Diagnostic Assessment Report commissioned by the NIHR HTA Programme on behalf of the National Institute for Health and Clinical Excellence – final protocol

1. Title of project

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

2. Name of External Assessment Group (EAG) and project lead

EAG:
Peninsula Technology Assessment Group (PenTAG)

Project leads:
Ruben Mujica-Mota and Chris Hyde
PenTAG
Peninsula College of Medicine and Dentistry
University of Exeter
Veysey Building
Salmon Pool Lane
EXETER EX2 4SG
Email: ruben.mujica-mota@pcmd.ac.uk
christopher.hyde@pcmd.ac.uk

Administrative contact:
Sue Whiffin
PenTAG
Peninsula College of Medicine and Dentistry

University of Exeter
Veysey Building
Salmon Pool Lane
EXETER EX2 4SG
Phone: 01392 726056

Email: sue.whiffin@pms.ac.uk

3. Plain English Summary

The RD-100i OSNA System ("OSNA") and Metasin are new tests to help identify whether a breast cancer has spread to the lymph nodes in the armpit (axilla). Except where there are occasional isolated cells, if there are breast cancer cells in any of these lymph nodes, all the lymph nodes in the armpit need to be removed in an operation called axillary lymph node dissection. This can be associated with later side-effects such as swelling, pain, numbness and difficulty moving the arm, and so should not be undertaken unless necessary.

Normally, in the NHS, the test to check if the breast cancer has spread to the lymph nodes in the armpit takes several days and involves cutting very thin slices of one to three key or "sentinel" lymph nodes and carefully examining them under a microscope (histopathology). This means that if breast cancer cells are found in these lymph nodes, there is a delay until this is discovered and that the axillary lymph node dissection has to be done separately from the first operation to remove the breast cancer itself.

There are claims that OSNA and Metasin can be performed sufficiently quickly that spread to the lymph nodes can be detected during the first operation to remove the breast cancer, and that, if required, the axillary lymph node dissection can be done at the same time without delay. It is further claimed that these new tests are as accurate as examination under a microscope as well as being quicker.

This project will check these claims by examining published research evidence, extending to unpublished evidence where no published evidence exists. In this way their effectiveness and cost-effectiveness relative to histopathology will be established.

4. Decision problem

4.1. Objective

To evaluate if the RD100i OSNA system ("OSNA") and Metasin would be clinically effective and cost effective if used in the NHS in England.

4.2. Intervention technologies

4.2.1 OSNA (the referred technology)

The RD100i OSNA system is an automated molecular test that uses one-step nucleic acid amplification technology to indicate if cancer has spread to the axillary lymph nodes in people diagnosed with breast cancer. The test analyses and amplifies genetic material (mRNA) from solubilised biopsy samples of sentinel lymph node tissue and detects the presence of the Cytokeratin 19 (CK19) gene, a biological marker associated with breast cancer and not normally present in lymph node tissue. It is claimed that the RD 100i OSNA test will provide a result within a short time and therefore, can be used during breast surgery to determine if other lymph nodes should be removed at the same time as the initial tumour. This could avoid a second operation for the patient and enable subsequent treatments such as chemotherapy to begin earlier. Doing the axillary lymph node dissection during the first operation may simplify this procedure and reduce complication rates, but may also limit the opportunity for a multi-disciplinary team to consider the appropriateness of axillary dissection taking account of all the information that would be available at the time of the second operation.

OSNA does not require the mRNA to be extracted and purified from the tissue before being analysed. The expression level of CK19 mRNA correlates with the size of the metastatic foci. Since the metastatic foci may not be evenly distributed throughout the

node, the system provides more accurate results if more of the node is analysed because there is less risk of tissue allocation bias (sample bias). The result is most accurate if the entire node is used, but then no follow-up histopathology is possible. The system can be used with half of the lymph node (one piece or alternate slices), allowing for the possibility of follow-up histopathology but potentially decreasing the accuracy of the results due to the increased risk of tissue allocation bias. The time to results is dependent on the number of lymph nodes analysed, but the test takes approximately 30 - 45 minutes. The OSNA test result is expressed both quantitatively and qualitatively; - for lymph node negative test results, + (> 250 copies of CK19 m RNA / µl) for lymph nodes with a micro-metastatic tumour burden and ++ (>5000 copies of CK19 m RNA / µl) for lymph nodes with a macro-metastatic tumour burden. The analyser amplifies and detects the CK19 mRNA by using 6 different primers which have been specifically designed to avoid the amplification of CK19 pseudogenes or their transcripts; amplification of these would lead to false positive results. Undesired amplification of genomic DNA is avoided by precipitation of DNA at low pH during sample preparation and the isothermal reaction temperature of 65°C.

The manufacturer estimates that 1% of breast tumours do not express CK19 mRNA and therefore, if cancer spreads to the lymph nodes from these tumours, CK19 mRNA will not be detected even though the lymph nodes are metastatic. Pre-screening of tumour biopsies for CK19 expression could be carried out before using the RD100i OSNA test to reduce the small risk of false negative results for metastatic sentinel lymph nodes.

A (Conformitée Européenne) CE mark has been obtained for this technology.

4.2.2 Metasin

This test was identified during the scoping phase and the claims for its effect on management of patients with breast cancer are similar to those for OSNA .

The Metasin test is an intraoperative molecular test developed within the NHS at the Princess Alexandra Hospital in Harlow, Essex. The test has similarities to a

discontinued commercial test (Veridex Genesearch BLNA assay) and uses the technique of quantitative reverse transcriptase PCR (qRT-PCR) to detect 2 predictive markers of metastases, CK19 and mammaglobin. Mammaglobin is expressed mainly by breast epithelial cells and high levels of mammaglobin are associated with breast cancer. A reference gene, PBGD, is used to confirm the validity of the mRNA used in the test and two other controls, positive and negative, are also included. The test uses reagents that can be purchased from Roche and Qiagen and can be used on any platform (PCR machine). This in-house test differs from the discontinued commercial test by using distinctly different and unique primer-probe combinations to detect the CK19 and mammaglobin genes. The test is reported to take 26 minutes to results after 6-10 minutes for extracting and purifying mRNA from the tissue.

Pre-screening of tumour biopsies for CK19 mRNA and mammaglobin mRNA expression could be carried out before using the Metasin test because like the CK19 biomarker, mammaglobin is not expressed in all breast tumours. The proportion of breast cancer tumours that do not express mammaglobin mRNA is not known.

Metasin is used as an in-house test, and so does not require a CE mark.

4.2.3 Other technologies

No other technologies have so far been identified in the scoping phase despite specific enquiry.

4.3. Population

Individuals with invasive breast cancer who undergo a sentinel lymph node biopsy.

This will be during the primary operation to excise the suspected breast cancer and will have been preceded by investigations such as clinical examination, needle biopsy of the suspected breast cancer and ultrasound of the axilla (with needle biopsy of any suspicious lymph nodes). Individuals may have originally identified a breast lump or

other abnormality themselves or have been referred from a breast screening programme. Sentinel lymph node biopsy is a technique in which a radioactive solution and a blue dye are injected into the breast before surgery to identify the first lymph node(s) to which the breast drains lymph in a particular individual. These sentinel lymph node(s) are the ones removed during the first operation to see if the cancer has spread from the original site. Removal of up to three sentinel nodes is a minor procedure in comparison with axillary lymph node dissection and is not associated with the side-effects noted for total axillary dissection, particularly lymphoedema which follows axillary lymph node dissection in 7-8% of cases.

4.4. Relevant comparators

Post-operative standard histopathology of the fresh biopsy tissue from sentinel lymph node biopsy alone.

This is the usual approach currently used in the NHS where the sentinel lymph node(s) is fixed in paraffin blocks, sliced very thinly to produce sections which are mounted on slides, stained and then examined under a microscope by a consultant histopathologist. The time to receive results from histopathology is usually between 5 and 15 working days in the NHS. Individuals who have macrometastases or micrometastases detected in their sentinel lymph node are regarded as lymph node- positive, and axillary lymph node dissection will be performed on them. Individuals who have isolated tumour cells in their sentinel lymph node are regarded as lymph node-negative and will not receive axillary lymph node dissection.

Either OSNA or Metasin can be used as a replacement for current normal practice, in which case the sentinel lymph nodes can be used in their entirety. However, OSNA or Metasin could also be used adjunctively where half of each lymph node (one piece or alternate slices) is used for the OSNA testing and the remaining half examined using standard histopathology. Where OSNA has been introduced in practice the first model is the most commonly followed as it maximises the claimed benefits of the new technology.

Other intra-operative approaches to examining tissue for sentinel lymph node biopsy were also considered as potential comparators during the scoping phase including frozen section examination and Touch imprint cytology, but were rejected because they were not felt to be sufficiently feasible for widespread implementation. The key issue in this respect was identified as whether OSNA and Metasin were effective and cost-effective relative to standard histopathology.

4.5 Healthcare setting

Secondary and tertiary care settings.

4.6 Health outcomes

The intermediate measures for consideration include:

- Test failure rate
- Diagnostic test accuracy
- Discordant test results
- Time to test result
- Duration of anaesthesia/ time in operating theatre
- Number of repeat operations (except for re-excision of positive margins)
- Time to start and nature of adjuvant therapy

The clinical outcomes for consideration include:

- Patient anxiety associated with waiting time for result and not knowing the extent of surgery prior to operation
- Adverse events from false test results including patient distress and sequelae
- Morbidity and mortality from biopsies, axillary dissections, first and second operations and treatment of cancer

5. Report methods for assessing clinical effects and effectiveness

A series of systematic reviews will be conducted to summarise the evidence on the clinical effects of OSNA and Metasin for the intra-operative analysis of breast cancer metastases in sentinel lymph nodes. Systematic review methods will follow the principles outlined in the Centre for Reviews and Dissemination guidance for undertaking reviews in health care [1] and NICE Diagnostic Assessment Programme methods statement [2].The protocol will be registered in the PROSPERO database.

The general approach is outlined below, but its modification will be necessary to deal with the different types of study design likely to provide the most valid information on the different aspects of outcome listed in the preceding section. Thus:

- Impact on patient outcome - experimental study designs e.g. RCTs and controlled trials are likely to be the most relevant included studies
- Impact on process - quasi-experimental study designs e.g. pre-post studies and observational studies are likely to be the most relevant included studies
- Test accuracy and discordance - test accuracy studies e.g. cross-sectional studies with delayed verification are likely to be the most relevant included studies

5.1. Inclusion and exclusion criteria

These will be as dictated by the elements of the decision problem indicated above.

5.1.1 Participants

Individuals with invasive breast cancer who undergo a sentinel lymph node biopsy during the primary operation to excise a suspected breast cancer.

5.1.2 Interventions

OSNA or Metasin as used at the thresholds recommended by the manufacturer or designer.

5.1.3 Comparators/reference standard

Where appropriate to the study design the comparator will be standard histopathology. Again where appropriate the reference standard will be the results of standard histopathology, with careful attention to the number of sections taken and the experience of the histopathologist undertaking the examination.

5.1.4 Outcomes

No study will be excluded on the basis of outcomes provided it appears relevant to those listed in the decision problem

5.1.5 Study design

The following study/publication types will not be considered:

- Pre-clinical and animal
- Reviews, editorials, and opinion pieces
- Case reports
- Studies with <10 participants

Beyond this no study design will be excluded unless evidence on the intervention and outcome of interest is already available from study designs less open to bias as judged with reference to standard hierarchies of evidence [1].

5.2. Search strategy

Search strategies will be based on target condition and intervention, as recommended in the Centre for Reviews and Dissemination guidance for undertaking reviews in health care and the Cochrane Handbook for Diagnostic Test Accuracy Reviews.[1 3] They will be prepared and implemented by information specialists, and refined in consultation with experts particularly with respect to descriptors and trade names of the interventions. No restrictions on language or publication status will be applied. Sample search strategies are included in Appendix 1.

The following databases will be searched for relevant studies from inception to the present:

- MEDLINE (OvidSP)
- MEDLINE In-Process Citations and Daily Update (OvidSP)
- EMBASE (OvidSP)
- Cochrane Database of Systematic Reviews (CDSR) (Internet)
- Cochrane Central Register of Controlled Trials (CENTRAL) (Internet)
- Database of Abstracts of Reviews of Effects (DARE) (Internet)
- Health Technology Assessment Database (HTA) (Internet)
- Science Citation Index (SCI) (Web of Science)
- NIHR Health Technology Assessment Programme (Internet)

Completed and ongoing trials will be identified by searches of the following resources (up to 2011):

- NIH ClinicalTrials.gov (http://www.clinicaltrials.gov/)
- Current Controlled Trials (http://www.controlled-trials.com/)
- WHO International Clinical Trials Registry Platform (ICTRP) (http://www.who.int/ictrp/en/)
- EU Clinical Trials Register (https://www.clinicaltrialsregister.eu/)

Identified references will be downloaded in Endnote software for further assessment and handling.

References in retrieved articles and relevant systematic reviews will be scrutinised as will all material received from the companies or groups sponsoring each of the technologies. Some targeted Internet searching via Google will also be employed, including examination of the test producers' web-sites.

5.3. Data extraction strategy

Two reviewers will independently screen titles and abstracts of all reports identified by searches and discrepancies will be discussed. Full copies of all studies deemed

potentially relevant, after discussion, will be obtained and two reviewers will independently assess these for inclusion; any disagreements will be resolved by consensus or discussion with a third reviewer.

Data relating to study details, participants, intervention and comparator tests, reference standard, and outcome measures will be extracted by one reviewer, using a piloted, standard data extraction form. A second reviewer will check data extraction and any disagreements will be resolved by consensus or discussion with a third reviewer.

5.4. Quality assessment strategy

The methodological quality of included studies will be assessed using standard tools appropriate to the study designs being included. Thus for experimental studies, particularly RCTs, the Cochrane Collaboration quality assessment checklist will be used as detailed in the Table below:

Table: The Cochrane Collaboration's Tool for Assessing Risk of Bias [4]

Domain	**Item**	**Description**
Sequence Generation	Was the allocation sequence adequately generated?	The method used to generate the allocation sequence should be described in sufficient detail to allow an assessment of whether it should produce comparable groups.
Allocation Concealment	Was allocation adequately concealed?	The method used to conceal the allocation sequence should be

		described in sufficient detail to determine whether intervention allocations could have been foreseen in advance of, or during, enrolment.
Blinding of participants, personnel and outcome assessors. Assessments will be made for each main outcome (or class of outcomes).	Was knowledge of the allocated intervention adequately prevented during the study?	All measures used, if any, to blind study participants and personnel from knowledge of which intervention a participant received, should be described. Any information relating to whether the intended blinding was effective should also be reported.
Incomplete outcome data Assessments will be made for each main outcome (or class of outcomes).	Were incomplete outcome data adequately addressed?	The completeness of outcome data for each main outcome should be described, including attrition and exclusions from the analysis. The authors should report any attrition and exclusions, the numbers in each intervention group (compared with total randomized participants), reasons for attrition/exclusions

		and any re-inclusions in analyses.
Selective outcome reporting	Are reports of the study free of suggestion of selective outcome reporting?	The study should be free of the possibility of selective outcome reporting.
Other sources of bias	Was the study apparently free of other problems that could put it at a high risk of bias?	Overall, the study should be free from any important concerns about bias (i.e. bias from other sources not previously addressed by the other items).

Each study will be awarded a 'yes', 'no' or 'unclear/unknown' rating for each individual item in the checklist. Any additional clarifications or comments will also be recorded.

QUADAS-2 [5] will be used for test accuracy studies, noting that amendments need to be made should comparative accuracy studies be included.

All quality assessment will be carried out independently by two reviewers. Any disagreements will be resolved by consensus. The results of the quality assessment will be used for descriptive purposes to provide an evaluation of the overall quality of the included studies and to provide a transparent method of recommendation for design of any future studies. In addition, if enough data are available from the included studies, each of the quality components will be included as explanatory variables in a meta-regression analysis to investigate the association of each of these components with study results as a way of explaining possible heterogeneity. Based on the findings of the quality assessment, recommendations will be made for the conduct of future studies.

5.5. Methods of analysis/synthesis

The main method of analysis will be narrative synthesis, with conclusions based on patterns of results across all included studies clearly presented in tables. Careful account will be taken of issues of generalisability and study quality.

Where sufficient data are available, meta-analysis will be undertaken using techniques appropriate to the different types of study design. Test accuracy requires a different approach and the methods suggested by the Cochrane Collaboration will be employed [3].

6. Report methods for synthesising evidence of cost-effectiveness

6.1 Existing cost-effectiveness studies

A review of published economic evaluation studies of intra-operative molecular assessment for metastasis in early breast cancer will identify evidence relevant to current NHS practice. In addition to electronic databases searched in the effectiveness section, the NHS Health Economic Evaluation Database (NHS HEED) and EconLit will be searched for cost, cost-effectiveness and cost-utility studies.

Information on characteristics of relevant studies, including technologies investigated, study design, patient population, time-frame, analytical method (including modelling technique), measure of costs and benefits, measure of synthesis of costs and benefits and degree and sources of uncertainty will be extracted. The quality of the identified studies will be evaluated according to NICE methodological guidance [2].

6.2 Evaluating costs and benefits from the English NHS perspective

A model of costs and benefits of intra-operative molecular diagnosis of the sentinel node will be developed for early breast cancer patients undergoing sentinel lymph node biopsy (Figure 1). The following diagnostic options will be compared:
a) Molecular diagnosis using half node followed by post-operative histopathology
b) Molecular diagnosis using the whole node alone
c) Post-operative histopathology

With each diagnostic option, a positive results leads to axillary dissection, whereas a negative result signifies no axillary dissection, except for patients subject to intra-operative diagnosis employing half the node (b), which require confirmatory post-operative histopathology; if the latter is positive, a second operation would be required to clear the axilla.

Results will be presented for two time horizons. In one analysis, the model timeframe will be the diagnostic phase, and results will be synthesised in the form of incremental costs, incremental benefits and incremental costs per unit of benefit gained (e.g. additional case correctly diagnosed). An extended analysis will model the consequences of diagnostic outcomes, i.e. false and true negative and positive states, in terms of expected lifetime costs and QALYs. Because of the short duration of the diagnostic phase and the possibility that false negative cases be identified at subsequent, undetermined stages in the treatment pathway, the incremental cost, incremental QALY and, if applicable, incremental cost per QALY gain estimates from this analysis are likely to be highly uncertain. Deterministic sensitivity analysis will be undertaken to illustrate the limitations of life time analysis.

Populating cost parameters in the model

Information on model parameters will be identified from literature relevant to current practice in the English NHS. Direct and Indirect healthcare costs of the diagnostic phase will be measured. Direct costs include those for surgeons, ward, specialty administration and junior doctors. Indirect costs comprise the costs of theatre, including staff, anaesthesia, consumables, doctor training, overheads, physiotherapy and multidisciplinary team inputs, and the costs to the NHS Trust, including clinical support and facilities management, diagnostic costs and overheads. Standard unit costs from national representative sources will be used, including the NHS Reference costs and Personal and Social Services Research Unit, for healthcare, and the British National Formulary, for drugs.

Fixed capital costs of infrastructure required to establish a new diagnostic option will be sought from the scientific literature, relevant information from industry submissions or publicly available sources that report economic costing methods. Complementary information on costs from a breast cancer centre in the South West of England will also be sought.

Health care costs in the treatment phase will be obtained from modelling studies evaluating costs, cost-effectiveness or cost-utility, of patient management in breast cancer identified in 6.1. Cost parameters for which information is not available will be assigned values according to plausible assumptions on patterns of care and patients' prognosis as determined in consultation with clinical experts.

Populating health benefit parameters

In general parameters relating to health effects will be taken from the systematic reviews described in section 5, provided these effects have been the subject of research. Additional reviews beyond those described in section 5 are likely to be required to address questions such as:

- Possible inaccuracy of sentinel lymph node biopsy
- The consequences of false positive results especially the side-effects of axillary lymph node dissection
- The consequences of false negatives especially outcome in the absence of axillary lymph node dissection , which may be informed by a number of recent randomised trials

As part of the literature search in 6.1, valuations of health related quality of life (HRQoL) associated with contingent health states in the diagnostic options depicted in Figure 1 will be obtained from the literature, to be complemented by plausible values based on expert opinion. In particular the HRQoL benefit of earlier diagnosis and avoided need for second operation, and the negative impact of false positive and false negative results of intra-operative testing will be analysed. Unless evidence is found in the literature to suggest otherwise, the model will assume no difference across diagnostic options during the treatment phase, conditional on the outcome of the diagnostic phase: i.e. true positive, true negative, false positive or false negative. This assumption implies, for example, no survival effect of any potential earlier treatment initiation with intra-operative diagnosis. The assumption also implies no effect of intra-operative diagnosis on patient management that is independent of the test accuracy outcome.

Discounting

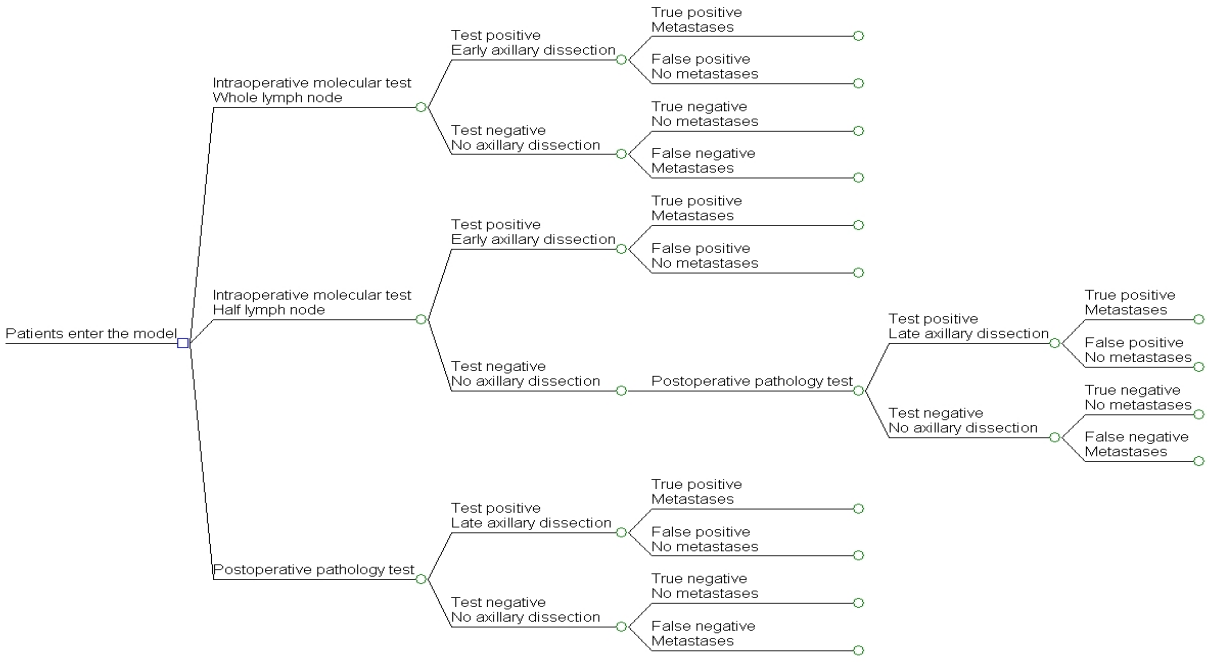
Costs and benefits for the extended model analysis that incorporates the treatment phase will be discounted at a 3.5% annual rate.

Sensitivity Analysis

The uncertainty in the estimated incremental costs per diagnostic outcome unit gained will be analysed using both probabilistic and deterministic methods. Probabilistic analysis will account for sampling uncertainty in parameter estimates. Deterministic methods will address the effect of varying key assumptions relating, for example, to the interpretation of discordant cases relative to post-operative histopathology in studies of diagnostic accuracy, opportunity costs of operation with peri-operative diagnosis and of operation following post-operative diagnosis, and the HRQoL effect of earlier diagnosis and avoiding a second operation.

The lifetime analysis will use deterministic methods to put the diagnostic outcomes into the wider context of breast cancer survival and QALY outcomes in England. Threshold analysis will explore the potential economic significance of diagnostic outcomes.

Figure 1 **Decision tree model**



7. Handling of information from the companies

All data submitted by the manufacturers/sponsors will be considered if received by the EAG no later than 01/10/2012. Data arriving after this date will not be considered. If the data meet the inclusion criteria for the review they will be extracted and quality assessed in accordance with the procedures outlined in this protocol.

Any ‘commercial in confidence’ data provided by manufacturers, and specified as such, will be highlighted in blue and underlined in the assessment report (followed by company name in parentheses). Any ‘academic in confidence’ data provided by manufacturers, and specified as such, will be highlighted in yellow and underlined in the assessment report. Any confidential data used in the cost-effectiveness models will also be highlighted.

8. Competing interests of authors

None

9. Timetable/milestones

Milestones	Completion data
Draft protocol	14/06/2012
Final protocol	03/07/2012
Progress report	15/10/2012
Draft assessment report	23/11/2012
Final assessment report	14/01/2013

Appendix 1

Clinical effectiveness search

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 below is the draft scoping search, formatted for the database Medline via OVID.

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

Host: Ovid

Data Parameters: 1946 to Present

Date Searched: Monday, July 2nd 2012

Search Strategy:

#	Searches	Results
1	Sysmex.mp.	488
2	(RD100i or RD-100i or (RD adj1 100i) or OSNA).mp.	25
3	1 or 2	511
4	Metasin.mp.	0
5	"98/79/EC".tw.	16
6	3 or 4 or 5	527
7	Cytokeratin 19.mp.	1257
8	(CK19 adj5 (gene or lymph)).mp.	47

9	Mammaglobin B/ or Mammaglobin A/	178
10	mammaglobin.mp.	245
11	7 or 8 or 9 or 10	1487
12	6 or 11	2004
13	Sentinel Lymph Node Biopsy/	6826
14	exp Lymph Nodes/	65221
15	(lymph$ adj3 node$).mp.	173135
16	13 or 14 or 15	174920
17	12 and 16	208

Appendix 2 Glossary of terms

Axillary lymph node	Lymph node located in the armpit
Frozen section	A technique in which a section of tissue is snap-frozen, stained and sliced before being viewed by a consultant histopathologist
Lymphoedema	Swelling caused by a build-up of lymph fluid in the tissues of the body
Lymph node	Small round mass of supported lymphatic tissue that is filled with white blood cells (lymphocytes) and acts as a filter to trap bacteria and foreign particles from lymph fluid. Lymph nodes are critical for the immune system and are principal sites where many immune reactions are initiated.
Messenger RNA	Messenger RNA is transcribed from a DNA template and carries coding information to sites of protein synthesis, at which the messenger RNA is translated into protein.
One step Nucleic Acid Amplification	A technique that uses a process called reverse-transcription-loop-mediated-isothermal-amplification (RT-LAMP) to rapidly amplify genes without extracting or purifying the genes from tissue.
Pseudogene	A DNA sequence that resembles a gene but lacks essential components that are necessary for function.

Polymerase chain reaction	A scientific technique that amplifies a few copies of a segment of DNA into millions of copies.
Quantitative reverse transcriptase polymerase chain reaction	A technique in which RNA is reverse transcribed into DNA and the resulting DNA is amplified using the polymerase chain reaction.
Reverse Transcription Loop Mediated Isothermal Amplification	A technique that enables amplification and detection of the target sequence to be completed in a single step.
Sentinel lymph nodes	The first lymph nodes to which cancer cells are most likely to have spread from the primary tumour
Touch Imprint Cytology	A technique in which tissue is sliced and the cut tissue surface imprinted on to a slide. The sample slide is then stained and viewed by a consultant histopathologist.

References

[1] Centre for Reviews and Dissemination. Systematic Reviews: CRD's guidance for undertaking reviews in health care [Internet]. York: University of York, 2009 Available from: http://www.york.ac.uk/inst/crd/SysRev/!SSL!/WebHelp/SysRev3.htm

[2] National Institute for Health and Clinical Excellence. Diagnostics Assessment Programme Manual [Internet]. London: NICE, Deecember 2011. Available from: http://www.nice.org.uk/media/A0B/97/DAPManualFINAL.pdf

[3] Cochrane Diagnostic Test Accuracy Working Group. Handbook for DTA Reviews [Internet]: Cochrane Collaboration, 2011. Available from: http://srdta.cochrane.org/handbook-dta-reviews

[4] Higgins JPT, Green S, eds. Cochrane handbook for systematic reviews of interventions [Internet]. Version 5.1.0 [updated March 2011]: The Cochrane Collaboration, 2011. Available from: http://www.cochrane-handbook.org/

[5] Whiting PF, Rutjes AW, Westwood ME, Mallett S, Deeks JJ, Reitsma JB, Leeflang MM, Sterne JA, Bossuyt PM. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. Ann Intern Med 2011:155(8):529-36.