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Diagnostic Accuracy of Intraoperative Techniques for Margin Assessment in Breast Cancer Surgery: A Meta-Analysis

--Manuscript Draft--

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Please list the potential conflicts of interest. as follow-up to "Do you or any of your coauthors have any potential conflicts of interest you need to declare?"	The authors are working on emerging technologies for margin assessment in breast cancer surgery.
Was this submission funded by an outside agency (e.g., business, government agency, etc.)	No funding was received in support of this work.
If your submission contains figures, are any of them in color?	My submission has only black & white figures.
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31st May 2016

Dear Professor Keith D. Lillemoe, Editor in Chief of Annals of Surgery

RE: "***Diagnostic Accuracy of Intraoperative Techniques for Margin Assessment in Breast Cancer Surgery: A Meta-Analysis***"

Authors: Edward Robert St John, Rashed Al-Khudairi, Hutan Ashrafian, Thanos Athanasiou, Zoltan Takats, Dimitri John Hadjiminas, Ara Darzi and Daniel Richard Leff.

Thank you for your recent comments regarding the above titled manuscript. The authors have addressed the comments by the editor and the reviewers to make the manuscript more acceptable for publication in the Annals of Surgery.

The authors' address each of the reviewer's comments overleaf in point counter-point format, and we highlight the change to the revised manuscript to make it easier for you and the editorial team to see the further amendments the co-authors have made.

On behalf of all co-authors, I declare that this manuscript has not been previously submitted for publication nor has it been published elsewhere.

We sincerely hope that this revised submission is successful and we look forward to hearing from you.

Yours sincerely,

Mr Daniel R Leff
Senior Clinical Lecturer and Consultant in Oncoplastic Breast Surgery
Imperial College London

Response to Editor and Reviewers: Revised Manuscript V2

Reviewer #1:
The authors have provided an adequate revision. The paper remains very statistically "heavy" but provides data for future technology comparisons.

Authors' Response:
We thank you for your review and we acknowledge the strong statistical component of this meta-analysis which we believe is necessary to provide robust evidence for our findings. We agree that the paper provides data for future technology comparisons and we consider this to be necessary and relevant due to the large number of emerging IMA techniques.

Reviewer #3:
Thank you very much for responding to my comments. I only have one minor question:
On page 11, 'Meta-analysis of the raw data .... since there was only one study in each of these groups resulting in a total of 35 studies included for final meta-analysis.' This reads a bit confusing. Why a total of 35 studies make it unfeasible for meta-analysis? I suggest that the authors make changes to make this sentence less confusing.

Authors' Response:
We have amended the sentence mentioned by the reviewer to make it less confusing. We now state as follows (page 11, line 16) : *"To enable meta-analysis at least two studies were required per IMA group, therefore MP and MCT groups were excluded as they only contained one study each. This resulted in a total of 35 studies included for final meta-analysis of five IMA techniques (i.e. CYT, FS, SR, IOUS, OPT)."*

MINI-ABSTRACT

Intraoperative margin assessment techniques (IMA) aim to diagnose residual cancer at resection margins for real-time decision-making regarding the need for re-excision. Diagnostic accuracy of existing IMA techniques are compared by meta-analysis to clarify the accuracy of existing technologies and provide pooled data against which emerging IMA techniques can be evaluated.

STRUCTURED ABSTRACT

Objective: To conduct a systematic review and meta-analysis to clarify the diagnostic accuracy of intraoperative breast margin assessment (IMA) techniques against which the performance of emerging IMA technologies may be compared.

Summary Background Data: IMA techniques have failed to penetrate routine practice due to limitations including slow reporting times, technical demands and logistics. Emerging IMA technologies are being developed to reduce positive margin and re-excision rates and will be compared to the diagnostic accuracy of existing techniques.

Method: Studies were identified using electronic bibliographic searches up to January 2016. MESH terms and all-field search terms included "Breast Cancer*" AND "Intraoperative*" AND "Margin*". Only clinical studies with raw diagnostic accuracy data as compared to final permanent section histopathology were included. A bivariate model for diagnostic meta-analysis was used to attain overall pooled sensitivity and specificity.

Results: 838 unique studies revealed 35 studies for meta-analysis. Pooled sensitivity (Sens), specificity (Spec) and area under the receiver operating characteristic curve (AUROC) values were calculated per group (Sens, Spec, AUROC): frozen section = 86%, 96%, 0.96 (n=9); cytology = 91%, 95%, 0.98 (n=11); intraoperative ultrasound = 59%, 81%, 0.78 (n=4); specimen radiography = 53%, 84%, 0.73 (n=9); optical spectroscopy = 85%, 87%, 0.88 (n=3).

Conclusions: Pooled data suggests frozen section and cytology have the greatest diagnostic accuracy. However, these methods are resource intensive and turnaround times for results have prevented widespread international adoption. Emerging technologies need to compete with the diagnostic accuracy of existing techniques whilst offering advantages in terms of speed, cost and reliability.

Systematic Review and Meta-Analysis Submission to Annals of Surgery

Manuscript Title

"Diagnostic Accuracy of Intraoperative Techniques for Margin Assessment in Breast Cancer Surgery: A Meta-Analysis"

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Running Head: ***Meta-Analysis of Intra-Operative Margin Assessment Techniques***

AUTHOR CONTRIBUTIONS

The motivation and need for the study was conceived and developed by ERS and DRL. The study design was developed by ERS, RA, HA, DRL. ERS and RA conducted the literature search and data collection. Statistical analysis was conducted by ERS, DJL, HA and TA. Data interpretation was performed by ERS, RA, HA, in consultation with TA, ZT, AD and DRL. The manuscript was drafted by ERS, RA, HA and DRL. Critical editing of the manuscript was performed by TA, ZT, DJH, AD and DRL.

Disclosure: The authors are working on emerging technologies for margin assessment in breast cancer surgery.

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Supplementary information: Figures 3, Tables = 4

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MAIN MANUSCRIPT

INTRODUCTION:

Breast cancer is the commonest cancer in females worldwide.[1] On both sides of the Atlantic, breast conserving surgery (BCS) is the most commonly performed surgical treatment for women with early stage breast cancer.[2, 3] Unfortunately, on average, approximately 20% of patients undergoing BCS require a re-operation for close or positive resection margins.[3, 4] Positive margins following excision of breast cancer correlates with a two-fold increase in ipsilateral breast tumour regional recurrence (IBTR).[5] This risk is not eliminated by the use of radiotherapy, systemic chemotherapy or endocrine therapy.[6] The reasons for high reoperation rates and ergo high positive or close margin rates following attempted BCS are multifactorial and include regional variation in histopathological definitions of margin status, ability to adequately image and localize impalpable disease, and the 3D perceptual skills of the operating surgeon as well as the desire to maintain cosmesis which may cause certain operators to be excessively conservative.

Re-operative breast cancer surgery has physical, psychological and economic sequelae. It delays to receipt of adjuvant therapy, and has been associated with an elevated risk of local and distant disease relapse.[7] Re-operation results in greater body trauma, is associated with a higher incidence of post-operative wound complications[8] and leads to a greater volume of excised breast tissue all of which have been linked to impaired cosmetic outcome.[9, 10] Anxiety, depression, body image, sexuality and self-esteem correlate with the perception of cosmetic outcome[11], hence reoperation resulting in poor cosmesis has consequences for psychosexual function.

The increasing demand for BCS, relatively high average re-operation rates for positive margins[3, 4], link between positive margins and risk of IBTR, and the negative impact of re-operation on cosmesis generate demand for reliable intraoperative margin assessment (IMA) tools. IMA techniques provide information about the neoplastic potential of the margins of tissue resection and/or walls of the resection cavity within a timeframe that facilitates re-excision at index surgery. Established IMA techniques include frozen section (FS), cytology (CYT), intraoperative ultrasound (IOUS) and specimen radiography (SR), which all have unique limitations. Therefore, to date, no single IMA technique has gained universal international adoption.

Pathological techniques are operator dependent, resource intensive, and results are slow to turnaround.[12, 13] Only limited sampling points are achievable with FS, and tissue can suffer destructive, freezing and compression artifacts.[14] CYT techniques (e.g. touch/imprint/scrape) are unable to distinguish *in situ* from invasive carcinoma and do not provide information regarding margin width.[15] SR is unable to detect non-calcified lesions and benign calcifications may be misinterpreted as malignant. IOUS is operator dependant, requires additional training and suspicious calcifications are often invisible on ultrasound**.**

To address the limitations of existing techniques, presently there has been growing interest in the development of novel IMA tools for breast surgery. These can broadly be categorized into four groups as follows: A) 'imaging' (high resolution scanners provide enhanced images for specimen interpretation e.g. microcomputed CT (MCT)[16], high frequency ultrasound[17], MRI[18]). B) 'optical' (light, at a variety of wave lengths ranging

from visible to infra-red, is either directed onto tissues or detected within tissues enabling visualization or producing spectra unique to tissue type e.g. raman spectroscopy[19], optical coherence tomography[20], confocal microscopy[21]). C) 'bioimpedance' / 'radiofrequency' (radiofrequency spectroscopy subjects tissue to an electric field and measures and quantifies electromagnetic frequencies to determine tissue specific spectral signatures e.g. MarginProbe™ (MP)[22], ClearEdge[23]). D) 'mass spectrometry' (mass spectrometers measure the mass to charge ratio of ions and various techniques exist that are capable of measuring the tissue specific ionic content linked to cellular metabolism e.g. rapid evaporative ionization mass spectrometry (REIMS)[24, 25] and desorption electrospray Ionization (DESI)[26]). Whilst re-excision rates are the preferred clinical outcome measure, this end-point is sub-optimal for the assessment of emerging technologies that lack clinical data. On the other hand, metrics of diagnostic accuracy as compared to gold standard permanent section histopathology can be used for the comparison of both existing and emerging IMA techniques.

Successful translation of emerging techniques to clinical practice will rely upon achieving diagnostic accuracy at least comparable to existing methods, whilst also providing benefits such as rapid diagnosis, improved cost effectiveness, and reduced re-operation rates. Critically, for investigators evaluating new devices, the accuracy of existing techniques appears to vary markedly between studies. For example, the sensitivity of FS has been demonstrated to range from 64.8% [27]– 95.8%[28], and IOUS from 28.6%[29] to 80.0%[30]. Selective comparison of new IMA techniques to individual studies with lower diagnostic accuracy values risks over inflating the benefits of the emerging technologies. Moreover, there have been no meta-analyses of the available IMA literature and hence investigators lack pooled diagnostic accuracy data. Meta-

analysis offers superior diagnostic summaries from multiple studies when compared to simple averages of sensitivity and specificity as it weights for precise components of study results based on data distribution, dispersion and study size.[31] The aim of this study was to perform a systematic review and meta-analysis to evaluate pooled diagnostic accuracy for IMA techniques that have been evaluated in clinical practice, as a benchmark against which the performance of emerging technologies can be compared.

METHODS:

IMA tools used during BCS in patients with breast cancer were identified to compare the diagnostic accuracy of the technique with permanent section histopathology. The primary outcome measure was diagnostic accuracy. Secondary outcomes included: (a) positive margin rates; (b) cavity re-excision rates; (c) re-operation rates; (d) turnaround time for IMA results.

Literature search:

This systematic review was conducted in accordance with the guidelines for the "Preferred Reporting Items for Systematic Reviews and Meta-Analyses" (PRISMA).[32] Relevant studies were identified using electronic bibliographic searches (up to date as of January 2016) in Pubmed, Cochrane Library, Scopus and EMBASE. MESH terms and all-field search terms were searched for "breast cancers" (breast neoplasm* (MESH), breast cancer*, breast tumour*, breast tumor*, mammary neoplasm*, mammary cancer*, breast carcinoma*) AND "Intraoperative" (intraoperative period(MESH), intraoperative care(MESH), intraoperative*, intra-operative*, intra operative*) AND "margin" (margin*). The search included all study designs. Further studies not captured by the search strategy were identified through bibliographic cross-referencing of included studies and performing searches in Google Scholar until no further relevant studies were identified. Two investigators (E.R.S, R.A.) independently screened titles and abstracts and selected all relevant citations for full-text review. Disagreement regarding article inclusion was resolved by consensus in discussion with the senior author (DRL).

Inclusion Criteria:

Studies written in English that comprised margin assessment data, acquired from one or more IMA techniques used during BCS for breast cancer (invasive or in situ) were eligible. Only studies that stated sensitivity and specificity data compared to permanent section histopathology or in whom sensitivity and specificity data could be calculated from raw data were included. Study participants were adults (mean age>18 years). No limitations were placed on date range and the last search was performed in January 2016.

Exclusion Criteria:

Articles were excluded if the full text article was not written in English. Abstracts, conference articles, opinions, case studies, reviews and meta-analysis were not considered. Studies not performed in humans or not in a clinical environment were excluded. Studies that failed to report on sensitivity and/ or specificity data as compared to permanent section histopathology were excluded, however, data regarding positive predictive values (PPV), negative predictive values (NPV) and overall accuracy were not mandatory (where possible these data were calculated from raw data).

Study Quality:

Two investigators (E.R.S and R.A.) assessed study methodological quality using two validated scoring systems as follows: (a) Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2) checklist to evaluate the risk of bias and applicability of primary diagnostic studies scored out of 14,[33] and (b) the Strength Of Recommendation Taxonomy (SORT) numerical scale for diagnostic studies scored out of 3.[34] Both checklists were independently completed by the two investigators (E.R.S and R.A.) with

agreement and therefore subsequently used to rate all included studies. All seven QUADAS-2 questions were considered relevant for inclusion. For each question, a score of 1 was given for a low risk of bias and 2 was given for a high or unclear risk of bias. To consider the conduct or interpretation of the index test adequate we required study authors to report the number of margins obtained per specimen, as well as provide information on the embedding, sectioning and staining of the derived specimen. The specialty of the operator (i.e. pathologist, surgeon or radiologist) interpreting the results had to be stated. We defined "bias" of the reference standard as occurring when the precise width (mm) between tumour and healthy tissue defining a 'positive margin' was not reported. We determined a "bias" in flow and timing to have occurred when the number of patients selected for inclusion did not correspond to the number of patients included in the statistical analysis, with the exception of reports for which all patients were accounted for despite not being included in outcome data. The SORT scoring system was used to assess the diagnostic quality of the studies according to a numerical scale from 1-3 as described in Supplementary Table C.

Data Collection:

Two investigators (E.R.S and R.A.) extracted study demographic and accuracy data using a pre-defined electronic spreadsheet. Specifically, data was extracted on (1) the first author; (2) year of publication; (3) study design; (4) number of patients or samples; (5) mean age of patients; (6) diagnostic accuracy raw data- false negative (FN), false positive (FP), true negative (TN), true positive (TP); (7) percentages of sensitivity, specificity, positive predictive value, negative predictive value, diagnostic accuracy; (8) cavity re-excision rates; (9) positive margin rates; (10) re-operation rates; and finally (11) turnaround time for results.

Where possible, the two investigators extracted data independently and calculated from raw data the number of true and false positives / negatives. Diagnostic accuracy figures were calculated to 1 decimal point. An inadequate margin of 1cm by ultrasound or radiological criteria was used when a variety of distances were given for the interpretation of diagnostic accuracy using IOUS and SR. When interpretation of SR was performed by either a surgeon or radiologist, the scores from the radiologist were used to determine diagnostic accuracy results.[35] Mean age was recorded when available otherwise median age was used. If a choice of dimensions was given for SR, the results for two-dimensional imaging were used.

Meta-Analysis:

A bivariate model for diagnostic meta-analysis was used to compute pooled sensitivity and specificity data.[36] The relationship between sensitivity and specificity was assessed using a hierarchical summary receiver operating characteristic (SROC) model. SROC curves were utilized to convey the diagnostic test performance for each intra-operative technique. A prediction region (within the prediction curves) was also produced and represents the probability of including the true sensitivity and specificity of a future study. Heterogeneity was assessed using I^2 (25-49% was considered a low level of heterogeneity, 50-74% was considered moderate heterogeneity, and >75% was considered as a high degree of heterogeneity).

Trapezoidal integration was used to calculate the pooled area under the curve (AUC), where 0.5 implies that a test was equally likely to diagnose a positive result as either positive or negative and a value of 1.0 indicates a 'perfect' test that gives a 100% correct

diagnosis. Pragmatically, tests will have a variable pooled AUC value and tend towards 1.0 as diagnostic accuracy improves; a pooled AUC of greater than 0.75-0.92 represents a good degree of diagnostic accuracy (as opposed to 0.93-0.96 which is considered very good). We utilized Stata version 13 (StataCorp, College Station, Texas) for all statistical analyses.

Meta-regression analysis was performed incorporating the number of patients or samples, number of surgical resections, entry criteria (see Table 2), mean age, accepted margin distance for determination of positive margin, cavity re-excision at index procedure, positive margin rate after index procedure, re-operation rate by subsequent procedure, turnaround time for delivery of IMA result, QUADAS quality scoring system-individual questions and total score, and finally the SORT quality scoring system result.

This study was undertaken in accordance with reported guidance for diagnostic test meta-analyses.[37] Our meta-analysis calculated the diagnostic odds ratio of each intra-operative technique. This is a measure of the efficacy of a binary diagnostic test that evaluates the odds of test positivity in the presence of disease relative to the odds of test positivity in the absence of disease. Cook's distance was calculated to assess for influential studies and Deek's test was calculated to assess for publication bias.[31]

RESULTS:

Search and study selection:

Our electronic literature searches identified a total of 1675 citations (Figure 1) and 9 additional papers were found through extended bibliographic searches. Once duplicates were removed, 838 unique records remained. Of these, 695 did not fulfill inclusion criteria based on title and abstract. 143 full manuscripts were individually assessed and 88 were excluded because: (1) sensitivity and/or specificity data were missing; (2) IMA techniques were not assessed; (3) the paper was published in abstract format only; (4) the paper was a literature review; and /or (5) experimental study with no intraoperative data and/or was not in humans. 55 papers (Table 1 and Supplementary Table A) fulfilled inclusion criteria for the systematic review and contained data required for sensitivity and specificity: 12=FS, 14 CYT, 6=IOUS, 13=SR, 4=MP, 6=OPT, 1 =MCT. One study[14] contributed data to two IMA techniques (FZ and SR). Raw diagnostic accuracy data (true/false positive and true/false negative) was unavailable in 18 papers (Supplementary Table A) but was available in 37 papers (Table 1): 11=CYT[15, 38-47], 9=FS[13, 14, 28, 48-53], 9=SR[14, 35, 54-60], 4=IOUS[29, 30, 61, 62], 3=OPT[20, 63, 64], 1=MP[22], 1 =MCT[16]. To enable meta-analysis at least two studies were required per IMA group, therefore MP and MCT groups were excluded as they only contained one study each. This resulted in a total of 35 studies included for final meta-analysis of five IMA techniques (i.e. CYT, FS, SR, IOUS, OPT). Quality scores (QUADAS2 and SORT) are listed for studies with (Table 2) and without (Supplementary Table C) raw diagnostic accuracy data.

Study Demographics:

In total, 55 studies (35 prospective, 20 retrospective) were included in the systematic review and all contained percentage sensitivity and specificity data. Results from the

systematic review that did not contain sufficient raw data to enable meta-analysis are detailed in the supplementary information (Supplementary Tables A and B).

Results are listed for the 35 studies included in the meta-analysis containing raw diagnostic accuracy data (true/false positive and true/false negative). 21 papers were prospective studies and 14 were retrospective. Prospective studies defined a set length of time after surgery when results were recorded in each patient. The publication dates for all the included studies spanned 25 years from 1990 – 2015. Mean or median age was available in 22 studies with a range between 44.9-66 years. Accepted distances for positive margins varied from 1-5mm with a mode of 2mm. Cavity re-excision rates (CRR) performed within the same operation, positive margin rates (PMR) and re-operation rates (ROR) performed at an additional operation are detailed in Table 2. Reported turnaround times listed in Table 2 highlight the additional time required for FZ results relative to IOUS and MP. Documented or calculable percentage sensitivity, specificity, positive predictive value, negative predictive value and overall diagnostic accuracy for each study are listed in Table 1.

Meta-analysis:

Multivariable meta-regression was possible for datasets with two or more studies in the group (i.e. FS, CYT, IOUS, SR, OPT) and demonstrated that patient number significantly contributed to the variation in specificity outcomes for these modalities. Overall, we did not identify a consistently significant variable contributing to sensitivity or specificity outcomes. Deeks' Funnel Plot Asymmetry test was performed on all intra-operative group results and demonstrated no significant small study effects or publication bias. Sensitivity analysis did not offer distinguishing diagnostic evaluation in our analysis due

to the small numbers of studies within some intra-operative technique subgroups and the similarity of scores for QUADAS-2 and SORT within these groups. Sensitivity and specificity data including pooled values for each IMA group are detailed in Figure 2 and Table 3.

Frozen Section:

9 studies report diagnostic accuracy data for FS. Pooled sensitivity was 0.86 (95% CI 0.78-0.91) and pooled specificity was 0.96 (95% CI 0.92-0.98). Overall heterogeneity was significant with an I^2 of 97% (95% CI 94-99) and a Cochran Q of 58.240. The pooled positive likelihood ratio was 21.6 (95% CI 10.4-44.8) with a pooled negative likelihood ratio of 0.15 (95% CI 0.09-0.23). The diagnostic Odds Ratio (OR) was 147 (95% CI 73-297). The pooled, weighted AUC was 0.96 (95% CI 0.94-0.97). Figure 3a illustrates the bivariate summary receiver operating characteristic (SROC) graph with the 95% confidence region and 95% prediction region.

Cytology:

11 studies reported on diagnostic accuracy data for CYT. Pooled sensitivity was 0.91 (95% CI 0.71-0.97) and a pooled specificity of 0.95 (95% CI 0.90-0.98). Overall heterogeneity was significant with an I^2 of 94% (95% CI 90-99) and a Cochran Q of 35.225. The pooled positive likelihood ratio was 18.9 (95% CI 9.2-38.9) with a pooled negative likelihood ratio of 0.10 (95% CI 0.03-0.34). The diagnostic Odds Ratio (OR) was 193 (95% CI 46-816). The pooled, weighted AUC was 0.98 (95% CI 0.96-0.99). Figure 3b illustrates the bivariate summary receiver operating characteristic (SROC) graph with the 95% confidence region and 95% prediction region.

IOUS:

4 studies reported on diagnostic accuracy data for IOUS. Pooled sensitivity was 0.59 (95% CI 0.36-0.79) with a pooled specificity of 0.81 (95% CI 0.66-0.91). Overall heterogeneity was significant with an I^2 of 96% (95% CI 91-99%) and a Cochran Q of 41.344. Pooled positive likelihood ratio was 3.2 (95% CI 2.0-5.2) with a pooled negative likelihood ratio of 0.50 (95% CI 0.32-0.80). The diagnostic Odds Ratio (OR) was 6 (95% CI 3-13). The pooled, weighted AUC was 0.78 (95% CI 0.75-0.82). Supplementary Figure A illustrates the bivariate summary receiver operating characteristic (SROC) graph with the 95% confidence region and 95% prediction region.

Specimen Radiography:

9 studies reported on diagnostic accuracy data for SR. Pooled sensitivity was 0.53 (95% CI 0.45-0.61) with a pooled specificity of 0.84 (95% CI 0.77-0..89) Overall heterogeneity was not significant with an I^2 of 0% (95% CI 0-100%) and a Cochran Q of 0.188. Pooled positive likelihood ratio was 3.3 (95% CI 2.1-5.0) with a pooled negative likelihood ratio of 0.56 (95% CI 0.46-0.69). The diagnostic Odds Ratio (OR) was 6 (95% CI 3-11). The pooled, weighted AUC was 0.73 (95% CI 0.69-0.77). Supplementary Figure B illustrates the bivariate summary receiver operating characteristic (SROC) graph with the 95% confidence region and 95% prediction region.

Optical imaging:

3 studies reported on diagnostic accuracy data for OPT. Pooled sensitivity was 0.85 (95% CI 0.74-0.91) with a pooled specificity of 0.87 (95% CI 0.65-0.96). Overall heterogeneity was not significant with an I^2 of 48% (95% CI 0-100%) and a Cochran Q of 3.861. Pooled positive likelihood ratio was 6.7 (95% CI 2.1-21.4) with a pooled negative

likelihood ratio of 0.18 (95% CI 0.10-0.33). The diagnostic Odds Ratio (OR) was 37 (95% CI 8-187). The pooled, weighted AUC was 0.88 (95% CI 0.85-0.90). Supplementary Figure C illustrates the bivariate summary receiver operating characteristic (SROC) graph with the 95% confidence region and 95% prediction region.

DISCUSSION:

This systematic review and meta-analysis compares the diagnostic accuracy of intraoperative techniques for breast cancer margin assessment and thereby provides data against which the performance of emerging techniques may now be compared. The findings suggest CYT (pooled sensitivity 0.91, pooled specificity 0.95) and FS (pooled sensitivity 0.86, pooled specificity 0.96) are the most accurate IMA techniques with AUC values of 0.98 and 0.96 respectively. However, despite this level of accuracy, routine adoption and diffusion has been poor, and these techniques are infrequently employed. Regarding UK practice, we surveyed the major breast screening centers in London under the breast national external quality assessment scheme to enquire about routine histopathological IMA usage in their individual hospitals. Responses collated from lead pathologists (n=18) of these centers (89% response rate) confirmed that in no institutions were IMA techniques routinely used during breast cancer surgery (supplementary Table D). Interestingly, we were unable to gauge national uptake of IMA methods in the USA neither from the published literature nor from direct correspondence with the American Society of Breast Surgeons. However, we anticipate that outwith of leading cancer centres uptake of IMA technology is likely to mirror UK specialist practice.

The reasons for poor FS or CYT IMA uptake are likely to be multifactorial but may include slow turnaround times (e.g. average increase of 29 minutes for FS), disruption to surgical workflow, inter-departmental logistical challenges, and resource requirements. In our experience pathological IMA techniques are labour intensive, costly and time consuming. Only high volume centres with suitably large pathology teams are able to routinely staff a service to the required level. Skills in CYT reporting have been

decreasing over the past years due to the decline in routine cytological assessment which makes analysis by appropriately experienced cytologists more troublesome. Furthermore, in our experience the interpretation of FS specimens is more difficult than permanent section histology and as such requires a senior pathologist to produce confident reports. In our department it costs approximately £300 ($=430) to run FS axillary lymph node analysis, taking into considering histopathologists' time, technician support and materials. Despite this, total surgical costs per patient are reduced with IMA use from an estimated $22,013 +/- $13,821 to $15,341 +/- $4,328.[65] More specifically, the use of FS was demonstrated to be cheaper to the payor and provider when re-excision rates were above 26% and 36% respectively.[66] Beyond cost-effectiveness, reoperation rates are clinically important and will therefore influence the usage of any IMA technique.

The diagnostic accuracy values of histopathological techniques correlate with a significant reduction in reoperation rates with a previous pooled analysis comparing the use of permanent section histopathology (35 ±3 %) to FS (10 ± 6 %) and CYT (11 ± 4 %) ($p \leq 0.0001$).[67] More recently data from the National Surgical Quality Improvement Program at the Mayo clinic suggests that a 70% relative risk reduction (RRR) is achievable when FS methods are employed (3.6% reoperation rate compared to a national rate of 13.2%).[68] Although statistically significant, an even greater reduction in reoperation rate might be anticipated given the degree of diagnostic accuracy demonstrated in the current meta-analysis. The accepted distance for the classification of a positive margin may play a role with certain IMA techniques being unable to detect tumour further away from the surface of a margin. Other independent factors such as tumour multi-focality may also affect re-excision rates.[69]

Interestingly, two-dimensional specimen mammography, a technique known to be in use routinely in many cancer institutions in the USA[70] has substantially inferior diagnostic accuracy when compared to histopathological techniques (pooled sensitivity=0.53, pooled specificity=0.84, AUC= 0.73). As expected of a technique with lower diagnostic accuracy, the reduction in re-operation rates using specimen x-ray (RRR 0%[71] to 56%[54]) is generally more modest and variable than with the use of histopathological techniques. Furthermore, a significant reduction in reoperation rates using FS has been demonstrated compared to using SR.[14] Constructing a three-dimensional image of the resected specimen such as with MCT[16] may improve the accuracy of radiological techniques but there was insufficient data upon which to perform a meta-analysis. Importantly, SR can be used within the operating room itself, provides results that are interpretable by the surgeon[35] and as such avoids the need for additional personnel. Indeed, the current analysis demonstrates IMA techniques that depend on the surgeon acquiring additional skills by learning to interpret images or use devices to analyse tissue,[20, 30, 72] provide competitive diagnostic accuracy results. For example, here IOUS is observed to have a higher diagnostic accuracy (AUC=0.78) compared to SR. Additionally, despite the limited data available for meta-analysis[20, 63, 64], pooled accuracy of optical spectroscopy is promising (AUC=0.88). Success has been demonstrated with similar probe based technologies that are applied to the edge of resected breast tissues, such as radiofrequency (RF) spectroscopy (e.g. MarginProbe™)[72] that measures tissue specific electromagnetic properties. Interestingly, whilst there was insufficient data for meta-analysis here, clinical studies have identified significant reductions in re-operations rates using the RF technique (Control=39%, RF Device=17%, RRR=56%),[72] despite modest diagnostic accuracy (Table 4). Technologies that can be used by the surgeon either during dissection or

shortly after resection minimize disruption to the surgical workflow, are time-efficient and in our opinion likely to represent clinically actionable tools, as long as the accuracy is comparable to existing IMA techniques.

Preliminary data from a number of developing technologies suggest that it is possible to compete with the accuracy of pathological methods, whilst also providing rapid diagnostic information to the surgeon. For example, the diagnostic accuracy of raman spectroscopy (sensitivity=95.6% and specificity=96.2%)[19] for distinguishing cancerous from normal and benign ex-vivo breast tissues is comparable to the pooled accuracy of FS and Cyt as computed here. Advances have been made in confocal microscopy with surgeons being able to accurately distinguish the appearance breast carcinoma from normal breast tissues based on cellular and subcellular imaging features (sensitivity=97% specificity=86%).[21] REIMS has recently reported sensitivities of 93.0% and specificities of 91.9% for classification of tumour to normal breast tissue.[24] Emerging IMA technologies will need to compete with the pooled diagnostic accuracy of existing techniques that have been demonstrated to reduce reoperation rates, whilst offering additional advantages in terms of speed, cost and reliability. Based on the results of this meta-analysis (Table 3) techniques should aim for at least comparable sensitivity (SR 53% to CYT 91%) and specificity (IOUS 81% to FS 96%) whilst ultimately demonstrating significant reductions in reoperation rates.

Any discussion regarding the use of IMA techniques needs to be framed in the context of a changing landscape as to what defines 'close-positive margins' and the lack of an international consensus on this definition. For example, the American Society for Radiation Oncology released consensus guidelines in 2014 stating that a negative

margin should be considered as “no ink on tumour”.[6] Whilst in the UK, a consensus agreement from the Association of Breast Surgery (ABS) re-defined a positive margin as cancer within 1mm from the resection margin.[73] Therefore, as ever narrower margins are being accepted, it is conceivable that the positive margin rates will steadily decline. This along with evidence that a reduction in re-excision rates can be performed through simple routine cavity shaves[74], understandably calls into question the need for IMA techniques. However, routine cavity shaves were found to result in significant increases in volume of tissue excised[74] and there are concerns regarding the long-term cosmetic outcomes that are yet to be published. Finally, the decrease in acceptable margin width favor emerging techniques as these approaches have limited penetrance of tissue interrogation. For example, REIMS[13], DESI[14], MarginProbe[10] and confocal microscopy[3] have a depth resolution of <200 μm, whilst others can penetrate up to a couple of millimeters e.g. optical coherence tomography 200 μm-2mm[8] raman 1-2mm[12] and ClearEdge 1-2mm.[15] These technologies therefore, provide data comparable to the “inked” radial resection margin which is commensurate with the emerging guidelines on negative margins. Although the positive margin rate is likely to be curtailed by such changes in definitions, re-operation rates are unlikely to disappear, and we therefore predict the need for further accurate and acceptable IMA devices.

Our meta-analysis provides appreciation of the comparative benefit of IMA techniques for BCS. To achieve this we were able to employ a quantifiable assessment of each modality utilizing diagnostic accuracy meta-analytical techniques. Overall the studies scored relatively low levels of bias and applicability concerns on QUADAS2 scoring (supplementary Table C) whilst only the emerging techniques of OPT and MCT had a poorer SORT score of 3. To our surprise, approximately a third of the papers (33%)

lacked raw diagnostic accuracy data (18 out of 55, supplementary Table A) and we would encourage the inclusion of these results as mandatory for studies reporting on IMA techniques in the future. As a minimum requirement we suggest future IMA studies report on sensitivity and specificity in relation to the gold standard of final section histopathology. The raw numbers used to calculate diagnostic accuracy values should be detailed in full and it should be clear if the numbers used are per biopsy, per specimen, per patient or any other measure. We suggest that a "confusion matrix" should be included in all future IMA studies so the reader can quickly assess the number of patients in each group and the classification accuracy.

Previous reviews have assessed IMA diagnostic accuracy by calculating average sensitivity and specificity.[67, 75] For completeness we have also documented studies containing this data. However, in our opinion this does not offer a robust assessment of diagnostic accuracy and for comparison we have listed the discrepancies between the results if simple averages are computed versus meta-analytical pooling as described here (Table 4). With the exception of optical spectroscopy the sensitivity and specificity values for every other IMA technique were underestimated using pooled simple averages alone when compared to the results from the meta-analysis.

Our results do however suffer from a number of inherent limitations. There are only small number of studies in each IMA group, particularly noted in IOUS and OPT, which renders the derivation of high quality summary scores and discernment of subgroup analysis difficult. Ensuring consistency within and between groups was challenging due to significant heterogeneity and variation in methodology, which is a common limitation in surgical studies. The clinical application of these results may not be directly

applicable to laboratory based emerging techniques. Even in systematic assessment of the literature there is variation in description of outcome measures. Some papers reported diagnostic accuracy results per patient whilst others reported per sample. The diagnostic criteria and accepted margin distances also varied between studies.

Conclusion:

In summary, this meta-analysis provides pooled diagnostic accuracy for IMA techniques that evaluate resection margin status following breast cancer surgery. The results demonstrate that the diagnostic accuracy of FS and CYT are currently unparalleled. In order to become a disruptive technology, emerging techniques will need to compete with this level of accuracy and yet will also need to provide significant improvements such as the rapidity of results, cost-effectiveness and accessibility of information to the surgeon for fast, accurate and appropriate operative decision making.

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Figure legends

Main Manuscript:

Figure 1 Title: PRISMA flow diagram

Figure 1 Legend: Flow chart of studies included in the qualitative and quantitative analysis.

Figure 2 Title: Pooled meta-analysis forest plots per group

Figure 2 Legend: Sensitivity and specificity data for all studies included for meta-analysis, organized per IMA group with pooled data displayed above.

Figure 3a Title: Meta-analysis SROC Curve for Frozen Section

Figure 3b Title: Meta-analysis SROC Curve for Cytology

Figure 3 Legend: Showing pooled SROC curve for frozen section (a) and Cytology (b). Here the horizontal axis demonstrates specificity (true negative) and the vertical axis demonstrates sensitivity (true positives). The curve demonstrates the true positive rate of each test at each true negative value. The diagnostic accuracy of each modality is highest when the results of both axes tend closer to 100% (or 1) for both true positives and true negatives.

Supplementary Figures:

Supplementary Figure A Title: Meta-analysis SROC Curve for Intraoperative Ultrasound

Supplementary Figure A Legend: Showing pooled SROC curve for intraoperative ultrasound

Supplementary Figure B Title: Meta-analysis SROC Curve for Specimen Radiography

Supplementary Figure B Legend: Showing pooled SROC curve for specimen radiography.

Supplementary Figure C Title: Meta-analysis SROC Curve for Optical Spectroscopy

Supplementary Figure C Legend: Showing pooled SROC curve for optical spectroscopy.

Table 1: Diagnostic Accuracy – Studies containing raw diagnostic accuracy data for meta-analysis.

Type	Author	Meta	TP	FP	TN	FN	N_Tot	Sensitivity	Specificity	PPV	NPV	Accuracy
FS	Noguchi et al.[28]	Yes	23	12	64	1	100	95.8	84.2	65.7	98.5	87
	Ikeda et al.[49]	Yes	17	4	34	1	56	94.4	89.5	81	97.1	91.1
	Olson et al.[12]	Yes	57	5	1228	21	1311	73.1	99.6	91.9	98.3	98
	Weber et al.[14]	Yes	32	5	35	8	80	80	87.5	86.5	81.4	83.8
	Rusby et al.[53]	Yes	39	15	495	8	557	83	97	72.2	98.4	96
	Caruso et al.[48]	Yes	5	3	44	1	53	83	93	62	97	94
	Jorns et al.[31]	Yes	12	0	28	6	46	66.7	100	100	82.4	87
	Osako et al.[13]	Yes	259	53	955	60	1327	81.2	94.7	83	94.1	91.5
	Kikuyama et al.[51]	Yes	287	18	440	18	763	94.1	96.1	94.1	96.1	95.3
CYT	Ku et al.[43]	Yes	17	2	68	0	87	100	97.1	89.5	100	97.7
	Cox et al.[40]	Yes	22	3	86	0	111	100	96.6	88	100	97.3
	Tohnosu et al.[45]	Yes	27	16	156	1	200	96.4	90.7	62.8	99.4	91.5
	Muttalib et al.[15]	Yes	6	6	15	0	27	100	71.4	50	100	77.8
	Creager et al.[41]	Yes	12	18	104	3	137	80	85.3	40	97.2	85
	Valdes et al.[46]	Yes	1	1	59	11	72	8.3	98.3	50	84.3	83.3
	Valdes et al.[47]	Yes	3	11	53	1	68	75	82.8	21.4	98.2	82.4
	Bakhshandeh et al.[38]	Yes	30	7	472	1	510	97	99	81.1	99.8	98.4
	Blair et al.[39]	Yes	3	0	115	1	119	75	100	100	99.1	99.2
	D'Halluin et al.[42]	Yes	71	26	304	9	410	88.6	92.2	73.6	97	91.5
	Sumiyoshi et al.[44]	Yes	14	4	136	6	160	70	97.1	77.8	95.8	93.8
IOUS	Mesurolle et al.[61]	Yes	30	8	33	10	81	75	80.5	79	76.7	77.8
	Londero et al.[29]	Yes	8	24	132	20	156	28.6	84.6	25	86.8	76.1
	Ramos et al.[30]	Yes	24	79	116	6	225	80	59.5	95.1	23.3	62.2
	Moschetta et al.[62]	Yes	16	6	90	20	132	44.4	93.8	72.7	81.8	80.3
SR	Graham et al.[57]	Yes	62	1	18	38	119	62	95	98	32	67.2
	Saarela et al.[60]	Yes	9	8	31	18	66	33	79	53	63	61
	McCormick et al.[58]	Yes	6	10	72	5	93	54.6	87.8	37.5	93.5	83.9
	Coombs et al.[35]	Yes	12	4	25	11	52	52.2	86.2	75	69.4	
	Ciccarelli et al.[56]	Yes	25	9	55	13	102	65.8	85.9	73.5	80.9	
	Weber et al.[14]	Yes	12	6	9	8	35	60	60	66.7	52.9	60
	Prueksadee et al.[59]	Yes	3	3	1	5	12	37.5	25	50	16.7	33.3
	Bathla et al.[54]	Yes	24	5	56	17	102	58.5	91.8	82.8	76.7	78.4
	Chagpar et al.[55]	Yes	14	12	44	20	90	41.2	78.6	53.9	68.8	64.4
OPT	Nguyen et al.[20]	Yes	9	2	9	0	20	100	81.8	81.8	100	90
	Keller et al.[64]	Yes	29	6	139	5	179	85.3	95.9	82.9	96.5	94
	Brown et al.[63]	Yes	27	7	14	7	55	79.4	66.7	79.4	66.7	74.6
MP	Karni et al.[22]	No	30	88	184	12	314	71.4	67.7	25.4	93.9	68.2
MCT	Tang et al.[16]	No	5	1	18	1	25	83.3	94.7	83.3	94.7	92

Legend: FS=frozen section, CYT=Cytology, IOUS=Intraoperative Ultrasound, SR=Specimen radiography, OPT=Optical spectroscopy, MP=Margin probe, MCT=Micro-computed tomography, Meta=Included for meta-analysis, TP=True positive, FP=False positive, TN=True negative, FN=False negative, N_Tot=Total number of patients/samples, PPV=Positive predictive value, NPV=Negative predictive value. Accuracy=Overall diagnostic accuracy.

Table 2: Demographics and secondary outcome data - Studies containing raw diagnostic accuracy data for meta-analysis.

Type	Author	Year	N_PT	N_Res	N_Mar	Method	E-Crit	AGE	M_Dist	CRR	PMR	ROR	TAT	Q_tot	SORT
FS	Noguchi et al.[28]	1995	95	100		Prospective	1			35	24			9	2
	Ikeda et al.[49]	1997	54	56		Retrospective	1	44.9	0	35.7	12.5	10.7		8	2
	Olson et al.[12]	2007	290	292	1404	Retrospective	1	57.2		24.1		11.4	*25*	11	2
	Weber et al.[14]	2008		80		Retrospective	1	59.6	1		22.5	12.5		8	2
	Rusby et al.[53]	2008	115		557	Prospective	5	49.5	5	4.4	7	2.6	*20*	9	2
	Caruso et al.[48]	2011	50	52		Retrospective	5		2	10	10		*20*	9	2
	Jorns et al.[31]	2014	46			Prospective	2	57.4	2	23.9	39.1	19.6	*22*	9	2
	Osako et al.[13]	2015	1029	1327		Retrospective	1		5	30.3	30.3	0.1	*50*	9	2
	Kikuyama et al.[51]	2015	220		763	Prospective	1	51.3						9	2
CYT	Ku et al.[43]	1990		87		Prospective	1						*15*	10	2
	Cox et al.[40]	1991	111	111		Prospective	1	58.4					*15*	10	2
	Tohnosu et al.[45]	1998	50		200	Prospective	1	52.9	5					8	2
	Muttalib et al.[15]	2004	26	27		Prospective	1		1		22.2		22.5	8	2
	Creager et al.[41]	2002	137	141	758	Retrospective	1	58	2				*20*	8	2
	Valdes et al.[46]	2007	12		72	Prospective	3			23		33.3	*15*	9	2
	Valdes et al.[47]	2007	30		68	Prospective	2						*15*	9	2
	Bakhshandeh et al.[38]	2007		100	510	Retrospective	1						*20*	10	2
	Blair et al.[39]	2007	20	20	120	Prospective	1							10	2
	D'Halluin et al.[42]	2009	396	400		Prospective	1	58.6	2	38.3		13.3	*10*	9	2
	Sumiyoshi et al.[44]	2010	160			Prospective	1	58.1						9	2
IOUS	Mesurolle et al.[61]	2006		81		Retrospective	1	59.1	2		17.4		3-6	8	2
	Londero et al.[29]	2010		46	184	Prospective	1	53	2				3-6	8	2
	Ramos et al.[30]	2013	223	225		Prospective	1	59.5	2	45.7		4		8	2
	Moschetta et al.[62]	2015	132			Prospective	1	51	2					8	2
SR	Graham et al.[57]	1994		119		Prospective	6		1					8	2
	Saarela et al.[60]	2001	64	66		Prospective	6	55	0	74.2	16.7			9	2
	McCormick et al.[58]	2004	93			Retrospective	5			18		5	*15*	9	2
	Coombs et al.[35]	2006	101	52		Retrospective	1	58.2	5		19.7	9.3		8	2
	Ciccarelli et al.[56]	2007	102			Retrospective	6		2	31.4	22.5	20		9	2
	Weber et al.[14]	2008		35		Retrospective	1	57.5	1		42.9	37.1		9	2
	Prueksadee et al.[59]	2009	12			Retrospective	5	59.3	2	50	25			8	2
	Bathla et al.[54]	2011	99	102		Retrospective	1	58.6	1	28.4	17.6	14.7		8	2
	Chagpar et al.[55]	2015	90			Prospective	1	60	1	28.9	30	10		8	2
OPT	Nguyen et al.[20]	2009	20	20	210	Prospective	1	66	2					8	3
	Keller et al.[64]	2010	40		179	Prospective	7		1					8	3
	Brown et al.[63]	2010	57		55	Prospective	1		2				*20*	8	3
MP	Karni et al.[22]	2007	57	57	314	Prospective	1		1	15.8	38.6		*7*	9	2
MCT	Tang et al.[16]	2013	6		25	Prospective	1	55	2				*10*	8	3

Legend: FS=frozen section, CYT=Cytology, IOUS=Intraoperative Ultrasound, SR=Specimen radiography, OPT=Optical spectroscopy, MP=Margin probe, MCT=Micro-computed tomography, N_Pt=Number of patients, N_Res=Number of resections/operations/specimens, N_Mar=Number of margins/biopsies, E-Crit=Entry criteria (1=Breast conserving surgery (BCS) for breast cancer (BC), 2=Re-excision of BC after positive margins, 3=BCS for Invasive lobular carcinoma, 4=BCS for ductal carcinoma in-situ, 5=Therapeutic mammoplasty/partial mastectomy (Mx), 6=BCS for impalpable BC, 7=Mx or BCS for BC), M_Dist=Margin +ve distance in mm, CRR= Cavity re-excision rate % (same operation), PMR=Positive margin rate %, ROR=Re-operation rate % (additional operation), TAT=Turnaround time for result, Q_tot = Total QUADAS2 score, SORT=Total SORT score.

Table 3: Meta-analysis - Pooled diagnostic accuracy data of IMA techniques.

IMA technique:	No. of Studies	Sensitivity	Specificity	AUROC
Frozen section	9	0.86 [0.78-0.91]	0.96 [0.92-0.98]	0.96 [0.94 - 0.97]
Cytology	11	0.91 [0.71-0.97]	0.95 [0.90-0.98]	0.98 [0.96 - 0.99]
Intraoperative Ultrasound	4	0.59 [0.36-0.79]	0.81 [0.66-0.91]	0.78 [0.75 - 0.82]
Specimen Radiography	9	0.53 [0.45-0.61]	0.84 [0.77-0.89]	0.73 [0.69 - 0.77]
Optical Spectroscopy	3	0.85 [0.74-0.91]	0.87 [0.65-0.96]	0.88 [0.85 - 0.90]

Legend: AUROC=Area under the receiver operating characteristic curve.

Table 4: Simple averages of percentage sensitivity and specificity data of IMA techniques compared to pooled meta-analysis.

		Sensitivity			Specificity		
IMA technique:	No. of Studies: MA* / All**	Pooled MA	Average – MA studies	Average – All studies	Pooled MA	Average – MA studies	Average – All studies
Total Studies ✣	35✣ / 55✣	35✣	35✣	55✣	35✣	35✣	55✣
Frozen section	9 / 12	**86%**	83.5%	81.0%	**96%**	93.5%	94.4%
Cytology	11 / 14	**91%**	80.9%	82.9%	**95%**	91.9%	93.5%
Intraoperative Ultrasound	4 / 6	**59%**	57%	55.7%	**81%**	79.6%	84.4%
Specimen Radiography	9 / 13	**53%**	51.6%	54.8%	**84%**	76.6%	73.9%
Optical Spectroscopy	3 / 6	**85%**	88.2%	81.7%	**87%**	81.5%	81.9%
Margin Probe	0 / 4			70.4%			58.5%
MicroCT	0 / 1			83.3%			94.7%

Legend: *=Same studies as meta-analysis, **=All studies from systematic review, ✣=One study contributed data to two different IMA techniques, MA=Meta-analysis, All studies=all studies included in systematic review.

Supplementary Table A: Diagnostic Accuracy – Studies missing raw diagnostic accuracy data but included in systematic review.

Type	Author	Meta	TP	FP	TN	FN	N_Tot	Sensitivity	Specificity	PPV	NPV	Accuracy
FS	Cendan et al.[1]	No						64.8	100	100	94.4	96
	Cabioglu et al.[2]	No						77.8	91.7			87.4
	Fukamachi et al.[3]	No						78.6	100	100	94	95.1
CYT	Cox et al.[4]	No						99	98	98	99	
	Klimberg et al.[5]	No						96.4	100	100	99.3	99.3
	Bukhari et al.[6]	No						75	100	100	84	95
IOUS	Olsha et al.[7]	No						58	89	27	97	
	Eggemann et al.[8]	No						48.4	99.1	81	96.2	
SR	Mazouni et al.[9]	No						75	41	68	49	
	Britton et al.[10]	No						64	73			
	Layfield et al.[11]	No						58.1	80.8	56.3	81.9	
	Rua et al.[12]	No						50	76.8	36	85.5	
OPT	Brown et al.[13]	No						74	86	85	75	80
	Zysk et al.[14]	No						55.0-65.0	68.0-70.0	8.0-18.0	94.0-97.0	
	Erickson-Bhatt et al.[15]	No						91.7	92.1	78.6	97.2	92
MP	Pappo et al.[16]	No						70	70			
	Schnabel et al.[17]	No						75.2	46.4			
	Thill et al.[18]	No						65	50			

Legend: FS=frozen section, CYT=Cytology, IOUS=Intraoperative Ultrasound, SR=Specimen radiography, OPT=Optical spectroscopy, MP=Margin probe, MCT=Micro-computed tomography, Meta=Included for meta-analysis, TP=True positive, FP=False positive, TN=True negative, FN=False negative, N_Tot=Total number of patients/samples, PPV=Positive predictive value, NPV=Negative predictive value. Accuracy=Overall diagnostic accuracy.

Supplementary Table B: Demographics and secondary outcome data - Studies missing raw diagnostic accuracy data but included in systematic review.

Type	Author	Year	N_PT	N_Res	N_Mar	Method	E-Crit	AGE	M_Dist	CRR	PMR	ROR	TAT	Q_tot	SORT
FS	Cendan et al.[1]	2005	97	116	628	Retrospective	1	59.4		25.8		18.6	*13*	10	2
	Cabioglu et al.[2]	2007	145			Retrospective	1		2					10	2
	Fukamachi et al.[3]	2010	122			Retrospective	1	56	5		27		*53*	9	2
CYT	Cox et al.[4]	1997	114	116		Prospective	4	58				6.6		10	2
	Klimberg et al.[5]	1998	428	428		Prospective	1	54.8		7.2			*15*	10	2
	Bukhari et al.[6]	2009		78		Prospective	1							10	2
IOUS	Olsha et al.[7]	2011	45	48	179	Prospective	1		2	31		7		10	2
	Eggemann et al.[8]	2014	147			Prospective	1	59	1	13.6	14.3	8.8		10	2
SR	Mazouni et al.[9]	2006	164			Retrospective	6	55	1	22				9	2
	Britton et al.[10]	2011	106			Retrospective	1	60	4		34			9	2
	Layfield et al.[11]	2012	104			Retrospective	1	59.6	2	30.8	29.8	29.8		10	2
	Rua et al.[12]	2012		87		Retrospective	6	60.1	1	46		12.6		9	2
OPT	Brown et al.[13]	2013	70	88		Prospective	1	59.6	2			27.1	*22.5*	9	3
	Zysk et al.[14]	2015	46		229	Prospective	1	62	0-2		17.4			8	2
	Erickson-Bhatt et al.[15]	2015	21			Prospective	1	61	1		13.6			8	3
MP	Pappo et al.[16]	2010	76		753	Prospective	7							10	2
	Schnabel et al.[17]	2014	298			Prospective RCT	6	60.3	1	11	30.9	19.8		8	1
	Thill et al.[18]	2014	42			Prospective	4		1			17	*5*	10	2

Legend: FS=frozen section, CYT=Cytology, IOUS=Intraoperative Ultrasound, SR=Specimen radiography, OPT=Optical spectroscopy, MP=Margin probe, MCT=Micro-computed tomography, N_Pt=Number of patients, N_Res=Number of resections/operations/specimens, N_Mar=Number of margins/biopsies, E-Crit=Entry criteria (1=Breast conserving surgery (BCS) for breast cancer (BC), 2=Re-excision of BC after positive margins, 3=BCS for Invasive lobular carcinoma, 4=BCS for ductal carcinoma in-situ, 5=Therapeutic mammoplasty/partial mastectomy (Mx), 6=BCS for impalpable BC, 7=Mx or BCS for BC), M_Dist=Margin +ve distance in mm, CRR= Cavity re-excision rate % (same operation), PMR=Positive margin rate %, ROR=Re-operation rate % (additional operation), TAT=Turnaround time for result, Q_tot = Total QUADAS2 score, SORT=Total SORT score.

Supplementary Table C: Quality scoring for all studies.

		QUADAS2: Risk of Bias				QUADAS2: Applicability Concerns				
Type	**Author**	**Patient Selection**	**Index Test**	**Reference Standard**	**Flow and Timing**	**Patient Selection**	**Index Test**	**Reference Standard**	**Q_tot**	**SORT**
FS	Noguchi et al.	High	Low	Unclear	Low	Low	Low	Low	9	2
	Ikeda et al.	High	Low	Low	Low	Low	Low	Low	8	2
	Cendan et al.	High	Low	Unclear	Unclear	Low	Low	Low	10	2
	Olson et al.	High	High	Unclear	High	Low	Low	Low	11	2
	Cabioglu et al.	High	Low	High	Unclear	Low	Low	Low	10	2
	Weber et al.	High	Low	Low	Low	Low	Low	Low	8	2
	Rusby et al.	High	High	Low	Low	Low	Low	Low	9	2
	Fukamachi et al.	High	Low	Low	Unclear	Low	Low	Low	9	2
	Caruso et al.	High	Low	Low	High	Low	Low	Low	9	2
	Jorns et al.	High	High	Low	Low	Low	Low	Low	9	2
	Osako et al.	High	Low	Low	High	Low	Low	Low	9	2
	Kikuyama et al.	High	Low	Unclear	Low	Low	Low	Low	9	2
CYT	Ku et al.	High	Unclear	Unclear	Low	Low	Low	Low	10	2
	Cox et al.	High	Unclear	Unclear	Low	Low	Low	Low	10	2
	Cox et al.	High	Low	Unclear	Unclear	Low	Low	Low	10	2
	Tohnosu et al.	High	Low	Low	Low	Low	Low	Low	8	2
	Klimberg et al.	High	Low	Unclear	Unclear	Low	Low	Low	10	2
	Creager et al.	High	Low	Low	Low	Low	Low	Low	8	2
	Muttalib et al.	High	Low	Low	Low	Low	Low	Low	8	2
	Valdes et al.	High	Low	Unclear	Low	Low	Low	Low	9	2
	Valdes et al.	High	Low	Unclear	Low	Low	Low	Low	9	2
	Bakhshandeh et al.	High	Low	Unclear	Unclear	Low	Low	Low	10	2
	Blair et al.	High	Low	Unclear	High	Low	Low	Low	10	2
	D'Halluin et al.	High	Low	Low	High	Low	Low	Low	9	2
	Bukhari et al.	High	Low	Unclear	Unclear	Low	Low	Low	10	2
	Sumiyoshi et al.	High	Low	Unclear	Low	Low	Low	Low	9	2
IOUS	Mesurolle et al.	High	Low	Low	Low	Low	Low	Low	8	2
	Londero et al.	High	Low	Low	Low	Low	Low	Low	8	2
	Olsha et al.	High	Unclear	Low	Unclear	Low	Low	Low	10	2
	Ramos et al.	High	Low	Low	Low	Low	Low	Low	8	2
	Eggemann et al.	High	Unclear	Low	Unclear	Low	Low	Low	10	2
	Moschetta et al.	High	Low	Low	Low	Low	Low	Low	8	2
SR	Graham et al.	High	Low	Low	Low	Low	Low	Low	8	2
	Saarela et al.	High	High	Low	Low	Low	Low	Low	9	2
	McCormick et al.	High	Low	Unclear	Low	Low	Low	Low	9	2
	Coombs et al.	High	Low	Low	Low	Low	Low	Low	8	2
	Mazouni et al.	High	Low	Low	Unclear	Low	Low	Low	9	2
	Ciccarelli et al.	High	Unclear	Low	Low	Low	Low	Low	9	2
	Weber et al.	High	Unclear	Low	Low	Low	Low	Low	9	2
	Prueksadee et al.	High	Low	Low	Low	Low	Low	Low	8	2

	Bathla et al.	High	Low	Low	Low	Low	Low	Low	8	2
	Britton et al.	High	Low	Low	Unclear	Low	Low	Low	9	2
	Layfield et al.	High	Unclear	Low	Unclear	Low	Low	Low	10	2
	Rua et al.	High	Low	Low	Unclear	Low	Low	Low	9	2
	Chagpar et al.	High	Low	Low	Low	Low	Low	Low	8	2
OPT	Nguyen et al.	High	Low	Low	Low	Low	Low	Low	8	3
	Keller et al.	High	Low	Low	Low	Low	Low	Low	8	3
	Brown et al.	High	Low	Low	Low	Low	Low	Low	8	3
	Brown et al.	High	Low	Low	Unclear	Low	Low	Low	9	3
	Zysk et al.	High	Low	Low	Low	Low	Low	Low	8	2
	Erickson-Bhatt et al.	High	Low	Low	Low	Low	Low	Low	8	3
MP	Karni et al.	High	Low	Low	Unclear	Low	Low	Low	9	2
	Pappo et al.	High	Low	Unclear	Unclear	Low	Low	Low	10	2
	Schnabel et al.	Low	Low	Low	Unclear	Low	Low	Low	8	1
	Thill et al.	High	Unclear	Low	Unclear	Low	Low	Low	10	2
MCT	Tang et al.	High	Low	Low	Low	Low	Low	Low	8	3

SORT scoring criteria - A paper was classified as being level 1 (good quality patient oriented evidence) if it was a well-designed RCT with an adequate method of randomization, blinding and a large sample size. Level 2 evidence (limited quality patient oriented evidence) included RCTs with an inadequate or unclear method of randomization, blinding or sample size. Lower quality diagnostic cohort studies with poor follow up or case controlled studies were also classified as level 2. Papers were graded level 3 if they were an extrapolation from bench research.

Legend: FS=frozen section, CYT=Cytology, IOUS=Intraoperative Ultrasound, SR=Specimen radiography, OPT=Optical spectroscopy, MP=Margin probe, MCT=Micro-computed tomography, QUADAS2= Quality Assessment of Diagnostic Accuracy Studies 2. SORT= Strength Of Recommendation Taxonomy. Low risk of bias is scored 1, High or unclear risk of bias is scored 2. Q_tot=Total QUADAS 2 score.

Supplementary Table D: Routine usage of histopathological IMA techniques during breast surgery among London Hospitals.

Hospital	Routine usage of histopathological IMA techniques for BCS
St George's Hospital	No
Kingston Hospital	No
Queen Elizabeth Hospital	No
St Thomas' Hospital	No
Lewisham & Greenwich NHS Trust	Nil response
King's College Hospital	No
Royal Marsden Hospital	No
Charing Cross Hospital	No
Princess Royal University Hospital	No
Northwick Park Hospital	No
Hillingdon Hospital	No
University College Hospital	No
Ealing Hospital	No
Royal London Hospital	No
Barnet & Chase Farm Hospitals	Nil response
The Whittington Hospital	No
Queens Hospital	No
Royal Free Hospital	No

Legend: IMA=Intraoperative margin assessment, BCS=Breast conserving surgery.

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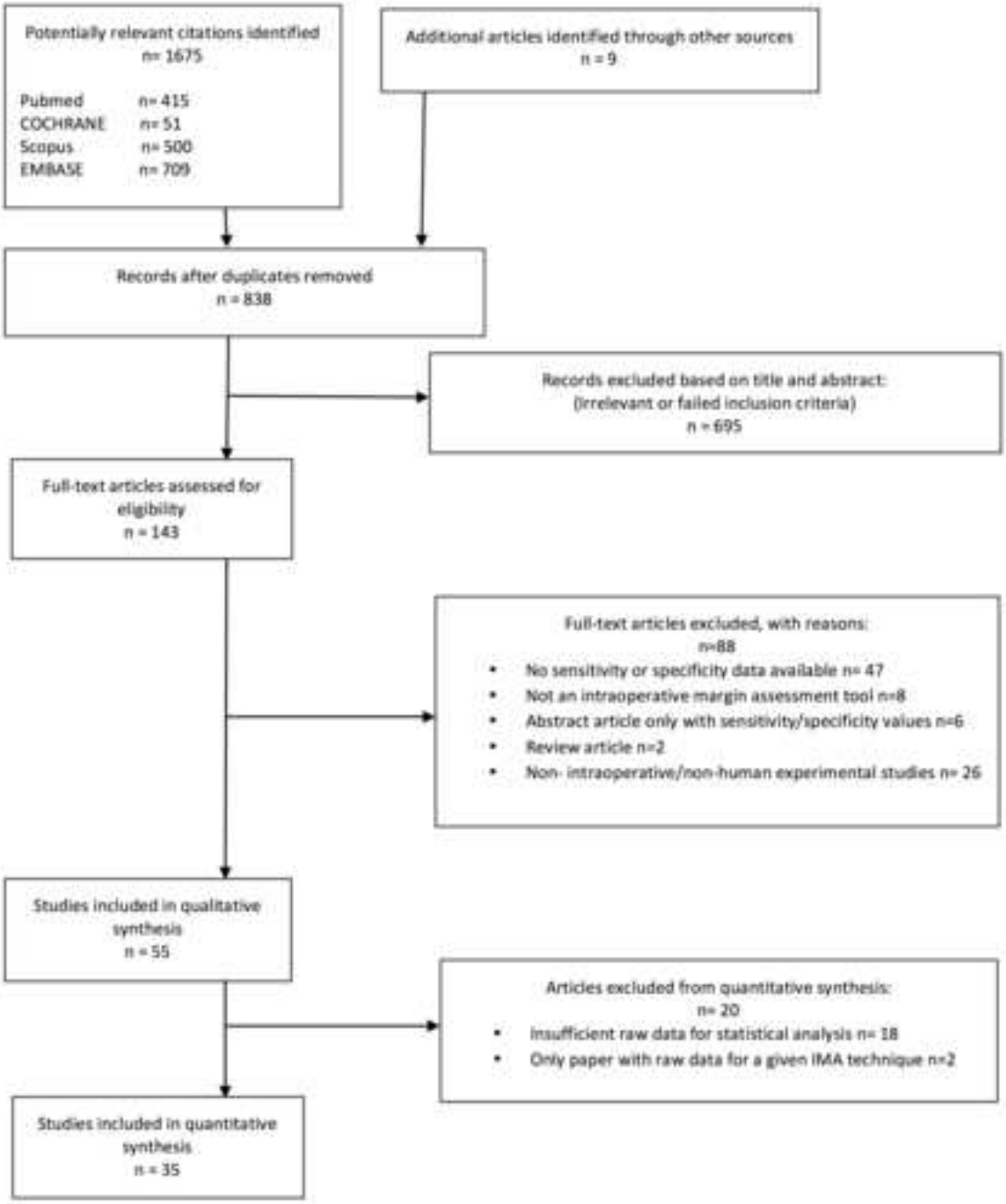

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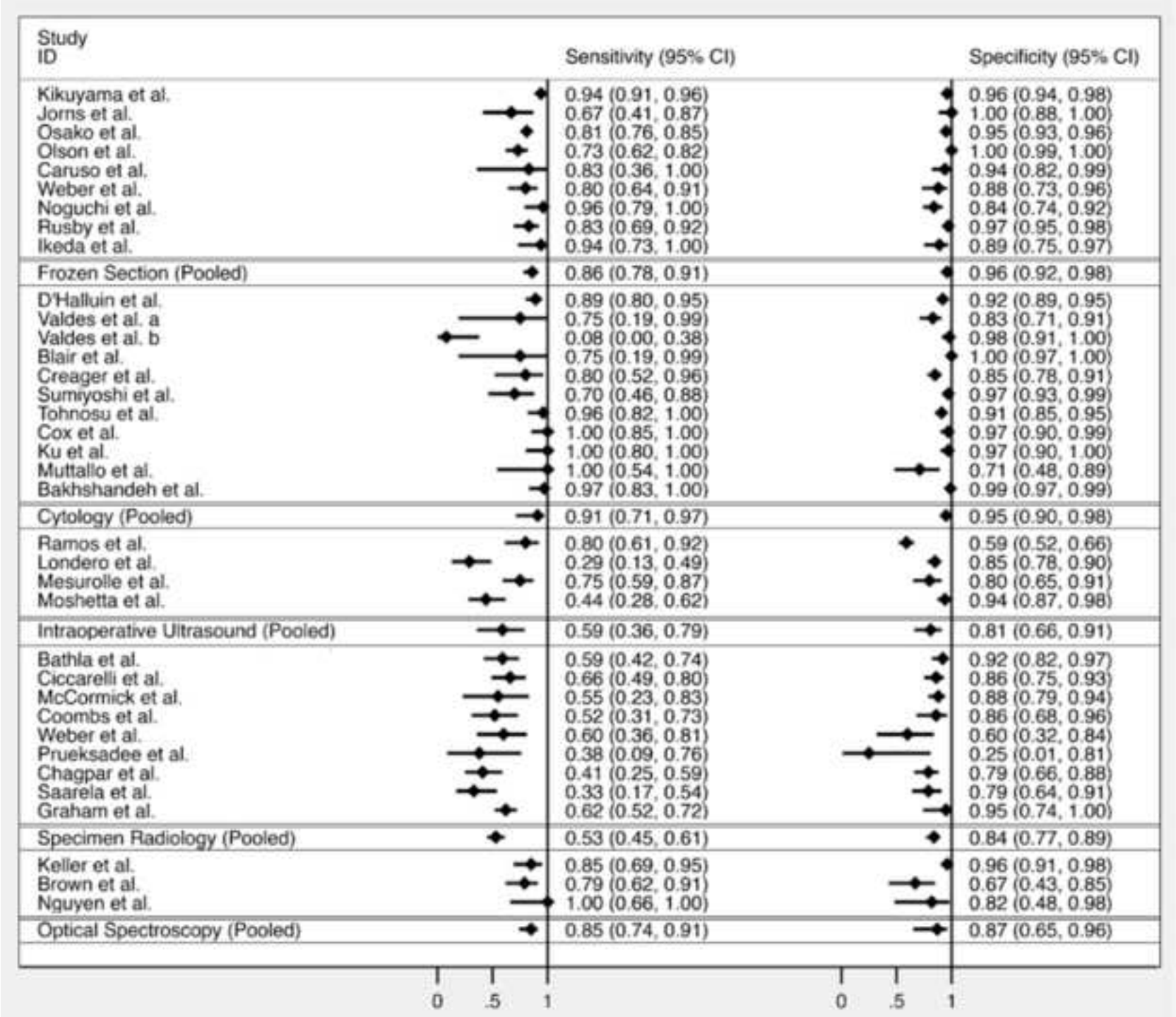

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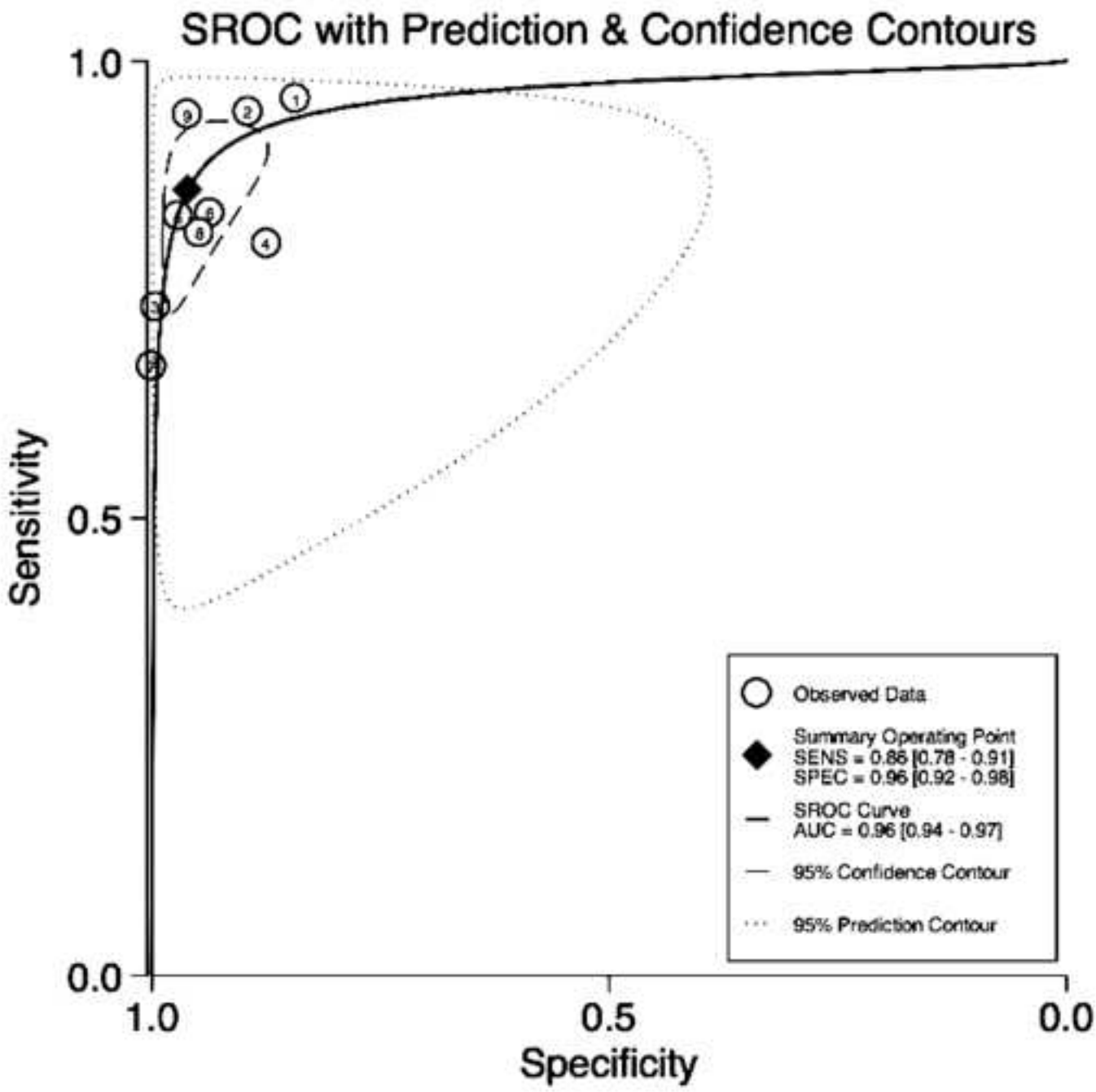

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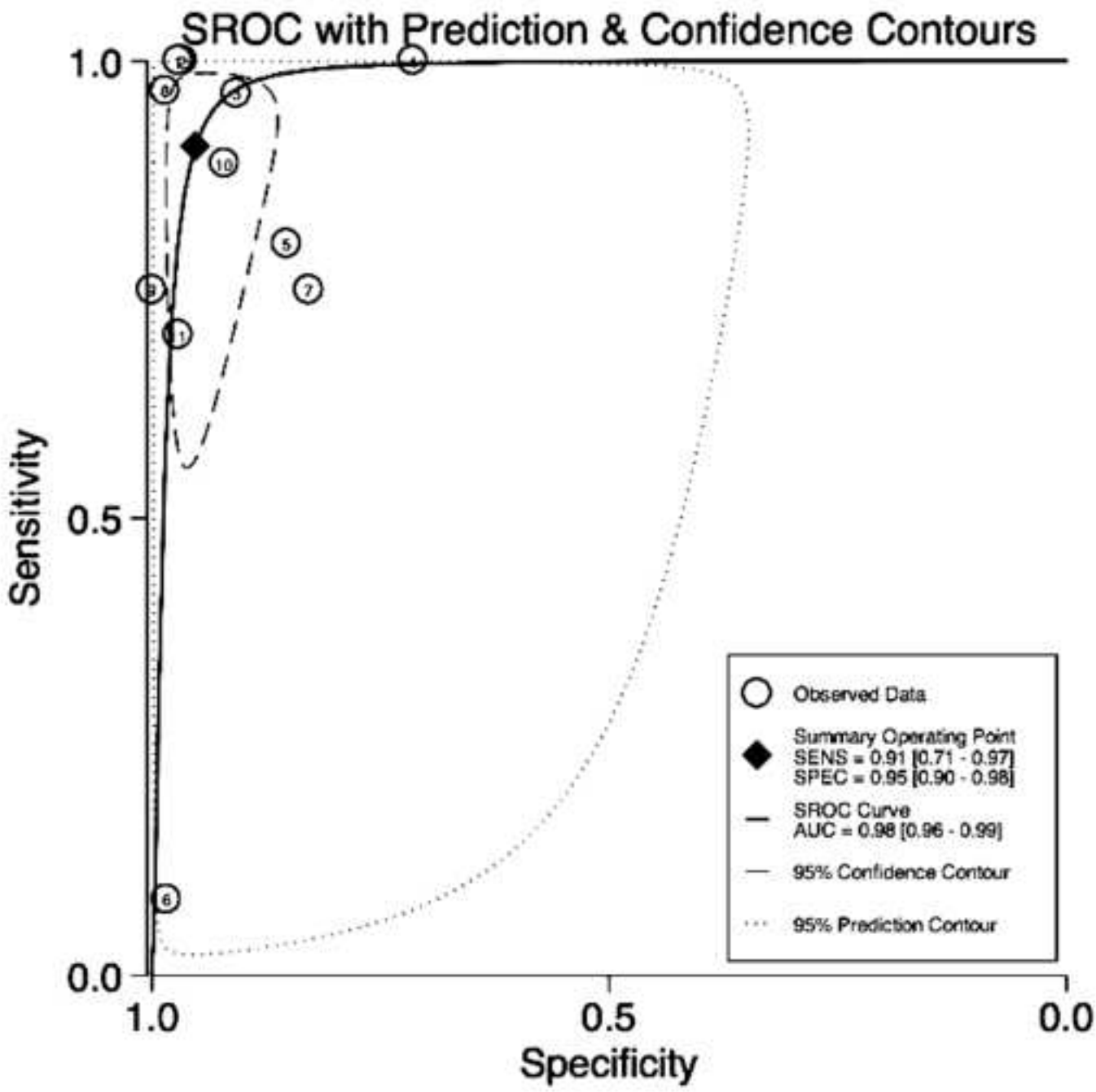

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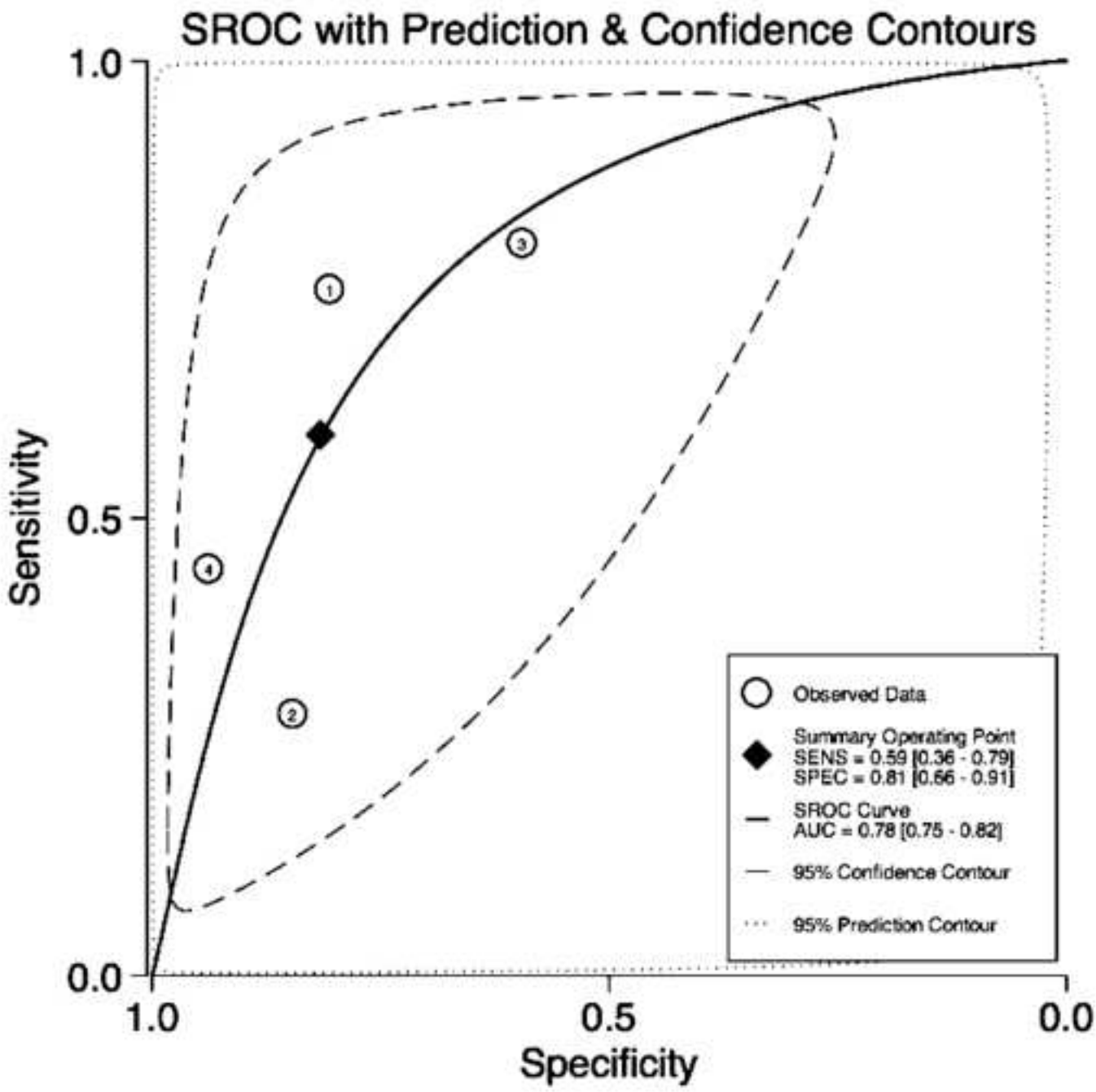

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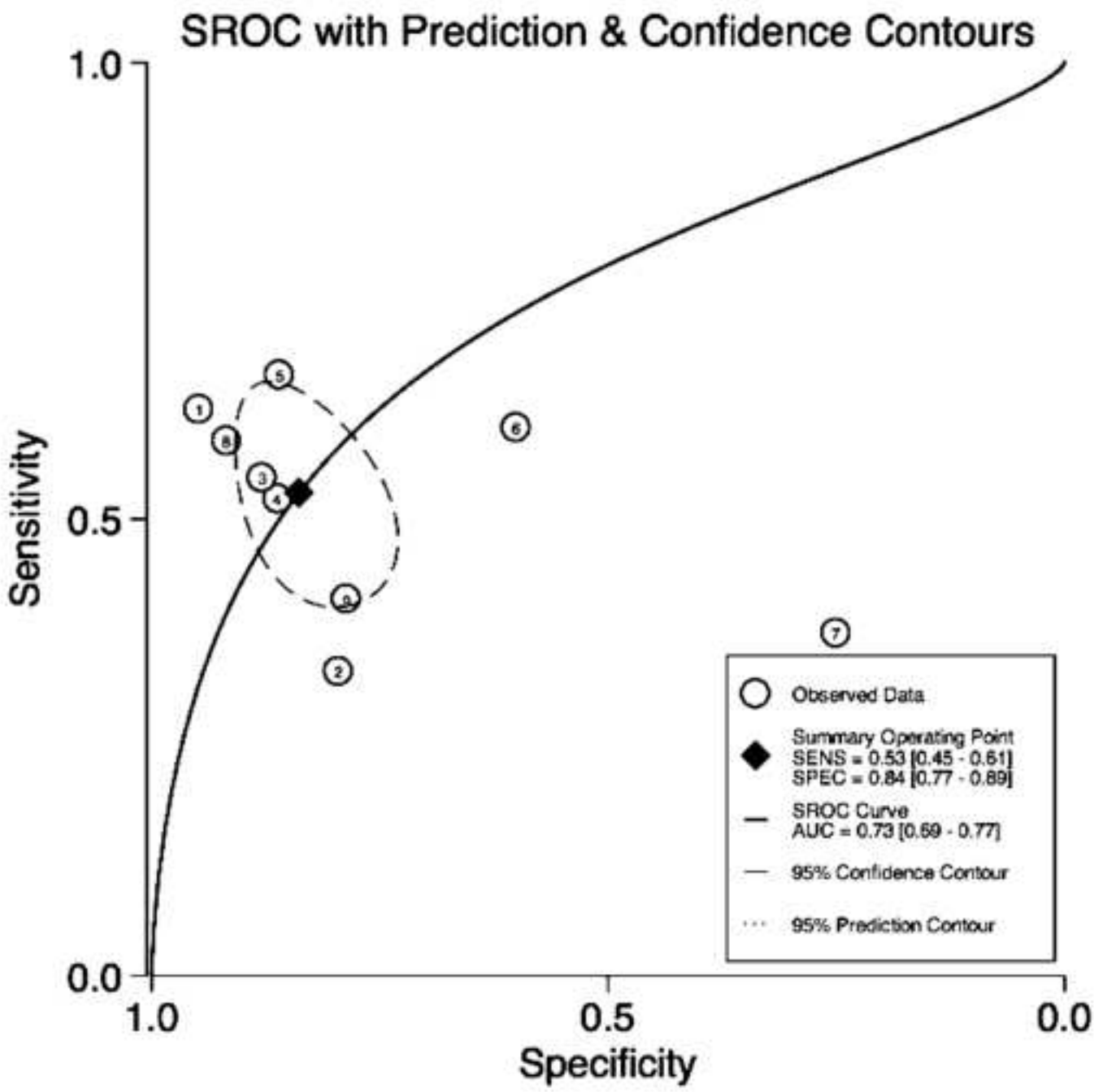

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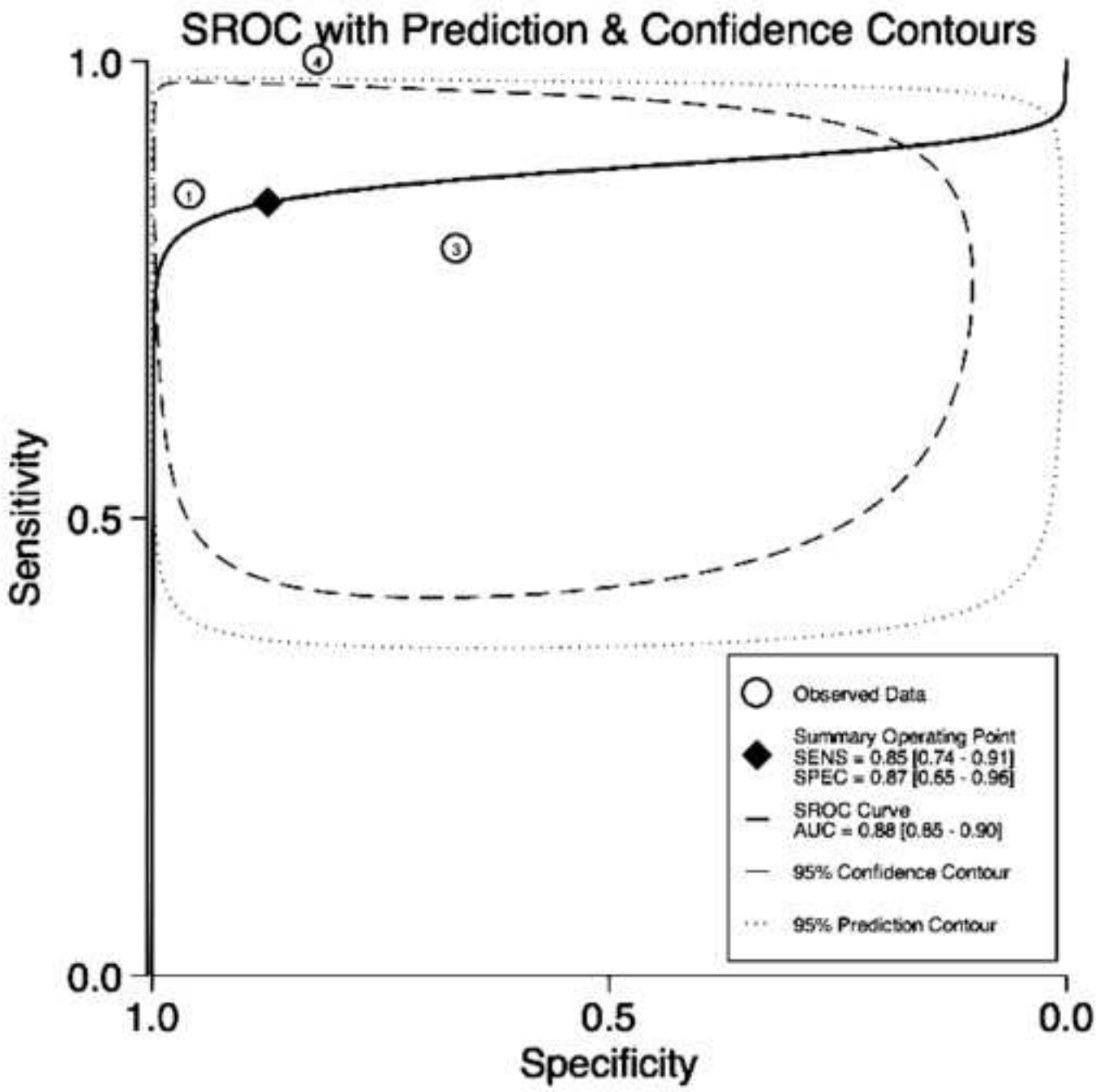

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