did all patients receive the same reference standard? (Y/N/U)	**Y**
Were all samples (that should have been) included in the analysis? (Y/N/U)	**Y**
Could the patient flow have introduced bias? (H/L/U)	**U**

Were samples suspected of TAB excluded from the analysis? (Y/N/U)	**NA**
Are there concerns about selective reporting of outcomes? (H/L/U)	**L**

Design	Participants	Tests	OUTCOMES
Le Frere Belda (2011) Objective: To assess one-step nucleic acid amplification (OSNA) for intraoperative sentinel lymph node (SLN) metastasis detection in breast cancer patients, using final histology as the reference standar Study design: Single gate Country: France No. of centres: 8 Funding: Laboratory consumables funded by Sysmex **Notes**	Number of participants: 233 Number of SLNs or ALNs: 503 samples from 456 SLNs Recruitment procedure:NR Inclusion criteria: All breast cancer patients scheduled for surgery with SLN biopsy were considered for enrolment. Exclusion criteria: Patients who had other types of cancer with metastatic spread, patients given neoadjuvant therapy or patients younger than 18 years of age. Sample attrition / dropout: NR	Index (technical details): Automated RT-LAMP of CK19 mRNA in the RD-100i detection system (Sysmex) was performed, without prior mRNA isolation and purification. The assay was performed in duplicate on a pure sample and on a diluted sample (1/10). Homogenates were then stored at -80oC. Results were automatically characterized by the CK19 mRNA copy number/uL of the original tissue homogenate. A positive result (++; CK19 mRNA copy number (greater than 5,000/uL) is associated with macrometastasis, A positive result (+; copy numbers between 250 and 5,000/uL) with micrometastasis, and a negative result (copy numbers no greater than 250/uL) with either ITCs or no tumor. Inhibition of amplification is a rare event detected as a positive result (+, micrometastasis) in the diluted sample, but not the pure sample. Reference standard (technical details): In five centers, the two slices (b and d) for the histological analysis were first used for intraoperative frozen section (one hematoxylin-eosin stained level) or touch imprint diagnosis, according to standard practice in those centers. For the sections, five ribbons were cut with a 200 um skip space. From each ribbon, three sections were prepared, one H&E staining and two for IHC. Macrometastasis	Accuracy outcomes: sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (LR+) and negative likelihood ratio (LR-) Process outcomes: Median time for OSNA testing Clinical outcomes:None reported Other: None reported Unit of analysis:Patient and node Discordant case analysis:Yes Test failures:Yes

		was defined as a tumour deposit > 2 mm and micrometastasis as a tumor **deposit larger than 0.2 mm, but no greater than 2 mm. Tumor deposits no greater than 0.2 mm were categorized as ITCs and recorded as histologically negative** **pN0 (i+) in this study.** **Details of SLN detection: NR** **Extraction and division of SLN: The excised SLNs were cut into four equal slices. Two alternate slices (a and c) were prepared for OSNA and the other two slices (b and d) were fixed in 4% buffered formaldehyde and embedded in a paraffin block.** **Discordance analysis:** **When OSNA was positive and histology negative, consecutive ribbons with 200-um skip space were cut until exhaustion of the remainder** **of the paraffin-embedded SLN slices. The sections were stained with hematoxylin-eosin and immunostained with CK19 and AE1/AE3. In all cases of discrepancies, the SLN homogenates were shipped to Sysmex and subjected to blind molecular analysis.** **QRT-PCR was performed for CK19 and the breast tissue specific markers SPDEF (SAM pointed domain containing ETS transcription factor) and FOXA1**	

		(forkhead box A1). CK19 protein expression was assessed using Western blot. **OSNA and intensive molecular investigation showing the same results (both negative or both positive) were taken to indicate TAB, that is, presence of tumour deposit in either the b and d slices used for histology or the a and c slices used** **for OSNA.** **Outcome assessor:NR** **Blinding:Yes**	

Participant characteristics

Intervention	OSNA
No.	233
Median age, yrs (range)	58 (30-93)
Clinical stage (%)	
0	41 (17.7)
I	175 (75.4)
II	13 (5.6)
III	2 (0.9)
IV	1 (0.4)
Nodal status (%)	
pN0	225 (97.0)
pN1	7 (3.0)
pN2	
pN3	
Histopathologic type (%)	
IDC	164 (70.4)
ILC	34 (14.6)
DCIS	23 (9.9)
Others	12 (5.2)
HER2 (%)	
Negative	
Positive	13 (6.3)

Results

After TAB exclusion:

n=503 SLN				
Five level histopathology				
OSNA	**Macrometastasis**	**Micrometastasis**	**ITC**	**Negative**
++	**37**	**6**	**0**	**3**
+	**5**	**3**	**1**	**23**
-	**3**	**9**	**27**	**386**

n=233 patients				
Five level histopathology				
OSNA	**Macrometastasis**	**Micrometastasis**	**ITC**	**Negative**
++	**22**	**6**	**0**	**3**
+	**2**	**3**	**3**	**17**
-	**2**	**7**	**17**	**151**

Before TAB per sample:

Sensitivity % (95%CI)	Specificity% (95%CI)	PPV% (95%CI)	NPV% (95%CI)	OSNA LR+	OSNA LR-	Discordance (%)
80.9 (69.0-89.8)	93.9 (91.2-96.0)	65.4 (53.7-75.8)	97.2 (95.1-98.6)	13.2	0.20	7.7

Before TAB per patient:

Sensitivity % (95%CI)	Specificity% (95%CI)	PPV% (95%CI)	NPV% (95%CI)	OSNA LR+	OSNA LR-	Discordance (%)
78.6 (63.1-89.1)	88.0 (82.4-92.3)	58.9 (44.9-71.9)	94.9 (90.5-97.7)	6.5	0.20	7.5

Nodes (n)	Median time to analysis, min
1	33

2	**40**
3	**48**
4	**54**

Test failures – 1 sample excluded due to manipulation error

Methodological issues	
Recruitment: Unclear whether patients were recruited consecutively or randomly **Analysis: Five centres re-used frozen section samples which may impair integrity for final histology.** **Outcome assessment: Unclear whether histology was assessed by more than one independent pathologist** **Conflict of interest: Laboratory consumables purchased by Sysmex**	
Quality appraisal	
Was a consecutive or random sample of patients enrolled? (Y/N/U)	**U**
Was a cohort study design avoided?(Y/N/U)	**Y**
Did the study avoid inappropriate exclusions? (Y/N/U)	**Y**
Could the selection of patients have introduced bias? (H/L/U)	**U**
Concerns that the included patients do not match the review question? (H/L/U)	**L**
Were the index test results interpreted without knowledge of the results of the reference standard? (Y/N/U)	**U**
If a threshold was used, was it pre-specified? (Y/N/U)	**Y**
Could the conduct or interpretation of the index test have introduced bias? (H/L/U)	**L**
Are there concerns that the index test, its conduct, or interpretation differ from the review question? (H/L/U)	**L**
Is the reference standard likely to correctly classify the target condition? (Y/N/U)	**Y**
Were the reference standard results interpreted without knowledge of the results of the index test? (Y/N/U)	**Y**
Could the reference standard, its conduct, or its interpretation have introduced bias? (H/L/U)	**U**
Are there concerns that the target condition as defined by the reference standard does not match the review question?	**L**
Did all patients receive a reference standard? (Y/N/U)	**Y**

Did all patients receive the same reference standard? (Y/N/U)	**Y**
Were all samples (that should have been) included in the analysis? (Y/N/U)	**Y**
Could the patient flow have introduced bias? (H/L/U)	**L**
Were samples suspected of TAB excluded from the analysis? (Y/N/U)	**Y**
Are there concerns about selective reporting of outcomes? (H/L/U)	**L**

Design	**Participants**	**Tests**	**OUTCOMES**
[redacted]	[redacted]	[redacted]	[redacted]
Notes			
Participant characteristics			
Results			
[redacted]			

[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	
Histology		
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]

	Sensitivity (%)	Specificity (%)	Discordance (%)
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]

[redacted]

Methodological issues
Randomisation and allocation: **Conflicts of interest:**
Quality appraisal
Was a consecutive or random sample of patients enrolled? (Y/N/U)
Was a cohort study design avoided?(Y/N/U)
Did the study avoid inappropriate exclusions? (Y/N/U)
Could the selection of patients have introduced bias? (H/L/U)
Concerns that the included patients do not match the review question? (H/L/U)
Were the index test results interpreted without knowledge of the results of the reference standard? (Y/N/U)
If a threshold was used, was it pre-specified? (Y/N/U)
Could the conduct or interpretation of the index test have introduced bias? (H/L/U)
Are there concerns that the index test, its conduct, or interpretation differ from the review question? (H/L/U)
Is the reference standard likely to correctly classify the target condition? (Y/N/U)
Were the reference standard results interpreted without knowledge of the results of the index test? (Y/N/U)
Could the reference standard, its conduct, or its interpretation have introduced bias?(H/L/U)
Are there concerns that the target condition as defined by the reference standard does not match the review question?
Did all patients receive a reference standard? (Y/N/U)
Did all patients receive the same reference standard? (Y/N/U)
Were all samples (that should have been) included in the analysis? (Y/N/U)
Could the patient flow have introduced bias? (H/L/U)
Were samples suspected of TAB excluded from the analysis? (Y/N/U)

Are there concerns about selective reporting of outcomes? (H/L/U) [redacted]

Design	Participants	Tests	OUTCOMES
Choi (2010) Objective: To assess the clinical utility and applicability of OSNA assay in breast cancer treatment in Korea by comparing it with histopathological examination Study design: Single gate Country: Korea No. of centres: 1 Funding: Sysmex	Number of participants: 199 (after exclusion – see below) Number of SLNs or ALNs: 284 SLNs Recruitment procedure: NR Inclusion criteria: Included patients were suspected as negative for lymph node metastasis from initial clinical assessment, and scheduled for SLN biopsies. Exclusion criteria: The patients receiving neoadjuvant therapy before undergoing SLN biopsy, and those who had already undergone SLN biopsy were excluded from the study. Sample attrition / dropout: One patient was excluded because she was finally diagnosed as not having breast cancer but large B cell lymphoma.	Index (technical details): Each lymph node was homogenized in glycine buffer. The solutions (10-time diluted and 100-time diluted solution) and the gene amplification reagent Lynoamp BC (Sysmex, Kobe, Japan) were set in dedicated device (RD-100i; Sysmex) and the following steps were automatically done. The solutions were mixed with six different CK19 primers, four deoxynucleoside triphosphates, reverse transcriptase, DNA synthetase and magnesium sulfate. The resulting solution reacted at a constant temperature of 65^{o}C. cDNA was synthesized from CK19 mRNA in the lymph node homogenized solution using reverse transcriptase. The gene amplification was preceded by DNA synthetase based on the synthesised cDNA. The degree of DNA amplified product was calculated by calibration curve, with standards of known CK19 mRNA concentrations. Negative - when both CK19 mRNA concentrations of 10 x diluted solution and that of 100 x diluted solution were <250 copies/µL. OSNA assay can classify the positive result into 3 categories: (++), (+), and (+I; positive with reaction	Accuracy outcomes: Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and concordance rate Process outcomes: The rapidity of OSNA assay was investigated by measuring the turnaround time, i.e., the time between starting homogenization and obtaining the results of OSNA assay Clinical outcomes:NR Other: None reported Method of assessment: Unit of analysis:Patient Discordant case analysis:Yes Test failures:NR
Notes			

		inhibited). Positive (++) was the case when CK19 mRNA concentration in the 10-time diluted solution was ≥5,000 copies/µL. Positive (+) – when CK19 mRNA concentration in the 10 x diluted solution <5,000 and ≥250 copies/µL. Positive (+I) – when CK19 mRNA concentration in the 10 x diluted solution was <250 copies/µL and CK19 mRNA concentration in the 100 x diluted solution was ≥250 copies/ µL. **Reference standard (technical details):** **Each SLN was cut along its longitudinal axis into sections of 1.5-2.0 mm thickness. For the postoperative histopathological examination, three level sections were prepared at 200 µm intervals. And three sections were obtained at each level for H&E staining, anti-cytokeratin antibody (AE1/ AE3) immunohistochemical (IHC) staining and unstaining.** **Presence/absence of metastases was judged by observing H&E staining and AE1/3 staining slides.** **In accordance with the TNM classification of AJCC 7th edition, metastatic deposits were recorded as isolated**	

		tumor cells (ITC) if their largest diameter was smaller than 0.2 mm, as micrometastases if they were larger than 0.2 mm but not larger than 2 mm, and as macrometastases if they were larger than 2 mm. In concordance with the TNM designation of ITC as pN0 (i+), lymph node samples were only regarded as positive if at least one micrometastasis **or macrometastasis was found. Consequently, lymph nodes with ITC were considered as negative in this study. Macrometastasis or micrometastasis was confirmed** **by both or either of intraoperative histopathological examination of frozen section specimens and postoperative histopathological examination with permanent tissue** **specimens.** **Details of SLN detection: For the detection of sentinel node, both radioisotope and blue dye was used in 159 patients, and radioisotope only in 40 patients.** **One to six hours prior to surgery, subareolar intradermal** **injection of Tc99m-antimony sulfate colloid (0.4 mCi)** **was performed in the quadrant where the tumour was** **located. After approximately 40-50 min, numbers and** **locations of SLN were checked**	

		with a gamma camera. **Subareolar intradermal injection of 0.8% indigiocarmine** **(0.8 cc) in four parts of the periareola was performed** **immediately prior to surgery. SLN was defined as any** **blue-stained nodes or any nodes with radioactive counts** **of 10% or great.** **Extraction and division of SLN: Resected lymph nodes were equally sectioned into blocks along their long axis at 2 mm intervals . Blocks a and c were subjected to OSNA assay, and blocks b and d to intra- and postoperative histopathological examination. If lymph nodes** **were less than 4 mm in the short axis, they were cut in** **half. One half was subjected to OSNA assay, and the other** **half to histopathological examination. Each lymph node** **was subjected to OSNA assay and histopathological examination** **Outcome assessor: NR** **Blinding: Unclear** **Discordant case analysis: In discordant cases, clinical** **information, status of non-SLNs, and expression of CK19** **protein in lymph node**	

		metastasis foci were evaluated on a patient basis.	

Participant characteristics

Intervention	O+H
No.	199
Median age, yrs (range)	40-49
Clinical stage (%)	
0	11 (5.5)
I	132 (66.3)
II	54 (27.1)
III	2 (1.0)
IV	11 (5.5)
Clinical tumour classification (%)	
T0	
Tis	8 (4.0)
T1	129 (64.8)
T2	56 (28.1)
T3	2 (1.0)
T4	
Tx	4 (2.0)
Nodal status (%)	
pN0	153 (76.9)
pN1	37 (18.6)
pN2	5 (2.5)
pN3	4 (2.0)
Histopathologic type (%)	
IDC	165 (82.9)
ILC	9 (4.5)
DCIS	9 (4.5)
Others	16 (8.1)

Results

n=199 pts				
Three level histopathology				
OSNA	Macrometastasis	Micrometastasis	ITC	Negative
++	19	2	1	1
+	3	3	0	4
+i	1	0	0	0
-	4	4	3	154

	Sensitivity (%)	Specificity (%)	Discordance (%)
n=199 pts	77.8 (0.60-0.90)	96.3 (0.92-0.99)	7

Nodes (n)	Mean time to analysis, min
1	35.2
2	44.8
3	50.4
4	50.0

Overall, 39.0 mins

Methodological issues

Recruitment: Unclear

Replicates: Unclear whether replicate samples were analysed

Outcome assessment: Unclear whether the histology was checked by more than one independent pathologist

Conflict of interest: The study was funded by Sysmex

Quality appraisal

Was a consecutive or random sample of patients enrolled? (Y/N/U)	U

Was a cohort study design avoided?(Y/N/U)	**Y**
Did the study avoid inappropriate exclusions? (Y/N/U)	**Y**
Could the selection of patients have introduced bias? (H/L/U)	**U**
Concerns that the included patients do not match the review question? (H/L/U)	**L**
Were the index test results interpreted without knowledge of the results of the reference standard? (Y/N/U)	**U**
If a threshold was used, was it pre-specified? (Y/N/U)	**Y**
Could the conduct or interpretation of the index test have introduced bias? (H/L/U)	**L**
Are there concerns that the index test, its conduct, or interpretation differ from the review question? (H/L/U)	**L**
Is the reference standard likely to correctly classify the target condition? (Y/N/U)	**Y**
Were the reference standard results interpreted without knowledge of the results of the index test? (Y/N/U)	**U**
Could the reference standard, its conduct, or its interpretation have introduced bias? (H/L/U)	**U**
Are there concerns that the target condition as defined by the reference standard does not match the review question?	**L**
Did all patients receive a reference standard? (Y/N/U)	**Y**
Did all patients receive the same reference standard? (Y/N/U)	**Y**
Were all samples (that should have been) included in the analysis? (Y/N/U)	**Y**
Could the patient flow have introduced bias? (H/L/U)	**U**
Were samples suspected of TAB excluded from the analysis? (Y/N/U)	**N**
Are there concerns about selective reporting of outcomes? (H/L/U)	**L**

Design	Participants	Tests	OUTCOMES
Feldman (2011) **Objective: To compare the performance of the OSNA system with that of a detailed histopathological examination of the lymph node and with IC for the detection of metastatic carcinoma in axcillary SLNs in patients who had early stage breast cancer** **Study design: Single gate** **Country: USA** **No. of centres: 11** **Funding: Sysmex** **Notes**	**Number of participants: 496** **Number of SLNs or ALNs: 1044 SLNs** **Recruitment procedure: NR** **Inclusion criteria: Patients aged >18 years with clinical tumor in situ (Tis), T1, or T2 primary breast cancer who were awaiting lymphatic mapping and SLN biopsy were eligible for enrollment** **Exclusion criteria: Locally advanced breast cancer (tumors classified as T3 or T4), ductal carcinoma in situ in patients who were undergoing breast-conserving surgery, clinically palpable suspicious axillary lymph nodes, previous diagnosis of another type of carcinoma, previous breast or axillary surgery, and preoperative neoadjuvant therapy** **Sample attrition / dropout: NR**	**Index (technical details):** **The SLN slices were homogenized in 4 mL of OSNA lysis buffer and centrifuged according to the manufacturer's directions. A 1:10 dilution of the RNA-rich middle layer was transferred into the analyzer, which automatically performed the amplification reaction and analysis. The device was calibrated to designate samples that contained ≥250 copies per lL of CK19 mRNA as positive for metastatic tumor. Cutoff values, system calibration, and calculation of the CK19 mRNA level of the sample from the calibration curve were determined as described previously (Tsujimoto et al., 2007). A negative control was analyzed during the calibration and sample analysis to check for contamination issues, and a positive control was analyzed to check for any reagent quality or instrument issues** **Reference standard (technical details): Slices of the SLNs that were selected for histopathology were fixed in formalin and embedded in paraffin. Pathologists at the individual clinical sites evaluated the SLNs according to the standard protocol established at each site for clinical management. Paraffin blocks of the SLNs subsequently were cut at 200-lm intervals (levels) until all tissue was depleted. At each level, three 5-lmsections were cut; the first section for each level was stained with hematoxylin and eosin (H&E), and the third section from the third level was stained immunohistochemically using pan-CK antibodies. The remaining sections were blanks to be used for additional staining, if needed. All slides, including the H&E-stained, pan-CKimmunostained, and blank sections, were sent to a central reference pathology laboratory (Quest Diagnostics, Terterboro, NJ) for evaluation by at least 2 independent pathologists who were blinded to the histopathology results from the clinical sites and the results from the OSNA system. Tumor deposits in the SLNs were classified according to American Joint Committee on**	**Accuracy outcomes: Sensitivity and specificity; agreement; NVP and PPV** **Process outcomes: Time to analysis** **Clinical outcomes:NR** **Other: None** **Method of assessment:** **Unit of analysis:SLN** **Discordant case analysis: Yes** **Test failures:NR**

		Cancer guidelines **Details of SLN detection: Blue dye used in 34 pateints (6.9%), technetium 99m sulfur colloid radiocolloid used in 107 patients (21.6%), and both used in 355 patients (71.6%).** **Extraction and division of SLN: SLNs only included if 4mm-20mm along 4 the long axis with a thickness ranging from 4 mm to 10 mm. SLNs were cut using a proprietary, 5-blade lymph node cutter with an interblade distance of 1mm, which sectioned the SLNs into an average of 6 pieces along the long axis. Although the central pieces were cut uniformly into 1-mm slices, the edges could be ≥ 2 mm in thickness, in which case, they were manually bisected. Alternate slices of the lymph node were subjected either to analysis with the OSNA system or to detailed histopathologic examination** **Outcome assessor: 2 independent pathologists** **Blinding: Yes** **Discordant case analysis: Performed by Western blotting and QRT-PCR**	

Participant characteristics

Intervention	O+H
No.	496
Median age, yrs (range)	58.8 (28-88)
Clinical tumour classification (%)	
T0	
Tis	21 (4.2)
T1	327 (65.9)

T2	124 (25)
T3	5 (1)
T4	
Tx	19(3.8)
Nodal status (%)	
pN0	387 (78)
pN1	84 (16.9)
pN2	14 (2.8)
pN3	4 (0.8)
pNx	7 (1.4)
Histopathologic type (%)	
IDC	348 (70.2)
ILC	40 (8.1)
DCIS	
Others	109 (21.7)
HER2 (%)	
Negative	
Positive	

Results

n=1044 SLN				
Three level histopathology				
OSNA	Macrometastasis	Micrometastasis	ITC	Negative
++	77	9	1	8
+	9	12	0	29
-	9	22	14	854

	Sensitivity (%)	Specificity (%)	Discordance (%)
n=1044 SLN	77.5 (69.7-84.2)	95.8 (94.3-97.0)	6.8

Nodes (n)	Interquartile mean time to analysis, min
1	33.0
2	39.6
3	45.2

Methodological issues	
Recruitment: Unclear whether recruitment was consecutive or randomised **Patient flow: The number of SLNs after discordance (1018) does not comply with the numbers before discordance (1044) minus the resolved cases (28).** **Replicates: Unclear whether replicate samples were analysed** **Conflict of interest: The study was funded by Sysmex**	
Quality appraisal	
Was a consecutive or random sample of patients enrolled? (Y/N/U)	**U**
Was a cohort study design avoided?(Y/N/U)	**Y**
Did the study avoid inappropriate exclusions? (Y/N/U)	**Y**
Could the selection of patients have introduced bias? (H/L/U)	**U**
Concerns that the included patients do not match the review question? (H/L/U)	**L**
Were the index test results interpreted without knowledge of the results of the reference standard? (Y/N/U)	**Y**
If a threshold was used, was it pre-specified? (Y/N/U)	**N**
Could the conduct or interpretation of the index test have introduced bias? (H/L/U)	**L**
Are there concerns that the index test, its conduct, or interpretation differ from the review question? (H/L/U)	**L**
Is the reference standard likely to correctly classify the target condition? (Y/N/U)	**Y**
Were the reference standard results interpreted without knowledge of the results of the index test? (Y/N/U)	**Y**
Could the reference standard, its conduct, or its interpretation have introduced bias? (H/L/U)	**L**
Are there concerns that the target condition as defined by the reference standard does not match the review question?	**L**
Did all patients receive a reference standard? (Y/N/U)	**Y**
Did all patients receive the same reference standard? (Y/N/U)	**Y**
Were all samples (that should have been) included in the analysis? (Y/N/U)	**Y**
Could the patient flow have introduced bias? (H/L/U)	**U**

Were samples suspected of TAB excluded from the analysis? (Y/N/U)	**Y**
Are there concerns about selective reporting of outcomes? (H/L/U)	**L**

Design	Participants	Tests	OUTCOMES
Bernet Vegue (2012) **Objective: Description of the results of B-CLOSER-I with regard to staging** **Study design: Single gate** **Country: Spain** **No. of centres: 8** **Funding: Sysmex** **Notes**	**Number of participants: 55, after exclusions** **Number of SLNs or ALNs: 567 ALNs** **Recruitment procedure: Consecutive** **Inclusion criteria: In all cases, tumors were confirmed as CK19 positive by immunohistochemistry before SLN biopsy. All patients had** **undergone ALND after positive SLN biopsy diagnosed by OSNA.** **Exclusion criteria: Patients were excluded if they had metastatic disease, had received neoadjuvant therapy, or were judged unsuitable because of concomitant disease, and if fewer than 10 axillary lymph nodes were obtained by ALND** **Sample attrition / dropout: 2 patients with < 10 axillary nodes excluded**	**Index (technical details): The lymph node tissue was homogenized in 4mL of lysis buffer (Lynorhag, Sysmex) for 90 seconds and centrifuged for 1 minute at 10,000g. CK19 mRNA was then amplified by reverse-transcription loop-mediated amplification with a ready-to-use reagent kit (Lynoamp, Sysmex) in an RD-100i apparatus (Sysmex) according to the manufacturer's instructions. Results were classified according to the following cutoff values for CK19 mRNA copy number: <100 copies/mL, negative; 100 to 250 copies/ mL, negative (low expression); 250 to 5000 copies/mL, micrometastasis; and >5000 copies/mL, macrometastasis.** **Reference standard (technical details): The central tissue slice was then fixed and embedded in paraffin for histopathologic analysis and the remaining tissue was stored at -80oC before analysis by OSNA assay. A 5-mm paraffin section was obtained from each central slice and stained with hematoxylin-eosin. Macrometastases, micrometastases, and ITCs were classified according to AJCC TNM criteria. When ITCs were identified by histopathology, serial sections were taken from the remainder of the block to rule out the presence of micrometastases or macrometastases.** **Details of SLN detection: NR** **Extraction and division of SLN: Lymph nodes obtained by ALND were dissected away from the surrounding fat and weighed. In nodes weighing >50mg (the cutoff for validity using the OSNA method), a central longitudinal 1-mm slice was taken from each node using a fresh scalpel. 1 mm for histology, the remainder of the**	**Accuracy outcomes: Concordance** **Process outcomes: NR** **Clinical outcomes:NR** **Other: NR** **Method of assessment:** **Unit of analysis:Patient and ALN** **Discordant case analysis:Cases reported but no further analysis** **Test failures:NR**

		node for OSNA Outcome assessor: NR Blinding: NR Discordant case analysis: No further analysis	

Participant characteristics

Intervention	O+H
No.	55
Median age, yrs (range)	59 (23-87)
Clinical stage (%)	
0	
I	21 (38.2)
II	22 (40)
III	12 (21.8)
IV	
Unknown	
Histopathologic type (%)	
Invasive ductal carcinoma	44 (80.0)
Invasive lobular carcinoma	7 (12.7)
Ductal carcinoma in situ	1 (1.8)
Others	3 (5.5)
HER2 (%)	
Negative	49 (89.1)
Positive	6 (10.9)

Results

(n=567 non-SLN)

One level histopathology

OSNA	Macrometastasis	Micrometastasis	Negative
++	1	4	14
+	0	1	25
+i/low expression	0	0	8
-	0	0	514

Methodological issues	
Recruitment: Small sample size with relatively large number of ALNs Replicates: Unclear whether replicate samples were analysed Outcome assessment: Unclear whether the histology was checked by more than one independent pathologist Conflict of interest: The study was funded by Sysmex	
Quality appraisal	
Was a consecutive or random sample of patients enrolled? (Y/N/U)	Y
Was a cohort study design avoided?(Y/N/U)	Y
Did the study avoid inappropriate exclusions? (Y/N/U)	Y
Could the selection of patients have introduced bias? (H/L/U)	L
Concerns that the included patients do not match the review question? (H/L/U)	L
Were the index test results interpreted without knowledge of the results of the reference standard? (Y/N/U)	U
If a threshold was used, was it pre-specified? (Y/N/U)	Y
Could the conduct or interpretation of the index test have introduced bias? (H/L/U)	U
Are there concerns that the index test, its conduct, or interpretation differ from the review question? (H/L/U)	L
Is the reference standard likely to correctly classify the target condition? (Y/N/U)	Y
Were the reference standard results interpreted without knowledge of the results of the index test? (Y/N/U)	U
Could the reference standard, its conduct, or its interpretation have introduced bias? (H/L/U)	U
Are there concerns that the target condition as defined by the reference standard does not match the review question?	L
Did all patients receive a reference standard? (Y/N/U)	Y
Did all patients receive the same reference standard? (Y/N/U)	Y
Were all samples (that should have been) included in the analysis? (Y/N/U)	Y
Could the patient flow have introduced bias? (H/L/U)	L

Were samples suspected of TAB excluded from the analysis? (Y/N/U)	**L**
Are there concerns about selective reporting of outcomes? (H/L/U)	**L**

Design	Participants	Tests	OUTCOMES
Godey (2012) **Objective: To present first OSNA results in a routine clinical setting as compared with histology** **Study design: Single gate embedded in cohort** **Country: France** **No. of centres: Unclear** **Funding: NR** **Notes**	**Number of participants: 722** **Number of SLNs or ALNs: 810 SLN** **Recruitment procedure: NR** **Inclusion criteria: Clinically node negative early stage breast cancer undergoing axillary SLN procedure** **Exclusion criteria: NR** **Sample attrition / dropout:**	**Index (technical details): After removing extranodal tissue and lipid, the SLN is homogenised and centrifuged according to the manufacturer's instructions (Sysmex, Kobe, Japan). SLNs weighing more than 600 mg were cut and analysed separately with two or more molecular analyses. OSNA analysis was carried out in duplicate with a pure and a diluted sample (1/10) of SLN lysates without prior isolation and purification of mRNA. After a 16 min amplification time, the CK19 mRNA copy number per µl of lysate determined the node status defined as follows: copy number <250 = no metastasis, copy number 250–5000 = micrometastasis and copy number>5000 = macrometastasis. The OSNA assay discriminated macrometastasis from micrometastasis well but was not calibrated to detect isolated tumour cells.** **If copy numbers were >250 in the diluted preparation only, the OSNA result was designated as positive with inhibition of the amplification reaction; the SLN metastasis cannot be semi-quantified because of potential interference with the molecular detection. In our study, patients with at least one SLN macrometastasis were classed as macrometastatic, those with at least one SLN micrometastasis as micrometastic, and those with at least one metastasis with inhibition as metastatic.** **Reference standard (technical details): The final histological examination consisted of a detailed analysis of the SLN tissue sections embedded in paraffin blocks, and sectioned every 250 µm until the block was completely cut. Each level was initially stained with standard H&E. If no metastasis were revealed by conventional staining, then immunohistochemical (IHC) labelling was carried out using an anti-pancytokeratin antibody (AE1/ AE3 clones, Dako, Trappes, France): the SLN was examined by IHC labelling of all levels. Final examination of axillary non-SLNs was investigated by permanent**	**Accuracy outcomes: Positivity rate** **Process outcomes: Time for analysis** **Clinical outcomes:NR** **Other:NR** **Method of assessment:** **Unit of analysis: Patient** **Discordant case analysis: N/A** **Test failures:Issues with 3 samples for OSNA, no further details**

		histology (each 2 mm section of the lymph node was analysed with H&E staining) in both the OSNA and historical cohort. Details of SLN detection: The localisation of the sentinel node was identified using the combined method: 99mtechnetium-labelled colloid (Nanocoll, Amersham Swan, Eindhoven, the Netherlands) injected the day before surgery and 3 h after axillary lymphoscintigraphy, then, on the day of the procedure, subcutaneous injection of 2 ml of patent blue dye (Guerbet Patent Blue V, Guerbet Laboratory, Aulnay-sous-Bois, France). SLNs were cut by the pathologist and touch imprints were performed intraoperatively. Extraction and division of SLN: A 1 mm thick central slice was stained for postoperative histology. The remaining portion of the node was used for OSNA analysis intraoperatively. Outcome assessor: NR Blinding: NR Discordant case analysis: NR	

Participant characteristics

Intervention	O	H
No.	258	355
Median age, yrs (range)	56.8	56.9
Clinical tumour classification (%)		
T0		
Tis		

T1 a, b or c	**19, 93,146**	**16, 125, 214**
T2		
T3		
T4		
Tx		
Histopathologic type (%)		
IDC	**212**	**313**
ILC	**46**	**42**
DCIS		
Others		

Results

OSNA positive rate of 24.4%, histology 24.8%
Technical problems with OSNA for 3 patients, no further details

Nodes (n)	Mean time to analysis, min (std)
1	32.9 (4.9)
2	36.4 (4.5)
3	41.6 (5.2)
4	48.5 (8.7)

Methodological issues

Replicates: Unclear whether replicate samples were analysed
Outcome assessment: Unclear whether the histology was checked by more than one independent pathologist

Quality appraisal

Was a consecutive or random sample of patients enrolled? (Y/N/U)	**U**
Was a cohort study design avoided?(Y/N/U)	**N**
Did the study avoid inappropriate exclusions? (Y/N/U)	**N**
Could the selection of patients have introduced bias? (H/L/U)	**U**
Concerns that the included patients do not match the review question? (H/L/U)	**L**
Were the index test results interpreted without knowledge of the results of the reference standard? (Y/N/U)	**NA**
If a threshold was used, was it pre-specified? (Y/N/U)	**Y**
Could the conduct or interpretation of the index test have introduced bias? (H/L/U)	**U**
Are there concerns that the index test, its conduct, or interpretation differ from the review question? (H/L/U)	**U**
Is the reference standard likely to correctly classify the target condition? (Y/N/U)	**Y**

Were the reference standard results interpreted without knowledge of the results of the index test? (Y/N/U)	**U**
Could the reference standard, its conduct, or its interpretation have introduced bias? (H/L/U)	**U**
Are there concerns that the target condition as defined by the reference standard does not match the review question?	**U**
Did all patients receive a reference standard? (Y/N/U)	**N**
Did all patients receive the same reference standard? (Y/N/U)	**N**
Were all samples (that should have been) included in the analysis? (Y/N/U)	**Y**
Could the patient flow have introduced bias? (H/L/U)	**U**
Were samples suspected of TAB excluded from the analysis? (Y/N/U)	**NA**
Are there concerns about selective reporting of outcomes? (H/L/U)	**H**

Single gate results not reported.

Design	Participants	Tests	OUTCOMES
Bernet (2011) **Objective: To compare the results of OSNA with conventional histology and evaluate the feasibility of OSNA for intraoperative evaluation of SN in breast cancer surgery** **Study design: Observation** **Country: Spain** **No. of centres: 1 (for Trial 2)** **Funding: NR**	**Number of participants: 55** **Number of SLNs or ALNs: Unclear** **Recruitment procedure: NR** **Inclusion criteria: NR** **Exclusion criteria: NR** **Sample attrition / dropout: NR**	**Index (technical details): The OSNA protocol consisted of homogenization of tissue in a mRNA-stabilizing solution (Lynorhag, pH3.5; Sysmex, Barcelona, Spain) and subsequent isothermal** **(65°C) amplification of cytokeratin 19 (CK19) using the Lynoamp amplification kit (Sysmex)** **through a reverse transcriptase–loop-mediated isothermal** **amplification assay (RT–LAMP) in a gene amplification** **detector RD-100i (Sysmex) in compliance with the protocol described above.5,6 The technique uses six primers, which increase the specificity and** **speed of the reaction. Tissue homogenates from each** **lymph node were kept frozen at -80°C as a back-up for** **possible future studies.** **Reference standard (technical details): N/A** **Details of SLN detection:NR** **Extraction and division of SLN: The entire node was** **submitted to the OSNA assay in all cases, except in nine cases, where alternate slices were studied by both methods.**	**Accuracy outcomes: N/A** **Process outcomes:** **Time from receipt of node to analytical report** **Method of assessment:** **Unit of analysis: Node** **Discordant case analysis: N/A** **Test failures: NR**
Notes **Trial 1 not included in this review due to excluded comparator. Trial 2 was included for process outcomes**			

		Outcome assessor: N/A **Blinding: N/A** **Discordant case analysis: N/A**	
Participant characteristics			
NR			
Results			

Nodes (n)	Mean time to analysis, min (range)
1	39.6 (26-70)

Methodological issues	
Replicates: Unclear whether replicate samples were analysed	
Quality appraisal	
Was a consecutive or random sample of patients enrolled? (Y/N/U)	**N**
Was a cohort study design avoided?(Y/N/U)	**NA**
Did the study avoid inappropriate exclusions? (Y/N/U)	**U**
Could the selection of patients have introduced bias? (H/L/U)	**U**
Concerns that the included patients do not match the review question? (H/L/U)	**L**
Were the index test results interpreted without knowledge of the results of the reference standard? (Y/N/U)	**U**
If a threshold was used, was it pre-specified? (Y/N/U)	**Y**
Could the conduct or interpretation of the index test have introduced bias? (H/L/U)	**L**
Are there concerns that the index test, its conduct, or interpretation differ from the review question? (H/L/U)	**L**
Is the reference standard likely to correctly classify the target condition? (Y/N/U)	**Y**
Were the reference standard results interpreted without knowledge of the results of the index test? (Y/N/U)	**U**
Could the reference standard, its conduct, or its interpretation have introduced bias? (H/L/U)	**L**
Are there concerns that the target condition as defined by the reference standard does not match the review question?	**L**
Did all patients receive a reference standard? (Y/N/U)	**N**
Did all patients receive the same reference standard? (Y/N/U)	**NA**
Were all samples (that should have been) included in the analysis? (Y/N/U)	**U**
Could the patient flow have introduced bias? (H/L/U)	**U**

Were samples suspected of TAB excluded from the analysis? (Y/N/U)	**NA**
Are there concerns about selective reporting of outcomes? (H/L/U)	**U**

Design	Participants	Tests	OUTCOMES
Guillen-Paredes (2011) Objective: To analyse the economic costs of intraoperative OSNA compared to conventional deferred histological and immumohistochemical assay carried out in the hospital Study design: Cohort Country: Spain No. of centres: 1 Funding: Foundation for Healthcare Research and training of Murcia, FFIS	Number of participants: Histology – 45 patients OSNA – 35 patients Number of SLNs or ALNs: 114 SLNs Recruitment procedure: Patients were recruited from an Access database that recorded all sentinel node biopsies (SNBS) reported by the pathology department since the implementation of this technique in hospital in 2002 for the study of sentinel nodes in breast cancer patients Inclusion criteria: patients with breast cancer stages pT1/2 N0 M05 with clinically and ultrasound negative axillary lymph nodes, who underwent SNBS along with appropriate breast cancer surgery in the same intervention by the breast unit of our hospital, during the period between 15 October 2008 and 15 December 2009. Exclusion criteria: patients who had received neoadjuvant treatment, those who refused to sign the informed consent, patients who could not undergo the planned surgery due to high	Index (technical details): The sentinel node was sent fresh to the pathology department (if there were more than one lymph node then all were sent at once). The fat was separated from the lymph node and sectioned if it weighed more than 600 mg. The samples were then lysed by adding 4 ml of the reagent Lynorhag1 and centrifuged. The liquid phase of the mixture was placed in the OSNA RD100i, an analysis machine for automatic pipetting, amplification and detection. Results were obtained in approximately 30 min. Data are expressed quantitatively according to the number of CK19 mRNA copies per tumour cell: no metastasis (<2.5_102 CK19 mRNA copies per µl), micrometastasis (from 2.5_02 to 5_103 CK19 mRNA copies per µl) and macrometastasis (>5_103 CK19 mRNA copies per µl). Reference standard (technical details): After initial preparation of the lymph node using 4 mm sections fixed in formalin and embedded in paraffin, 15 x 4µm thick serial sections were cut and stained with haematoxylin–eosin and	Accuracy outcomes: NR Process outcomes: Operative time, days in hospital, hospital costs Clinical outcomes: Complications, lymphadenectomy Method of assessment: Unit of analysis: Patient Discordant case analysis: N/A Test failures: NR
Notes			

	anaesthetic risk, patients who underwent previous extensive breast surgery, patients who underwent a SNBS with local anaesthesia before the definitive breast surgery (as they were candidates for immediate reconstruction or for receiving neoadjuvant chemotherapy with clinical N0 in order to reduce tumour size), pregnant women and males. Sample attrition / dropout:	immunohistochemistry for cytokeratins. All preparations were examined by a pathologist using a conventional optical microscope, establishing the following stages: negative (no metastatic cells), isolated tumour cells (focus of malignant cells <0.2 mm), micrometastasis (>0.2 mm and _2 mm) and macrometastasis (>2 mm). Results were obtained within two weeks after surgery. Details of SLN detection: Breast cancer diagnoses were performed in our outpatient clinics, scheduling operations within 2 weeks after assessing the preoperative anaesthesia. The morning of the surgery, patients attended a nuclear medicine centre, where the breast lesions were located via ultrasound or stereotaxis. Radio-guided needles were placed at the centre of the lesions, through which a radiopharmaceutical agent was injected (0.5 mCi to 1 mCi of 99mTc albumin nanocolloid). After 2–3 h, control lymphoscintigraphies were performed. Subsequently, patients were admitted to our hospital to complete the preparation for surgery, which would be performed on that same afternoon. The	

		intervention involved a nuclear medicine specialist who traced the axillary region using a gamma detection probe. The sentinel node was defined as that which had an activity greater than 10% of the maximum activity detected. Extraction and division of SLN:Details only as above . Outcome assessor: Pathologist using a conventional optical microscope Blinding: N/A Discordant case analysis: N/A	

Participant characteristics

Intervention	O	H
No.	35	45
Median age, yrs (range)	55.54	61.89
Clinical tumour classification (%)		
T0		
Tis	2	3
T1	16	13
T2	17	29
T3		
T4		
Tx		
Histopathologic type (%)		
IDC	31	37
ILC	2	5
DCIS	1	2
Others	1	1

Results

	Mean Intervention Time, mins (sd)			Mean Days in Hospital (sd)		
	1st Operation	2nd	Total	1st Admission	2nd Admission	Total
Histology	**57.11 (23.93)**	**78.33 (NR)**	**78 (48.02**	**1.8 (2.04)**	**2.41 (1.09)**	**2.44(0.78)**
OSNA	**62.14 (48.02)**	**NA**	**62.14(21.93)**	**1.54(0.78)**	**NA**	**1.54(0.78)**

	Complications in 1st intervention			Complications in 2nd intervention		
	None	Minor	Major	None	Minor	Major
Histology	28	17	0	4	8	0
OSNA	24	10	1	N/A	N/A	N/A

Methodological issues

Replicates: Unclear whether replicate samples were analysed
Recruitment: Patients do not appear to have been recruited consecutively or randomly
Analysis: Unclear whether histopathology results were checked by an independent pathologist

Quality appraisal

Was a consecutive or random sample of patients enrolled? (Y/N/U)	**U**
Was a cohort study design avoided?(Y/N/U)	**N**
Did the study avoid inappropriate exclusions? (Y/N/U)	**Y**
Could the selection of patients have introduced bias? (H/L/U)	**U**
Concerns that the included patients do not match the review question? (H/L/U)	**L**
Were the index test results interpreted without knowledge of the results of the reference standard? (Y/N/U)	**NA**
If a threshold was used, was it pre-specified? (Y/N/U)	**Y**
Could the conduct or interpretation of the index test have introduced bias? (H/L/U)	**L**
Are there concerns that the index test, its conduct, or interpretation differ from the review question? (H/L/U)	**L**
Is the reference standard likely to correctly classify the target condition? (Y/N/U)	**Y**

Were the reference standard results interpreted without knowledge of the results of the index test? (Y/N/U)	**NA**
Could the reference standard, its conduct, or its interpretation have introduced bias? (H/L/U)	**U**
Are there concerns that the target condition as defined by the reference standard does not match the review question?	**L**
Did all patients receive a reference standard? (Y/N/U)	**NA**
Did all patients receive the same reference standard? (Y/N/U)	**NA**
Were all samples (that should have been) included in the analysis? (Y/N/U)	**Y**
Could the patient flow have introduced bias? (H/L/U)	**U**
Were samples suspected of TAB excluded from the analysis? (Y/N/U)	**NA**
Are there concerns about selective reporting of outcomes? (H/L/U)	**L**

Design	Participants	Tests	OUTCOMES
Khaddage (2011) **Objective: To evaluate the intraoperative performance of OSNA in comparison to post-op histology and then to introduce the technique into routine practice.** **Study design: Single gate** **Country: France** **No. of centres: Multi-centre. Number not reported** **Funding: Sysmex** **Notes**	**Number of participants: Validation study - 46 patients** **Routine study – 197 patients** **Number of SLNs or ALNs: Validation study – 80 SLNs** **Routine study - unclear** **Recruitment procedure: NR** **Inclusion criteria: For both patient cohorts inclusion criteria were a minimum age of 18 years and assignment for SLN biopsy** **Exclusion criteria: Neoadjuvant treatment and the presence of metastatic disease other than breast carcinoma** **Sample attrition / dropout: NR**	**Index (technical details): OSNA was performed according to the manufacturer's instructions (Sysmex, Kobe, Japan). The SLN slices were homogenised in 4 ml homogenising buffer Lynorhag (Sysmex). Afterwards, the homogenate was briefly centrifuged and directly used as a template for reverse transcription loop-mediated isothermal amplification (RT-LAMP) . Amplification of CK19 mRNA was automatically performed in an RD-100i instrument (Sysmex) with a ready-to-use reagent kit Lynoamp (Sysmex) consisting of a primer-nucleotide-mix, enzymes and CK19 mRNA calibrators as well as positive and negative controls.** **Prior to the sample run, three different calibrators with defined CK19 mRNA copy concentrations were used to establish a standard curve on the RD-100i. All the results were presented on the RD-100i in qualitative categories (++, +, –) and further specified by CK19 mRNA copy number/µl: 0-249 copies (–), 250-5000 copies (+), and copy number >5000 (++). A result indicating a (+) was comparable to the presence of a micrometastasis and (++) to a macrometastasis.** **Reference standard (technical details): For the clinical study, slices b and d were embedded**	**Accuracy outcomes: Concordance, sensitivity, specificity** **Process outcomes: Time to analysis** **Clinical outcomes:** **Method of assessment:** **Unit of analysis: Node and patient** **Discordant case analysis: Yes** **Test failures: NR**

		in paraffin and post-operatively cut at 200 µm intervals (5 levels). Each level was subjected to H&E and IHC staining for CK19 protein (Clone RCK108, Dako; Glostrup, Denmark) as well as IHC with AE1/AE3 (Clones AE1/AE3, Dako; Glostrup, Denmark) as a pan-cytokeratin marker. For routine use, a central slide of 1 mm from each SLN was analysed by 1 level of H&E staining and 1 level IHC (AE1/AE3). Non-SLNs (NSLN) were cut into 2 mm slices and 1 level of H&E staining was performed for each slice. **Tumour deposits were classified according to the TNM classification of the Union for International Cancer Control (UICC 6th edition) and the American Joint Committee on Cancer (AJCC 6th edition). The presence of a macrometastasis or micrometastasis was recorded as a positive histological result, isolated tumour cells (ITC), or a tumour-free SLN as a negative histological result.** **Details of SLN detection: NR** **Extraction and division of SLN: During the clinical study, nodes were defatted after SLN biopsy and intra-operatively cut into four equal slices (a, b, c, d) of 1 to 2 mm thickness. Two alternate slices were analysed by OSNA (a and c), slices b and d were subjected to histology.**	

		Outcome assessor: NR **Blinding: The results of OSNA were not known to the investigator of histology and vice versa.** **Discordant case analysis: DCI consisted of quantitative reverse-transcriptase polymerase chain reaction (QRT-PCR) for CK19 mRNA and two breast cancer-specific markers (SAM pointed domain containing ETS transcription factor, SPDEF, forkhead box A1, FOXA1) as well as beta-actin for RNA control. RNA was extracted from 200 µl of the homogenate. The cut-off levels for each marker were determined according to the QRT-PCR results of a series of histologically positive and negative lymph nodes from breast cancer patients.**	

Participant characteristics

Intervention	O+H Validation	O+H Routine
No.	46	197
Median age, yrs (range)		
Clinical tumour classification (%)		
T0	7	1
Tis	0	21
T1	34	141
T2	2	30
T3	2	1
T4	1	1
Tx		
Histopathologic type (%)		
IDC	36	148
ILC	5	16
DCIS	5	21
Others	0	12

Results

n=80 SLN				
5 Level Histopathology – validation study				
OSNA	**Macrometastasis**	**Micrometastasis**	**ITC**	**Negative**
++	11	2	-	0
+	2	0	-	1
-	0	0 (2)	2	60 (62)
n=46 patients – validation study				
5 Level Histopathology				
OSNA	**Macrometastasis**	**Micrometastasis**	**ITC**	**Negative**
++	6	2	0	0
+	0	0	0	1
-	0	0 (2)	2	33 (35)
n=197 patients				
1 Level Histopathology – routine use				
OSNA	**Macrometastasis**	**Micrometastasis**	**ITC**	**Negative**
++	9	1	-	3
+	8	7	-	14
-	0	0	-	155

	Sensitivity (%)	Specificity (%)	Discordance (%)
Validation study			
n=46 patients before TAB	80.0	97.2	3.7

n = 46 patients after TAB	**100**	**97.2**	
n= 80 SLN before TAB	**88.2**	**98.4**	
n= 80 SLN after TAB	**100**	**98.4**	

Median time to analysis for 2 nodes – 37 min

Methodological issues	
Replicates: Unclear whether replicate samples were analysed Recruitment: Unclear whether patients been recruited consecutively or randomly Analysis: Unclear whether histopathology results were checked by an independent pathologist	
Quality appraisal	
Was a consecutive or random sample of patients enrolled? (Y/N/U)	U
Was a cohort study design avoided?(Y/N/U)	Y
Did the study avoid inappropriate exclusions? (Y/N/U)	Y
Could the selection of patients have introduced bias? (H/L/U)	U
Concerns that the included patients do not match the review question? (H/L/U)	L
Were the index test results interpreted without knowledge of the results of the reference standard? (Y/N/U)	Y
If a threshold was used, was it pre-specified? (Y/N/U)	Y
Could the conduct or interpretation of the index test have introduced bias? (H/L/U)	L
Are there concerns that the index test, its conduct, or interpretation differ from the review question? (H/L/U)	L
Is the reference standard likely to correctly classify the target condition? (Y/N/U)	Y
Were the reference standard results interpreted without knowledge of the results of the index test? (Y/N/U)	Y
Could the reference standard, its conduct, or its interpretation have introduced bias? (H/L/U)	L
Are there concerns that the target condition as defined by the reference standard does not match the review question?	L
Did all patients receive a reference standard? (Y/N/U)	Y
Did all patients receive the same reference standard? (Y/N/U)	Y
Were all samples (that should have been) included in the analysis? (Y/N/U)	Y
Could the patient flow have introduced bias? (H/L/U)	L

Were samples suspected of TAB excluded from the analysis? (Y/N/U)	**Y**
Are there concerns about selective reporting of outcomes? (H/L/U)	**L**

Design	Participants	Tests	OUTCOMES
Osako (2011) Objective: To determine the performance of the OSNA assay as an accurate nodal staging tool in comparison with routine histological examination Study design: Cohort Country: Japan No. of centres: 1 Funding: Sysmex contributed to funding of laboratory consumables **Notes**	Number of participants: 183 Number of SLNs or ALNs:NR Recruitment procedure: Consecutive Inclusion criteria: Patients with clinically and ultrasonographically node-negative pT1-2 breast cancer who had undergone CALND after a positive SN biopsy with OSNA between April 2009 and September 2010. Exclusion criteria: Patients with 3 or more positive SNs 1) SN identification without using the radioisotope tracer 2) Previous excision of primary tumour 3) heterchronous ipsilateral breast cancer recurrence 4) Neoadjuvant drug therapy Sample attrition / dropout: NR	Index (technical details): After removal of the extranodal tissue, whole lymph nodes were homogenised with 4 ml lysis buffer solution (Lynorhag, Sysmex) and centrifgued at 10000 x g at RT. 2 µl of the supernatant was analysed using the RD-100i system (Sysmex), an automated molecular detection system using a reverse transcription loop-mediated isothermal amplification method and with the LynoampBC kit (Sysmex). The degree of amplification was detected using a by-product of the reaction, pyrophosphate. The resulting change in turbidity upon precipitation of magnesium pyrophosphate was in turn correlated with CK19 mRNA copy number per µl of the original lysate by a standard curve, which was established beforehand with thre calibrators containing different CK19mRNA copy numbers. A standard positive control containing 5000 copies per ul of CK19 mRNA and a negative conreol with no CK19 mRNA were used for quality assurance in each run. Lymph nodes that exceeded the specified maximum wieght of 600 mg were cut into two or more pieces and processed as separate nodes. The number of CK19mRNA copies per ul was calculated and the result assessed in accordance with the cutoff	Accuracy outcomes: Positive rate Process outcomes: Clinical outcomes: Method of assessment: Unit of analysis: Patient Discordant case analysis: N/A Test failures: NR

		level. CK19 mRNA (copy per µl) **≥5000 = Positive (++)** **250-5000 = Positive (+)** **≥250 = Positive with reaction inhibited (+i)** **<250 = Negative** **All SNs and a small number of non-SNs were assessed intraoperatively. Almost all non-SNs in CALND specimens were assessed post-operatively after frezzing at -80oC. The frozen non-SNs were assessed in the same manner as fresh nodes at a later date.** **Reference standard (technical details): All non-SNs were sliced in half along the long axis after formalin fixation. One of the cut surfaces was examined after H&E staining. Approx 5-7 nodes were embedded in paraffin in one casette. IHC was not used for evaluation of non-SNs.** **The non-SN specimens were classified into 3 categories according to the 7th AJCC Staging Manual: positive, micrometastasis; negative, ITC (<0.2mm) or no tumour cell. When cells were observed in multiple lymph nodes, the priority order was macrometastasis then micrometastasis.** **Details of SLN detection: The RI**	

		tracer used was 1.5mCl/ml of 99mTc-phytate. One day before surgery, the tracer was injected into the intraderal and subdermal space in the area of the tumour and the retro-tumoural space. In all cases, lymphoscintography was performed 1 hr after the injection. In addition, 2-3ml of vital dye, indigocarmine, was injected into the peri-tumoural space or areola at the time of surgery. Extraction and division of SLN: All non-SNs were sliced in half along the long axis after formalin fixation. Discussion refers to three-level histology, although this is not clear. Outcome assessor: NR Blinding: N/A. Discordant case analysis: N/A	

Participant characteristics

Intervention	O	H
No.	119	64
Median age, yrs (range)	53 (27-86)	56 (39-81)
Nodal status (%)		
pN0		
pN1	115 (96.6)	62 (96.9)
pN2	3 (3.4)	2 (3.1)
pN3		
Histopathologic type (%)		
Invasive ductal carcinoma	110 (92.4)	57 (89.1)
Invasive lobular carcinoma	4 (3.4)	2 (3.1)
Ductal carcinoma in situ		
Others/special type	5 (4.2)	5 (7.8)
HER2 (%)		
Negative	106 (89.1)	55 (85.9)
Positive	13 (10.9)	9 (14.1)

Results

Positive rate for non-SNs (%)	
Histology	20.3 (11.7-32.6)
OSNA	55.5 (46.1-64.5)

SLN stage	Overall axillary stage for histology									
		pN1mi		pN1a		pN2a		pN3a		Upstaging rate (%)
	No	No	%	No	%	No	%	No	%	
pN1 mi (sn)	21	19	90.5	1	4.8	1	4.8	0	0	9.5
pN1a (sn)	41	-	-	34	82.9	4	9.8	3	7.3	17.1
pN2a(sn)	2	-	-	-	-	2	100	0	0	0
All	64	19	29.7	35	54.7	7	10.9	3	4.7	14.1

SLN stage	Overall axillary stage for OSNA									
		pN1mi		pN1a		pN2a		pN3a		Upstaging rate (%)
	No	No	%	No	%	No	%	No	%	
pN1 mi (sn)	50	43	86.0	6	12.0	0	0	1	2.0	14.0
pN1a (sn)	65	-	-	54	83.1	9	13.8	2	3.1	16.9
pN2a(sn)	4	-	-	-	-	2	50.0	2	50.0	50.0
All	119	43	36.1	60	50.4	11	9.2	5	4.2	16.8

Methodological issues

Replicates: Unclear whether replicate samples were analysed

Analysis: Unclear whether histopathology results were checked by an independent pathologist

Conflict of interest: Consumables funded by Sysmex

Quality appraisal

Was a consecutive or random sample of patients enrolled? (Y/N/U)	Y
Was a cohort study design avoided?(Y/N/U)	N
Did the study avoid inappropriate exclusions? (Y/N/U)	Y

Could the selection of patients have introduced bias? (H/L/U)	**L**
Concerns that the included patients do not match the review question? (H/L/U)	**L**
Were the index test results interpreted without knowledge of the results of the reference standard? (Y/N/U)	**U**
If a threshold was used, was it pre-specified? (Y/N/U)	**Y**
Could the conduct or interpretation of the index test have introduced bias? (H/L/U)	**L**
Are there concerns that the index test, its conduct, or interpretation differ from the review question? (H/L/U)	**L**
Is the reference standard likely to correctly classify the target condition? (Y/N/U)	**Y**
Were the reference standard results interpreted without knowledge of the results of the index test? (Y/N/U)	**U**
Could the reference standard, its conduct, or its interpretation have introduced bias? (H/L/U)	**L**
Are there concerns that the target condition as defined by the reference standard does not match the review question?	**L**
Did all patients receive a reference standard? (Y/N/U)	**N**
Did all patients receive the same reference standard? (Y/N/U)	**Y**
Were all samples (that should have been) included in the analysis? (Y/N/U)	**Y**
Could the patient flow have introduced bias? (H/L/U)	**L**
Were samples suspected of TAB excluded from the analysis? (Y/N/U)	**NA**
Are there concerns about selective reporting of outcomes? (H/L/U)	**L**

Design	Participants	Tests	OUTCOMES
Schem (2009) **Objective: To evaluate the performance of OSNA in comparison to histology** **Study design: Single gate** **Country: Germany** **No. of centres: 2** **Funding: Sysmex** **Notes**	**Number of participants: 93** **Number of SLNs or ALNs: 343 ALNs** **Recruitment procedure: NR** **Inclusion criteria: NR** **Exclusion criteria: NR** **Sample attrition / dropout: NR**	**Index (technical details): The lymph node slices a&c** **were homogenized together in 4 ml of homogenizing buffer Lynorhag, pH 3.5, (Sysmex, Kobe, Japan) on ice. Twenty** **microliters of this homogenate were further used for automated amplification of CK19 mRNA via reverse transcription loop-mediated isothermal amplification (RTLAMP). Real-time amplification was accomplished with the Lynoamp Kit (Sysmex, Kobe, Japan) on the RD-100i (Sysmex, Kobe, Japan). Four lymph nodes can be analyzed in one run. The degree of amplification was detected via a by-product of the reaction, pyrophosphate. The resulting change in turbidity, upon precipitation of magnesium pyrophosphate, was in turn correlated to CK19 mRNA copy number/µL of the original lysate via a standard curve which was established beforehand with three calibrators containing different CK19 mRNA copy numbers.** **Since no isolation or purification of RNA was required for OSNA, results were available after a total of 30–40 min. The lymph node lysates were stored at −80°C until further use. If the CK19 mRNA copy number/µL lysate was less than 250 copies/µL, the result was regarded as (−); copy numbers between 250 and 5,000/µL were regarded as (+),**	**Accuracy outcomes: Concordance, sensitivity, specificity** **Process outcomes:** **Clinical outcomes:** **Method of assessment:** **Unit of analysis: Node** **Discordant case analysis: Yes** **Test failures: NR**

		and copy numbers larger than 5,000/µL as (++). **Reference standard (technical details): Lymph node slices b&d were fixed with neutral buffered formaldehyde and embedded in the same paraffin block. Each slice was identified by color coding. Two initial H&E sections (representing frozen sections of SN), one initial level, and four additional levels with a 0.1-mm skip space were cut from the 343 blocks.** **Each level consisted of four 4 µm sections: one was used for H&E staining, one for immunohistochemistry (IHC) with the pan anticytokeratin antibody LU5 (T-1302, Dianova, Germany), one for CK19 IHC (M0888, clone RCK 108, DAKO, Germany), and one spare section.** **For the specificity study, the paraffin blocks of 120 histologically negative samples, as judged by five-level histological work-up, were cut into further levels until no remnants remained. IHC was performed according to a standard protocol. Shortly, deparaffinised sections were cooked in a pressure cooker in Tris–ethylenediaminetetraacetic acid–sodium citrate buffer, pH 7.8, for 4 min. After blocking, incubation with the primary antibody was performed for 40 min and with the secondary**	

		antibody for 30 min. Visualization was done with diaminobenzidine tetrahydrochloride (Vector, Burlingame, CA, USA). Staining with the LU5 antibody was done using the NEXES staining automat and the I-View-Kit (Ventana, Illkirch, France). **Metastatic deposits were recorded, according to the TNM classification of UICC 6th and AJCC 6th edition [25, 26] as isolated tumor cells (ITC) if their largest diameter was smaller than 0.2 mm, as micrometastases if they were larger than 0.2 mm but not larger than 2 mm in** **diameter, and as macrometastases if they were larger than 2 mm in diameter. In concordance with the TNM designation of ITC as pN0(i+), lymph node samples were only regarded as positive if at least one micrometastasis or macrometastasis was found. Consequently, lymph nodes with ITC were considered as negative in this study.** **Details of SLN detection: NR** **Extraction and division of SLN: The 343 lymph node samples were longitudinally cut into four nearly equal slices (a, b, c, d) with a special cutting** **tool consisting of three blades being either 1 or 2 mm apart. ALN were categorized into groups according to their size:**	

		ALN with a minor axis smaller than 0.4 cm were excluded from the study; lymph nodes with a minor axis between 0.4 and 0.6 cm (group 1) were centrally cut into four slices with the 1–mm cutting tool; ALN between 0.6 and 1.0 cm (group 2) were centrally cut into four slices with a 2-mm cutting tool. Lymph nodes with a minor axis larger than 1.0 cm (group 3) were either halved or cut into several pieces, and each piece, depending on its size, was treated in a similar fashion as described for groups 1 and 2. Alternate slices were allocated to the OSNA method (a&c) and to histological work-up (b&d) at five levels. The slices used for OSNA (a&c) were shock frozen in liquid nitrogen and stored at −80°C before the analysis. Histological analysis was performed for slices b&d as outlined in a different section. **Outcome assessor: NR** **Blinding: Yes** **Discordant case analysis: If discordant results between the OSNA assay and five level histological examination occurred, the histological work-up of these cases was also extended until no tissue remained in the paraffin blocks. In addition, the homogenates of these discordant cases were also analysed by Western Blot and quantitative RT-PCR (QRT-PCR) as depicted in a different section. Provided that these supplemental analyses gave the same result as the OSNA assay, these samples were excluded from the study cohort because an uneven distribution of the metastases within pieces a, b, c, and d (tissue allocation bias) was likely to be the case.**	

Participant characteristics	
Intervention	**O+H**
No.	**93**
Clinical tumour classification (%)	
T0	
Tis	
T1	**6**
T2	**36**
T3	**4**
T4	**1**
Nodal status (%)	
pN0	**46**
pN1	**27**
pN2	**13**
pN3	**7**
Histopathologic type (%)	
Invasive ductal carcinoma	**68**
Invasive lobular carcinoma	**21**
Ductal carcinoma in situ	
Others (mixed)	**4**

Results

Before TAB exclusion

n=343 ALN				
Five level histopathology				
OSNA	Macrometastasis	Micrometastasis	ITC	Negative
++	90	7	0	9
+	7	-	1	16
-	0	2	2	209

	Sensitivity (%)	Specificity (%)	Discordance (%)
n= 343 ALN before TAB	98.1	91.7	8.2
N=330 ALN after TAB	100	95.6	4.5

Methodological issues

Replicates: Unclear whether replicate samples were analysed
Analysis: Unclear whether histopathology results were checked by an independent pathologist
Conflict of interest: Consumables funded by Sysmex

Quality appraisal

Was a consecutive or random sample of patients enrolled? (Y/N/U)	**U**
Was a cohort study design avoided?(Y/N/U)	**Y**
Did the study avoid inappropriate exclusions? (Y/N/U)	**U**
Could the selection of patients have introduced bias? (H/L/U)	**U**
Concerns that the included patients do not match the review question? (H/L/U)	**U**
Were the index test results interpreted without knowledge of the results of the reference standard? (Y/N/U)	**Y**
If a threshold was used, was it pre-specified? (Y/N/U)	**Y**

Could the conduct or interpretation of the index test have introduced bias? (H/L/U)	**L**
Are there concerns that the index test, its conduct, or interpretation differ from the review question? (H/L/U)	**L**
Is the reference standard likely to correctly classify the target condition? (Y/N/U)	**Y**
Were the reference standard results interpreted without knowledge of the results of the index test? (Y/N/U)	**U**
Could the reference standard, its conduct, or its interpretation have introduced bias?[f] (H/L/U)	**U**
Are there concerns that the target condition as defined by the reference standard does not match the review question?	**L**
Did all patients receive a reference standard? (Y/N/U)	**Y**
Did all patients receive the same reference standard? (Y/N/U)	**Y**
Were all samples (that should have been) included in the analysis? (Y/N/U)	**Y**
Could the patient flow have introduced bias? (H/L/U)	**U**
Were samples suspected of TAB excluded from the analysis? (Y/N/U)	**Y**
Are there concerns about selective reporting of outcomes? (H/L/U)	**L**

Design	Participants	Tests	OUTCOMES
Snook (2011) Objective: To evaluate OSNA as a potential intraoperative diagnostic tool via a multicentre prospective study which was undertaken to reassess the accuracy of OSNA diagnosis compared with intensive histopathological examination and to investigate the feasibility of intraoperative use of OSNA to diagnose lymph node metastases. Study design: Single gate Country: UK No. of centres: 4 Funding: The JuniperTrust and BUFFER (The BreastUnit Fund for Education and Research), both registered charities, funded the salary of clinical research fellow K.L.S., who was registered with the University of Surrey during the period of her MD research. There was no financial contribution from any commercial organization.	Number of participants: 204 Number of SLNs or ALNs: 393 lymph nodes, dissected to 417 samples Recruitment procedure: NR Inclusion criteria: SLNs from patients with a preoperative diagnosis of breast carcinoma, undergoing mastectomy or breastconserving surgery, were identified and removed surgically using the standard technique employed at each study site Exclusion criteria: Patients who had undergone neoadjuvant chemotherapy and those with a previous diagnosis of a potentially metastatic malignancy were excluded from the study Sample attrition / dropout: NR	Index (technical details): Lysates of homogenized lymph node samples were prepared manually before amplification. This involved mixing with the homogenizing reagent Lynorhag (Sysmex) followed by a short centrifugation step. The neat lysate sample and a diluted (1 : 10) lysate sample were analysed simultaneously using the OSNA/RD100i system (Sysmex) by reverse transcription–loop-mediated isothermal amplification (RT–LAMP)12 for the presence and amount of CK-19 mRNA. With OSNA, the user is provided with a qualitative result (++, + or −) and a quantitative result (copy numbers of CK-19 mRNA). (++) / >5000/ Macrometastasis (>2 mm) (+) /250–5000/ Micrometastasis (>0·2 to ≤2mm) (−) / 0–250/ negative (0) The time required for automated CK-19 mRNA amplification is 16 min, with variations in the preparation time according to the number of nodes to be processed. Simultaneous positive and negative controls are	Accuracy outcomes: Concordance, sensitivity, specificity Process outcomes: Time to test Clinical outcomes: Method of assessment: Unit of analysis: Patient and node Discordant case analysis: Yes Test failures: Yes
Notes			
The study was undertaken in two phases. The technical performance phase (TPP) was designed to familiarize each site with the molecular biological test. The technical performance and accuracy of the OSNA method of diagnosing breast cancer lymph node metastasis was compared with histology using both			

sentinel and non-sentinel axillary nodes for analysis. **Lymph node specimens for OSNA analysis were snap-frozen at −80° for analysis at a time suitable for the laboratory. Each site had to achieve a concordance of at least 80 per cent (94 per cent concordance was achieved across the four sites following discordant case analysis) in a minimum of 40 lymph nodes before starting the second phase. This next phase, the clinical equivalence study (CES), was designed to investigate the feasibility of intraoperative use of OSNA. Only SLNs were used for this phase. Lymph node specimens were analysed by OSNA immediately on arrival at the histopathology laboratory to simulate the intraoperative scenario.**		**performed** **with the specimen analysis to ensure quality control.** **Reference standard (technical details): One initial ribbon and additional ribbons with a 0·25-mm skip space were cut from slices b and d. From the initial ribbon and from the subsequent four ribbons (giving a total of 5 levels, equating to 10 levels of analysis in total as 2 separate slices of node were analysed), three 3-µm sections were used for haematoxylin and eosin staining, standard immunohistochemistry (IHC) with pancytokeratin clone** **AE1/AE3 (Dako, Glostrup, Denmark), and CK-19 IHC (clone RCK108; Dako). In the event of discordance, further levels were taken from the remaining ribbons, using the same protocol, until the entire paraffin block had been examined. Pathologists reporting on the histopathology slides were masked to the results of OSNA.** **Details of SLN detection: All sites used a combination of injected radioisotope, scintigraphy, hand-held γ probe and blue dye injection for identification of SLNs. Nodes were considered to be sentinel nodes as per the NEW START criteria (hot, blue, a**	

		combination of both, palpable and suspicious). **Extraction and division of SLN: Upon arrival in the histopathology laboratory, each node was categorized into one of four groups according to its size and sliced longitudinally with a purpose-designed cutting instrument into four 1- or 2-mm slices labelled a, b, c and d according to the criteria listed below.** **The total weight of each node for slicing could not exceed 1·2 g, so nodes weighing more than 1·2 g were divided into portions amenable to analysis by the technique (giving 2 or more node samples) before slicing. All nodal material subjected to slicing is herein referred to as the lymph node 'sample' and results reported according to sample numbers. Alternate slices (slices b and d) were colour coded, immediately fixed with neutral buffered formaldehyde and processed to paraffin blocks for histopathological analysis. Slices a and c were designated for OSNA analysis and snap-frozen at −80° (TPP) or analysed immediately for the clinical equivalence study.** **Outcome assessor: NR** **Blinding: Yes** **Discordant case analysis: When OSNA and histology results were discordant, the stored homogenate was**	

		analysed further by quantitative reverse transcriptase–polymerase chain reaction (qRT–PCR), as outlined below. If the discordant case investigation supported the OSNA result, it was concluded that metastases were confined either to the slices used for OSNA or to the slice or slices used for histology. This was defined as tissue allocation bias (TAB) and the samples were excluded because comparative evaluation of the two methods for this node was not possible. Total RNA was extracted from the homogenates of discordant samples with the RNeasy Mini Kit (Qiagen, Hilden, Germany). qRT–PCR for breast-cancer specific markers was then carried out with CK-19, SPDEF (SAM pointed domain containing Ets transcription factor) and FOXA1 (forkhead box A1). In addition, western blot analysis for CK-19 using 20 µl lysate was performed according to the procedure detailed elsewhere. At least one marker in addition to β-actin had to be positive to classify a result as truly discordant.	

Participant characteristics

Intervention	O+H
Clinical tumour classification (%)	
T0	
Tis	
T1	133
T2	60
T3	5
T4	
Histopathologic type (%)	
Invasive ductal carcinoma	160 (78.8)
Invasive lobular carcinoma	22 (10.8)
Ductal carcinoma in situ	
Others	16 (7.9)

Results

After TAB exclusion

n=395 SLN				
Five level histopathology				
OSNA	**Macrometastasis**	**Micrometastasis**	**ITC**	**Negative**
++	**48**	**1**	**0**	**0**
+	**8**	**9**	**0**	**10**
-	**4**	**2**	**20**	**293**

n=194 patients				
Five level histopathology				
OSNA	**Macrometastasis**	**Micrometastasis**	**ITC**	**Negative**
++	**33**	**1**	**0**	**0**
+	**5**	**5**	**1**	**7**
-	**4**	**1**	**11**	**126**

	Sensitivity (%)	**Specificity (%)**	**Discordance (%)**
n=417			
n = 194 patients after TAB	**89.8**	**94.5**	
n = 395 SLN after TAB	**91.7**	**96.9**	

Nodes (n)	**Median time to analysis, min (range)**
1	**32 (22-97)**
2	**42 (30-73)**
3	**51 (38-73)**

4	**62 (46-90)**

Test failures - 6 technical errors reported

Methodological issues	
Replicates: Unclear whether replicate samples were analysed **Analysis: Unclear whether histopathology results were checked by an independent pathologist**	
Quality appraisal	
Was a consecutive or random sample of patients enrolled? (Y/N/U)	**U**
Was a cohort study design avoided?(Y/N/U)	**Y**
Did the study avoid inappropriate exclusions? (Y/N/U)	**Y**
Could the selection of patients have introduced bias? (H/L/U)	**U**
Concerns that the included patients do not match the review question? (H/L/U)	**L**
Were the index test results interpreted without knowledge of the results of the reference standard? (Y/N/U)	**Y**
If a threshold was used, was it pre-specified? (Y/N/U)	**Y**
Could the conduct or interpretation of the index test have introduced bias? (H/L/U)	**L**
Are there concerns that the index test, its conduct, or interpretation differ from the review question? (H/L/U)	**L**
Is the reference standard likely to correctly classify the target condition? (Y/N/U)	**Y**
Were the reference standard results interpreted without knowledge of the results of the index test? (Y/N/U)	**Y**
Could the reference standard, its conduct, or its interpretation have introduced bias? (H/L/U)	**U**
Are there concerns that the target condition as defined by the reference standard does not match the review question?	**L**
Did all patients receive a reference standard? (Y/N/U)	**Y**
Did all patients receive the same reference standard? (Y/N/U)	**Y**
Were all samples (that should have been) included in the analysis? (Y/N/U)	**Y**
Could the patient flow have introduced bias? (H/L/U)	**U**

Were samples suspected of TAB excluded from the analysis? (Y/N/U)	**Y**
Are there concerns about selective reporting of outcomes? (H/L/U)	**L**

Design	Participants	Tests	OUTCOMES
Tamaki (2012) Objective: To determine the usefulness of the OSNA assay for clinical use in SLNB of breast cancer Study design: Single gate Country: Japan No. of centres: 11 Funding: Nakatani Foundation of Electronic Measuring Technology Advancement. **Notes**	Number of participants: 439 Number of SLNs or ALNs:775 Recruitment procedure: NR Inclusion criteria: The enrolment for this study comprised patients with tumor in situ (Tis) through T2, clinically lymph node negative primary breast cancer who underwent SLNB between August 2009 and December 2010 at 1 of the participating hospitals. Patients who had a preoperative diagnosis of ductal carcinoma in situ (DCIS) were enrolled in the study when a surgeon judged SLNB was needed. Patients who underwent SLNB before receiving preoperative systemic chemotherapy (PSCT) also were eligible for the analysis of sensitivity of the OSNA assay. Exclusion criteria: Those who received chemotherapy or hormone therapy before SLNB were excluded from the study. Men also were excluded. Sample attrition / dropout: Twenty-one of the originally enrolled patients were excluded	Index (technical details): An SLN was assessed with the OSNA assay according to the cutoff level of calculated CK19 mRNA copy numbers per microliter determined by Tsujimoto et al, and the results were reported according to the manufacturer's instructions: that is, as negative (<2.5 x 10^2 copies/uL), + positive (>2.5 x 10^2 and <5.0 x 10^3 copies/uL), ++ positive (>5.0 x 10^3 copies/uL), or positive +i(inhibited in the regular sample and >2.5 x 10^2 copies/uL in the diluted sample). Reference standard (technical details): A 1mm slice was cut from the longitudinal central part of the SLN, fixed as a permanent section for staining with H&E and examined postoperatively by a pathologist. Details of SLN detection: SLNs were detected using both radiocolloids and blue dye, radiocolloids only or blue dye only Extraction and division of SLN: Removed SLNs were assessed immediately with OSNA. Patients had axillary lymph node dissection recommended according to OSNA and/or other	Accuracy outcomes: Positive rate, concordance Process outcomes: Clinical outcomes: Method of assessment: Unit of analysis: Patient Discordant case analysis: Discussed but not analysed Test failures: Unclear – although 98.3 examined successfully with OSNA

	from the analysis because of significant violations against the study protocol, including 8 patients who received PSCT before SLNB, 10 patients who were not examined with the OSNA assay, 2 patients whose central sections of the SLN did not undergo pathologic examination as a permanent specimen for H&E staining, and 1 patient who was a man. Two patients who had benign intraductal papilloma confirmed after surgery, 1 who had with a clinical T4 tumor, and 2 who had clinically evident axillary lymph node metastases also were excluded because they did not meet the general criteria for SLNB candidates. Conversely, 2 patients who had T3 tumors that finally were diagnosed as DCIS and T1, invasive cancer were included. The final total enrolment was 413 patients who had 417 SLNBs eligible for analysis.	clinicopathologic factors. Non-SLNs were examined with a routine pathologic examination using H&E staining. Fat tissue surrounding the SLN was trimmed off. A 1 mm thick slice was then cut out from the longitudinal central part of the SLN, fixed as a permanent section for staining with H&E, and examined postoperatively by a pathologist at one of the hospitals. The remaining part of the lymph node was immediately examined with the OSNA assay by laboratory technicions . Outcome assessor: NR Blinding: Yes Discordant case analysis: Discussed but not analysed	

Participant characteristics

Intervention	O+H
No.	439
Men age, yrs (range)	56.1 (25-90)
Clinical stage (%)	
0	
I	183 (43.9)
II	110 (26.4)
III	70 (16.8)
IV	
Unknown	54 (12.9)
Clinical tumour classification (%)	
T0	
Tis	50 (12)
T1	254 (60.9)
T2	111 (26.6)
T3	2 (0.5)

T4	
Histopathologic type (%)	
Invasive ductal carcinoma	**305 (73.1)**
Invasive lobular carcinoma	**24 (5.8)**
Ductal carcinoma in situ	**53 (12.7)**
Others	**35 (8.4)**
HER2 (%)	
Positive	**51 (12.2)**
Negative	**334 (87.8)**

Results

n=417 patients (SLN)		
	One-level histopathology	
OSNA	Positive	Negative
Positive	58	36
Negative	8	315

	Discordance (%)
n=417	5.7

Methodological issues

Replicates: Unclear whether replicate samples were analysed
Analysis: Unclear whether histopathology results were checked by an independent pathologist
Recruitment: Unclear if recruitment was consecutive or random

Quality appraisal

Was a consecutive or random sample of patients enrolled? (Y/N/U)	**U**
Was a cohort study design avoided?(Y/N/U)	**Y**
Did the study avoid inappropriate exclusions? (Y/N/U)	**Y**
Could the selection of patients have introduced bias? (H/L/U)	**U**
Concerns that the included patients do not match the review question? (H/L/U)	**L**
Were the index test results interpreted without knowledge of the results of the reference standard? (Y/N/U)	**U**
If a threshold was used, was it pre-specified? (Y/N/U)	**Y**
Could the conduct or interpretation of the index test have introduced bias? (H/L/U)	**U**
Are there concerns that the index test, its conduct, or interpretation differ from the review question? (H/L/U)	**L**

Is the reference standard likely to correctly classify the target condition? (Y/N/U)	**Y**
Were the reference standard results interpreted without knowledge of the results of the index test? (Y/N/U)	**U**
Could the reference standard, its conduct, or its interpretation have introduced bias?[f] (H/L/U)	**U**
Are there concerns that the target condition as defined by the reference standard does not match the review question?	**L**
Did all patients receive a reference standard? (Y/N/U)	**Y**
Did all patients receive the same reference standard? (Y/N/U)	**U**
Were all samples (that should have been) included in the analysis? (Y/N/U)	**Y**
Could the patient flow have introduced bias? (H/L/U)	**L**
Were samples suspected of TAB excluded from the analysis? (Y/N/U)	**N**
Are there concerns about selective reporting of outcomes? (H/L/U)	**L**

Design	Participants	Tests	OUTCOMES
Tamaki (2009) **Objective: To develop a more efficient method for intraoperative detection of lymph node metastasis** **Study design: Single gate** **Country: Japan** **No. of centres: 6** **Funding: Not known - Sysmex had some involvement in the study** **Notes** **T1 – full histology** **T2 – frozen section then full histology**	**Number of participants: Two trials** **T1 n = 36 pts; n =149 nodes** **T2 n = 185 pts; n = 551 nodes** **Recruitment procedure: Unknown** **Inclusion criteria: NR** **Exclusion criteria: NR** **Sample attrition / dropout:** **T1 – 5 nodes, patient withdrew** **19 nodes – lack of lymphatic tissue** **1 node – technical error** **T2 – 8 nodes, 3 patients withdrew** **26 nodes, 6 pts had neoadjuvant chemotherapy** **36 nodes, lack of lymphatic tissue** **31 nodes did not meet study spec.**	**Index (technical details): Pieces obtained from ALN were homogenised with 4mL of lysis buffer solution and centrifuged at 10,000 x g at room temperature. Two microlitres of the supernatant were analysed with the RD-100i system. A standard positive control sample containing 5 x 10^3 copies/µL of CK19 mRNA and a negative control sample containing 0 copy/µL of CK19 mRNA were used for calibration in every assay. The lymph node was assessed as negative when there were less than 2.5 x 10^2 copies/µl of CK19 mRNA and positive when there were 2.5 x 10^2 copies/µL or more.** **Reference standard (technical details): In the case of LN from pN0 patients, blocks b and d were further sliced at 0.2mm intervals, followed by staining each alternate slice with H&E and CK19 IHC. A total of 144 lymph nodes, in which neither micrometastases or macrometastases were observed were used for the false positive study for OSNA.** **Details of SLN detection: NR** **Extraction and division of SLN: A fresh LN with a short axis of 4 to 12 mm was divided into 4 blocks at 1 or 2 mm intervals using a lymph node cutting device. Blocks a and c were used for OSNA. Two slices were cut**	**Accuracy outcomes: Concordance, sensitivity, specificity** **Process outcomes:** **Clinical outcomes:** **Method of assessment:** **Unit of analysis:** **Discordant case analysis: Yes** **Test failures: 1 tech error**

		from each of the three cutting surfaces and used for the permanent three-level histopathological examination with H&E and CK19 IHC. Outcome assessor: Histology checked by 3rd party pathologists Blinding: NR Discordant case analysis (T2 only): When discordance between OSNA and 3-level histopathology occurred, a histopathologic analysis of blocks b and d was repeated. All slides were examined and evaluated by three third party pathologists. All results of histopathologic examinations were finally determined by a study group comprised of representatives from the different facilities.	

Participant characteristics

Intervention	O+H T1	O+H T2
No.	36	185
Mean age, yrs (range)	55.9	54.7
Clinical stage (%)		
0	2 (6)	14 (9)
I	8 (24)	51 (31)
II	A-14 (41) B-3 (9)	A-64 (40)
III	5 (15)	7 (4)
IV	0	0
Unknown	2 (6)	0
Histopathologic type (%)		
Invasive ductal carcinoma	32 (94)	130 (79)
Invasive lobular carcinoma	1 (3)	7 (4)
Ductal carcinoma in situ	0	18 (11)
Others	1 (3)	9(5)

Results

n=124 ALN Trial 1				
0.2 mm Section histopathology				
OSNA	Macrometastasis	Micrometastasis	ITC	Negative

Positive	**16**	**3**		**3**
Negative	**0**	**1**		**101**

n=551 ALN Trial 2

Three-level histopathology

OSNA	**Macrometastasis**	**Micrometastasis**	**ITC**	**Negative**
Positive	**64**	**6**		**22**
Negative	**4**	**6**		**348**

Before TAB exclusions

	Sensitivity (%)	**Specificity (%)**	**Discordance (%)**
Trial 1 n=124 ALN	**95 (75.1-99)**	**97.1 (91.8-99.4)**	
Trial 2 n= 450 ALN	**87.5 (78.5-93.8)**	**94.1 (91.0-96.3)**	**7.1**

Methodological issues	
Replicates: Unclear whether replicate samples were analysed Analysis: Unclear whether histopathology results were checked by an independent pathologist Recruitment: Unclear if recruitment was consecutive of random Conflict of interest: The study was funded by Sysmex	
Quality appraisal	
Was a consecutive or random sample of patients enrolled? (Y/N/U)	U
Was a cohort study design avoided?(Y/N/U)	Y
Did the study avoid inappropriate exclusions? (Y/N/U)	Y
Could the selection of patients have introduced bias? (H/L/U)	U
Concerns that the included patients do not match the review question? (H/L/U)	L
Were the index test results interpreted without knowledge of the results of the reference standard? (Y/N/U)	Y
If a threshold was used, was it pre-specified? (Y/N/U)	Y
Could the conduct or interpretation of the index test have introduced bias? (H/L/U)	L
Are there concerns that the index test, its conduct, or interpretation differ from the review question? (H/L/U)	L
Is the reference standard likely to correctly classify the target condition? (Y/N/U)	Y
Were the reference standard results interpreted without knowledge of the results of the index test? (Y/N/U)	Y
Could the reference standard, its conduct, or its interpretation have introduced bias?[f] (H/L/U)	L
Are there concerns that the target condition as defined by the reference standard does not match the review question?	L
Did all patients receive a reference standard? (Y/N/U)	Y
Did all patients receive the same reference standard? (Y/N/U)	Y
Were all samples (that should have been) included in the analysis? (Y/N/U)	Y
Could the patient flow have introduced bias? (H/L/U)	L

Were samples suspected of TAB excluded from the analysis? (Y/N/U)[c]	**Y**
Are there concerns about selective reporting of outcomes? (H/L/U)	**L**

Design	Participants	Tests	OUTCOMES
Tsujimoto (2007) **Objective: To develop a more efficient method for intraoperative detection of lymph node metastasis** **Study design: Single gate** **Country: Japan** **No. of centres: 6** **Funding: NR** **Notes**	**Number of participants: 101 patients (81 SLN from 49 patients)** **Number of SLNs or ALNs:325 SLN and ALN, 81SLN** **Recruitment procedure: NR** **Inclusion criteria: NR** **Exclusion criteria: NR** **Sample attrition / dropout:NR**	**Index (technical details): A histopathologically negative lymph node (≤600 mg) was homogenised in 4 mL of lysis buffer for 90s on ice. The homogenate was centrifuged at 10,000 x g for 1 min at room temperature. A 20 µl sample of supernatant was subject to the RT-LAMP reaction in a gene amplification detector, RD-100i** **Reference standard (technical details): Two slices were cut from each of the three cutting surfaces and used for permanent three-level histology with H&E and CK19. Macrometastasis and micrometastasis were defined according to TNM classification of the Unio Internationale Contra Cancrum sixth and AJCC sixth edition. All samples for histopathology were examined by third party pathologists. Conflicting results were settled consensually.** **Details of SLN detection: NR** **Extraction and division of SLN: A fresh LN with a short axis of 4 to 12 mm was divided into 4 blocks at 1 or 2 mm intervals using a lymph node cutting device. Blocks a and c were used for OSNA. Two slices were cut from each of the three cutting surfaces and used for the permanent three-level histopathological examination with H&E and CK19 IHC.**	**Accuracy outcomes: Concordance, sensitivity, specificity** **Process outcomes: Time to analysis** **Clinical outcomes:** **Method of assessment:** **Unit of analysis: Node** **Discordant case analysis: Yes** **Test failures: NR**

		Outcome assessor: Three third party pathologists. Conflicting results were settled consensually. **Blinding: Unclear, although blinded in paper by Tamaki, which is same trial** **Discordant case analysis: When discordance between OSNA and 3-level histopathology occurred, a histopathologic analysis of blocks b and d was repeated. All slides were examined and evaluated by three third party pathologists. All results of histopathologic examinations were finally determined by a study group comprised of representatives from the different facilities.** **In the analysis of discordant cases, QRT-PCR and CK19 Western blot analysis of the lysates were carried out. (Further details of this process in paper). A cutoff value for CK19 protein expression between histopathologically positive and negative lymph nodes was determined by Western blot analysis of 37 histopathologically negative LN from 16 pN0 pts, 54 histopathologically negative LN from 17 pN1-3 pts and 22 histopathologically positive LN from 12 pts. The cutoff value was determined by statistical analysis of the amount of CK19 measured by Western blot analysis of 37 histopathologically negative LN from 16 pN0 pts.**	

Participant characteristics

Intervention	O+H
No.	101
Median age, yrs (range)	NR
Clinical stage (%)	
0	5
I	41
II	49
III	5
IV	1
Unknown	5
Nodal status (%)	
pN0	60
pN1	35
pN2	2
pN3	4

Histopathologic type (%)	
Invasive ductal carcinoma	87
Invasive lobular carcinoma	4
Ductal carcinoma in situ	5
Others	5

Results

n= 325 SLN and ALN

Three level histopathology

OSNA	Macrometastasis	Micrometastasis	ITC	Negative
++	34	0	0	0
+	6	3	0	4
-	0	2	13	263

n= 81 SLN

Three level histopathology

OSNA	Macrometastasis	Micrometastasis	ITC	Negative
++	11	0	0	0
+	1	2	0	1
-	0	2	3	61

n= 144 SLN from pN0 pts

0.2mm Interval histopathology

OSNA	Macrometastasis	Micrometastasis	ITC	Negative
++	0	0	0	0
+	0	0	0	0
-	0	0	3	141

	Sensitivity (%)	Specificity (%)	Discordance (%)
325 nodes (ALN or SLN)	91.1	NR	7.4

Time to analysis - <30 min

Methodological issues	
Replicates: Unclear whether replicate samples were analysed **Analysis: Histopathology results were checked by an independent pathologist** **Recruitment: Unclear if recruitment was consecutive of random**	
Quality appraisal	
Was a consecutive or random sample of patients enrolled? (Y/N/U)	**U**
Was a cohort study design avoided?(Y/N/U)	**Y**
Did the study avoid inappropriate exclusions? (Y/N/U)	**U**
Could the selection of patients have introduced bias? (H/L/U)	**U**
Concerns that the included patients do not match the review question? (H/L/U)	**L**
Were the index test results interpreted without knowledge of the results of the reference standard? (Y/N/U)	**Y**
If a threshold was used, was it pre-specified? (Y/N/U)	**Y**
Could the conduct or interpretation of the index test have introduced bias? (H/L/U)	**L**
Are there concerns that the index test, its conduct, or interpretation differ from the review question? (H/L/U)	**L**
Is the reference standard likely to correctly classify the target condition? (Y/N/U)	**Y**
Were the reference standard results interpreted without knowledge of the results of the index test? (Y/N/U)	**U**
Could the reference standard, its conduct, or its interpretation have introduced bias? (H/L/U)	**L**
Are there concerns that the target condition as defined by the reference standard does not match the review question?	**L**
Did all patients receive a reference standard? (Y/N/U)	**Y**
Did all patients receive the same reference standard? (Y/N/U)	**Y**
Were all samples (that should have been) included in the analysis? (Y/N/U)	**Y**
Could the patient flow have introduced bias? (H/L/U)	**U**

Were samples suspected of TAB excluded from the analysis? (Y/N/U)	**N**
Are there concerns about selective reporting of outcomes? (H/L/U)	**U**

Design	Participants	Tests	OUTCOMES
Sundaresan (unpub) **Objective:** **Study design: To describe the validation of Metasin, a novel real time PCR assay for the detection of metastatic cancer in sentinel lymph nodes from breast cancer patients.** **Country: UK** **No. of centres: 6 centres** **Funding: NR**	**Number of participants: 1265** **Number of SLNs or ALNs: 2279 SLNs** **Recruitment procedure: NR** **Inclusion criteria: NR** **Exclusion criteria: NR** **Sample attrition / dropout: NR**	**Index (technical details): The initial early beta study of internal validation of the Metasin-BLNA (M-BLNA) assay for preliminary use was carried out on a series of 245 cases. This high level of determination of the cut off values was carried out against the Veridex data set and morphology, enabling the verification of the thresholds for macro-metastasis (>2mm) and micro-metastasis (<2mm & more than 0.2mm) determination. The Cp values were determined for Ck19 (Cp values <25) and for Mammaglobin (<25.9). Thresholds for micro-metastasis were similarly determined (CK19>25 and <32) and for MGB the micro-metastasis were identified (Cp>25.9 and <32).** **The detailed methodology for the assay is presented in a companion manuscript (Ramadhani et al, manuscript in preparation) detailing PCR primers and PCR machine assay conditions. For RNA extraction and quantification, the protocol was adopted from the Genesearch assay. BMS staff were trained over a 3 day period.** **Reference standard (technical details):** **Sentinel lymph nodes were sectioned at 3 levels/steps of 150um.**	**Accuracy outcomes: Sensitivity, specificity and concordance** **Process outcomes:** **Clinical outcomes: NR** **Other:NR** **Unit of analysis: Patient** **Discordant case analysis: Yes** **Test failures: 1.2% - insufficient RNA**
Notes			

		Nodal micro-metastasis (<2 mm and >0.2 mm) and macro-metastatic disease (>2 mm) were interpreted as positive for histologically confirmed positive disease **Details of SLN detection: Sentinel nodes were identified by a combination of the use of blue dye and radiation: as per established conventional protocol following NEW START.** **Extraction and division of SLN: Six centres contributed tissue homogenates and RNA from patients treated for breast cancer. Two centres were only able to provide frozen RNA. The remaining institutions contributed lymph node homogenates stored at -80C.** **Lymph nodes were serially sliced in the longitudinal plane into an even number of approximately 2 mm slices. Alternate slices were submitted for conventional histopathological analysis and for homogenization and RNA preparation.** **Discordance analysis: Cases with discrepancy were further followed up by examination of the block by extra levels and selectively examined with MNF116 immunostaining.** **Cases deemed discordant if molecular assay was positive but histology negative were subject to a further round of analysis, subject to availability of homogenates for analysis. RNA was re-extracted where possible and was examined by an independent panel of markers.** **Retrospective discordant case analysis could not be uniformly followed in view of the lack of a formal process for informing patients of the different outcome if deeper levels were positive for tumour on the histological sections** **Outcome assessor: NR** **Blinding: NR**	

Participant characteristics

NR

Results

	n = 1265 patients	
	Three level histopathology	
Metasin	Positive	Negative
Positive	249	26/34/38/56 – various numbers reported – prob 36 with 20 fails
Negative	20	940

	Sensitivity (%)	Specificity (%)	Discordance (%)
n=1265 patients	92	97	4.4

Nodes (n)	Median time to analysis (min)
1	36
2	42
3	46

Test failure – 1.2% due to insufficient mRNA in sample

Methodological issues	
See STARD table	
Quality appraisal	
Was a consecutive or random sample of patients enrolled? (Y/N/U)	U
Was a cohort study design avoided?[a](Y/N/U)	Y
Did the study avoid inappropriate exclusions? (Y/N/U)[g]	U
Could the selection of patients have introduced bias? (H/L/U)	U
Concerns that the included patients do not match the review question? (H/L/U)	L
Were the index test results interpreted without knowledge of the results of the reference standard? (Y/N/U)	U
If a threshold was used, was it pre-specified? (Y/N/U)	Y
Could the conduct or interpretation of the index test have introduced bias? (H/L/U)[e]	U
Are there concerns that the index test, its conduct, or interpretation differ from the review question? (H/L/U)	L
Is the reference standard likely to correctly classify the target condition? (Y/N/U)	Y
Were the reference standard results interpreted without knowledge of the results of the index test? (Y/N/U)	U
Could the reference standard, its conduct, or its interpretation have introduced bias?[f] (H/L/U)	U
Are there concerns that the target condition as defined by the reference standard does not match the review question?	L
Did all patients receive a reference standard? (Y/N/U)	Y
Did all patients receive the same reference standard? (Y/N/U)	Y
Were all samples (that should have been[b]) included in the analysis? (Y/N/U)	Y
Could the patient flow have introduced bias? (H/L/U)	U

Were samples suspected of TAB excluded from the analysis? (H/L/U)[c]	**N**
Are there concerns about selective reporting of outcomes? (H/L/U)	**L**

Design	Participants	Tests	OUTCOMES
Visser (2008) Objective: To test the suitability of OSNA for intraoperative SN analysis Study design: Single gate Country: The Netherlands No. of centres: 2 Funding: Sysmex	Number of participants: 32 Number of SLNs or ALNs: 346 ALN and SLN Recruitment procedure: NR Inclusion criteria: NR Exclusion criteria: NR Sample attrition / dropout: NR	Index (technical details): NR Reference standard (technical details): Lymph nodes were cut using special cutters. The blades of this device were 1 mm apart for lymph nodes with a minor axis of 4–6 mm and 2 mm apart for lymph nodes with a minor axis of 6–10 mm. Lymph nodes with a minor axis larger than 10 mm were halved, and the resulting pieces were then cut either with the 1 mm or 2 mm cutting device depending of the size of the pieces. Of the slices b and d initially three 4-lm thick sections were stained with H&E, CAM5.2 and an anti-CK19 antibody, respectively. If the initial sections were tumour positive no further sections were cut. Otherwise, additional sections (n = 3) at further levels at an interval of 250 lm (usually 4) were cut and analyzed. Immunostaining was performed with an antibody against cytokeratin 8 (CAM5.2) as well as CK19. Separate sections containing nonneoplastic epithelial cells were included in each staining procedure and served as a positive control for both antibodies. The size of a metastasis was determined by measuring its largest diameter and categorized as isolated tumour cells (ITC: <0.2mm), micrometastasis (tumor deposits larger than 0.2 mm but smaller than 2.0 mm), or macrometastasis (tumor deposits	Accuracy outcomes: Concordance, sensitivity, specificity Process outcomes: NR Clinical outcomes: NR Unit of analysis: ALN Discordant case analysis: Yes Test failures: NR
Notes			

		equal to or larger than 2.0 mm).19 **. Histology was regarded positive if at least 1** **micrometastasis or macrometastasis was detected in 1 of the sections.** **Lymph nodes containing isolated tumor cells were recorded** **as lymph node negative and designated as N0(i1) according to** **the 6th UICC TNM classification.** **Details of SLN detection: NR** **Extraction and division of SLN: Lymph node samples were cut in 4 equal slices (a, b, c, d) with a special cutting device.18 Two of these slices (a&c) were snapfrozen in liquid nitrogen and stored at -80°C until OSNA analysis was performed. The remaining 2 slices (b&d) were fixed in 4% buffered formaldehyde and embedded in a single paraffin block for histological examination at 5 levels since this was the standard in-house method for sentinel node investigation in both breast cancer and melanoma patients** **Outcome assessor: Microscopic evaluation was done by 2 pathologists without prior knowledge of the results** **of the OSNA method.** **Blinding: No**	

		Discordant case analysis: To investigate whether these figures might be influenced by a **sampling bias caused by limited investigation of the material the** **histologic work-up was extended to all levels in the first 120 histologically negative lymph node samples. The same was done for** **paraffin blocks of discordant cases. In addition, the homogenised lymph node lysates of samples with discordant OSNA versus histology results were subjected to quantitative reverse-transcriptase polymerase chain reaction (QRT-PCR) and Western Blot analysis. In case these investigations yielded a result compatible with a positive** **OSNA result these samples were excluded from the final** **analysis because of a strong indication for sampling bias.**	

Participant characteristics

Intervention	O+H
No.	32
Median age, yrs (range)	NR
Clinical stage (%)	
0	0
I	8
II	15
III	7
IV	2
Unknown	
Nodal status (%)	
pN0	14
pN1	10
pN2	6
pN3	2
Histopathologic type (%)	
Invasive ductal carcinoma	30
Invasive lobular carcinoma	2
Ductal carcinoma in situ	
Others	

Results

n=346 ALN				
Five level histopathology				
OSNA	**Macrometastasis**	**Micrometastasis**	**ITC**	**Negative**
++	**50**	**4**	**0**	**2**
+	**2**	**5**	**0**	**13**
-	**1**	**2**	**3**	**264**

	Sensitivity (%)	Specificity (%)	Discordance (%)
n = 346 ALN before TAB	**95.3**	**94.7**	**5.2**
n = 339 ALN after TAB	**95.3**	**97.1**	**3.2**

Methodological issues	
Replicates: Unclear whether replicate samples were analysed Recruitment: Unclear if recruitment was consecutive of random Conflict of interest: Consumables funded by Sysmex	
Quality appraisal	
Was a consecutive or random sample of patients enrolled? (Y/N/U)	U
Was a cohort study design avoided?(Y/N/U)	Y
Did the study avoid inappropriate exclusions? (Y/N/U)	Y
Could the selection of patients have introduced bias? (H/L/U)	U
Concerns that the included patients do not match the review question? (H/L/U)	L
Were the index test results interpreted without knowledge of the results of the reference standard? (Y/N/U)	U
If a threshold was used, was it pre-specified? (Y/N/U)	Y
Could the conduct or interpretation of the index test have introduced bias? (H/L/U)	L
Are there concerns that the index test, its conduct, or interpretation differ from the review question? (H/L/U)	L
Is the reference standard likely to correctly classify the target condition? (Y/N/U)	Y
Were the reference standard results interpreted without knowledge of the results of the index test? (Y/N/U)	U
Could the reference standard, its conduct, or its interpretation have introduced bias? (H/L/U)	U
Are there concerns that the target condition as defined by the reference standard does not match the review question?	L
Did all patients receive a reference standard? (Y/N/U)	Y
Did all patients receive the same reference standard? (Y/N/U)	Y
Were all samples (that should have been) included in the analysis? (Y/N/U)	Y
Could the patient flow have introduced bias? (H/L/U)	U

Were samples suspected of TAB excluded from the analysis? (Y/N/U)	**Y**
Are there concerns about selective reporting of outcomes? (H/L/U)	**L**

Appendix 5: Clinical effectiveness: excluded studies

Papers excluded	Reason for exclusion
Abdul-Rasool (2006)	Exclude on intervention
Aihara (1999)	Exclude on population
Allende (2008)	Exclude on study design
Al-Ramadhani (2011)	Exclude on study design
Babar (2011)	Exclude on study design
Backus (2005)	Exclude on intervention
Bedrosian (2011)	Exclude on study design
Belda (2008)	Exclude on comparator
Berger (2006)	Exclude on intervention
Basu (2012)	Exclude on outcomes
Bernet (2010)	Exclude on comparator
Bernet (2011)	Exclude on comparator
Blumencranz (2008)	Exclude on intervention
Branagan (2002)	Exclude on intervention
Campbell (2012)	Exclude on study design
Cannone (2006)	Exclude on comparator
Cano Munanoz (2010)	Exclude on comparator
Cepedello Boiso (2011)	Exclude on comparator
Croner (2008)	Exclude on comparator
Cserni (2003)	Exclude on study design
Cutress (2010)	Exclude on intervention
Daniele (2009)	Exclude on intervention
Dauplat (2010)	Exclude on language

Dell'Orto (2006)	Exclude on comparator
Denninghoff (2008)	Exclude on intervention
Fisher (2010)	Exclude on intervention
Gillanders (2004)	Exclude on intervention
Gimbergues (2007)	Exclude on intervention
Gorgens (2011)	Exclude on comparator
Guillen-Paredes (2011)	Exclude on language
Hasui (2008)	Exclude on study design
Inokuchi (2003)	Exclude on intervention
Laia (2011)	Exclude on comparator
Le Frere Belda (2012)	Exclude on comparator
Le Frere Belda (2008)	Exclude on language
Jackson (2012)	Exclude on comparator
Ghaffari (2006)	Exclude on intervention
Madani (2010)	Exclude on comparator
Madani (2010)	Exclude on study design
Mansel (2009)	Exclude on study design
Manzotti (2001)	Exclude on intervention
Nishimura (2009)	Exclude on outcomes
Nissan (2006)	Exclude on intervention
Osako (2011)	Exclude on comparator
Pinero (2010)	Exclude on study design
Rebollo-Aguirre (2012)	Exclude on comparator
Remoundos (2012)	Exclude on outcomes
Rothe (1995)	Exclude on population

Saha (2010)	Exclude on intervention
Sanchez-Mendez (2012)	Exclude on comparator
Sansano (2012)	Exclude on comparator
Sapino (2011)	Exclude on comparator
Schimanski (2012)	Exclude on comparator
Schroder (2003)	Exclude on intervention
Span (2010)	Exclude on study design
Sua (2012)	Exclude on intervention
Unknown (2010)	Exclude on intervention
Unkown (2011)	Exclude on population
Verbanac (2010)	Exclude on intervention
Velasco (2011)	Exclude on study design
Vieites (2010)	Exclude on comparator
Vilardell (2011)	Exclude on comparator
Wang (2012)	Exclude on intervention
Wallwiener (2011)	Exclude on intervention
Whisker (2012)	Exclude on intervention
Woefl (2012)	Exclude on study design

Appendix 6. Table of abstracts

Study ID	First Author	Year	Design	SLNs	Level of histology	Outcomes
001Garcia	Garcia-Estepa, R.	2010	SR			Concordance 91.7-98.2%, sensitivity 87.5%-98.1%, specificity 89 to 98.5%
002Buglioni	Di Filippo, F.	2009	Single gate	247 SLN	6	Concordance 96.7%, specificity 96.8%, sensitivity 96.4%
	Buglioni, S.	2009		228 SLN	6	Concordance 96.9%, specificity 97.2%, sensitivity 96.1%
	Buglioni, S.	2010		416 SLN	6	Concordance 95%, specificity 95%, sensitivity 94%
004Tsujimoto	Kaneko, T.	2007	Single gate	141 ALN 469 ALN		Specificity 96.9, 95%CI, 92.3-99.3 Concordance 93.0%, 95% CI 90.3-95.1
	Masuda, N.	2007		178 ALN		Specificity 97.5%, 95%CI 93.5-99.3. Positivity rate 17.9% Concordance 94%, 95% CI 91.9-95.8
	Tsuda, H.	2006		144 SLN		Concordance with 3 level histology 98%, specificity 100%.
005Tamaki	Sato, N.	2011	Single gate	415 pts		PPV of OSNA++ for non-SLN metastases 44.0% PPV of OSNA + for non-SLN metastases 17.6% (p=0.01)
				417 pts		PPV of OSNA++ for non-SLN metastases 44.0% PPV of OSNA + for non-SLN metastases 17.6% (p=0.01)
	Takabatake, D.	2011				
006Snook	Peston, D.	2009	Single gate	100 SLN	NR	Concordance 96%, sensitivity 92%, specificity 97% OSNA results achieved, on average, within 30 min for two nodes.
						DTA
	Snook, K. L.	2007		45 ALN	5	Concordance 93.3%, sensitivity 100%, specificity 91.2%
	Snook, K. L.	2008				Median time for analysis of 1 SLN, 32 mins
	Snook, K. L.	2007		87 ALN	5	Concordance 90.8%, sensitivity 90%, specificity 91%
	Kissin, M.W.	2009		396 SLN	5	Concordance 96.2%, sensitivity 91.5%, specificity 97.2, PPV 87.7%, NPV 98.1% Minimum time to reach a result on a single node was 22 min
007Nizar	Nizar, S.	2010	Single gate	31 pts		Specificity 82%, sensitivity 50% OSNA requires investment of nearly £60000, with regular servicing and replenishment of reagents costing nearly £1500 per month.
008Chaudhry	Chaudhry, A.	2011	Single gate	251 SLN		Sensitivity 93%, specificity 89%, increased to 94% if accounting for TAB

	Massey, E.	2011	Cohort			Mean time 40.5, 51.8, 54 and 61.5 mins for 1, 2, 3 and 4 nodes, respectively. Operation time was prolonged by -48 to +65 mins (median 20 mins)
009Choi	Choi, Y.	2010	Single gate			DTA
010Beitsch	Beitsch, P.	2007	Single gate	58 SLN		Concordance 94.8%,
011Tomlins	Tomlins, A.	2011	Single gate	62 SLN	NR	Concordance 95%
012Iqbal	Iqbal, M.	2012	Observation	99 SLN	N/A	Mean time SLN analysis 49.7 min (range 37-94 min) A second operation saved for 33% of patients
013Ng	Ng, V.	2011	Observation	100 pts	N/A	Median time for SLNB to be performed 12 min (range 2-57 min) Median time for telephone result 44 min (28-75 min) In 54%, the operation had finished prior to results coming back, median waiting time of 3 mins.
	Ng, V.	2011	Cohort	200 pts	NR	OSNA positive rate 39% Histology positive rate 19%
	Ng, V.	2011	Observation	100 pts	N/A	Median time for SLNB to be performed 12 min (range 2-57 min) Median time for telephone result 44 min (28-75 min) In 54%, the operation had finished prior to results coming back, median waiting time of 3 mins.
	Ng, V.	2011	Cohort	200 pts	NR	OSNA positive rate 39% Histology positive rate 19%
	Remoundos, D.	2012	Cohort	602 SLN	NR	OSNA positive rate 21% + 19% Histology positive rate 19% + 2 %
	Remoundos, D.	2012	Cohort	602 SLN	NR	OSNA positive rate 21% + 19% Histology positive rate 19% + 2 %
014Bilous	Bilous, M	2012	Single gate	211 SLN		Specificity 96.3%, sensitivity 95.8%
015Godey	Godey, F.	2011	Cohort	344 SLN	NR	OSNA positivity rate 21.3% Histology positivity rate 25% Concordance 138/160 pts Median time to analysis 35 min for 2 SLN and ~ 5 min per additional node
			Cohort	367 pt	NR	OSNA positive rate 24.32% Histology positive rate 24.79
016Khaddage	Peoch, M.	2011	Single gate	197 pt	1	OSNA positivity rate 21.3% Median time for analysis for 2 SLN, 37 min.
	Khaddage, A.	2009	Single gate	80 SLN	200um	Sensitivity 100%, concordance (after adjusting for TAB) 97.7%,

					intervals 1	specificity 97.1% Concordance 94.9%, specificity 92.9% Median time for analysis for 2 SLN, 37 min.
	Godey, F.	2009	Single gate	175 SLN	1	OSNA positivity rate 18% 7/91 cases discordant Median time for analysis for 2 SLNs, 35-37 min
	Godey, F.	2010	Single gate			DTA, time to analysis
017Feldman	Levine, E.	2010	Single gate			DTA
	USA study group	2010	Single gate			DTA
018Schem	Schem, C.	2007	Single gate	188 LN	4µm sections	DTA
	Schem, C.	2009	Single gate			DTA
			Single gate	343		Concordance 95.5%, sensitivity 100%, specificity 95.6%
	Schem, C.	2010	Single gate	335		Concordance 94%, specificity 96.5%, sensitivity 100% (after adjusting for TAB)
019Schem	Schem, C.	2010	Single gate			OSNA positivity rate 24.5%
	Schem, C.	2010	Single gate			OSNA positivity rate 24.5%
021Bernet	Bernet, L.	2010	Observation	87 SLN	N/A	Operating room to pathology department, mean 48.5 min Reception, macroscopic study and processing until amplification, mean 37.9 min Amplification to diagnostic report, mean 31min
	Bernet Vegue, L.	2010	Cohort	473 SLN	NR	OSNA positive rate 24.3 %(reviewers' authors calculations) Histology positive rate 18.6%
	Cano Munoz, R.	2010	Observation			Sensitivity 100%, specificity 97.2%. Mean of 31 minutes to evaluate up to 4 nodes.
022Jimbo	Jimbo, K.	2012	Single gate			Concordance 91.5%, sensitivity 90.3%, specificity 93.3%
023Suzuki	Suzuki, M.	2011	Single gate			Concordance 95.1% , specificity 96.9%
024Rai	Rai, Y.	2012	Observation			Av time to result – 36 min 703pts 581 (83%) were OSNA - and 56 (8.0%) + 66 (9.4%) were OSNA+ PPV OSNA++ 57.6% OSNA+17.9%
025Capadello	Capadello Boiso, I	2011	Cohort study			130 SLN Histology 27 positive 83 negative 146 SLN OSNA 23 positive 87 negative

027Wahab	Wahab, T.	2012	Single gate	196 SLN	NR	Sensitivity 94%, specificity 96.6%, concordance 96%
028Siso	Siso, C.	2012	Observation	49 pts		? excl
032Krish-mamurthy	Krishmamurthy, S.	2009	Single gate	279 ALN	NR	Kappa coefficient between histology and OSNA was 0.87% (95%CI, 0,72-1.00)
034Mizoo	Mizoo, T.	2012	Single gate	36 SLN	N/A	Mean time to analysis 38.9 min (34.9 for 1 node, 46.4 for 2 nodes, 55 for 3 to 4 nodes)
	LeFrere Belda	2008	Single gate	509 SLN	200 um intervals	Concordance 93.96%, sensitivity 94.3%, specificity 93.9%

Appendix 7: Cost-effectiveness: quality appraisal

Criteria	Cutress 2010	Guillen-Paredes 2011	Burke 2010	Cooper 2011 Meng 20012	Classe 2012
	✓	✓	✓	✓	✓
(DRUMMOND 1996)					
Study design					
The research question is stated	✓	✓	✓	✓	✓
The economic importance of the research question is stated	✓	✓	✓	✓	✓
The viewpoint(s) of the analysis are clearly stated and justified	✓	✓	✓	✓	✓
The rationale for choosing alternative programmes or interventions compared is stated	partial	✓	✓	✗	✗
The alternatives being compared are clearly described	✓	✓	✓	✓	✓
The form of economic evaluation used is stated	✓	✓	✓	✓	✓
The choice of form of economic evaluation is justified in relation to the question addressed	✗	✗	✓	✓	✗
Data collection					
The source(s) of effectiveness estimates used are stated	✓	✓	✓	✓	✗
Details of the design and results of effectiveness study are given (if based on a single study)	✓	✓	✓	n/a	✓
Details of the methods of synthesis or meta-analysis of estimates are given (if based on a synthesis of a number of effectiveness studies)	n/a	n/a	n/a	✓	n/a
The primary outcome measure(s) for the economic evaluation are clearly stated	partial	✓	partial	✓	✓
Methods to value benefits are stated	✗	✗	✗	✗	✗
Details of the subjects from whom valuations were obtained were given	n/a	n/a	n/a	n/a	n/a
Productivity changes (if included) are reported separately	✗	✗	✗	✗	✗ attempted
The relevance of productivity changes to the study question is discussed	n/a	n/a	n/a	n/a	n/a
Quantities of resource use are reported separately from their unit costs	✓	✓	✓	✓	✓

Methods for the estimation of quantities and unit costs are described	partial	partial	partial	✓	✓
Currency and price date are recorded	✓	partial	✓	✓	✓
Details of currency of price adjustments for inflation or currency conversion are given	✗	✗	✗	partial	✗
Details of any model used are given	n/a	n/a	✓	✓	n/a
The choice of model used and the key parameters on which it is based are justified	n/a	n/a	✓	✓	n/a
Analysis and interpretation of results					
Time horizon of costs and benefits is stated	✓	✓	✓	✓	✓
The discount rate(s) is stated	n/a	n/a	n/a	✓	n/a
The choice of discount rate(s) is justified	n/a	n/a	n/a	✓	n/a
An explanation is given if costs and benefits are not discounted	n/a	n/a	✓	n/a	n/a
Details of statistical tests and confidence intervals are given for stochastic data	✗	✓	✗	✓	✓
The approach to sensitivity analysis is given	n/a	n/a	✓	✓	✓
The choice of variables for sensitivity analysis is justified	n/a	n/a	✓	partial	✗
The ranges over which the variables are varied are justified	n/a	n/a	✗	✓	✗
Relevant alternatives are compared	✓	✓	✓	✓	✓
Incremental analysis is reported	✗	✗	✗	✓	✓
Major outcomes are presented in a disaggregated as well as aggregated form	✓	✓	✗	✓	✓
The answer to the study question is given	✓	✓	partial	✓	✓
Conclusions follow from the data reported	✓	✓	✓	✓	✓
Conclusions are accompanied by the appropriate caveats	✗	✗	✓	✓	✗

Note: Only full articles were critically assessed.

Appendix 8: Cost-effectiveness: excluded studies

Papers excluded	Reason for exclusion
Cooper et al., (2011);	Intervention not intra-operative diagnosis
Meng et al., (2012)	Intervention not intra-operative diagnosis
Classe et al., (2012)	Intervention not relevant to evaluation

Appendix 9: Expert Advisors

Title	Name	Specialty	Affiliation
Mr	Simon Pain	Consultant Breast and Endocrine Surgeon	Norfolk & Norwich University Hospital
Mr	Zenon Rayter	Consultant Surgeon	Bristol Royal Infirmary
Professor	Ian Kunkler	Consultant and Hon Professor in Clinical Oncology	Edinburgh Cancer Centre
Professor	Graham Layer	Consultant surgeon and director of professional standards	The Royal Infirmary of Edinburgh
Dr	Abeer Shaaban	Consultant Pathologist	St James's University Hospital Leeds
Dr	Deirdre Ryan	Consultant Pathologist	Barts Health NHS Trust
Mr	Simon Pain	Consultant Breast and Endocrine Surgeon	Norfolk & Norwich University Hospital
Mr	Zenon Rayter	Consultant Surgeon	Bristol Royal Infirmary