

# Can serum autoantibodies be a potential early detection biomarker for breast cancer in women? a diagnostic test accuracy review and meta-analysis

**Thejas Kathrikolly**

Kasturba Medical College Manipal <https://orcid.org/0000-0002-4492-5254>

**Sreekumaran Nair**

JIPMER: Jawaharlal Institute of Postgraduate Medical Education and Research

**Aju Mathew**

MOSC Medical College Hospital: Malankara Orthodox Syrian Church Medical College and Hospital

**Prakash Saxena**

Kasturba Medical College Mangalore

**Suma Nair (✉ [suma.nair@manipal.edu](mailto:suma.nair@manipal.edu))**

Jawaharlal Institute of Postgraduate Medical Education & Research (JIPMER) <https://orcid.org/0000-0002-1843-075X>

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## Research

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## Abstract

## Background

The increasing incidence of breast cancer necessitates the need to explore alternate screening strategies that circumvent the setbacks of conventional techniques specially among population that report earlier age at diagnosis. Serum autoantibodies is one such potential area of interest. However, their ubiquitous presence across cancer types limits its applicability to any one specific type of cancer. This review was therefore carried out to explore and consolidate available evidence on autoantibodies for early detection of breast cancer and to identify those that demonstrated a higher sensitivity.

## Methods

A diagnostic test accuracy (DTA) review was carried out to ascertain serum autoantibodies that could be used for early detection of breast cancer among women. All relevant articles that investigated the role of autoantibodies in early detection of breast cancer were included for the review. MEDLINE, Scopus, ProQuest, Ovid SP and Cochrane Library were searched extensively for eligible studies. Quality of the included studies was assessed using Quality Assessment of Diagnostic Accuracy Studies (QUADAS) -2 tool. RevMan 5.3 was used for exploratory and MetaDTA 2019 for hierarchical analyses. The review helped identify the most frequently investigated autoantibodies and a meta-analysis further consolidated the findings.

## Results

A total of 53 articles were included for the final analysis that reported over a 100 autoantibodies that were studied for early detection of breast cancer in women. P53, MUC1, HER2, HSP60, P16, Cyclin B1 and c-Myc were the most frequently investigated autoantibodies. Of these P53, MUC1, HER2 and HSP60 exhibited higher summary sensitivity measures. While the individual pooled sensitivity estimates ranged between 10–56%, the panel sensitivity values reported across studies were higher with an estimated range of 60–87%.

## Conclusion

Findings from the review indicate a higher sensitivity for an autoantibody panel in comparison to individual assays. A panel comprising of *P53, MUC1, HER2 and HSP60 autoantibodies* has the potential to be investigated as an early detection biomarker for breast cancer.

## Background

Over the past decade, the scenario of breast cancer burden in India has become a cause for concern with available data suggesting it to be the foremost reason for female cancer deaths in the country [1]. For every two women newly diagnosed with breast cancer in India, one woman dies of it signifying a high mortality to incidence ratio [1]. Findings underscore that certain factors, particularly lack of awareness and poor access to effective screening methods, along with delayed diagnosis are likely significant contributors to the high mortality.

Most breast cancers are amenable to treatment if detected early enough [2]. Screening strategies that aid early detection is the key to reduce mortality rates. However, in the Indian setting where the median age of diagnosis of breast cancer is nearly a decade younger when compared to the western population, existing strategies such as mammography is seldom effective [1]. Clearly, there is a need to devise and implement alternate screening strategies that could circumvent the setbacks of conventional screening techniques. One such promising approach is the field of cancer immunology that has brought forth the potential of serum autoantibodies as early detection markers for cancer.

Serum antibody against tumour associated antigens may have a potential as an early detection strategy in breast cancer as it is detected in circulation even before the clinical manifestations of the disease. In addition, it is produced in considerable amount and remain in circulation for a longer period due to limited clearance [3]. Nonetheless, universal presence of certain auto antibodies across different types of cancers has limited its applicability in early detection of any one specific cancer.

This systematic review was carried out with an aim to ascertain serum autoantibodies that could be used for early detection of breast cancer among women. Since research on autoantibodies for early detection has consistently advocated a panel or a multiplex of autoantibodies rather than one autoantibody for enhanced sensitivity [2, 4], this systematic review also aimed at identifying an autoantibody multiplex comprising of the most sensitive autoantibodies.

## Materials And Methods

We conducted a diagnostic test accuracy (DTA) review to identify specific autoantibodies and their role in early detection of breast cancer. We had two primary objectives for this review - (a) To compile available evidence on serum autoantibodies used for early detection of breast cancer and (b) to identify autoantibodies that have demonstrated a higher sensitivity in detecting early breast cancer.

The protocol was developed using the PRISMA-P (Preferred Reporting Items for Systematic review and Meta-Analysis Protocols) checklist [5]. We complied with all the 17 recommended items in the checklist. A framework of all the steps was put in place for easy reference and the review was carried out over a period of one year.

## Criteria for considering studies for this review

### Inclusion criteria

Diagnostic test studies using serum autoantibodies for breast cancer diagnosis in women of any age group. ELISA (Enzyme- Linked Immunosorbent Assay) as the preferred method of analysis either standalone or in combination with other techniques such as phage display, bio-panning, 2D electrophoresis (2DE), Western Blot (WB), proteomic analysis, Mass Spectrophotometry (MS) etc.

#### Exclusion criteria

Studies were excluded if (a) they were animal studies (b) the sample used for analysis was not blood (c) samples were analysed post-surgery or post-therapy and (d) autoantibody detection was not applied for early detection/ diagnosis.

### Search Criteria

A search strategy was conceived using a combination of Medical Subject Headings (MeSH) and controlled vocabulary to identify peer-reviewed articles on our topic of interest. Pubmed /MEDLINE, Scopus, Proquest, Ovid SP and Cochrane Library were searched for relevant articles. Key words used were 'breast', 'breast cancer', 'breast carcinoma', 'tumour associated antigens', 'antibodies', 'serum', 'biomarker', 'blood', 'screening', 'detection', 'early detection' and 'diagnosis'. MeSH terms for the keywords such as 'breast neoplasm', 'neoplasm antigens', 'autoantibodies', 'carcinogen markers' and 'cancer screening' were also utilised for expanding the search. These were combined with appropriate Boolean operators so as to generate relevant results. As the use of special filters like 'diagnostic study', 'sensitivity' and specificity' for retrieval of diagnostic test studies may cause to overlook relevant studies, these were avoided [6, 7]. The search was updated till December 2020.

### Study Selection and Data Extraction

Studies were initially screened for eligibility by their titles and abstracts. Full text of the eligible articles was retrieved and reviewed prior to including them in the final analysis. The process of screening each potentially relevant study for inclusion in the review was carried out independently by two authors Suma Nair (SN) and Thejas Kathrikolly (TK) using the eligibility form based on the inclusion criteria. We excluded studies that did not meet the eligibility criteria and listed the reasons for exclusion in the 'Characteristics of excluded studies' table (Additional file 1). Data from the selected studies were extracted using a data extraction form (Additional file 2). Disagreements were resolved through discussion with other co reviewers Sreekumaran Nair (SNN), Prakash Saxena (PU) and Aju Mathew (AM).

### Quality Assessment of included studies

Full text articles of the selected studies were assessed for quality with the help of a tailored Quality Assessment of Diagnostic Accuracy Studies (QUADAS) -2 tool [6, 8] (Additional file 3).

### Data Analysis

Primary exploratory analyses were carried out with RevMan 5.3 [9]. This included quality assessment summary of eligible studies, producing paired forest plots of sensitivity and specificity for autoantibodies and summary receiver operating characteristic (SROC) curves. We carried out meta-analysis for the

common autoantibodies - P53, MUC1, HER2, HSP60, Cyclin B1, c-Myc and P16. Since heterogeneity is inherent in DTA studies, hierarchical models were used for analysis viz., HSROC (Hierarchical Summary Operating Characteristic) curve to estimate pooled sensitivity and specificity. MetaDiSC version 1.4. and MetaDTA 2019, were used for the analysis [10].

## Results

A total of 10100 published articles were retrieved in all. After excluding the duplicates, 7213 articles were screened by their titles and abstracts to look for their relevance for inclusion. Of these, 7056 articles were rejected as they did not concur with our inclusion criteria. The remaining 157 articles were selected for full text retrieval to check their eligibility to be included for the final analysis. Of these, articles that did not focus on early- stage patients or autoantibodies for early detection were excluded and, in the end, we had 53 articles that met the inclusion criteria of the review and were included for the final analysis. The PRISMA chart illustrating the search results is shown in Fig. 1.

Figure 1 PRISMA chart with the search results

## Characteristics of Included Studies

Details of the included studies are presented in Tables S1 and S2 (Additional file 4). Majority of the studies were diagnostic studies with a reversed flow design. Details about study design and population characteristics, type of sample used for analysis, detection techniques and autoantibodies investigated are tabulated in Table S1. Description of index tests, reference tests, definition of threshold and diagnostic measures reported in each study are depicted in *Table S2*.

Study characteristics were further entered in RevMan 5.3 to generate a risk of bias summary table representing the quality of studies as illustrated in Fig. 2.

Figure 2 Risk of bias summary table (Methodological quality summary): review authors' judgements about each methodological quality item for each included study

Studies were assessed based on four domains of the QUADAS-2 tool and showed low risk of bias and applicability concerns.

## Population included in the studies

Majority of the studies had recruited women barring three that reported male participants [19, 52, 55]. Five studies reported the role of autoantibodies in other types of cancers in addition to breast cancer [12, 19, 20, 45, 53]. Almost all the diagnostic test studies used the case control study design except the one by Regele et al. that was cross sectional in nature [50].

## Tests, sample and methods of analysis used in the studies

There was considerable variation among the studies with respect to index tests, reference tests, samples and methods used for investigating autoantibodies. Biopsy proven diagnosis and sometimes radiological and clinical examination were used to confirm the breast cancer status of participants, while routine health check and follow-up, detailed medical history, routine mammograms and radiological examination were the procedures used to group the participants as healthy as shown in (Table S2, Additional file 4).

Although our pre-defined inclusion criteria specified serum as the preferred sample for detecting autoantibodies, we also included studies that used plasma for analysis, as they contained significant information about autoantibodies for early detection [15, 18, 24, 37, 44, 54, 57]. One study by Yi et al. had used urine as a sample source of breast cancer tumour proteins in addition to serum [31].

Majority of the studies used ELISA as the method of analysis. However, many studies had combined ELISA with Western blotting, immunohistochemistry, proteomics and microarrays to gain better sensitivity at autoantibody detection as presented in (Table S1 Additional file 4).

## **Autoantibodies**

A total of 100 autoantibodies (Additional file 5) were investigated for their role in early detection and diagnosis of breast cancer. Most frequently studied autoantibodies were P53 [2, 12–14, 28, 32, 34, 36, 41, 43, 48–50, 54, 55, 58], MUC1 [2, 4, 16, 21, 51, 61], HER2 [2, 34, 43], HSP60 [4, 22, 29], Cyclin B1 [39, 43, 49, 58], c-Myc [2, 39, 41, 48, 58] and P16 [18, 38, 41, 49, 58]. The diagnostic measures of individual autoantibodies were investigated by all studies. Ten studies also reported the measures for autoantibody panels [2, 4, 17, 34, 39, 41, 48, 54, 58, 61].

## **Threshold**

Studies adopted three common measures of threshold to define a positive value for autoantibodies, an absorbance value greater than the mean + 2, +3 or + 4 standard deviations (SDs) of the normal cohort (Additional file 6). Some studies reported a pre-defined threshold specific to the method of analysis [12, 13, 15, 21, 23, 28, 32, 45, 55, 57] while some others did not report any threshold for a positive cut-off [4, 14, 17–19, 26, 27, 29–31, 35, 37, 39, 41, 44, 46, 50, 59, 61].

## **Reporting of diagnostic measures**

Studies have reported the applicability of autoantibodies for early detection and diagnosis in the form of sensitivity, specificity and other diagnostic measures like area under the curve (AUC) values. Efforts were made to extrapolate the percentage of autoantibody positivity in patient and healthy groups to derive sensitivity and specificity values with the help of 2 X 2 tables (Additional file 7). Three studies reported measures in odds ratio [24, 27, 36]. Results from studies that investigated individual autoantibodies in addition to autoantibody panels suggested that although the sensitivity values were less for individual autoantibodies, such autoantibodies when combined in a panel demonstrated higher sensitivity values.

Most frequently investigated autoantibodies were identified and their sensitivity measures when used as a stand-alone or when included in a multiplex were compared. These autoantibodies were P53, MUC1,

HER2, HSP60, Cyclin B1, c-Myc and P16. We found 16 studies that investigated the P53 autoantibody and another eight that studied MUC1 autoantibodies in early detection of breast cancer. C-Myc and P16 were investigated by five studies each, while four studies investigated HER2, HSP60 and Cyclin B1 autoantibodies. Individual and panel values of these autoantibodies are illustrated in Table 1 that clearly illustrate a higher sensitivity for the panel.

## Meta- analysis

Coupled forest plots of sensitivity and specificity for the most frequently investigated autoantibodies, P53, MUC1, HER2, HSP60, Cyclin B, c-Myc and P16 were developed using RevMan 5.3 and are

Table 1  
Comparison of individual and panel sensitivity values

<i>Autoantibody</i>	<i>Autoantibody panel (Sensitivity %)</i>	<i>Stand-alone Sensitivity %</i>
P53	<i>P53, Cyclin B1, p16, p62, 14-3-3ξ (78)</i>	3.4–47
	<i>P53, HSP60, FKBP52, PRDX2, PP1A, MUC1, GAL3, PAK2, CCNB1, PHB2, RACK1, RUVBL1, HER2 (70–77)</i>	
	<i>P53, c-Myc, NY-ESO-1, BRCA-1, BRCA-2, HER-2, MUC1 (64)</i>	
	<i>P53, c-Myc, survivin, cyclin, B1, cyclin D1, p62, p12, CDK2 (61)</i>	
MUC1	<i>MUC1, LGALS3, Phb2, GK2, CA 15 – 3 (87)</i>	20–50
	<i>MUC1, ANGPTL4, DKK1, EPHA2, GAL1, HER-2, IGFBP2, LAMC2, SPON2, CST2, SPINT2, SSR2 (73)</i>	
	<i>MUC1, HSP60, FKBP52, PRDX2, PP1A, P53, GAL3, PAK2, CCNB1, PHB2, RACK1, RUVBL1, HER-2 (70–77)</i>	
	<i>MUC1, P53, c-myc, NY-ESO-1, BRCA-1, BRCA-2, HER-2 (64)</i>	
HER2	<i>HER-2, MUC1, ANGPTL4, DKK1, EPHA2, GAL1, IGFBP2, LAMC2, SPON2, CST2, SPINT2, SSR2 (73)</i>	13–17
	<i>HER2, HSP60, FKBP52, PRDX2, PP1A, MUC1, GAL3, P53, PAK2, CCNB1, PHB2, RACK1, RUVBL1 (70–77)</i>	
	<i>HER-2, c-Myc, NY-ESO-1, BRCA-1, BRCA-2, P53, MUC1 (64)</i>	
	<i>HSP60, FKBP52, PRDX2, PP1A, MUC1, GAL3, PAK2, CCNB1, PHB2, RACK1, RUVBL1, HER2, P53 (70–77)</i>	
HSP60	<i>HSP60, MUC1, PPIA, PRDX2, FKBP52 (61)</i>	33–48
	<i>c-Myc, cyclin B1, Imp1, p16, Koc, survivin (67)</i>	
	<i>c-Myc, NY-ESO-1, P53, BRCA-1, BRCA-2, HER-2, MUC1 (64)</i>	
	<i>c-Myc, P53, survivin, cyclin, B1, cyclin D1, p62, p12, CDK2 (61)</i>	
c-Myc	<i>Cyclin B1, P53, p16, p62, 14-3-3ξ (78)</i>	12 -18.4
	<i>Cyclin B1, Imp1, P16, Koc, survivin, c-Myc (67)</i>	
	<i>Cyclin B1, c-Myc, survivin, P53, cyclin D1, p62, p12, CDK2 (61)</i>	
Cyclin B1		
P16	<i>P16, CyclinB1, P53, p62, 14-3-3ξ (78)</i>	12.2–30.3

depicted in Fig. 3. Their corresponding summary ROC (SROC) curves are shown in Additional file 8. This was the preliminary step in assessing heterogeneity of the studies to guide further hierarchical analysis.

<b>Autoantibody</b>	<b>Autoantibody panel (Sensitivity %)</b>	<b>Stand-alone Sensitivity %</b>
	P16, Cyclin B1, Imp1, Koc, survivin, c-Myc (67)	
depicted in Fig. 3. Their corresponding summary ROC (SROC) curves are shown in Additional file 8. This was the preliminary step in assessing heterogeneity of the studies to guide further hierarchical analysis.		

Figure 3 Coupled forest plots of autoantibodies

As observed from the forest plots, there is considerable heterogeneity, which could be attributed to the choice of threshold employed in the studies. In view of this, hierarchical analyses and HSROC curves were used to estimate pooled sensitivity and specificity and these are illustrated in Figs. 4, 5 and 6.

Figure 4 HSROC curve for P53

Figure 5 HSROC curve for MUC1

Figure 6 HSROC curve for c-Myc

Owing to a small number of studies for hierarchical analysis, summary curves were avoided for HER2, HSP60, Cyclin B1 and P16. However, their diagnostic summary estimates were obtained using random effects model with the help of MetaDTA software. Pooled estimates of individual autoantibodies show a higher specificity as illustrated in Table 2. The corresponding Diagnostic Odds Ratio (DOR) show good discriminatory power.

Table 2  
Pooled estimates of autoantibodies

<b>Autoantibody</b>	<b>Sensitivity (95% CI)</b>	<b>Specificity (95% CI)</b>	<b>Diagnostic Odds Ratio (95% CI)</b>
P53	0.184 (0.093–0.332)	0.962 (0.912–0.984)	5.760 (4.029–8.220)
MUC1	0.498 (0.209–0.789)	0.691 (0.257–0.935)	2.215 (0.440–11.143)
HER2	0.453 (0.064–0.910)	0.794 (0.294–0.973)	3.200 (1.700–6.025)
HSP60	0.558 (0.210–0.857)	0.802 (0.316–0.973)	5.122 (2.145–12.229)
P16	0.216 (0.142–0.314)	0.941 (0.878–0.972)	4.373 (2.329–8.209)
Cyclin B1	0.149 (0.105–0.207)	0.977 (0.946–0.990)	7.455 (2.816–19.733)
c-Myc	0.103 (0.060–0.172)	0.966 (0.919–0.986)	3.230 (1.071–9.740)

## Sensitivity analysis

Sensitivity analysis was carried out with respect to P53, MUC1 and HSP60 as there were differences in certain measures of analysis such as threshold, method and sample used (Table 3).

Table 3  
Sensitivity analysis of P53, MUC1 and HSP60 autoantibodies

<b>Variable</b>		<b>Autoantibody</b>	<b>Sensitivity (95% CI)</b>	<b>Specificity (95% CI)</b>
Threshold	<i>Irrespective of the threshold defined (n = 13)</i>	P53	0.184 (0.093–0.332)	0.962 (0.912–0.984)
	<i>Investigator defined - mean + 2SD (n = 6)</i>		0.227 (0.059–0.580)	0.954 (0.794–0.991)
	<i>Irrespective of the threshold defined (n = 5)</i>	MUC1	0.498 (0.209–0.789)	0.691 (0.257–0.935)
	<i>Investigator defined - mean + 2SD (n = 4)</i>		0.548 (0.196–0.858)	0.615 (0.149–0.936)
Index test	<i>ELISA + immunohistochemistry (n = 4)</i>	HSP60	0.558 (0.210–0.857)	0.802 (0.316–0.973)
	<i>ELISA (n = 3)</i>		0.197 (0.097–0.358)	0.979 (0.965–0.988)

In the case of P53 and MUC1, there was a marginal difference in the overall sensitivity measures based on if the threshold used for analysis was investigator defined or manufacturer defined. HSP60 showed higher sensitivity when ELISA was combined with immunohistochemistry. On the other hand, type of sample used for analysis (plasma and serum) did not reflect any difference in overall sensitivity measures between the groups.

## Discussion

### Summary of evidence

Ongoing research in the field of cancer immunology has proposed the utility of serum autoantibodies for early detection of breast cancer [2, 62]. Due to their inherent properties of immune response to tumour associated antigens, these serum autoantibodies have been investigated for their role as biomarkers in cancer [63, 64].

Few of the most investigated autoantibodies are P53, NY-ESO-1, HER2/neu, MUC1, heat shock proteins and cyclins [63]. Nevertheless, information is sparse with respect to autoantibodies that are specific to early detection of breast cancer. This was the basis to conduct this systematic review, an initial step in identifying serum autoantibodies as biomarkers for early detection of breast cancer. The aim of this review was to select autoantibodies based on their sensitivity and specificity measures and thus, we planned a DTA review.

Considering its applicability in early detection and hence the need for considerable sensitivity, autoantibody studies have summarily concluded that a panel of autoantibodies demonstrates higher

sensitivity than any one individual autoantibody. An example of this is a study by Liu et al. on sera from breast cancer cases and healthy controls wherein a gradual increase in sensitivity was noted from 18.4% for one autoantibody to 67.3% for a panel of six autoantibodies [39]. A similar study by Ye et al. proposed a panel of ten autoantibodies having a sensitivity of 61% in comparison to only 22% for one autoantibody [58]. These findings suggest a clear potential of autoantibody panels in early detection of the disease and thus through this review, we also aimed at selecting a panel of autoantibodies instead of any one individual autoantibody.

This review reports P53, HER2, MUC1, HSP60, P16, Cyclin B1 and c-Myc as the most frequently investigated autoantibodies. A similar review on autoantibodies in breast cancer by Xia et al. reported P53, HER2, MUC1 and Cyclin B1 as autoantibodies with a potential for early detection of breast cancer [65]. Our results further reiterate the role of tumour suppressor and oncogenic proteins such as P53 and c-Myc, respectively in breast cancer. Over-expression of transmembrane mucin proteins such as MUC1 and growth factor like HER2 in breast cancer are sufficiently documented and our results on them support such findings. Of late, Cyclin B1 that is primarily involved in cell-cycle regulation has been investigated for its over-expression in breast and cervix cancers.

A review by Tang et al. who focused on autoantibody signatures in cancer, highlighted c-Myc, P53, NY-ESO-1, HER2, MUC1 and annexin-1 among other autoantibodies as those investigated for early detection of lung cancer [66]. Such findings on autoantibodies in different types of cancer necessitates focused analytical study designs to ascertain autoantibodies that are specific to a particular type of cancer.

We observed that pooled diagnostic odds ratio (DOR) of all the autoantibodies was greater than one implying good discriminating capacity of these autoantibodies. However, the corresponding beta parameter derived from the HSROC model was not equal to zero, suggesting high heterogeneity and therefore the DOR values must be interpreted with caution. Autoantibodies that were most frequently investigated and showed relatively higher pooled sensitivity values namely, HSP60 (55.8 %), HER2 (45.3%), MUC1 (49.8%) and P53 (18.4%) were considered for inclusion into a panel for further validation.

## Limitations

Based on the applicability of the review question, we tailored QUADAS-2. On assessment, we observed that most studies were unclear in the domains of 'reference standard' and 'flow and timing'. Although the applicability concern in these domains was 'low', this brings to light the shortcomings in reporting of diagnostic studies. This can be attributed to the observation that QUADAS-2 has been designed for diagnostic studies that are cross-sectional in nature. However, the studies in this review have followed a 'case-control' design which report elevated values of diagnostic measures.

As heterogeneity is the norm in DTA reviews, DTA experts recommend the use of hierarchical models for meta-analysis and similar other papers on autoantibodies for early detection have adopted this based on their respective review queries [65–68]. Although we used MetaDisc for analysis, considering certain

issues with its ongoing software update, we also used the recently developed online application, MetaDTA [10].

## Conclusion

Findings from the review indicate a higher sensitivity for an autoantibody panel in comparison to individual assays. Based on the higher pooled sensitivity values *P53*, *MUC1*, *HER2* and *HSP60* have the potential to be investigated as an early detection biomarker panel for breast cancer. However, as the findings of this review resulted from a heterogenous group of studies especially in terms of population and patient groups, their generalizability for clinical application must be exercised with caution.

## Abbreviations

DTA: Diagnostic Test Accuracy; PRISMA-P - Preferred Reporting Items for Systematic review and Meta-Analysis Protocols; MeSH - Medical Subject Headings; ELISA - Enzyme- Linked Immunosorbent Assay; 2DE-2 Dimension Electrophoresis; WB - Western Blot; MS - Mass Spectrophotometry; QUADAS-2 - ; Quality Assessment of Diagnostic Accuracy Studies-2; ROC curve–Receiver Operating Characteristic curve; AUC–Area under the ROC curve; SROC–Summary ROC; HSROC - Hierarchical Summary Operating Characteristic; SD - Standard Deviation; DOR–Diagnostic Odds Ratio

## Declarations

### *1. Ethics approval and consent to participate*

Not applicable

### *b. Consent for publication*

Not applicable

### *c. Availability of data and materials*

The datasets used and/or analysed during the current study are available from the corresponding author on request.

### *d. Competing interests*

The authors declare that they have no competing interests

### *e. Funding*

There was no external funding for this study. It was carried out as a part of the preliminary research work of an approved thesis on breast cancer and early detection among rural women.

#### *f. Authors' contributions*

SN and TK co-planned the preliminary version of the protocol, conducted the search and screened the studies. They also independently extracted data and assessed quality of included studies with inputs from AM and PU. TK, SN and SNN analysed the data and wrote the initial draft of the review. Final manuscript was scrutinised and approved by all authors.

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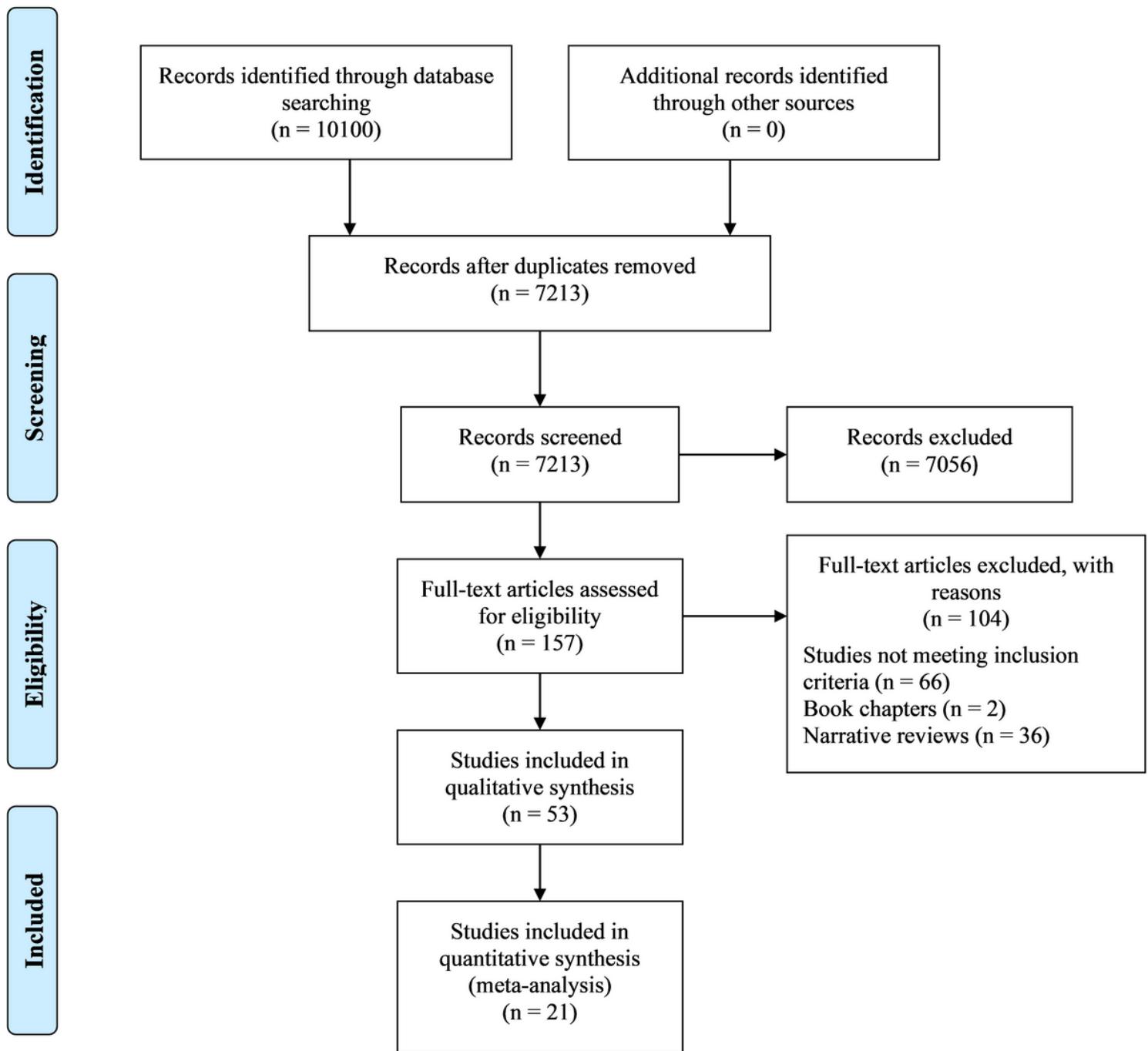
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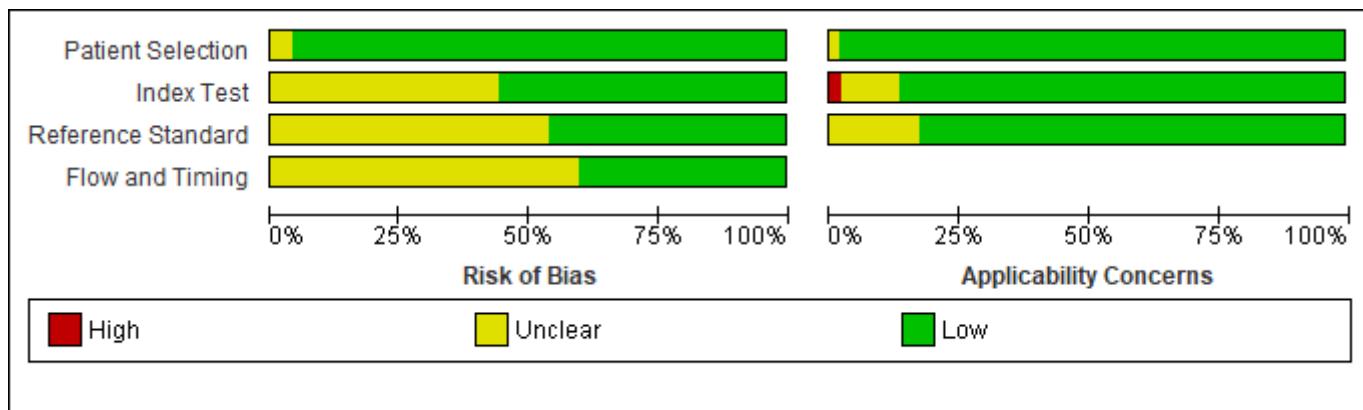
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## Figures



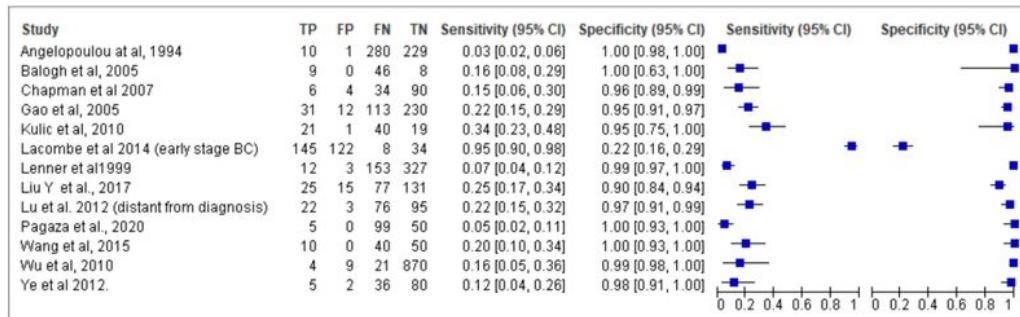
**Figure 1**

PRISMA chart with the search results

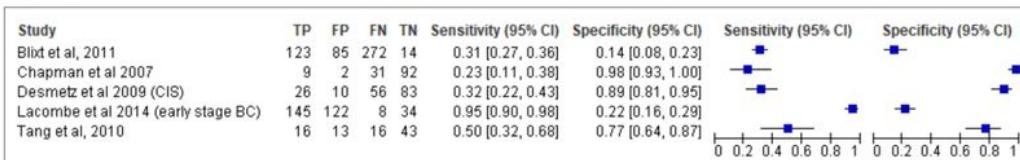


**Figure 2**

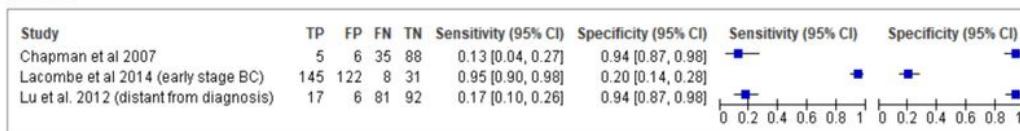
Risk of bias summary table (Methodological quality summary): review authors' judgements about each methodological quality item for each included study



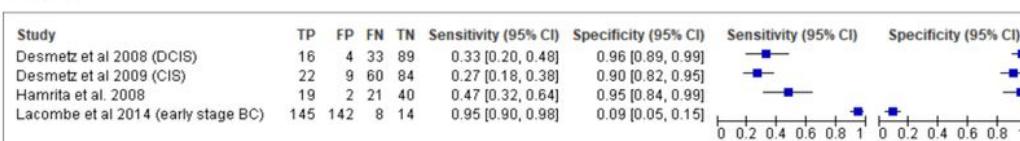
b. MUC1



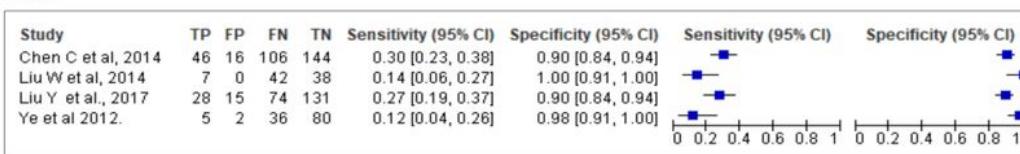
c. HER2



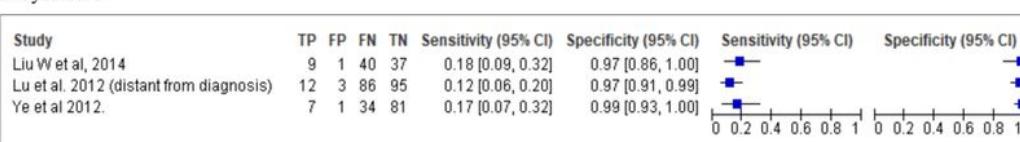
d. HSP60



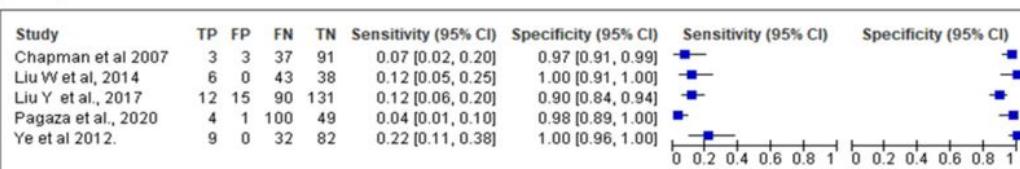
e. P16



f. Cyclin B1

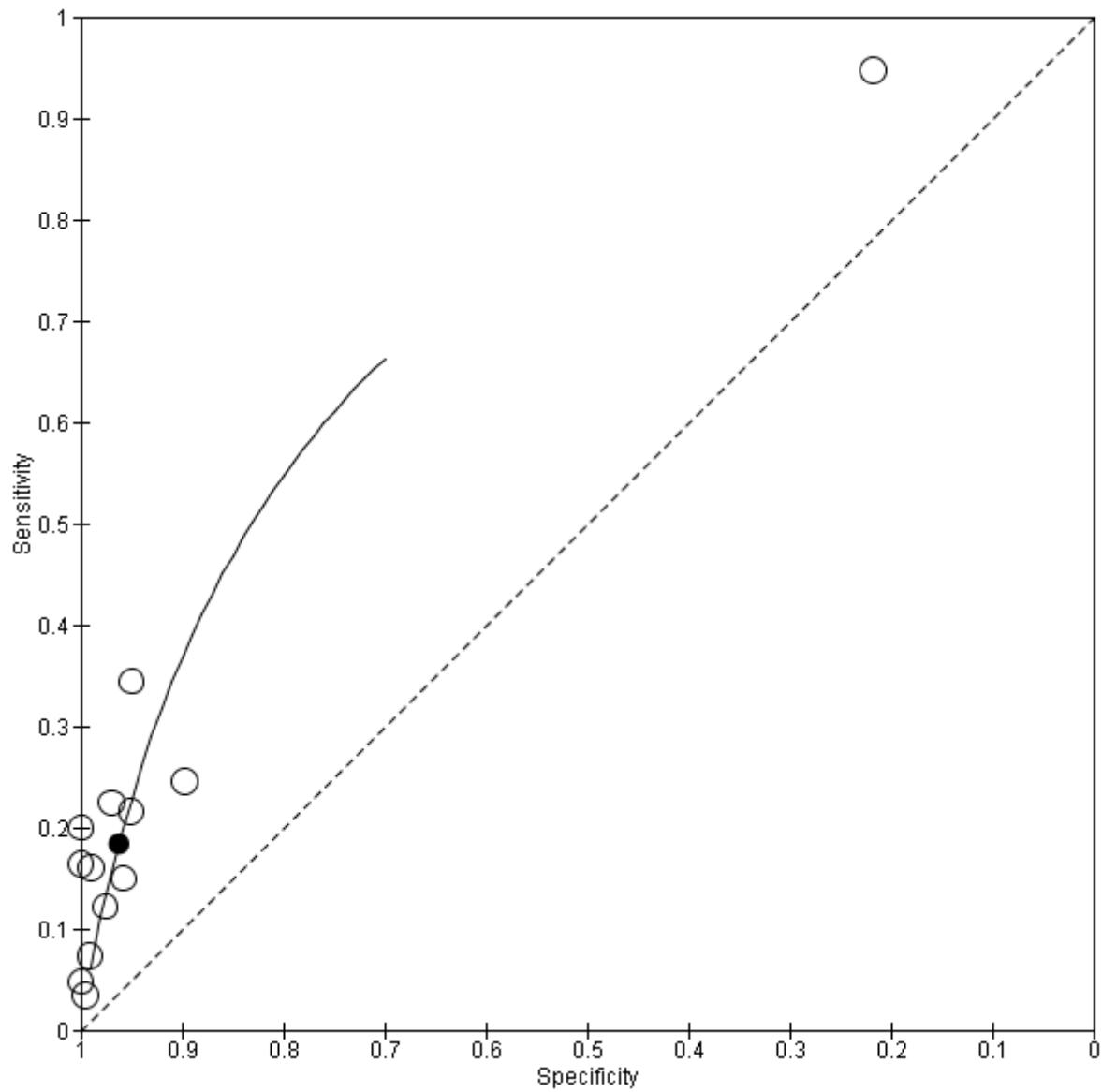


g. c-Myc



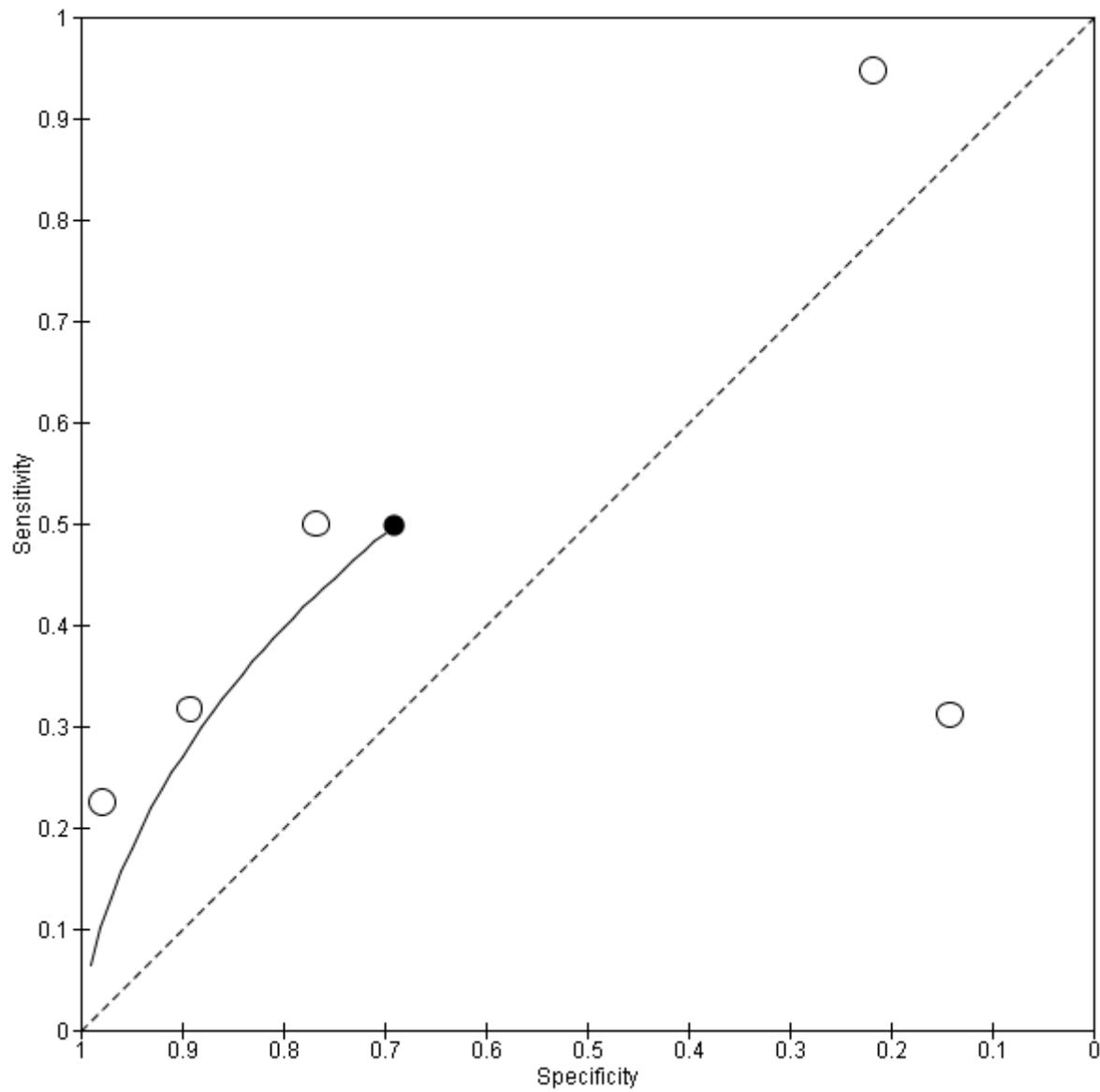
**Figure 3**

Coupled forest plots of autoantibodies



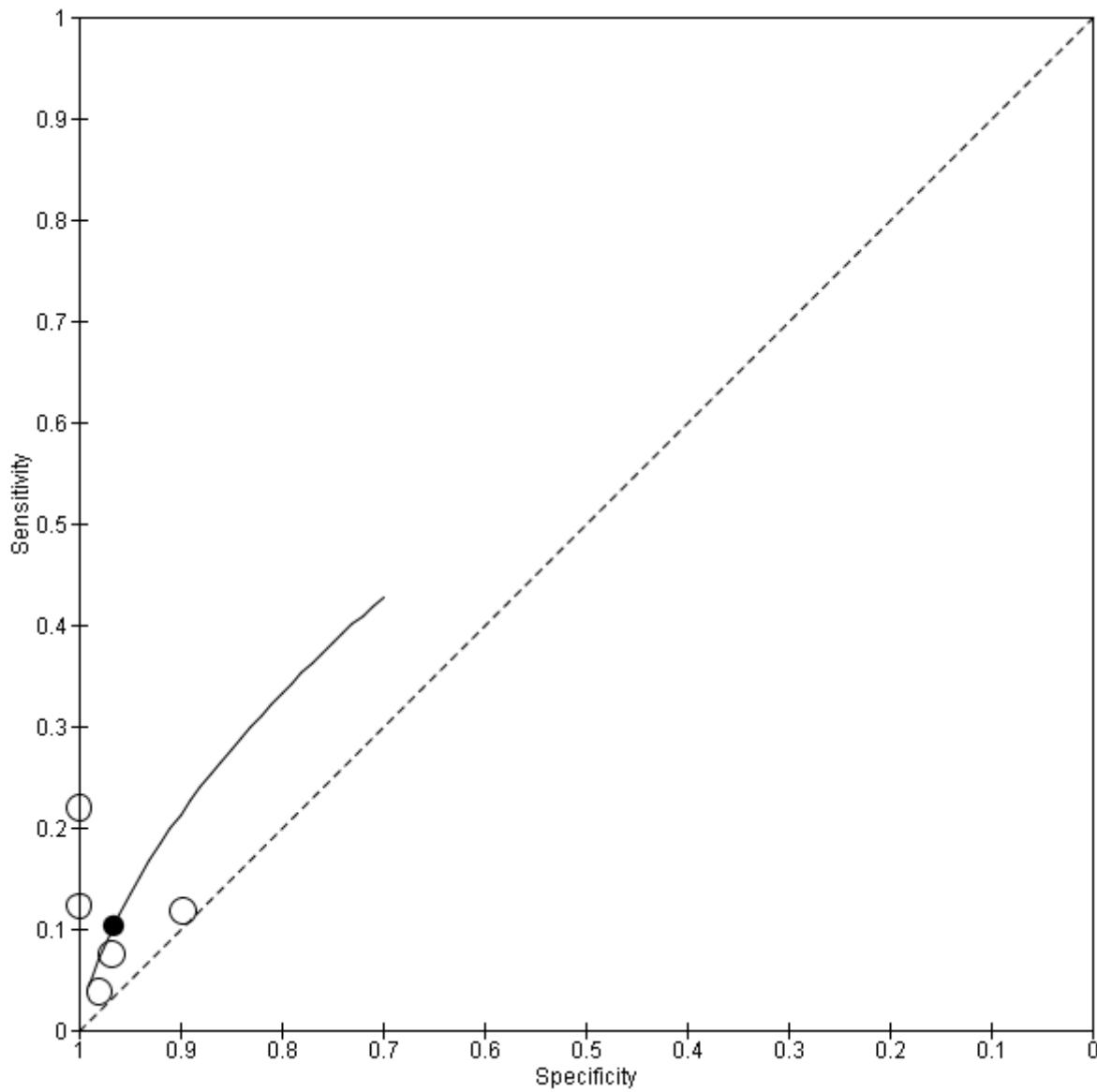
## Figure 4

## HSROC curve for P53



**Figure 5**

HSROC curve for MUC1



**Figure 6**

HSROC curve for c-Myc

## Supplementary Files

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