Cryptococcal antigen in serum and cerebrospinal fluid for detecting cryptococcal meningitis in adults living with HIV: systematic review and meta-analysis of diagnostic test accuracy studies

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Summary:

In patients with symptoms suspicious of HIV-associated cryptococcal meningitis (CM), a positive serum CrAg is highly presumptive of culture confirmed CM and a positive cerebrospinal fluid CrAg is diagnostic of first episode of CM

ABSTRACT

Cryptococcal antigen (CrAg) detection could direct timely initiation of antifungal therapy. We searched MEDLINE and EMBASE for studies where CrAg detection in serum/cerebrospinal fluid (CSF) and CSF fungal culture were done on HIV-positive adults with suspected cryptococcal meningitis (CM). With QUADAS-2, we evaluated risk of bias (RoB) of 11 included studies on 3,600 participants and used random-effects meta-analysis to obtain summary sensitivity and specificity of serum and CSF CrAg as well as agreement between CSF CrAg and CSF culture. Summary sensitivity and specificity of serum CrAg was 99.8% (88.4 – 100) and 95.2% (88.7 – 98), respectively; of CSF CrAg was 98.8% (96.2 – 99.6) and 99.3% (96.7 – 99.9), respectively. Agreement between CSF CrAg and CSF culture was 97% (96 – 99). In HIV-adults with CM symptoms, serum CrAg-negativity may rule out CM, positivity should prompt induction antifungal therapy if lumbar puncture is not feasible. In first episode of CM, CSF CrAg-positivity is diagnostic.

Key words: Cryptococcus, antigen, diagnosis, latex agglutination, lateral flow assay

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BACKGROUND

Cryptococcal meningitis (CM), a life-threatening systemic opportunistic fungal infection, occurs mainly in patients with defective cellular immunity [1, 2]. Consequent to acquired profound immune depression associated with the human immune deficiency virus (HIV) pandemic [3, 4], there has been a surge in the burden of CM, especially in low- and middle-income countries (LMIC) where more than 90% of CM is HIV-related [5]. In 2014, an estimated 223,100 cases of CM occurred, of which 181,100 were fatal, hence accounting for about 15% of all-cause HIV-associated mortality [6].

The reference standard for diagnosing CM is the direct identification of the encapsulated yeast *Cryptococcus* spp. by microscopy of Indian ink-stained preparations of cerebrospinal fluid (CSF) or of yeast colonies cultured from CSF on Sabouraud's dextrose agar [7, 8]. Consequently, confirmation of the diagnosis of CM requires specialised equipment, clinical and technical expertise, which are not always available in most LMICs. More so, patients' acceptance of lumbar puncture (LP) in such settings is not guaranteed [9-12]. Therefore, poor outcome associated with delayed diagnosis emphasizes the need for alternative and reliable methods for timely diagnosis of CM [8].

Cryptococcus spp. is characterised by the presence of a polysaccharide capsule containing cryptococcal antigen (CrAg) surrounding the cell wall. CrAg is shed into biological milieus during infection and constitutes a biomarker of cryptococcosis. Within the last half-century, growing interest in CrAg detection has resulted in the development of commercial CrAg tests, each based on antibody-antigen interactions, using latex agglutination (LA) assays, enzyme-linked immunosorbent assays (ELISA) [13, 14], or more recently, immunochromatographic lateral flow assays (LFA) [15, 16].

In 2011, the United States Food and Drug Administration approved a point of care (POC) immunochromatographic CrAg LFA test [15]. CrAg LFA is affordable (about 2.5 USD per test) [17], detects all cryptococcal serotypes, has no constraints on reactant storage or technical expertise, and provides results within ten minutes [15, 16]. This POC CrAg test is currently recommended by WHO for routine systematic screening for cryptococcosis in the blood of asymptomatic HIV patients presenting with less than 100 CD_4^+ cells/μL, before initiation of antiretroviral therapy (ART) [8].

A recent systematic review and meta-analysis evaluating the clinical utility of routine CrAg screening in asymptomatic HIV positive patients

without symptoms of central nervous system (CNS) disease revealed that up to a third of patients whose serum was CrAg positive had CM [11]. Such an evaluation in patients with symptoms suggestive of CNS disease could greatly improve the timeliness of clinical decision making and hence patient outcomes. This systematic review was designed to determine the diagnostic accuracy of CrAg detection in serum and CSF, as well as the prevalence of culture-confirmed CM in HIV-positive adults with symptoms suggestive of CM.

METHODS

This systematic review was registered at PROSPERO (www.crd.york.ac.uk/PROSPERO) as CRD42017069664, conducted according to Cochrane guidelines [18] and reported following the Preferred Reporting Items for a Systematic Review and Meta-analysis of Diagnostic Test Accuracy Studies statement (Appendix 1) [19].

Eligibility criteria

We included randomised trials, cross-sectional and cohort (prospective and retrospective) studies irrespective of country, region, continent, or level of care (primary, secondary, or tertiary). In these studies, CrAg detection had to be performed in blood or CSF of adults (age >18 years) with confirmed HIV serology, presenting with signs and symptoms suggestive of CM, using either LA, ELISA or LFA. In these patients, the reference standard for establishing the diagnosis of CM was direct yeast identification by microscopy of CSF or of colonies cultured from CSF and stained with India Ink, as defined by the European Organisation for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycosis Study group (EORTC/MSG) Consensus Group [7]. Participants with a positive cryptococcal culture and/or Indian ink stain in CSF were considered as having proven CM; those with a negative cryptococcal culture and negative Indian ink stain were considered as not having CM. Studies published in English, French and Spanish were assessed for inclusion and those published in other languages were considered for translation into English. Case-control studies were excluded due to

their high risk of bias (RoB) [20]. We included published and unpublished studies (e.g., conference abstracts).

Search strategy and study selection

A comprehensive search strategy was developed by a medical information specialist (R.S.) and adapted for MEDLINE (via PubMed) and EMBASE. Medical subject headings and other search words included: *cryptococcal antigen*, *cryptococcal surface polysaccharide*, *cryptococcal meningitis, HIV*, *AIDS*, *LA*, *ELISA*, and *LFA* (see search details in Appendix 2). Searches were run from 1981 (year of first HIV case description) through September 17th, 2019. We did not use methodological filters, to avoid omitting relevant studies [21]. We also searched for included studies on Google Scholar for reports that cited these studies. Conference proceedings of the International Conference on Cryptococcus and Cryptococcosis (ICCC), Conference on Retroviruses and Opportunistic Infections (CROI), and International AIDS Society (IAS) were screened from 2010 onwards.

During the study selection process, two review authors (E.T. and J.J.B.) independently screened citations for eligibility, first by perusing the title and abstracts. Studies irrelevant to the review question were excluded and the full text of relevant articles was retrieved for data extraction. Discrepancies were discussed and arbitrated by a third author (J.F.C.) to achieve consensus.

Data extraction

E.T. and J.J.B. independently extracted data from included studies into a previously piloted data collection form. Studies where more than one type of index test or the same index test on both serum and CSF had been evaluated were subdivided by index test and sample type into diagnostic cohorts (See Appendix 3 for detailed list). In this review, results of index tests and reference standards were considered as binary outcomes (positive or negative). Data on semi-quantitative CrAg titres or CSF fungal colony unit counts were not extracted because they were not relevant to the review question.

Information extracted from each study included study characteristics (first author, year of publication, design, setting), participant characteristics (number of participants, mean or median age, proportion of males, proportion of ART-naïve participants, mean or median CD_4 counts, survival history), CrAg test characteristics (commercial name, test principle [LA, ELISA, or LFA], types of biological samples used (serum, CSF, or both), total number of samples tested, technical specifications for testing [heat inactivation, pronase pre-treatment, and dilutions prior to testing]), reference standard characteristics (commercial name, underlying principle, technical specifications, component tests if a composite reference standard was used), data from 2 x 2 contingency tables (number of true positives, false positives, true negatives, and false negatives, number of indeterminate results when reported), and any other information of relevance (e.g., funding source).

Quality assessment

RoB was assessed using the Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2) tool [22]. This four-domain tool was adapted to suit the review question (Appendix 4). For each of the first three domains (patient selection, index test, and reference standard), the RoB as well as the applicability to the review question were evaluated and classified as either "low risk", "high risk" or "unclear" (if insufficiently reported details). For the fourth domain (flow and timing), only RoB was evaluated.

Statistical analysis and data synthesis

The prevalence of serum and CSF CrAg positivity as well as of culture-confirmed CM among patients with symptoms suggestive of CNS disease was estimated by standard random-effects meta-analysis for proportions using the Freeman-Tukey double arcsine transformation [23]. Then, we fitted bivariate random-effects models to obtain summary estimates of sensitivity and specificity of CrAg in serum and CSF, and their 95% confidence intervals. When the bivariate model could not be fitted because the number of studies was small (less than four), univariate random-effects models were used to obtain separate summary estimates of sensitivity and specificity. Meta-analysis results were

presented by CrAg test (LA, ELISA, or LFA) and sample type (serum, CSF, or both). Random-effects meta-analysis was also used to obtain summary estimates of agreement between CSF CrAg and CSF culture in the study population, i.e., the proportion of tests that gave similar results between CSF CrAg and culture. Heterogeneity was evaluated by inspecting the forest plots and ROC space, and by calculating I^2 statistics (when applicable). We performed meta-regression to investigate sources of heterogeneity across CrAg test (LA, ELISA, LFA) and sample types (serum vs. CSF), by incorporating covariates in the bivariate or univariate model as appropriate. We also performed sensitivity analyses using only studies judged as having "low" RoB. Statistical analysis involved use of Stata 16.0 (Statacorp, Texas, USA).

RESULTS

Results of the search

The electronic search performed on September 17th, 2019 identified 1972 citations (147 duplicates) of which 1794 were excluded based on title and abstract screening (Figure 1). Further assessment of 31 citations resulted in the inclusion of 11 studies [14, 24-33]. Non-electronic searches did not identify any additional study.

Study Characteristics

Studies included for meta-analyses were published between 1990 and 2018 and conducted in 8 countries (including 6 LMICs) on 3,600 adults living with HIV, clinically suspected of having CM (Table 1). The median number of participants per study was 146 (IQR: 99 – 465), and they were predominantly male (71%). When reported, median age and CD_4^+ count were 35.5 years and 27 cells/µL, respectively.

Across the 11 studies, the following commercial CrAg tests were evaluated: Pastorex (Sanofi Diagnostic Pasteur, France), Cryptococcal antigen latex agglutination system (CALAS, Meridian Biosciences, USA), Latex agglutination CrAg (IMMY Diagnostics, Oklahoma, USA), Crypto-Latex

agglutination (Crypto-LA, International Biological Labs, Cranberry, NJ, USA), Cryptococcal latex agglutination (Fumouze, France), CrAg LFA (IMMY Diagnostics, Oklahoma, USA), and StrongStep (Liming Bio, Nanjing, Jiangsu, China). Studies where more than one commercial CrAg tests were evaluated, were subdivided for data extraction for each test: three tests in two studies [14, 29] and two tests in one study [31]. These studies were further subdivided by sample type (serum or CSF) yielding a total of 24 diagnostic cohorts (8 on serum and 16 on CSF; Appendix 3).

In terms of CrAg detection technologies, 7 of 11 (63.6%) studies evaluated LA (613 participants) [14, 24, 25, 30-32], four (36.4%) evaluated LFA (2987 participants) [26-29, 33], and none evaluated ELISA (Table 1). CrAg was assessed in both serum and CSF of the same participants in five studies (1846 participants) [14, 25-28], only on serum, in one study (100 participants) [24], and only in CSF, in five studies (1654 participants) [29-33].

In all 11 studies, CSF fungal culture was the reference standard for confirming CM. Five studies (45.5%) used both culture and direct microscopy of CSF (1259 participants) [24, 25, 29, 31, 32]. However, in four studies (1433 participants) [26, 28, 29, 31], a composite reference standard comprising culture, India Ink staining, CrAg tests, or polymerase chain reaction (PCR) was considered. No study relied solely on India Ink positivity as the reference standard.

Methodological quality of included studies

One study (9%) was deemed at high RoB with respect to the patient selection process [14], two (18.2%) studies on how the index test was performed [14, 33], four studies (36.4%) [26, 28, 29, 31] on the reference standard (because of composite reference standards), and one study (9%) [29] on the flow and timing of tests (Appendix 5). The four studies (36.4%) [26, 28, 29, 31] which used composite reference standards were also judged at high risk of applicability concerns (Appendix 6). Overall, five studies (45.5%) were considered as low RoB [24, 25, 27, 30, 32].

Prevalence of CrAg positivity and culture-confirmed CM

The summary prevalence of serum CrAg in patients presenting with CNS symptoms was 63% (95% CI: 45 – 81, I^2 = 98.7%; Figure 2A). In CSF, the summary prevalence of CrAg was 37% (25 – 48, I^2 = 99.2%; Figure 2B). Across studies, the prevalence of culture-confirmed CM ranged between 6% [33] and 63% [29]. The summary prevalence of culture-confirmed CM was 43% (26 – 59, I^2 = 99.2%; Figure 3).

Diagnostic accuracy of CrAg

In serum, across 8 diagnostic cohorts of 1946 participants [14, 24-28], the sensitivity of CrAg detection ranged from 83 to 100%, and specificity ranged from 72 to 100% (Figure 4A). Summary estimates of sensitivity and specificity of serum CrAg for detecting CM were 99.8% (88.4 – 99.9) and 95.2% (88.8 – 98), respectively.

In CSF, across 16 diagnostic cohorts of 3500 participants [25-33], the sensitivity of CrAg detection ranged from 80 to 100%, and specificity from 82 to 100% (Figure 4B). Summary estimates of sensitivity and specificity of CSF CrAg for detecting CM were 98.8% (96.2 – 99.6) and 99.3% (96.7 – 99.8), respectively. In these 16 diagnostic cohorts (3500 participants) where CSF CrAg was compared with CSF culture, the summary agreement between CSF CrAg and CSF culture results was 97.0% (96 – 99) (Table 2).

Investigations of heterogeneity

In serum, LA (5 diagnostic cohorts, 256 participants) summary sensitivity was 100% (99.5 – 100) and summary specificity was 96.7% (93.8 – 98.9); while for LFA (3 diagnostic cohorts, 1690 participants) the summary sensitivity was of 94.4% (83.1 – 99.9) at a specificity of 89.1% (73.5 – 98.4). LA showed higher sensitivity in serum than LFA (p = 0.04) but there was no statistically significant difference in specificity (p = 0.14); Table 3.

In CSF, LA (10 diagnostic cohorts, 1810 participants) had a summary sensitivity of 97.1% (91.9 – 99.0) and a specificity of 99.1% (93.8 – 99.9) and LFA (6 diagnostic cohorts, 3099 participants) had a

summary sensitivity of 99.5% (97.2 – 99.9) and specificity of 99.5% (94.2 – 99.9). Though there was some weak statistical evidence that LFA may have better sensitivity in CSF (p = 0.07) than LA, their specificities were comparable (p = 0.54); Table 3.

In 7 diagnostic cohorts comprising 1846 participants [14, 25-28], CrAg detection was performed in both serum and CSF in the same participants, which allowed a direct head-to-head comparison. There was no evidence that sensitivity and specificity differed between CrAg in serum and CrAg in CSF (sensitivity 99.7% (86.8 – 100) and 99.9% (97.1 – 100), respectively, p = 0.33; specificity 95.2% (87.7 – 98.2) and 99.5% (86 – 100), respectively, p = 0.77; Figure 5 and Appendix 7.

Sensitivity analysis

Sensitivity analysis using only studies judged to be of low RoB confirmed the robustness of results: in serum, CrAg sensitivity was 98.3% (90.3 – 100) and specificity was 93.8% (86.5 – 98.6) and in CSF, CrAg sensitivity was 99% (84 – 99.9) and specificity was 99.7 (91.9 – 100).

DISCUSSION

Main findings

In this systematic review encompassing 11 studies (24 diagnostic cohorts, 3600 participants), we investigated the diagnostic accuracy of CrAg for detecting CM in HIV-infected adults presenting with CNS symptoms. We found that: (1) the prevalence of serum CrAg is about 60%, (2) the prevalence of culture-confirmed CM is about 40%, (3) the sensitivity and specificity of serum CrAg are 99% and 95%, respectively (4) the sensitivity and specificity of CSF CrAg are 99% and 99%, respectively, (5) agreement between the results of CSF CrAg and CSF culture is 97%

Implications for practice

In routine practice, the utility of a medical test depends on its role in guiding clinical decisions that could impact patient outcomes. Tests used in CM, an extremely severe disease with a high fatality, must be highly sensitive to ensure timely initiation of induction antifungal therapy [34, 35].

Concomittantly, the high cost of currently recommended induction treatment as well as potential amphotericin B (AmB)-related severe adverse events, not easy to monitor and manage in LMICs [36, 37], requires these tests also to be highly specific.

Among HIV-patients with CNS symptoms, we found that serum CrAg was highly predictive of confirmed CM [38] and was able to rule-out CM when negative. As such, in LMIC settings with a high burden of CM and no facilities for CSF analysis, systematically screening symptomatic patients for serum CrAg should become routine practice. If serum CrAg is positive, empirical inductive antifungal combination therapy should be started, unless the patient was previously known to have had cryptococcal infection. Thus, treatment is not delayed, although a lumbar puncture is still required in order to measure and manage CSF pressure; and provides the opportunity to confirm the diagnosis, and to confirm or not active infection in previously treated cases. Currently, systematic serum CrAg screening is recommended only for ART-naïve patients, prior to ART initiation [8]. However, with long term ART-interruption and therapy failure accounting for the majority of CM cases among ART-experienced patients [39, 40], systematic serum CrAg in all CNS symptomatic HIV-patients is warranted. As such, among those with serum CrAg positivity and a negative CSF CrAg, other causes of CNS infection could be considered.

Relying on India Ink staining of CSF and/or culture for confirmation of the diagnosis of CM requires a laboratory setting, trained technicians and sustainable reagents and equipment. Moreover, Indian Ink staining of CSF, which showed relatively low sensitivity in some studies, (as low as 86% [29, 41]), is only positive in the presence of a high fungal count and requires CSF centrifugation for highest sensitivity. Fungal culture, though reliable, requires viable organisms in CSF and laboratory incubation at 30˚C for several days to ensure fungal growth. This is not always logistically feasible and may delay diagnosis and treatment. In this meta-analysis, a positive agreement between CSF CrAg and reference standard results was 97%. With such high accuracy, the increasing availability of the LFA CrAg test, and ease of performance, we suggest that in contexts where there is limited

ability to analyse CSF, CSF CrAg is an alternative to conventional fungal culture, especially for first episodes of CM.

Implications for research

In CrAg-positive HIV-patients with asymptomatic underlying CM, a serum CrAg titre of at least 1:160 is associated with culture-confirmed CM [9, 42]. Though we did not investigate serum CrAg titres in this review due to scarcity of data, its potential role as a biomarker of culture-confirmed CM in CNS symptomatic patients is of high clinical importance, warranting further evaluation.

Limitations

Our review had some limitations. The LFA assay, though very promising, was investigated in serum only in four studies, which may explain the apparent difference in sensitivity we found between LA and LFA in serum. Moreover, in some of the studies on CSF, diagnostic accuracy might have been over-estimated because of composite reference standards. Due to a low number of studies, we had to use univariate random-effects models to separately estimate the sensitivity and specificity of CrAg in serum as well as in the meta-regression analysis of sources of heterogeneity. Comparison of the performances of LA and LFA was indirect as only one study evaluated both tests in CSF, limiting our ability to draw firm conclusions.

Conclusions

On average, the accuracy of CrAg detection in serum and CSF of HIV-positive adults with signs and symptoms suggestive of CM is very high when compared with conventional fungal culture and microscopy following India Ink staining. In settings without facilities for CSF analysis or with low LP uptake, CrAg detection in serum may be sufficiently sensitive to rule-out CM, and sufficiently specific to start antifungal therapy in cases with a positive result. In settings where LP is feasible but where laboratory equipment is limited, CSF CrAg could replace culture and India Ink staining for establishing the diagnosis of first episodes of HIV-associated CM.

NOTES

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Authors contributions

E.T., O.L. designed the review question; E.T., O.L., T.H. and J.F.C designed the study and drafted the protocol. R.S. performed literature search. E.T., J.J.B. and J.F.C performed study selection and data extraction. E.T. and J.F.C performed statistical analyses. E.T., O.L., J.F.C. and T.H. drafted the manuscript which was proofread, edited for important intellectual content by A.L., T.S., T.C., J.P. and P.G.P. All authors approved the final manuscript to be submitted.

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Potential Conflicts of Interest

JP reports grants from Merck, Astellas, Minnetronix, Amplyx, and Pfizer; is on advisory boards for Merck, F2G, Scynexis, Amplli Amplyx, Minnetronix, and Matinas; provides consulting for Scynexis and Amplli, all outside the submitted work. JSH reports non-financial support from Immunomycologics during the conduct of the study. OL reports personal fees from Pfizer, Merck, Astellas, and Gilead, outside the submitted work and has been involved with the development of the Biosynex CryptoPS test with no associated patent nor royalties. All other authors have no potential conflicts.

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Figure legends:

Figure 1: Flow diagram of the study selection process

Figure 2. Prevalence of CrAg positivity in serum (2A) and CSF (2B) in HIV-positive adults with central nervous system symptoms

Figure 3. Prevalence of confirmed cryptococcal meningitis (CM) in HIV-positive adults with central nervous system symptoms

Figure 4. Forest plots of serum (8 cohorts) and CSF (16 cohorts) CrAg sensitivity and specificity for CM diagnosis in HIV-positive adults with central nervous system symptoms

Figure 5. Direct head-to-head comparisons of serum and CSF CrAg performed in the same patients (7 cohorts). Circles and diamonds represent serum and CSF CrAg, respectively. The curved lines represent the summary ROC curves of sensitivity and specificity

Appendix 5. Review authors' judgment on risk of bias (RoB) and applicability concerns across all included studies (n=11)

Appendix 6. Review authors' judgment on risk of bias (RoB) and applicability concerns for each study (n=11)

TABLES

Table 1. Characteristics of included studies (n = 11)

Author, year	Country	Population	CrAg test(s) evaluated	Reference standard considered for the review	No. of participants	Comments
Nelson, M.R. 1990 [25]	United Kingdom	Consecutive sample of HIV patients presenting with fever and meningism in a hospital setting.	LA system IMMY Diagnostics	Nigrosin, Gram stain and fungal culture in Sabouraud dextrose agar	828	CrAg on both serum and CSF.
Temstet, A. 1992 [14]	France	Consecutive sample of HIV patients from University hospitals with suspected CM.	• LA Meridian Biosciences • Crypto-LA International Biological Labs • Pastorex LA Sanofi Pasteur Diagnostics	Fungal culture	87	CrAg detection performances of three latex agglutination tests on both serum and CSF.
Asawavichienjinda, T. 1999 [24]	Thailand	Consecutive sample of HIV-infected patients suspected of CNS	Pastorex LA Sanofi Pasteur Diagnostics	Indian ink stain and/or culture of CSF	100	Serum CrAg to identify LA cut-off point for the screening and

		infections in a hospital setting.				diagnosis of CM.
Boulware, D.R. 2014 [29]	South Africa and Uganda	Stored samples from two cohorts of HIV patients suspected of CM.	• LA Meridian Biosciences • LA system IMMY Diagnostics • LFA IMMY Diagnostics	India ink and/or CSF fungal culture	832	Three index tests were evaluated in CSF. Use of a composite reference standard.
Kabanda, T. 2014 [31]	Uganda	Prospective cohort of HIV-patients suspected of CM in a hospital setting.	• LA Meridian Biosciences • LFA IMMY Diagnostics	Indian ink and/or fungal culture	112	Two index tests evaluated on CSF. Use of composite reference standard.
Lourens, A. 2015 [33]	South Africa	Consecutive sample of HIV-patients with signs and/or symptoms of meningitis.	• LA Remel Inc. Lenexa USA • LFA IMMY Diagnostics	CSF fungal culture	465	Two index tests were evaluated in CSF.
Williams, D.A. 2015 [28]	Uganda	Consecutive sample of HIV patients suspected of CM in a	LFA IMMY Diagnostics	CSF fungal culture	207	Index test evaluation on serum and CSF. Use of a composite

		hospital setting.				reference standard.
Kammalac, N.T. 2015 [32]	Cameroon	Consecutive sample of HIV-patients, suspected of CNS infections in a hospital setting.	LA Fumouze Diagnostics	Indian ink stain and culture	146	Index test evaluated on CSF.
Dharmshale, S.N. 2016 [30]	India	Sample of HIV patients with signs and symptoms suggestive of meningitis.	LA Meridian Biosciences	Indian ink stain, fungal culture and polymerase chain reaction	99	Index test evaluation on CSF.
Mpoza, E. 2018 [26]	Uganda	Consecutive sample of patients from four cohorts clinically suspected of meningitis.	LFA StrongStep Liming Bio China	CSF fungal culture	282	Evaluation of a new test in both serum and CSF. Use of a composite reference standard.
Ssebambulidde, K. 2018 [27]	Uganda	Consecutive sample of HIV patients suspected of meningitis.	LFA IMMY diagnostics	CSF fungal culture in Sabouraud dextrose agar	1201	Evaluation of diagnostic performance in serum and CSF.

Abbreviations: CrAg: cryptococcal antigen, CSF: cerebrospinal fluid, CNS: central nervous system, LA: latex agglutination, LFA: lateral flow assay

Table 2. Agreement between CSF CrAg and CSF culture results across diagnostic cohorts (n = 16)

	CrAg test	N	No. CrAg (+) and culture (+)	No. CrAg (-) and culture (+)	No. CrAg (+) and culture (-)	No. CrAg (-) and culture (-)	Raw agreement between CSF CrAg and CSF culture results, % (95%CI)
Nelson M.R. 1990	LA (IMMY)	69	16	0	0	53	100 (94.8 – 100)
Temstet A. 1990	LA (Pastorex)	77	30	2	0	45	97.4 (90.1 – 99.7)
	LA (International biological)	41	30	0	2	9	95.1 (83.4 – 99.4)
	LA (Meridian)	41	30	0	2	9	95.1 (83.4 – 99.4)
Boulware D.R. 2014	LFA (IMMY)	666	435	3	2	226	99.2 (98.3 – 99.8)
	LA (IMMY)	749	452	14	0	283	98.1 (96.9 – 99.0)
	LA (Meridian)	279	176	4	14	85	93.5 (90.0 – 96.1)
Kabanda T. 2014	LFA (IMMY)	112	47	0	0	65	100 (96.8 – 100)
	LA (Meridian)	112	47	1	0	64	99.1 (95.1 – 100)
Lourens A. 2014	LFA (IMMY)	465	23	3	8	431	97.6 (95.8 – 98.8)

	LA (Remel Inc.)	465	26	7	0	432	98.4 (96.9 – 99.4)
Kammalac N.T. 2015	LA (Fumouze)	185	40	10	1	134	94.1 (89.6 – 97.0)
Williams D.A. 2015	LFA (IMMY)	207	126	0	12	69	94.2 (90.1 – 97.0)
Dharmshale S.N. 2016	LA (Meridian)	99	42	0	5	52	94.9 (88.6 – 98.3)
Mpoza E. 2018	LFA (StrongStep)	142	101	0	0	41	100 (97.4 – 100)
Ssebambulidde K. 2018	LFA (IMMY)	1201	671	3	0	527	99.8 (99.3 – 100)
Random-effects meta-analysis	-	**3500**	-	-	-	-	**98.0% (97.0 – 99.0)**

Abbreviations: CrAg, cryptococcal antigen; LA, latex agglutination; LFA, lateral flow assay

Table 3. Summary of diagnostic accuracy findings

		Quantity of evidence		Summary estimates	
Sample	**Test type**	**Cohorts (n)**	**Participants (n)**	**Sensitivity, % (95%CI)**	**Specificity, % (95%CI)**
Serum	LA	5	256	100 (99.5-100)*	96.7 (93.8-98.9)*
	LFA	3	1690	94.4 (83.1-99.9)*	89.1 (73.5-98.4)*
	Overall serum CrAg	8	1946	99.8 (88.4- 99.9)**	95.2 (88.8-98.0)**
	p-value***	8	-	0.04	0.14
CSF	LA	10	1810	97.1 (91.9-99.0)**	99.1 (93.8-99.9)**
	LFA	6	3099	99.5 (97.2-99.9)**	99.5 (94.2-100)**
	Overall CSF CrAg	16	3500	98.8 (96.2-99.6)**	99.3 (96.7-99.9)**
	p-value***	16	-	0.07	0.54

Abbreviations: CrAg, cryptococcal antigen; LA, latex agglutination; LFA, lateral flow assay; CSF, cerebrospinal fluid; CI, confidence interval

*univariate random-effects model; **bivariate random-effects model; ***univariate logit-normal random-effects meta-regression model.

Figure 1

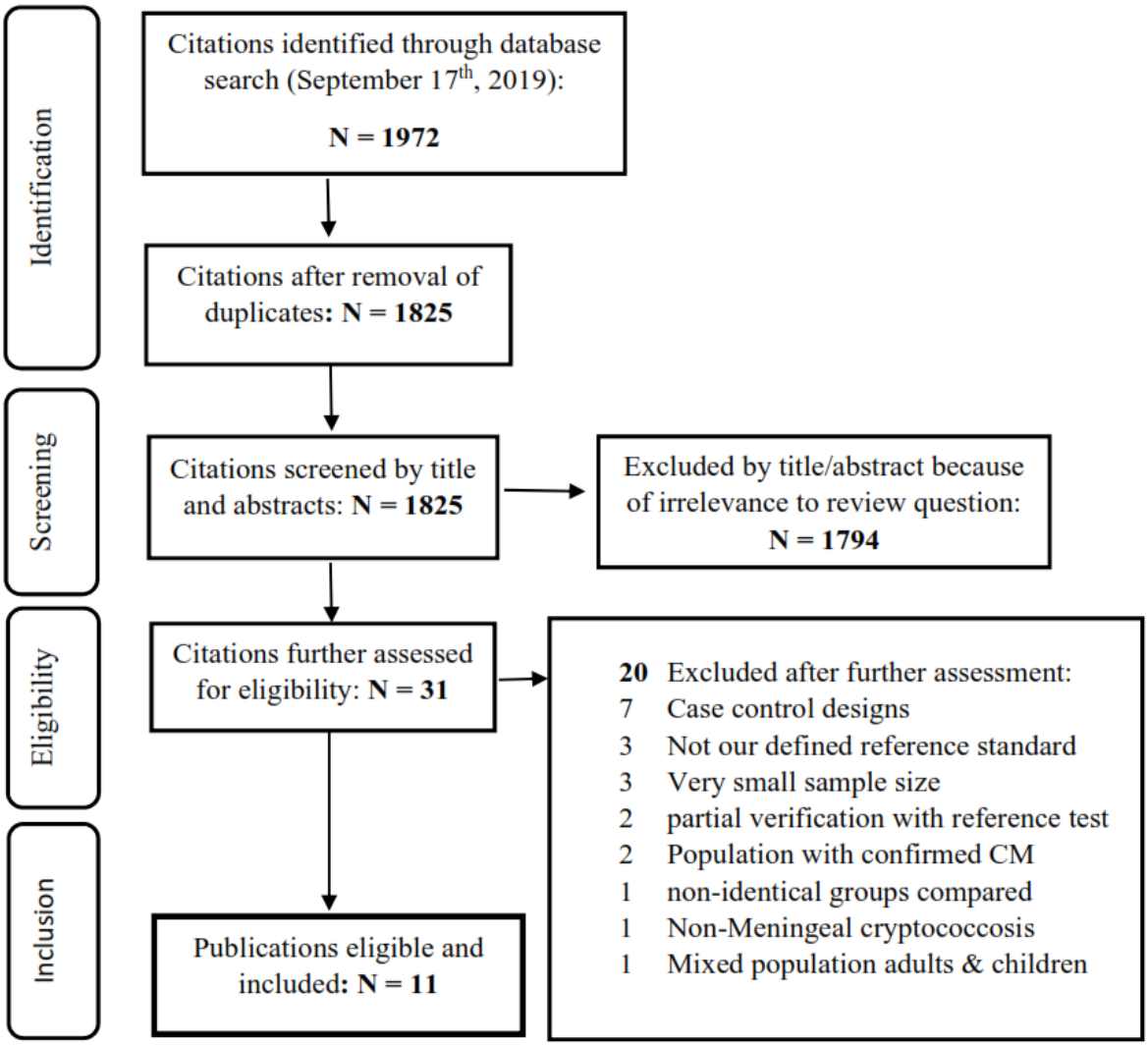


Figure 2A

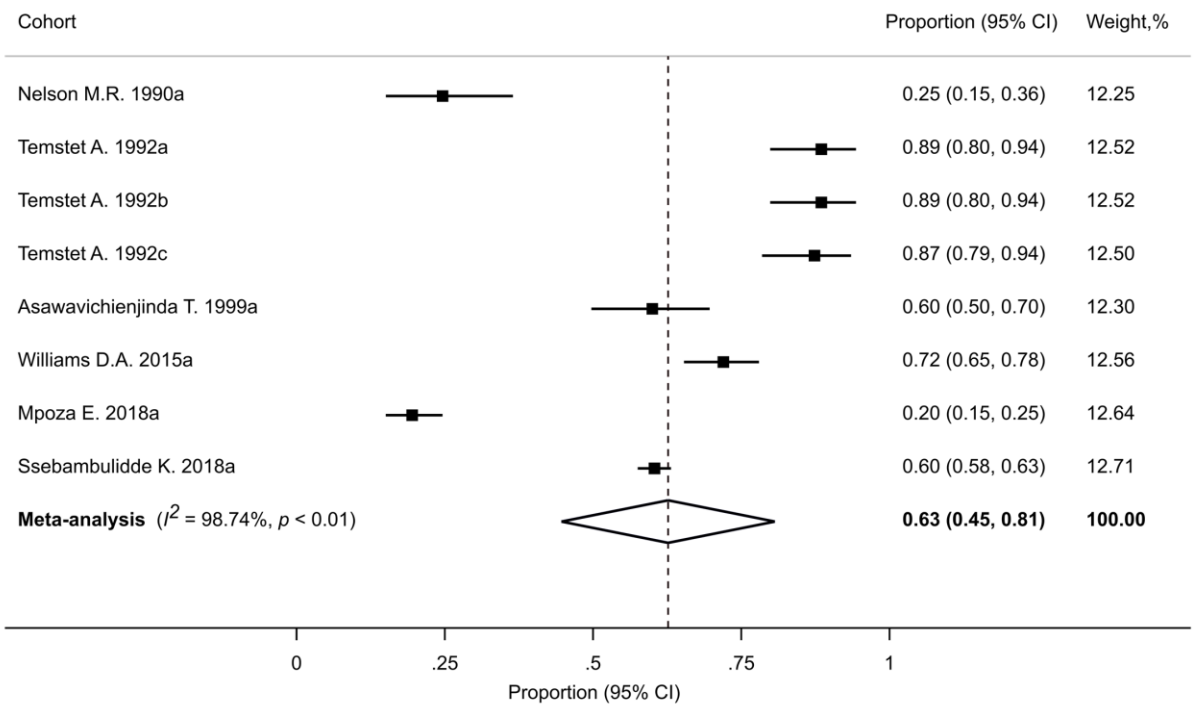


Figure 2B

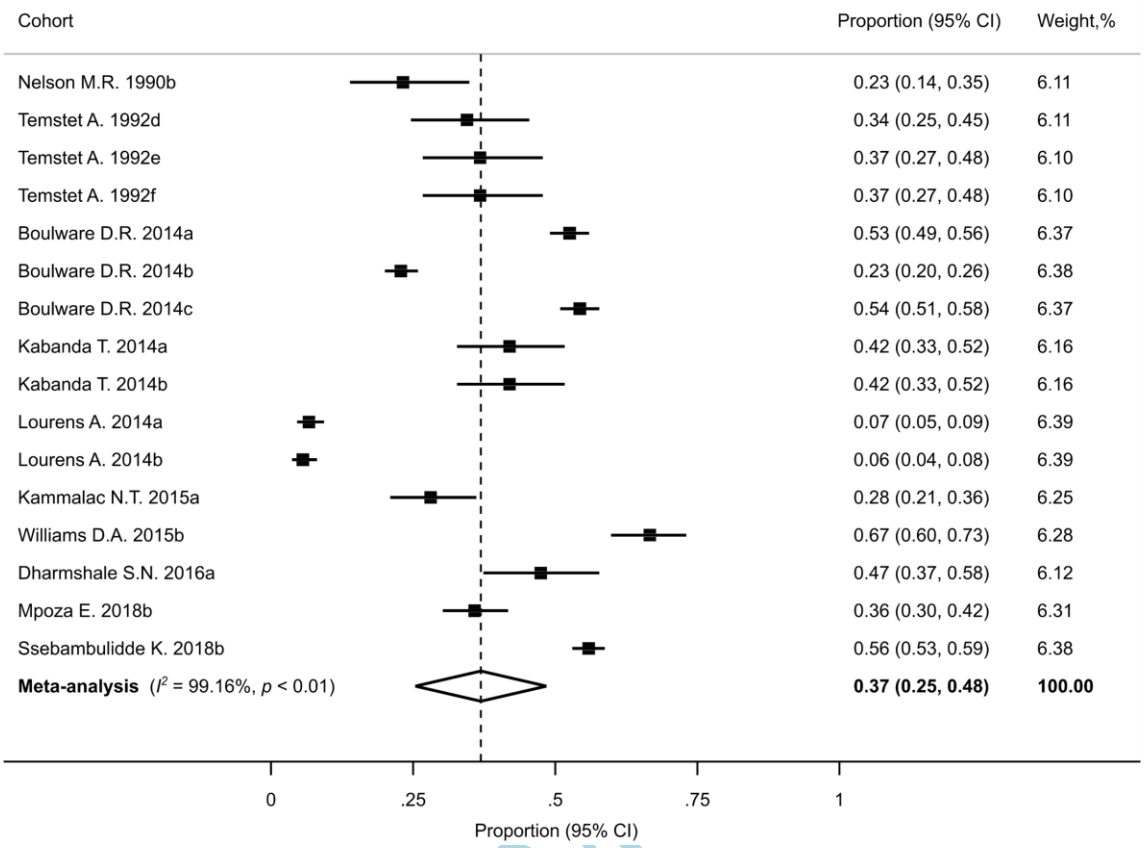


Figure 3

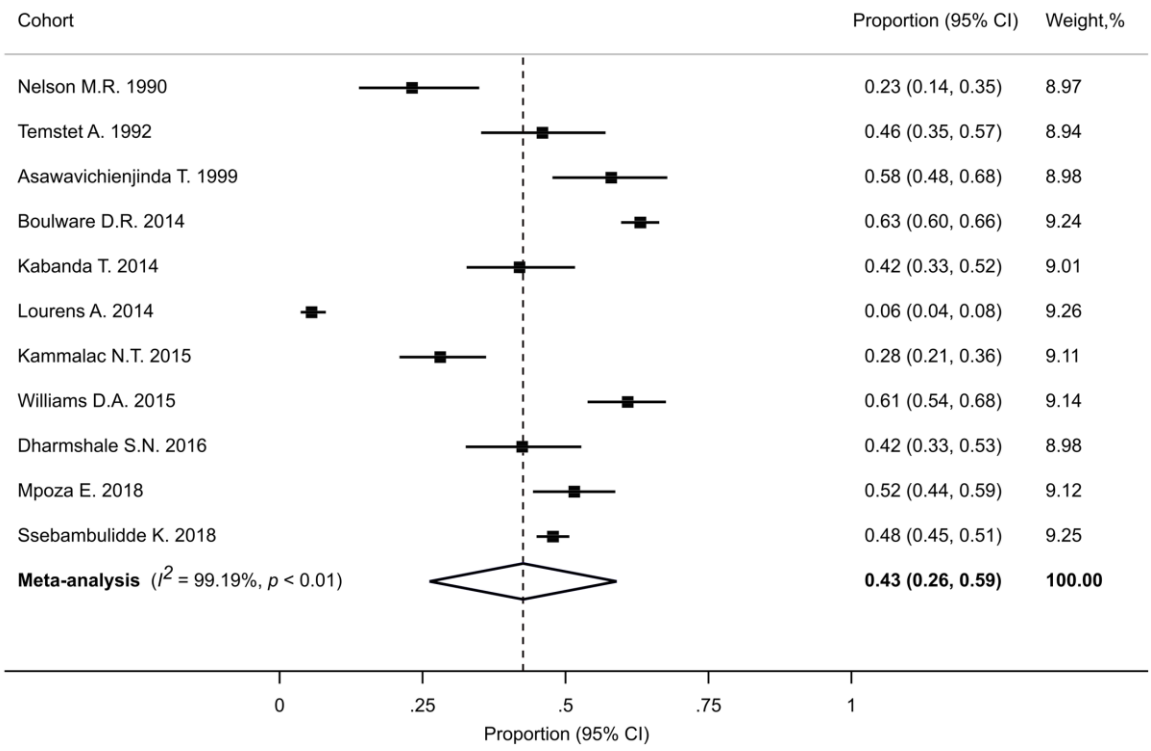
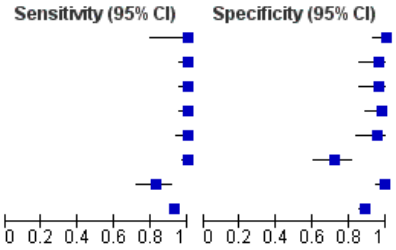


Figure 4

4A. Diagnostic accuracy of cryptococcal antigen (CrAg) in serum

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Nelson M.R. 1990a	16	0	0	53	1.00 [0.79, 1.00]	1.00 [0.93, 1.00]
Temstet A. 1992a	75	2	0	45	1.00 [0.95, 1.00]	0.96 [0.85, 0.99]
Temstet A. 1992b	75	2	0	45	1.00 [0.95, 1.00]	0.96 [0.85, 0.99]
Temstet A. 1992c	75	1	0	46	1.00 [0.95, 1.00]	0.98 [0.89, 1.00]
Asawavichienjinda, T. 1999a	58	2	0	40	1.00 [0.94, 1.00]	0.95 [0.84, 0.99]
Williams D.A. 2015a	126	23	0	58	1.00 [0.97, 1.00]	0.72 [0.60, 0.81]
Mpoza E. 2018a	54	1	11	101	0.83 [0.72, 0.91]	0.99 [0.95, 1.00]
Ssebambulidde K. 2018a	671	54	54	422	0.93 [0.90, 0.94]	0.89 [0.85, 0.91]



4B. Diagnostic accuracy of cryptococcal antigen (CrAg) in cerebrospinal fluid (CSF)

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Nelson M.R. 1990b	16	0	0	53	1.00 [0.79, 1.00]	1.00 [0.93, 1.00]
Temstet A. 1992d	30	0	3	45	0.91 [0.76, 0.98]	1.00 [0.92, 1.00]
Temstet A. 1992e	30	2	0	9	1.00 [0.88, 1.00]	0.82 [0.48, 0.98]
Temstet A. 1992f	30	2	0	9	1.00 [0.88, 1.00]	0.82 [0.48, 0.98]
Boulware D.R. 2014b	176	14	4	85	0.98 [0.94, 0.99]	0.86 [0.77, 0.92]
Boulware D.R. 2014c	452	0	14	238	0.97 [0.95, 0.98]	1.00 [0.98, 1.00]
Boulware D.R. 2014a	435	2	3	226	0.99 [0.98, 1.00]	0.99 [0.97, 1.00]
Kabanda T. 2014a	47	0	1	64	0.98 [0.89, 1.00]	1.00 [0.94, 1.00]
Kabanda T. 2014b	47	0	0	65	1.00 [0.92, 1.00]	1.00 [0.94, 1.00]
Lourens A. 2014a	26	7	0	432	1.00 [0.87, 1.00]	0.98 [0.97, 0.99]
Lourens A. 2014b	23	8	3	431	0.88 [0.70, 0.98]	0.98 [0.96, 0.99]
Kammalac N.T. 2015a	40	1	10	134	0.80 [0.66, 0.90]	0.99 [0.96, 1.00]
William D.A. 2015b	126	12	0	69	1.00 [0.97, 1.00]	0.85 [0.76, 0.92]
Dharmshale S.N. 2016a	42	5	0	52	1.00 [0.92, 1.00]	0.91 [0.81, 0.97]
Mpoza E. 2018b	101	0	0	41	1.00 [0.96, 1.00]	1.00 [0.91, 1.00]
Ssebambulidde K. 2018b	671	0	3	527	1.00 [0.99, 1.00]	1.00 [0.99, 1.00]

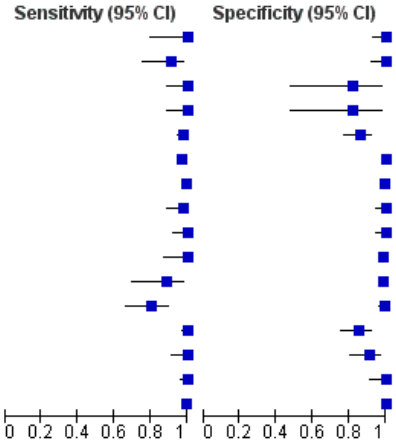
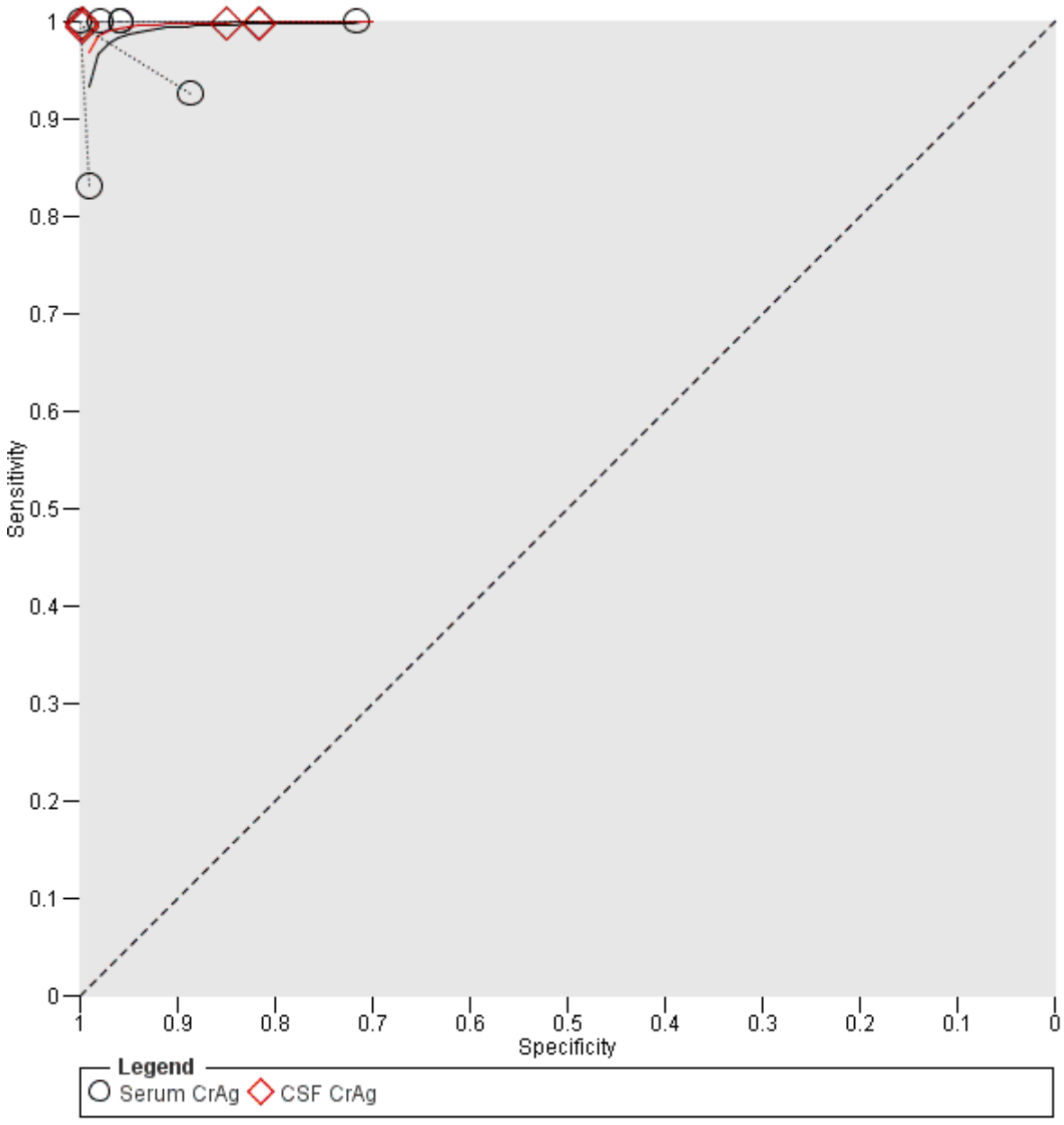
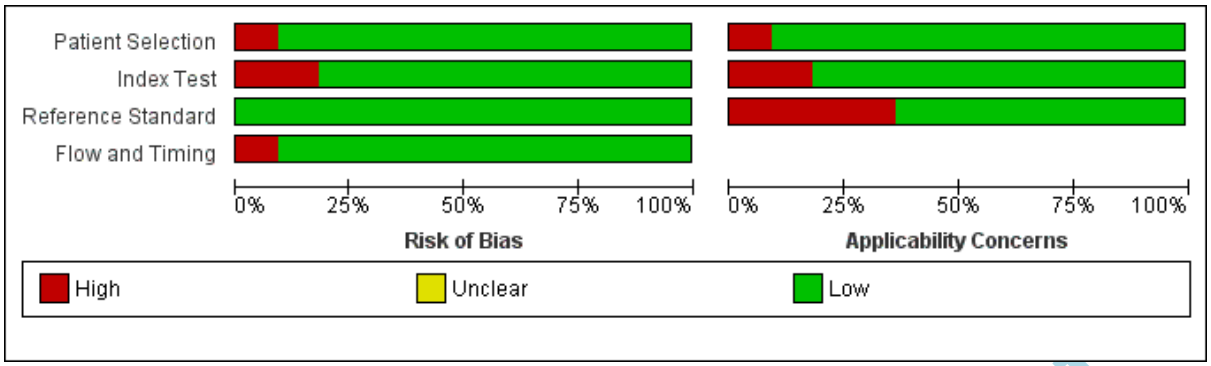


Figure 5



Appendix 5



Appendix 6