Polymerase Chain Reaction in the Diagnosis of Invasive Aspergillosis: A Meta-analysis of Diagnostic Performance

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Running Title: PCR for invasive aspergillosis
Abstract

Background: Invasive aspergillosis is a difficult to diagnose infection with high mortality that affects high risk groups such as patients with neutropenia and hematologic malignancies.

Methods: We performed a bivariate meta-analysis of diagnostic data for Aspergillus spp. PCR on blood specimens across high risk hematology patients. We included all studies involving human subjects that assessed the performance of any PCR assay for invasive aspergillosis on whole blood or serum and that used as reference standard the EORTC/MSG criteria. Three investigators independently searched the literature for eligible studies and extracted the data. Out of 37 total studies, 25 met strict quality criteria and were included in our evidence synthesis.

Results: Twenty five studies with 2,595 patients were analyzed. The pooled diagnostic performance of whole blood and serum PCR was moderate, with a sensitivity and specificity of 84% (95% CI 75%-91%) and 76% (95% CI 65%-84%), respectively, suggesting that a positive or negative result is unable on its own to confirm or exclude a suspected infection. The performance of PCR on serum was not significantly different from whole blood. Notably, at least two positive PCR test results were found to have a specificity of 95% and sensitivity of 64% for invasive infection, achieving a high positive likelihood ratio of 12.8. Importantly, the European Aspergillus PCR Initiative (EAPCRI) recommendations improved even further the performance of PCR when at least two positive specimens were used to define 'PCR positivity'.

Conclusions: Two positive PCR results should be considered highly indicative of an active Aspergillus spp. infection. Use of the EAPCRI recommendations by clinical laboratories can further enhance PCR performance.
Introduction

Despite advances in treatment and supportive care, invasive aspergillosis (IA) is associated with significant morbidity and mortality, especially among patients with hematologic malignancies and hematopoietic-stem cell transplant (HSCT) recipients (1, 2). Systemic antifungal prophylaxis is widely used in this patient population (3, 4) and patients with recurrent or persistent fever and prolonged neutropenia frequently require empiric coverage with antifungal agents (5). Timely and accurate diagnosis of an active infection is needed in order to initiate targeted antifungal therapy and avoid unnecessary antifungal treatment, which is often accompanied by a multitude of side effects and the cumulative risk of resistance.

There is a need for the development of newer diagnostic techniques that would ideally be able to identify IA rapidly, non-invasively and at an early stage. To aid in this endeavor, the European Organization for the treatment of Cancer/Mycoses Study Group (EORTC/MSG) developed specific criteria for the diagnosis of IA in 2002 (6), which were later revised in 2008 (7), in an attempt to provide the elusive “gold-standard” to which any newer diagnostic test should be compared. Dominant among the novel diagnostic methods is the polymerase chain reaction (PCR) which allows for pathogen DNA detection and species identification in a variety of clinical samples. A number of PCR assays are now available to detect Aspergillus spp. in various clinical specimens and have a high diagnostic yield in vitro but their clinical performance is debated due to contradictory reports from clinical trials. Previous systematic reviews have assessed the performance of Aspergillus spp. PCR with promising findings (8), but did not succeed in convincing the guideline issuing organizations to incorporate the test in their algorithms (6, 7). The main argument has been that differences in the PCR protocols among different studies do not allow sufficient interlaboratory comparisons to be made and thus preclude widespread implementation (9). In an effort to determine the value of Aspergillus spp. PCR in clinical diagnostics, as a screening or a confirmatory tool, and to evaluate different parameters that could contribute to contradictory reports regarding PCR performance from the existent literature, we performed a meta-analysis of clinical trials that evaluated the accuracy of PCR for IA performed on serum and whole blood of high risk patients.
Methods

Literature search

A systematic literature search of MEDLINE (1951-December 2013) and the Cochrane Central Register of Controlled Trials (2010-June 2013) databases was conducted to identify all published studies involving human subjects and evaluating the performance of a PCR assay for *Aspergillus* spp. using as a reference standard the EORTC/MSG criteria. We searched only full publications and not unpublished studies or conference abstracts. We used the search term “Aspergil* AND (PCR OR Polymerase chain reaction)”. Date of last access was January 20th 2014.

Three investigators (MA, IMZ, FNZ) independently identified and scrutinized studies for potential inclusion. Studies published in languages other than English were excluded. Our meta-analysis is in line with PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analysis) recommendations (10).

Eligibility criteria and definitions

We included all studies involving human subjects that assessed the performance of any PCR assay for invasive aspergillosis on whole blood or serum and that used as reference standard the EORTC/MSG criteria (either the 2002 (6) or the revised 2008 criteria (7)). Studies on series of patients with a positive only (probable/definite) or negative only (no infection) reference outcome were not considered because it is impossible to extrapolate all pertinent diagnostic estimates from these studies. We defined as “true positive” the cases that were positive by PCR and were classified as definite or probable by the EORTC/MSG criteria and as “true negative” the cases that were negative by PCR and were classified as possible or unlikely infection by the EORTC/MSG criteria. Possible IA cases were categorized as negative as per the recommendations of the Cochrane group (11). For our primary analysis, we defined as PCR positive any case that had at least one positive PCR result. In multi-arm studies that used the same patients more than once, we included the strata with the highest reported performance estimates. Also, in trials that examined the performance of PCR in both a high-risk group and a control group of patients at low risk for IA, we excluded the control arm to avoid a case-control design that could spuriously increase performance estimates.
Outcomes of interest

The primary outcome of interest was the summary sensitivity and specificity of *Aspergillus* PCR on blood products compared to the reference standard. Our secondary goal was to evaluate whether the choice of sample, the type of PCR, the choice of primers, or any of the methodological aspects proposed by the European *Aspergillus* PCR Initiative (EAPCRI) (12, 13) for optimum performance of PCR had a significant impact on the test’s accuracy. Finally, we performed a sensitivity analysis to evaluate how PCR performance would be affected if two positive results were used to determine a ‘PCR positive’ outcome.

Data extraction and Quality assessment

Data from eligible studies were independently extracted by three reviewers (MA, IMZ, FNZ). Discrepancies between authors were resolved by consensus. The methodological quality of each trial was evaluated independently by three authors (MA, IMZ, FNZ) for potential sources of bias using standard criteria. QUADAS 2 score was used to evaluate for potential sources of bias in each study (14). In order to have high quality estimates, we excluded from further analysis the studies with high-bias QUADAS 2 elements. Specifically, we excluded studies that had a non-random population or a case-control design, and we also excluded studies that did not have a pre-specified threshold for PCR positivity. We chose not to eliminate trials in which the investigators had knowledge of the EORTC/MSG classification of the patient before PCR interpretation because this knowledge cannot alter the interpretation of a positive or negative finding as long as the positivity threshold is pre-specified. Finally, we excluded studies that had a >10% loss to follow-up.

Statistical analysis

We calculated the independent sensitivity and specificity for each study using a 2×2 contingency table. Each table consisted of true positives (tp), true negatives (tn), false positives (fp) and false negatives (fn) according to the results of the *Aspergillus* spp. PCR (the index test) compared to the classification of the patient as definite/probable versus possible/unlikely aspergillosis based on the EORTC/MSG criteria (the reference standard). We estimated the combined (pooled) sensitivity, specificity, likelihood ratios and diagnostic accuracy (area under the curve, AUC) using a
bivariate mixed-effects binomial regression model, which accounts for both within
and between-study variability (15-17). We used the empirical threshold of >10 for
LR+ and <0.1 for LR- to rate the test as of high value in the decision to rule-in (i.e.
probable/definite aspergillosis) or rule-out infection (18). Publication bias was
assessed using the Deeks’ regression test for asymmetry, with a p<0.05 for the slope
coefficient denoting a significant asymmetry (19). We did not use heterogeneity tests
(16, 20) as such tests can mislead systematic reviews of diagnostic test accuracy and
are not recommended by the Cochrane diagnostic test accuracy group (16) and may
be misleading in diagnostic accuracy studies, since computing separate $I^2$ statistics for
sensitivity and specificity will usually overestimate statistical heterogeneity. Effects
were plotted as summary receiver operating characteristics (SROC) curves (15).

We calculated subgroup estimates to account for variation in laboratory PCR
methodologies, provided that we had at least 4 pertinent studies in each group to draw
the quadrature points, and provided data on the magnitude and difference of average
sensitivity and specificity estimates between groups. We also evaluated whether the
use of the old (2002) or the revised (2008) EORTC/MSG criteria as a reference
standard affected PCR performance estimated. The significance of difference between
average specificity or sensitivity was assessed using meta-regression and a p-value
was reported.

The bivariate meta-analysis was performed on Stata v11 (College Station, TX)
using the midas set of commands (21, 22). The meta-regression was performed on
MetaAnalyst Software (23).
Results

Two thousand and six non-duplicate studies were identified by the initial search, of which 37 met the inclusion criteria (Figure 1). Twenty three studies provided data for PCR using the 2002 EORTC/MSG criteria (24-46) and 14 using the revised (2008) criteria (47-60) (Table 1).

Quality Assessment

Most of the studies were of high quality as determined by the QUADAS 2 assessment tool (Table 2). After excluding all low-quality studies, twenty five studies remained and were further analyzed (25, 26, 28-37, 43-48, 50, 52-54, 56-58).

The 25 eligible studies (26 strata) included a total of 2,595 patients. The number of patients enrolled varied from 8 to 218 patients (median 91). The target patient population was patients with hematologic malignancies and/or hematopoietic stem cell transplant recipients in 17/25 studies, bone marrow transplant recipients in 1/25 and a mixed population of patients with hematologic malignancies and other immunocompromised individuals in 7/25. Six studies performed PCR on serum (23%) and the remaining used whole blood assays (77%). The setting was heterogeneous regarding quality laboratory criteria. Of note, only 3 studies (all 3 using whole blood PCR) were found to be fully compliant with all EAPCRI criteria, while 7 studies (5 using whole blood and 2 serum) were compliant with all but one criteria, 10 (9 using whole blood and 1 serum) deviated by 2 criteria and 6 (4 using whole blood and 2 serum PCR) deviated by 3 or more.

Pooled diagnostic estimates

Across the 25 eligible studies the individual sensitivity estimates ranged from 0 to 1.0, while the individual specificity estimates ranged from 0.29 to 0.98 (Figure 2). In bivariate meta-analysis the pooled sensitivity was 0.84 (0.75-0.91) and the corresponding pooled specificity was 0.76 (0.65-0.84) (Table 2; Figure 3). There was no evidence of significant funnel plot asymmetry (Deeks’ bias=-0.71, p=0.9), indicating that small study effects were not present. The combined effects show that Aspergillus spp. PCR has a positive likelihood ratio of 3.5 and a negative likelihood ratio of 0.21 thus suggesting that its diagnostic performance is moderate (61).
Specifically, in settings where the expected pre-test probability of IA is low (≤5%),
PCR will yield a positive predictive value (PPV) of 0.08 (0.06-0.11) and a negative
predictive value (NPV) of 0.99 (0.97-1.00), while in settings where IA is strongly
suspected (pre-test probability ≥10%) PCR is expected to yield a PPV of 0.76 (0.71-
0.81) and a NPV of 0.71 (0.66-0.77), respectively.

Subgroup analysis

We performed subgroup analysis to evaluate changes in the sensitivity and specificity
caused by a series of different parameters in each study. We also assessed whether the
effect of these covariates on the average sensitivity estimates were significant.
Specifically, across studies where the updated EORTC/MSG 2008 criteria were
implemented, the diagnostic estimates were 0.83 (0.72-0.90) for sensitivity and 0.79
(0.67-0.87) for specificity, respectively. These estimates did not differ to those of
studies using EORTC/MSG 2002 criteria as the reference index. Interestingly, studies
testing PCR on serum yielded higher specificity (85% vs. 73%) and lower sensitivity
estimates (78% vs. 86%) over whole blood assays, but these differences did not reach
statistical significance. It is of note that the average specificity for serum was
significantly higher compared to whole blood without bead-beating (p=0.04).

Regarding the assay methodology, no methodological parameter significantly
affected the performance of PCR (Table 3). Further, when comparing the studies that
deviated by no more than one of the EAPCRI criteria to studies that deviated by two
or more, no significant changes in sensitivity (87% vs 82% respectively) or specificity
(77% vs 75% respectively) were noted. Of note, we could not assess the effect of
white blood cell and red blood cell lysis steps as these were undertaken by almost all
studies on whole blood. We also were unable to evaluate the effect of the use of
mitochondrial vs. ribosomal primers because only 2 studies used the former (36, 44),
while the difference in PCR performance among different ribosomal primers (18S
erNA vs. others) was not significant.

Sensitivity analysis

Thirteen of our 26 studies (12 using whole blood and 1 using serum) included data on
the diagnostic performance of PCR when at least two positive PCR results were
considered “PCR positive”. By analyzing the performance estimates of these studies
using differential criteria for PCR positivity (one vs. two or more positive PCR results), we found that PCR specificity was greatly increased (73% vs. 95%, respectively) with the latter method, giving a high positive likelihood ratio of 12.8, whereas sensitivity decreased (85% vs 64%, respectively). Moreover, two or more positive assays for low risk individuals (≤5% pre-test probability) give a PPV and NPV of 0.23 and 0.99 respectively, while among high risk patients (≥10% pre-test probability), the PPV increases to 0.90, while the NPV drops to 0.62.

Interestingly, when comparing the effect of the EAPCRI recommendations on this sensitivity analysis (Table 4), we found that bead-beating significantly improved the specificity of the test (96% vs 86%, meta-regression p=0.006), with a non-significant drop in sensitivity (50% vs.73%). Further, we found that compliant studies (no more than one deviations from the EAPCRI criteria) showed higher specificity (98% vs. 85%, meta-regression p=0.003) with an additional non-significant increase in sensitivity (67% vs 61%).


Discussion

Despite the fact that multiple *Aspergillus* spp. PCR assays on blood specimens are now available, a consensual conclusion has yet to be reached on the role that this test can play in the diagnosis of IA. Shortly after its first introduction in the 1990s, it was thought that this method could in fact revolutionize diagnosis and management of this severe disease (62). However, the multitude of clinical studies performed since then failed to prove beyond doubt whether the test can rule-in or rule-out an active infection, thus often leaving clinicians baffled when trying to interpret a positive or negative result. Our findings indicate that whole blood and serum *Aspergillus* spp. PCR has a moderate diagnostic performance, which suggests that a positive or negative result is unable on its own to confirm or exclude a suspected infection in high risk patients. We should note however, that the pooled performance estimates for PCR in our study were not inferior to the performance estimates for serum galactomannan (sensitivity 71%, specificity 89%) (63) or beta glucan (sensitivity 76.8%, specificity 85.3%) (64) as reported by previous meta-analyses.

Our choice to define as PCR positive all episodes that had at least one PCR positive specimen, coupled with the fact that most studies tested more than one specimen per episode, may have overestimated the average sensitivity, and underestimated the specificity of the method. To see whether this is true, we performed a sensitivity analysis on studies that had extractable data about at least two vs. one PCR positive specimen per patient. Indeed, we found that, PCR specificity increased dramatically by this approach to 95%, which leads to a positive predictive value of 90% at high-risk individuals. Therefore, presence of at least two positive whole blood PCR specimens in a high risk patient should be considered very indicative, if not confirmatory of IA. On the other hand, the sensitivity of this approach was lower at 64%, which suggests that it would be more valuable as a confirmatory rather than a screening tool. Despite the fact that our results are in accordance with previous reports (8), we observed a significant variation of results reported by individual studies involved in our analysis, which led us to search for reasons behind these inconsistencies.

One of the potential explanations is the choice between different specimens, namely whole blood or serum, on which the PCR is performed. Two previous clinical
studies comparing *Aspergillus* spp. PCR performed on serum and whole blood of the same high risk patients failed to show significant differences in accuracy (49, 51), with one study suggesting a non-significant trend toward increased sensitivity and reduced specificity of whole blood PCR (51). In accordance with these studies, our results suggest that PCR assays performed on serum resulted in a non-significant decrease in sensitivity and increase in specificity compared to whole blood. Taking into consideration that serum PCR is faster and easier to perform and that serum can also be used for other biomarker diagnostic tests, such as galactomannan, serum has the potential to be the preferred specimen for PCR testing.

Another potential moderator of effect among different trials could be the use of different versions of the EORTC/MSG criteria for the diagnosis of IA. Compared to the old version, the revised criteria (7) kept the terminology but expanded the definition of probable while reducing the scope of possible IA. The adoption of the new classification may have an unclear impact on outcomes in future trials. Indeed, a recent retrospective evaluation stated that the majority of “possible” invasive aspergillosis cases can be downgraded to “unclassifiable”, but also probable cases showed 75% reductions (65). While this impact may or may not hold true in clinical practice, our data indicated that the performance of PCR was unaffected.

Finally, the use of different methodological parameters in DNA extraction and amplification is considered one of the most important sources of inconsistencies between different trials (66). In an effort to circumvent this problem and standardize *Aspergillus* spp. PCR among different laboratories, the EAPCRI has recently issued a series of recommendations on how to perform *Aspergillus* PCR on whole blood and serum based on the results of two multicenter studies on spiked samples (12, 13). These include the use of higher sample volume, lower elution volumes, presence of internal control, and thorough cell lysis with bead beating and enzymatic white and red blood cell lysis steps for whole blood. Notably, in the clinical setting we studied, none of these methodological characteristics significantly altered the sensitivity or specificity estimates in our primary analysis.

The interpretation of this finding is challenging, mainly because we don’t know in what form *Aspergillus* spp. circulate in the blood. Of note, many studies suggest that most of the circulating *Aspergillus* DNA is not in the form of conidia, but
rather as free DNA (67, 68). This provides an explanation to the fact that blood cultures have such a low sensitivity for aspergillosis (69), as well as to our finding that whole blood testing is not superior compared to serum. This would also explain why sequential cell lysis methods could not improve PCR performance in patients with IA, in contrast to what would be expected in samples spiked with *Aspergillus* spp. conidia (13). In addition, it is unclear whether any form of *Aspergillus* DNA can be found in the circulation at all times during an active infection. As suggested by a recent *in vitro* study, release of DNA is intermittent and happens only during certain stages of fungal growth (68). If in fact circulating fungal DNA is released intermittently only during mycelial breakdown, it is plausible that its levels in the blood would have such a wide fluctuation that could make differences in sample size or elution volume relatively insignificant.

Nevertheless, it is of note that compliance with the EAPCRI recommendations significantly increased the specificity of PCR and was also accompanied by an increase in sensitivity when at least two positive results were used to define PCR positivity. An explanation for this seemingly contradictory finding would be that other factors that we were unable to assess (such as PCR testing algorithms and the use of prophylactic antifungal agents) could have served as potential confounders in the assessment of the effect of the EAPCRI recommendations on our primary analysis and those factors should be evaluated in future trials. Compliance with these simple methodological guidelines should in fact be encouraged as a means that would allow interlaboratory comparisons and has the potential to improve PCR performance in clinical practice.

Several systematic reviews have been previously performed about *Aspergillus* spp. PCR on bronchoalveolar lavage fluid, with very promising findings (70). However, despite the large number of new studies on the issue, to our knowledge there is only one meta-analysis on whole blood and serum PCR performance to-date. This study by Mengoli et al. (8) reported that the performance of PCR is moderate, but specificity can be increased when two PCR results are used to define a ‘PCR positive’ finding. This study was published before the EAPCRI recommendations were issued and included a smaller number of trials, so the authors could not reach any conclusions regarding the performance of different PCR protocols. It is of note that our study confirms the finding that a single positive PCR result has moderate
diagnostic accuracy, whereas a strategy that uses at least two positive PCR results per suspicious episode to define a positive test is able to achieve superior specificity. Moreover, by investigating the effect of different protocols on PCR performance our study shows that serum PCR is not inferior compared to whole blood, and that compliance with the EAPCRI recommendations has the potential to improve PCR performance in clinical practice.

An important limitation of our study is the fact that only a subset of our included studies had extractable data regarding PCR performance for at least two positive specimens, and the vast majority of these studies used whole blood and not serum as their specimen. Therefore, the results of this sensitivity analysis should be interpreted with caution. Also, all studies included in our analysis enrolled individuals who were at high-risk for an invasive fungal infection, as this is the patient population in which the *Aspergillus* spp. PCR test will be of most value. Therefore, our performance estimates may not be accurate in the general population.

In summary, for high-risk individuals two whole blood or serum PCR specimens positive for IA should be considered very indicative, if not confirmatory for a clinically suspected infection. Given the superior specificity of this approach, it has the potential to be used along with other circulating biomarker detection assays, such as galactomannan, as a criterion to define a probable infection. Compliance with the EAPCRI recommendations for *Aspergillus* spp. PCR can further increase the specificity of this approach and will allow for better interlaboratory comparisons. However, given the complexity of IA diagnosis, it is unlikely that a single non-invasive test will be able to be used alone in clinical decision making. Consequently, the focus of future clinical trials should include the development of decision algorithms that would take into account multiple parameters to guide the management of high-risk groups. In this context, serum and whole blood PCR have a concrete potential to improve our ability to detect and diagnose IA.
Acknowledgments

A.M.C. is an active member of the Aspergillus Technology Consortium (AsTeC) group.

Conflict of Interest Statements

A.M.C. and E.M. have received financial support from T2 biosystems for research unrelated to this manuscript. All other authors report no potential conflicts of interest.
References


bone marrow transplant recipients using real-time PCR. J Glob Infect Dis 608:68-75.


Figure legends

Figure 1. Flow diagram

Figure 2. Forest plot for independent sensitivity and specificity estimates.

Figure 3. Summary receiver operating characteristics (ROC) curve for PCR performance
Flow diagram

Potentially relevant citations identified and screened for retrieval (N=2,022)
  MEDLINE: 2,004
  Cochrane trial registry: 18

Duplicate studies removed (N=16)

Studies screened after removal of duplicates (N=2,006)

Studies excluded (N=1,928) on title and abstract reading

Studies retrieved in full-text for more detailed evaluation (N=78):

Articles excluded (N=41)
  - Not a clinical trial: 4
  - Did not include cases of proven/probable IA: 4
  - Did not provide information about the reference standard or did not use EORTC criteria as standard: 23
  - Included samples other than whole blood or serum and did not differentiate the results among them: 4
  - Possible IA patients categorized together with proven/probable IA: 2
  - Did not include cases of unlikely IA: 4

Potentially appropriate studies to be included in the meta-analysis (N=37)

Studies excluded after quality assessment (N=12)

Studies included in analysis (N=25)
Table 1. Characteristics of included studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Study design</th>
<th>Patient population</th>
<th>Type of PCR</th>
<th>Specimen tested</th>
<th>Primaries used</th>
<th>Number of episodes (specific for IFI)</th>
<th>Number of patients (specific for IFI)</th>
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<tbody>
<tr>
<td>Nabili et al. 2013</td>
<td>Prospective</td>
<td>62 hematologic malignancy patients</td>
<td>Real time PCR</td>
<td>Whole blood</td>
<td>ITS1, 5.8S rDNA, 28S rRNA, 18S rDNA, rRNA gene</td>
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<td>Schwarzinger et al. 2013</td>
<td>Prospective</td>
<td>103 adult hematology patients at high risk for IA</td>
<td>Real time PCR</td>
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<td>ITS1, 5.8S rDNA, 28S rRNA, 18S rDNA, rRNA gene</td>
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<td>103</td>
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<td>Li et al. 2013</td>
<td>Prospective</td>
<td>72 patients with hematologic malignancies suffering from fever, 4 with normal temperatures and 10 healthy volunteers</td>
<td>Real time PCR</td>
<td>Serum</td>
<td>ITS1, 5.8S rDNA, 28S rRNA, 18S rDNA, rRNA gene</td>
<td>2</td>
<td>82</td>
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<tr>
<td>Rogers et al. 2013</td>
<td>Prospective</td>
<td>46 patients receiving either allogeneic SCT or myeloablative chemotheraphy</td>
<td>Real time PCR</td>
<td>Whole blood</td>
<td>ITS1, 5.8S rDNA, 28S rRNA, 18S rDNA, rRNA gene</td>
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<td>46</td>
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<td>Springer et al. 2013</td>
<td>Prospective</td>
<td>47 patients with proven/probable IA and 31 controls</td>
<td>Real time PCR</td>
<td>Whole blood</td>
<td>ITS1, 5.8S rDNA, 28S rRNA, 18S rDNA, rRNA gene</td>
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<td>Leung et al. 2013</td>
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<td>42 patients with at least one positive galactomannan test</td>
<td>Real time PCR</td>
<td>Whole blood</td>
<td>ITS1, 5.8S rDNA, 28S rRNA, 18S rDNA, rRNA gene</td>
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<td>Stone et al. 2013</td>
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<td>29 patients with at least one positive galactomannan test</td>
<td>Real time PCR</td>
<td>Whole blood</td>
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<td>Robins et al. 2013</td>
<td>Prospective</td>
<td>54 patients with cancer and pulmonary infiltrates</td>
<td>Real time PCR</td>
<td>Whole blood</td>
<td>ITS1, 5.8S rDNA, 28S rRNA, 18S rDNA, rRNA gene</td>
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<td>54</td>
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<td>Bernal et al. 2013</td>
<td>Retrospective</td>
<td>20 Patients with hematologic malignancies who had received or were at risk for IA</td>
<td>Real time PCR</td>
<td>Whole blood</td>
<td>ITS1, 5.8S rDNA, 28S rRNA, 18S rDNA, rRNA gene</td>
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<td>Lewis et al. 2013</td>
<td>Prospective</td>
<td>127 patients at risk for IA</td>
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<td>White et al. 2005</td>
<td>Prospective</td>
<td>77 hematologic malignancy patients or solid organ transplant recipients at high risk for IA</td>
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<td>Badiee et al. 2008</td>
<td>Prospective</td>
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<td>Whole blood</td>
<td>ITS1, 5.8S rDNA, 28S rRNA, 18S rDNA, rRNA gene</td>
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<td>Botterel et al. 2008</td>
<td>Prospective</td>
<td>29 adults and 36 children with febrile neutropenia, undergoing intensive chemotherapy</td>
<td>Real time PCR</td>
<td>Whole blood</td>
<td>ITS1, 5.8S rDNA, 28S rRNA, 18S rDNA, rRNA gene</td>
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<td>Badiee et al. 2003</td>
<td>Prospective</td>
<td>21 patients with hematologic malignancies suffering from fever and 21 controls</td>
<td>Real time PCR</td>
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<td>ITS1, 5.8S rDNA, 28S rRNA, 18S rDNA, rRNA gene</td>
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<td>Barnes et al. 2008</td>
<td>Prospective</td>
<td>125 hematology patients</td>
<td>Real time PCR</td>
<td>Whole blood</td>
<td>ITS1, 5.8S rDNA, 28S rRNA, 18S rDNA, rRNA gene</td>
<td>5</td>
<td>125</td>
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<tr>
<td>Suarez et al. 2008</td>
<td>Prospective</td>
<td>172 patients who received high dose chemotherapy</td>
<td>Real time PCR</td>
<td>Whole blood</td>
<td>ITS1, 5.8S rDNA, 28S rRNA, 18S rDNA, rRNA gene</td>
<td>15</td>
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<tr>
<td>Millon et al. 2005</td>
<td>Retrospective</td>
<td>54 patients with cancer and pulmonary infiltrates</td>
<td>Real time PCR</td>
<td>Whole blood</td>
<td>ITS1, 5.8S rDNA, 28S rRNA, 18S rDNA, rRNA gene</td>
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<td>Scotter et al. 2005</td>
<td>Prospective</td>
<td>12 patients with hematologic malignancies suffering from fever, 4 with normal temperatures and 10 healthy volunteers</td>
<td>Real time PCR</td>
<td>Whole blood</td>
<td>ITS1, 5.8S rDNA, 28S rRNA, 18S rDNA, rRNA gene</td>
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<tr>
<td>Kawazu et al. 2004</td>
<td>Prospective</td>
<td>20 Patients with hematologic malignancies who had received or were at risk for IA</td>
<td>Real time PCR</td>
<td>Whole blood</td>
<td>ITS1, 5.8S rDNA, 28S rRNA, 18S rDNA, rRNA gene</td>
<td>2</td>
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<tr>
<td>Pham et al. 2003</td>
<td>Prospective</td>
<td>15 children with hematologic malignancies who had received or were at risk for IA</td>
<td>Real time PCR</td>
<td>Whole blood</td>
<td>ITS1, 5.8S rDNA, 28S rRNA, 18S rDNA, rRNA gene</td>
<td>2</td>
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<tr>
<td>Pryce et al. 2003</td>
<td>Prospective</td>
<td>36 children with hematologic malignancies who had received or were at risk for IA</td>
<td>Real time PCR</td>
<td>Whole blood</td>
<td>ITS1, 5.8S rDNA, 28S rRNA, 18S rDNA, rRNA gene</td>
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<td>Costa et al. 2002</td>
<td>Retrospective</td>
<td>14 patients with hematologic malignancies who had received or were at risk for IA</td>
<td>Real time PCR</td>
<td>Whole blood</td>
<td>ITS1, 5.8S rDNA, 28S rRNA, 18S rDNA, rRNA gene</td>
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<td>Ferns et al. 2002</td>
<td>Prospective</td>
<td>32 patients with hematologic malignancies suffering from fever and 32 controls</td>
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<td>Whole blood</td>
<td>ITS1, 5.8S rDNA, 28S rRNA, 18S rDNA, rRNA gene</td>
<td>1</td>
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<tr>
<td>Bucheidt et al. 2001</td>
<td>Prospective</td>
<td>29 adults and 36 children with febrile neutropenia, undergoing intensive chemotherapy</td>
<td>Real time PCR</td>
<td>Whole blood</td>
<td>ITS1, 5.8S rDNA, 28S rRNA, 18S rDNA, rRNA gene</td>
<td>1</td>
<td>65</td>
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</tbody>
</table>

AML: Acute myeloid leukemia; GM: Galactomannan; IA: Invasive aspergillosis; IFI: Invasive fungal infection; PCR: Polymerase chain reaction
<p>| Study | Random patient selection | Case control design | Inappropriate exclusions | Test results without knowledge of standard | Prespecified threshold | Reference standard interpreted without knowledge of index test | Reference standard likely to correctly classify condition | Appropriate interval between index test and reference standard | Did all patients receive a reference standard | Was the reference standard the same for all patients | Were all patients included in the analysis | Are there concerns that the included patients do not match the review question | Are there concerns that the target condition as defined by the reference standard does not match the review question | Are there concerns that the index test differs from the review question |
|-------|--------------------------|---------------------|--------------------------|---------------------------------|------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Halliday et al. 2006 | yes | no | no | yes | yes | yes | yes | yes | yes | yes | yes | yes | low | yes | yes |
| badiee et al 2008 | unclear | no | unclear | unclear | yes | yes | yes | unclear | yes | yes | yes | low | yes | yes |
| Hummel et al. 2010 | yes | no | no | yes | yes | yes | yes | yes | yes | yes | yes | low | yes | yes |
| White et al. 2006 | yes | no | no | unclear | yes | yes | yes | yes | yes | yes | yes | low | yes | yes |
| Cesaro et al. 2008 | yes | no | no | no | yes | yes | yes | yes | yes | yes | yes | low | yes | yes |
| Ramirez et al. 2008 | yes | no | no | unclear | yes | yes | yes | yes | yes | yes | yes | low | yes | yes |
| badiee et al 2010 | yes | no | no | yes | yes | yes | yes | yes | yes | yes | yes | low | yes | yes |</p>
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<tr>
<th>Journal</th>
<th>Year</th>
<th>Included</th>
<th>Notes</th>
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<tr>
<td>Springer et al.</td>
<td>2012</td>
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<td>White et al.</td>
<td>2013</td>
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<tr>
<td>El-Mahallawy et al.</td>
<td>2006</td>
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<td>no</td>
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<td>Jordanides et al.</td>
<td>2005</td>
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<tr>
<td>Pryce et al.</td>
<td>2003</td>
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<td>no</td>
</tr>
<tr>
<td>White et al.</td>
<td>2005</td>
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<td>no</td>
</tr>
<tr>
<td>Scotter et al.</td>
<td>2005</td>
<td>yes</td>
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</tr>
<tr>
<td>Ferns et al.</td>
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<td>no</td>
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<td>Barnes et al.</td>
<td>2008</td>
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<td>Nabili et al.</td>
<td>2013</td>
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<td>Lopes Da Silva et al.</td>
<td>2010</td>
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<td>Badiee et al.</td>
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<td>Costa et al.</td>
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<td>Kawazu et al.</td>
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<td>Millon et al.</td>
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<td>Botterel et al.</td>
<td>2008</td>
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<td>Suarez et al.</td>
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<tr>
<td>Millon et al.</td>
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<td>Lewis White et al.</td>
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<td>Bernal-Martinez et al.</td>
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<td>Schwartzinger et al.</td>
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<td>Pham et al.</td>
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<td>no</td>
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<tr>
<td>El-Mahallawy et al.</td>
<td>2006</td>
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<td>no</td>
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<td>Table 3. Results of subgroup analysis</td>
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<td>--------------------------------------</td>
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<tr>
<td>At least one positive</td>
<td>Sensitivity (95%CI)</td>
<td>Specificity (95%CI)</td>
<td>LR+/LR-</td>
</tr>
<tr>
<td>All studies (26)</td>
<td>0.84 (0.75-0.91)</td>
<td>0.76 (0.65-0.84)</td>
<td>3.5/0.21</td>
</tr>
<tr>
<td><strong>Criteria</strong></td>
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<tr>
<td>2008 (10)</td>
<td>0.83 (0.72-0.90)</td>
<td>0.79 (0.67-0.87)</td>
<td>3.9/0.22</td>
</tr>
<tr>
<td>2002 (16)</td>
<td>0.85 (0.69-0.93)</td>
<td>0.74 (0.57-0.86)</td>
<td>3.2/0.21</td>
</tr>
<tr>
<td><strong>Setting</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Prospective (20)</td>
<td>0.86 (0.77-0.92)</td>
<td>0.76 (0.63-0.85)</td>
<td>3.5/0.18</td>
</tr>
<tr>
<td>Retrospective (6)</td>
<td>0.76 (0.49-0.91)</td>
<td>0.77 (0.55-0.90)</td>
<td>3.2/0.31</td>
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<tr>
<td><strong>Specimen</strong></td>
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<tr>
<td>Serum (6)</td>
<td>0.78 (0.69-0.85)</td>
<td>0.85 (0.70-0.93)</td>
<td>5.1/0.25</td>
</tr>
<tr>
<td>Whole Blood (10)</td>
<td>0.86 (0.73-0.93)</td>
<td>0.73 (0.59-0.83)</td>
<td>3.1/0.19</td>
</tr>
<tr>
<td><strong>Elution volume</strong></td>
<td></td>
<td></td>
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<tr>
<td>&lt;=100 (13)</td>
<td>0.86 (0.76-0.93)</td>
<td>0.72 (0.59-0.82)</td>
<td>3.1/0.19</td>
</tr>
<tr>
<td>&gt;100 (8)</td>
<td>0.82 (0.54-0.95)</td>
<td>0.75 (0.46-0.91)</td>
<td>3.3/0.24</td>
</tr>
<tr>
<td><strong>Sample volume</strong></td>
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<td></td>
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<tr>
<td>Large/&gt;=3 WB/&gt;=0.5 serum (18)</td>
<td>0.83 (0.70-0.91)</td>
<td>0.79 (0.68-0.87)</td>
<td>4.0/0.21</td>
</tr>
<tr>
<td>Small/&lt;3/&lt;0.5 (7)</td>
<td>0.89 (0.77-0.95)</td>
<td>0.64 (0.38-0.83)</td>
<td>2.4/0.17</td>
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<tr>
<td><strong>Internal Control</strong></td>
<td></td>
<td></td>
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<tr>
<td>Yes (19)</td>
<td>0.84 (0.73-0.91)</td>
<td>0.76 (0.63-0.85)</td>
<td>3.4/0.21</td>
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<tr>
<td>No (7)</td>
<td>0.79 (0.67-0.87)</td>
<td>0.77 (0.56-0.90)</td>
<td>3.4/0.27</td>
</tr>
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<td><strong>Type of Primer</strong></td>
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<td>18S (16)</td>
<td>0.84 (0.69-0.93)</td>
<td>0.80 (0.67-0.89)</td>
<td>4.3/0.19</td>
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<td>Other ribosomal (7)</td>
<td>0.85 (0.70-0.93)</td>
<td>0.74 (0.57-0.86)</td>
<td>3.3/0.20</td>
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<tr>
<td><strong>Beadbeating (for Whole Blood only)</strong></td>
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<tr>
<td>Yes (8)†</td>
<td>0.78 (0.62-0.89)</td>
<td>0.82 (0.64-0.92)</td>
<td>4.3/0.27</td>
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<td>0-1 deviate (10)</td>
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<td>0.77 (0.60-0.88)</td>
<td>3.8/0.17</td>
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<td>&gt;1 deviate (16)</td>
<td>0.82 (0.68-0.90)</td>
<td>0.75 (0.60-0.86)</td>
<td>3.3/0.24</td>
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All subgroup comparisons not significant.
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<th>Table 4. Results of sensitivity analysis</th>
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<td>At least two positive</td>
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<tr>
<td>All studies (13)</td>
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<td><strong>Criteria</strong></td>
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<td>2008 (5)</td>
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<td>2002 (8)</td>
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<td><strong>Setting</strong></td>
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<tr>
<td>Prospective (11)</td>
</tr>
<tr>
<td>Retrospective (2)</td>
</tr>
<tr>
<td><strong>Specimen</strong></td>
</tr>
<tr>
<td>Serum (1)</td>
</tr>
<tr>
<td>Whole Blood (12)</td>
</tr>
<tr>
<td><strong>Elution volume</strong></td>
</tr>
<tr>
<td>&lt;=100 (5)</td>
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<tr>
<td>&gt;100 (6)</td>
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<tr>
<td><strong>Sample volume</strong></td>
</tr>
<tr>
<td>Large&gt;=3 WB/&gt;=0.5 serum (8)</td>
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<tr>
<td>Small&lt;3/&lt;0.5 (5)</td>
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<td><strong>Internal Control</strong></td>
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<tr>
<td>Yes (11)</td>
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<td>No (2)</td>
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<tr>
<td><strong>Type of Primer</strong></td>
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<tr>
<td>18S (8)</td>
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<td>Other ribosomal (5)</td>
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<tr>
<td><strong>Beadbeating</strong></td>
</tr>
<tr>
<td>(for Whole Blood only)</td>
</tr>
<tr>
<td>Yes (6)†</td>
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<tr>
<td>No (6)</td>
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<tr>
<td><strong>Criteria Compliant</strong></td>
</tr>
<tr>
<td>0-1 deviate (7)‡</td>
</tr>
<tr>
<td>&gt;1 deviate (6)</td>
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</table>

†average specificity significantly improves for beadbeating (p=0.006). ‡average specificity significantly improves for the more compliant studies (p=0.003). All other subgroup comparisons not significant. n/a=not applicable (two few studies to pool).