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Galactomannan detection in broncho-alveolar lavage fluid for invasive aspergillosis in immunocompromised patients (Review)

de Heer K, Gerritsen MG, Visser CE, Leeflang MMG

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[Diagnostic Test Accuracy Review]

Galactomannan detection in broncho-alveolar lavage fluid for invasive aspergillosis in immunocompromised patients

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ABSTRACT

Background

Invasive aspergillosis (IA) is a life-threatening opportunistic mycosis that occurs in some people with a compromised immune system. The serum galactomannan enzyme-linked immunosorbent assay (ELISA) rapidly gained widespread acceptance as part of the diagnostic work-up of a patient suspected of IA. Due to its non-invasive nature, it can be used as a routine screening test. The ELISA can also be performed on bronchoalveolar lavage (BAL), allowing sampling of the immediate vicinity of the infection. The invasive nature of acquiring BAL, however, changes the role of the galactomannan test significantly, for example by precluding its use as a routine screening test.

Objectives

To assess the diagnostic accuracy of galactomannan detection in BAL for the diagnosis of IA in people who are immunocompromised, at different cut-off values for test positivity, in accordance with the [Cochrane Diagnostic Test Accuracy Handbook](#).

Search methods

We searched three bibliographic databases including MEDLINE on 9 September 2016 for aspergillosis and galactomannan as text words and subject headings where appropriate. We checked reference lists of included studies for additional studies.

Selection criteria

We included cohort studies that examined the accuracy of BAL galactomannan for the diagnosis of IA in immunocompromised patients if they used the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) classification as reference standard.

Data collection and analysis

Two review authors assessed study quality and extracted data. Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) was used for quality assessment.

Main results

We included 17 studies in our review. All studies except one had a high risk of bias in two or more domains. The diagnostic performance of an optical density index (ODI) of 0.5 as cut-off value was reported in 12 studies (with 1123 patients). The estimated sensitivity was 0.88 (95% confidence interval (CI) 0.75 to 1.00) and specificity 0.81 (95% CI 0.71 to 0.91). The performance of an ODI of 1.0 as cut-off value could

be determined in 11 studies (with 648 patients). The sensitivity was 0.78 (95% CI 0.61 to 0.95) and specificity 0.93 (95% CI 0.87 to 0.98). At a cut-off ODI of 1.5 or higher, the heterogeneity in specificity decreased significantly and was invariably >90%.

Authors' conclusions

The optimal cut-off value depends on the local incidence and clinical pathway. At a prevalence of 12% a hypothetical population of 1000 patients will consist of 120 patients with IA. At a cut-off value of 0.5 14 patients with IA will be missed and there will be 167 patients incorrectly diagnosed with IA. If we use the test at a cut-off value of 1.0, we will miss 26 patients with IA. And there will be 62 patients incorrectly diagnosed with invasive aspergillosis. The populations and results were very heterogeneous. Therefore, interpretation and extrapolation of these results has to be performed with caution. A test result of 1.5 ODI or higher appears a strong indicator of IA.

PLAIN LANGUAGE SUMMARY

Measuring galactomannan in lung-washing fluid to diagnose invasive aspergillosis in patients with an impaired immune system

Why is improving the accuracy of the diagnosis of invasive fungal disease important?

Some people have a weakened immune system; they are said to be 'immunocompromised'. People can be immunocompromised when they have leukaemia, or HIV/AIDS, and when using some forms of medication.

Immunocompromised means that the immune system is less able to fight infections. This can result in an infection that does not occur under normal conditions. This is called an opportunistic infection. An example is aspergillosis, an infection caused by a fungus. As the fungal infection is not stopped by the immune system, it spreads through the body. The fungus 'invades' the body - hence we call this invasive aspergillosis. This may be lethal. Early diagnosis allows timely treatment and stops the fungus from further invading the body.

What diagnostic test was studied in the review?

We wanted to find out how good 'galactomannan detection' is for diagnosing an infection of invasive aspergillosis.

Galactomannan detection is a diagnostic procedure. It works by passing an instrument through the mouth or nose into the airways. Fluid is flushed into a part of the lung. This fluid washes fungi and other microbes from the lungs and is collected and analysed. This process is called bronchoalveolar lavage (lung-washing), shortened to BAL. One of the things that may be in the collected BAL fluid is galactomannan, a component of the fungal cell wall.

The result of the BAL galactomannan test is part of international criteria used to differentiate between patients who are likely to have invasive aspergillosis and patients who are less likely to have invasive aspergillosis. If multiple criteria are fulfilled (e.g. imaging tests and clinical signs and symptoms), then the patients will be treated with antifungal medication.

What is the aim of this review?

The aim of this review was to determine the error rate of galactomannan testing on BAL. To this end, we searched for studies on this subject. The galactomannan test results in an 'optical density index' (ODI), not a yes or no answer. The studies differed with respect to the cut-off value above which a result was considered positive. Most considered a result above an ODI of 0.5 or 1.0 as positive.

What are the main results of this review?

Imagine a group of 1000 people with an impaired immune system where 120 (12%) have invasive aspergillosis. The results of this review indicate that in theory, if the BAL galactomannan test were to be used using an ODI of 0.5 or higher to decide which results are positive:

- an estimated 273 will have a test result indicating that they probably have invasive aspergillosis, and of these 167 will be incorrectly classified as having invasive aspergillosis; and
- of the 727 people with a result indicating that invasive aspergillosis is not likely to be present, 14 will be incorrectly classified as not having invasive aspergillosis.

If a higher cut-off value is used to determine test positivity, then more patients will have a false-negative result and fewer patients will have a false-positive result.

How reliable are the results of the studies in this review?

In the included studies, the diagnosis of invasive aspergillosis was made by assessing all patients with international criteria (known as the reference standard). This is likely to have been a reliable method for deciding whether patients really had invasive aspergillosis. The results of the various studies differed and had small numbers of patients with invasive aspergillosis. Therefore, the numbers are an average across the studies in the review and we cannot be sure that the test will always produce these results.

Who do the results of this review apply to?

The results are mainly applicable to adults who undergo intensive chemotherapy or a stem cell transplant for a form of cancer of the blood cells.

How up-to-date is this review?

The review authors searched for and used studies published up to 9 September 2016.

SUMMARY OF FINDINGS

Summary of findings 1. Summary of findings table

What is the diagnostic accuracy of the Platelia© *Aspergillus* ELISA for invasive aspergillosis in immunocompromised patients?

Patients/population: immunocompromised patients, mostly neutropenic haematology patients.

Prior testing: varied.

Index test: the Platelia© ELISA, a sandwich ELISA for galactomannan, an *Aspergillus* antigen.

Importance: currently regarded as the most accurate test to diagnose invasive aspergillosis.

Reference standard: the EORTC/MSG criteria.

Studies: cohort studies reporting all EORTC-MSG categories and of which the 2 × 2 or 4 × 2 table contained not more than one index test per participant.

Cut-off value	No. of participants	Summary estimates (95% CI)	Implications	Quality and Comments
0.5 ODI (12 studies)	229 with proven or probable IA	Sensitivity: 0.88 (95% CI 0.75 to 1.00)	At a prevalence of 12%, 120 out of 1000 patients will develop proven or probable IA. Of these, 14 will be missed.	All studies except one had a high risk of bias in two or more domains. For all studies the concerns regarding applicability were low.
	894 with possible or no IA	Specificity: 0.81 (95% CI 0.71 to 0.91)	Of the 880 patients with possible or no IA, 167 will have a false-positive test result and may be unnecessarily treated. Of all 273 patients with a positive test, 106 will indeed have proven or probable IA; of all 727 negative tests, 14 will have IA after all (2%).	
1.0 ODI (11 studies)	177 with proven or probable IA	Sensitivity: 0.78 (95% CI 0.61 to 0.95)	At a prevalence of 12%, 120 out of 1000 patients will develop proven or probable IA. Of these, 26 will be missed.	
	594 with possible or no IA	Specificity: 0.93 (95% CI 0.87 to 0.98)	Of the 880 patients with possible or no IA, 62 will have a false-positive test result and may be unnecessarily treated. Of all 156 patients with a positive test, 94 will indeed have proven or probable IA; of all 844 negative tests, 26 will have IA after all (3%).	

Note, the populations and results were very heterogeneous. Therefore, interpretation and extrapolation of these results has to be performed with caution.

CI: confidence interval; **IA:** invasive aspergillosis; **ODI:** optical density index

BACKGROUND

Target condition being diagnosed

Although it has been suggested that the mortality rate has decreased over recent years due to more effective therapy (Kwon 2012; Sun 2017), invasive aspergillosis (IA) is a life-threatening opportunistic mycosis. Published mortality rates range from 30% to 70% (Aisner 1977; Robenshtok 2007; von Eiff 1995). Invasive aspergillosis is caused by ubiquitous spores of *Aspergillus* species, inhaled by everyone on a daily basis, which can cause invasive disease in the case of a failing immune system (Chotirmall 2013). As IA mainly occurs in people with immune deficiency, the incidence varies depending on the degree of immune deficiency. In people who are highly immune deficient, such as people with acute myeloid leukaemia (AML) receiving highly intensive chemotherapy, the incidence during their treatment can exceed 5% (Robenshtok 2007). Mainly because of the increasing application of immunosuppressive treatments, especially highly intensive chemotherapy, prolonged corticosteroid therapy and stem cell transplantation (Kaneko 2001; Rubio 2009), the incidence of IA is increasing. Most often the lungs or airways (or both) are the site of infection. But IA can also occur in the nasal sinus, central nervous system, skin, gastro-intestinal tract, the deep structures of the eye, and prosthetic heart valves; or may give rise to a disseminated infection.

Index test(s)

Galactomannan is a cell wall component of *Aspergillus* species and *Penicillium* species (Latgé 1994). It is excreted by the fungus during the growth phase. It is suggested that the level of galactomannan antigenaemia is proportional to the fungal tissue load and has a prognostic value (Koo 2010).

Currently, galactomannan detection in bronchoalveolar lavage (BAL), usually performed as a result of a chest computerised tomography (CT) scan consistent with IA, is considered the most accurate test to diagnose or exclude IA. A meta-analysis in 2010 reported a sensitivity of 90% and a specificity of 94% (Guo 2010).

Currently, there is one commercially available assay for the detection of galactomannan in BAL, the Platelia™ sandwich enzyme-linked immunosorbent assay (ELISA) test. It can also be applied on serum, cerebrospinal fluid, pleural fluid and urine (Ascioglu 2001; Hope 2005). Results of the ELISA are given as an optical density index (ODI), which is the ratio of the optical density of (usually) 1 ng/mL galactomannan versus the optical density of the sample. In order to enhance the specificity of the test, the Food and Drug Administration (FDA) changed the recommended cut-off for positivity in the USA from an ODI of 0.5 to 1.0 (U. S. Food and Drug Administration 2011).

Clinical pathway

Indication

There is no generally accepted indication for galactomannan testing in BAL, and no generally accepted consequence of a specific test result.

Clinical signs and symptoms of IA are non-specific at an early stage. Establishing a diagnosis at an early stage of infection improves the chances of survival through subsequent earlier treatment (Upton

2007; von Eiff 1995). A definitive diagnosis can be made by two means. By autopsy, or "by a positive culture of *A.fumigatus* in a specimen obtained with a sterile procedure from a normally sterile site that is clinically or radiologically abnormal and consistent with an infectious disease process (excluding bronchoalveolar lavage fluid, a cranial sinus cavity specimen, and urine)" (De Pauw 2008). However, the person's clinical condition (frequently including thrombocytopenia (reduced numbers of platelets) because of preceding chemotherapy) often prohibits invasive techniques. Cultures are frequently false-negative. In fact, definitive diagnoses of IA are rare. In clinical practice one usually has to work with presumed diagnoses with a varying level of suspicion. In 2008, a committee consisting of the Invasive Fungal Infections Cooperative Group of the European Organization for Research and Treatment of Cancer (EORTC), the Mycoses Study Group (MSG) and the National Institute of Allergy and Infectious Diseases proposed to grade the diagnosis of IA with three levels of probability: 'proven', 'probable' and 'possible' IA (see Table 1; De Pauw 2008). There is also a fourth category of people without IA. The divisions are based on host factor criteria (a set of risk factors, which are designed to almost preclude IA if all are absent, an example being chemotherapy-induced prolonged neutropenia, clinical criteria (specific clinical and radiological signs such as a chest CT consistent with IA), and microbiological criteria (for example, *Aspergillus* species cultured from BAL). This consensus was a revision of a consensus reached in 2002, see Table 2. A positive galactomannan test, whichever fluid it is applied on, is a microbiological criterion in both versions. Clinical studies have shown that these criteria do not match autopsy results perfectly. This holds especially true for the 'possible' category. For clinical trials investigating the effect of treatment of IA, it is recommended that only people in 'proven' or 'probable' categories are considered to have IA (Borlenghi 2007; Subirà 2003).

Because of its invasiveness, the test is usually employed in the clinical pathway of a **person at risk of IA, who is also suspected of having IA based on clinical and/or radiological grounds**. The test is rarely used as a screening test in asymptomatic persons, whether they are at risk or not. In the setting of chemotherapy-induced prolonged neutropenia, the host factor that causes the majority of cases of IA, a full work-up is performed in most centres as a result of a number of indications, including most notably: fever not responsive to broad spectrum antibiotics, a positive galactomannan test on serum, or hyphae or moulds found in a respiratory specimen. This work-up will include a chest CT. In case it is consistent with IA, a BAL and the index test are performed, if the clinical condition permits.

Clinical consequence

The consequences of a positive or negative index test vary among hospitals and will usually depend on the EORTC/MSG classification after the full diagnostic work-up.

Despite a negative index test, a person can still be classified as having 'probable' IA (based on another microbiological criterion) or as having 'proven' IA. Also, he or she can still be classified as having 'possible' IA due to a clinical criterion. As BAL is usually performed because of a chest CT consistent with IA (a clinical criterion) in a patient at risk of IA (a host criterion), a classification of 'no' IA will not occur very frequently. For the same reason, in case of a positive index test a person will often be classified as having 'probable' IA.

Someone with 'probable' IA will usually be considered to have IA and treated accordingly. Although it is very well possible that a person classified as having 'possible' IA in fact has 'no' IA, the likelihood rises, and in light of the high mortality many clinicians will also prefer to treat such a person for IA. So it could seem the test has no clinical consequence. However, the duration of therapy with expensive and toxic antimycotics is usually longer in probable aspergillosis than in possible aspergillosis. It can also influence the choice of antimycotic, as the odds of an invasive mould infection other than aspergillosis decrease with a positive test result. Moreover, in case of side effects it is more likely a clinician would continue therapy in a patient with probable aspergillosis. Moreover, further immunosuppressive treatment such as chemotherapy or stem cell transplantation, which is often essential in curing a patient, is contra-indicated in people with IA.

Risk factors

There are other risk factors for IA besides chemotherapy-induced prolonged neutropenia that increasingly warrant the index test. Most notable are:

- allogeneic stem cell transplantation and/or graft-versus-host disease;
- intensive care unit (ICU) admittance;
- chronic pulmonary disease, such as chronic obstructive pulmonary disease (COPD) and bronchiectasis;
- acquired immune deficiency syndrome (AIDS);
- immunosuppressive drugs, such as tumour necrosis factor- α (TNF- α) inhibitors;
- long-term steroid use;
- inherited severe immunodeficiency;
- decompensated cirrhosis; and
- influenza.

The 2002 version of the EORTC/MSG criteria also contained the following host criteria:

- fever refractory to broad-spectrum antibacterial treatment in high-risk patients; and
- 'proven' or 'probable' invasive fungal infection during previous episode of neutropenia.

Alternative test(s)

There are many alternative tests, such as direct microscopy, histopathological examination, microbiological culture, and less frequently performed: beta-D-glucan testing and various polymerase chain reactions (PCRs). These can be performed on a number of specimens, such as respiratory samples, serum, BAL or tissue samples. The galactomannan ELISA can also be applied on other fluids, such as cerebrospinal fluid (CSF), pleural fluid and urine. None has a reported sensitivity or specificity comparable with the index test (Cuenca-Estrella 2011; Guo 2010; Karageorgopoulos 2011; Lamoth 2012; Pickering 2005; Zou 2012). There are published Cochrane Reviews on the index test performed on serum (Leeflang 2015), and on PCR blood tests (Cruciani 2015).

Rationale

There are already a number of meta-analyses on the diagnostic value of BAL galactomannan. However, the fact that the index test plays an important role in the set of criteria that forms the

reference standard confers a difficulty in interpreting such analyses. Our meta-analysis adds an evaluation of this and other sources of heterogeneity to the previous meta-analyses.

OBJECTIVES

To assess the diagnostic accuracy of galactomannan detection in bronchoalveolar lavage (BAL) for the diagnosis of invasive aspergillosis (IA) in people who are immunocompromised, at different cut-off values for test positivity, in accordance with the [Cochrane Diagnostic Test Accuracy Handbook](#).

Secondary objectives

Our secondary objective was to study several possible sources of heterogeneity:

- the effect of cut-off value,
- the effect of incorporation bias (the incorporation of the index test in the reference standard),
- the effect of pre-selection based on a positive serum galactomannan test,
- the effect of the revision of the reference standard,
- the effect of a protocol that defines the exact and explicit indication of the index test,
- study design,
- the effect of sponsoring of the study by the manufacturer of the commercially available galactomannan test,
- clinical subgroups.

See also '[Investigations of heterogeneity](#)'.

METHODS

Criteria for considering studies for this review

Types of studies

Eligible studies were those that assessed the diagnostic accuracy of galactomannan detection in bronchoalveolar lavage (BAL). The galactomannan enzyme-linked immunosorbent assay (ELISA) could be assessed alone or in comparison with (an)other test(s).

Participants

Immunocompromised participants who have been tested to diagnose, exclude or screen for invasive aspergillosis (IA) using galactomannan detection in BAL. We defined immunocompromised as the presence of one of the EORTC/MSG host criteria, either of the 2002 or 2008 version. The current version of the EORTC/MSG criteria was anticipated to be updated with two extra host criteria: chronic airway abnormalities and decompensated cirrhosis. We also included studies on participants with immune deficiencies as per the anticipated version of the EORTC/MSG criteria. If in more than 10% of the participants a EORTC/MSG host criterion was not present, a study was excluded.

Index tests

The commercially available galactomannan sandwich ELISA (Platelia™) performed on BAL was the test under evaluation. Studies addressing detection in serum and other body fluids were excluded.

Target conditions

The target condition of this review was invasive pulmonary aspergillosis.

Reference standards

The following reference standards were used to define the target condition:

- autopsy specimens, with histopathological evidence of aspergillosis and a positive culture;
- the EORTC/MSG criteria, see [Table 3](#) and [Table 4](#) for the criteria in full detail.

The results within each possible EORTC classification ('no', 'possible', 'probable' and 'proven' IA) had to be reported, or the results within the combined groups 'no/possible' and 'probable/proven' IA.

Search methods for identification of studies

Electronic searches

We searched Embase and MEDLINE through Ovid for aspergillosis and galactomannan as Medical Heading and text word, see [Appendix 1](#) for the exact terms. The appendix also lists the terms that were used to search the Web of Science Core Collection (1970 to date of search). The version of MEDLINE we searched was Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily and Ovid MEDLINE(R) 1946 to 9th September 2016. We searched the literature from 2000 onwards, as the reference standard was published in 2002.

We conducted the searches on 9 September 2016. All studies published after the date of the initial search will be included in the next update of the systematic review. The search for the update is planned for September 2021.

Searching other resources

To identify additional published studies, we:

- entered the included studies into PubMed and then used the Related Articles feature;
- searched the Science Citation Index to identify articles that cite the included studies;
- checked the reference lists of all included studies.

Data collection and analysis

Selection of studies

The first selection, based on title and abstract, was performed by two review authors (KdH, MG). We excluded articles that were judged to be highly unlikely to report the test performance of galactomannan detection in bronchoalveolar lavage, such as animal studies, plant studies, or studies on fungi other than *Aspergillus* species. Afterwards, we located the full paper of each potentially eligible article. Two of four review authors (KdH, MG, CV, ML) independently assessed all eligible articles for inclusion. We resolved disagreement by discussion. We included articles when we could not resolve disagreement.

We excluded studies:

- that were not published in an English peer-reviewed journal (we will include these in the first update of this review);
- that did not enrol patients consecutively;
- that were case-control studies;
- of which the 2×2 or 4×2 table contained more than one index test per participant;
- that (partially) contained the data of another study/publication;
- that did not report all index test results of the studied cohort, e.g. a study that does not report the results of the 'possible' IA and/or 'no' IA group;
- that contained an ostensible calculation error in the 2×2 or 4×2 table.

We contacted the study authors for more information before excluding studies on the last two grounds.

Data extraction and management

The following data were extracted:

- author, year of publication and journal;
- our application of the QUADAS-2 tool, see [Appendix 2](#), for which a data-extraction form and background document had been designed;
- the data for a 4×2 or 2×2 table.

The data-extraction form was accompanied by a background document that stated how each item on the form was to be interpreted. We standardised the form and piloted it on two studies. Four review authors (KdH, MG, CV, ML) extracted data and assessed quality. One review author (KdH) has a haematological background, one (MG) a medical background, one (CV) a microbiological background, and one (ML) a methodological background. The articles were randomly allocated to a pair of review authors. Disagreements were resolved by discussion.

Assessment of methodological quality

Study quality was assessed using the QUADAS-2 data-extraction form. For four domains a number of pre-defined signalling questions were scored as 'yes', 'no' or 'unclear', or had to be answered with free text. Then, per domain the risk of bias was judged to be either 'low', 'high' or 'unclear', based on the answers to these signalling questions ([Whiting 2011](#)). Results are presented in text, in a graph and in a table. We did not calculate a summary score estimating the overall quality of an article since the interpretation of such summary scores is problematic and potentially misleading ([Jüni 1999](#); [Whiting 2005](#)). We did not assess the concern about applicability for the patient domain, as we did not have a specific population of interest in mind and expected to find studies with a wide range of patients. After all, one cannot say that in a study on lung transplant patients (in which, quite understandably, the diagnostic accuracy is very different from the diagnostic accuracy which is found in a study on allogeneic stem cell transplantation patients) there is a problem with applicability. However, the results of the study are not applicable to patients undergoing allogeneic stem cell transplantation. Instead, we chose to carefully document the type of participants. For the index test and reference standard domains, we classified all studies to have a low applicability concern because our inclusion criteria stipulated that we only include studies that used the galactomannan ELISA performed on BAL as the index test and the EORTC/MSG criteria as reference

standard. Therefore no studies that were subject to applicability concerns on these two domains were included.

Statistical analysis and data synthesis

The EORTC/MSG criteria were our reference standards. They classify participants into one of four groups: 'proven' IA, 'probable' IA, 'possible' IA and 'no' IA. This results in a two-by-four table: a positive or negative galactomannan test result versus each of the four reference groups. To calculate test accuracy, we defined the 'proven' and 'probable' participants as having IA; and we defined 'no' IA and 'possible' IA participants as not having IA, in order to construct two-by-two tables. Other classifications of participants having and not having IA were assessed in a sensitivity analysis.

We calculated the sensitivity and specificity for each study per reported cut-off value. We also plotted the individual study results in forest plots and the receiver operating characteristic (ROC) space.

We used a bivariate random-effects approach for a meta-analysis of the pairs of sensitivity and specificity (Reitsma 2005; [Cochrane Diagnostic Test Accuracy Handbook](#)). Covariates were incorporated in the bivariate model to examine the effect of potential sources of bias and variation across subgroups of studies. Due to the bivariate nature of the model, effects of covariates on sensitivity and specificity could be modelled separately.

For the meta-analysis we used the two-by-two table of one cut-off value per study. Otherwise, the more cut-off values a study reports, the larger its impact on the outcome of the analysis becomes. We chose the thresholds of 0.5 or 1.0, if reported, because these are the positivity thresholds currently and formerly recommended. We expected most studies in our analysis to report either an optical density index (ODI) of 1.0 or 0.5. If both were reported, we selected one at random. As the random inclusion could have had an effect on the outcome of the meta-analysis, we performed a sensitivity analysis by including the two-by-two tables of an ODI of 0.5 **as well as an ODI of 1.0**, if both were reported.

All analyses were performed in SAS, version 9.4, using Proc NLMIXED.

Investigations of heterogeneity

Heterogeneity was investigated first through visual examination of the forest plots and the raw ROC plot of all data in the review, at all cut-off values. Then, the potential sources of heterogeneity were included in the meta-analysis as a covariate. We assumed no interaction between the sources of heterogeneity. We addressed the following possible sources.

a. Effect of cut-off value

A main source of heterogeneity in diagnostic test accuracy reviews are the differences in the applied cut-off values between studies. We first investigated what the influence of these cut-off values was on the sensitivity and specificity by including the cut-off value as a covariate in the meta-analysis.

b. Effect of incorporation bias (incorporation of the index test in the reference standard)

A positive galactomannan test is incorporated in the EORTC/MSG criteria. Studies that do not exclude this criterion from the reference standard might overestimate the diagnostic accuracy of the index

test. Vice versa, a study that does exclude this criterion from the reference standard might underestimate the diagnostic accuracy. We studied the effect of incorporation of the BAL and serum galactomannan test, as these are dependent tests.

c. Effect of pre-selection based on a positive serum galactomannan test

The indication for the index test in a study, and if applicable the clinical pathway in which it is embedded, are major determinants of the accuracy of the index test. We expected many studies not to contain a clinical pathway and the triggers to vary greatly, or even to be documented unclearly. We extracted as much detail as possible on the indication for the index test, the associated clinical pathway and the tests performed prior to the index test, but we expected that analysis of heterogeneity on this form of bias would not be feasible.

We did expect all studies to report whether a positive serum galactomannan could have been the indication for the index test. We studied the effect of this possibility.

d. Effect of the reference standard

The criteria of the EORTC/MSG were our reference standard. We studied the effect of the 2008 update of these criteria.

e. Effect of a protocol that defines the exact and explicit indication of the index test

We studied the effect of the presence of a formal indication for performing the index test in the study protocol.

f. Study design

We defined a study that uses a data set not created for the purpose of a research question as retrospective. We defined a study that includes patients consecutively after the formulation of a research question as prospective. We studied the heterogeneity as an effect of study design.

g. Effect of sponsoring

We studied the effect of sponsoring of the study by the manufacturer of the commercially available galactomannan test.

h. Clinical subgroups

We explored the possible influence of clinical subgroups by including the following additional covariates in the meta-analysis.

- children versus adult participants — we assumed a cut-off at age 16 to 18 to differentiate between children and adults;
- participants with neutropenia versus all other participant groups; we also performed a subgroup analysis comparing groups based on the different host criteria;
- routine use of anti-mould prophylaxis in a study (yes versus no);
- the department(s) where the patients were recruited.

Sensitivity analyses

a. Methodological quality

To assess whether methodological quality influenced the results, we performed a sensitivity analysis by leaving out the studies with a relatively low methodological quality.

b. Effect of positive and negative reference standard definition

Although it is uncertain whether a participant with 'possible' IA has invasive aspergillosis or not, we considered a participant with 'possible' IA as having no invasive aspergillosis and we combined these participants with those with 'no' IA when calculating sensitivity and specificity. We acknowledge the methodological difficulty of this choice and therefore studied its effect in a sensitivity analysis. We also examined the effect of ignoring 'possible' IA ('proven' and 'probable' versus 'no' IA).

RESULTS

Results of the search

Our search was performed on 9 September 2016 and resulted in 5844 hits. The initial screening, excluding the studies that were judged beyond any doubt not to contain original data on the diagnostic value of bronchoalveolar lavage (BAL) galactomannan in diagnosing invasive aspergillosis (IA), or that were not published in a peer-reviewed journal, resulted in 103 eligible reports of which the full text was retrieved (Figure 1). We excluded two non-English reports from the assessment which were studies eligible for inclusion. We will assess the translated versions at the first update of this review.

Figure 1. Study flow diagram

We sent corresponding authors an email if we thought it possible that a study could be included with additional information or data, i.e. if not all index test results were reported or if the report contained an ostensible error. This resulted in sending 29 emails, from which we received 10 replies.

After considering these 103 reports:

- 33 reports were judged not to be a study containing data on the diagnostic value of BAL galactomannan in the diagnosis of IA, or were not published in a peer-reviewed journal, or were a case-control study,
- 23 reports were judged not to use the EORTC/MSG criteria without significant modification as reference standard,
- 28 reports were judged not to report all EORTC/MSG categories (proven, probable, possible, no IA),
- for 4 reports, we were unable to repeat the statistical calculations,
- 2 reports did not report the index test specifically (but for example, as a composite with another test such as serum galactomannan),
- 3 reports were judged to be a double publication,
- 3 reports did not report on a per-patient basis,
- 1 report did not use the Platelia galactomannan assay as the galactomannan test.

This led to the exclusion of 87 reports, as more criteria could be applicable per study, and the inclusion of 16 reports. As one report contained two methodologically different studies, in total 17 studies were included.

No extra studies were found through additional searches or reference checking. Two studies were included after the receipt of additional data per email.

The studies comprised 1634 patients in total. 'Probable' or 'proven' IA was diagnosed in 366 patients, of which 34 were specifically denoted as having 'proven' IA and 177 as having 'probable' IA. Of the remaining patients, 222 were specifically denoted as having 'possible' IA and 538 as having 'no' IA. There were 479 patients with a positive BAL galactomannan and 1155 with a negative BAL galactomannan. On average, 90.8 patients were included in a study (range 30-210).

Methodological quality of included studies

Of the 17 studies, 10 were judged to have a high risk of bias with respect to the domain of Patient Selection. These studies were unclear about whether they enrolled participants consecutively.

Sixteen studies were judged to have a high risk of bias regarding the Index Test domain. In these studies the index test was either interpreted with knowledge of the reference standard, or using a cut-off value that was not predefined, or this was not specified.

Based on the signalling questions in the Reference Standard domain, 15 studies were considered at a large risk of bias. In these studies either the reference standard was interpreted with knowledge of the index test, or the reference standard was not strictly adhered to (e.g., the presence of a host criterion was not confirmed), or the authors were not specific on these points.

Finally, with respect to the domain of Flow and Timing, 10 studies were considered to be associated with a high risk of containing bias. In these studies the review authors judged that there was (a high probability of) a significant exclusion of study participants from the analysis.

These data are summarised in [Figure 2](#) and [Figure 3](#).

Figure 2. Risk of bias and applicability concerns graph: review authors' judgements about each domain presented as percentages across included studies

Figure 3. Risk of bias and applicability concerns summary: review authors' judgements about each domain for each included study

	<u>Risk of Bias</u>				<u>Applicability Concerns</u>		
	Patient Selection	Index Test	Reference Standard	Flow and Timing	Patient Selection	Index Test	Reference Standard
Becker 2003 -- part I	⊖	⊖	⊖	⊖	⊕	⊕	⊕
Becker 2003 -- part II	⊕	⊖	⊖	⊕	⊕	⊕	⊕
Bergeron 2010	⊕	⊖	⊖	⊕	⊕	⊕	⊕
Brownback 2013	⊖	⊖	⊖	⊖	⊕	⊕	⊕
Clancy 2007	⊖	⊖	⊕	⊖	⊕	⊕	⊕
de Mol 2013	⊖	⊕	⊖	⊖	⊕	⊕	⊕
Fisher 2014	⊕	⊖	⊖	⊕	⊕	⊕	⊕
Frealle 2009	⊖	⊖	⊖	⊖	⊕	⊕	⊕
Heng 2014	⊕	⊖	⊖	⊕	⊕	⊕	⊕
Hoenigl 2014	⊖	⊖	⊖	⊖	⊕	⊕	⊕
Maertens 2009	⊖	⊖	⊖	⊖	⊕	⊕	⊕
Nguyen 2011	⊕	⊖	⊕	⊕	⊕	⊕	⊕
Pasqualotto 2010	⊖	⊖	⊖	⊖	⊕	⊕	⊕
Penack 2008	⊕	⊖	⊖	⊕	⊕	⊕	⊕
Prattes 2014	⊖	⊖	⊖	⊖	⊕	⊕	⊕
Prattes 2015	⊖	⊖	⊖	⊖	⊕	⊕	⊕
Rose 2014	⊕	⊖	⊖	⊕	⊕	⊕	⊕

High
 Unclear
 Low

Findings

Raw data

In seven studies both the 0.5 optical density index (ODI) and 1.0 ODI data were reported (see [Figure 4](#)). For three of these, we even had the individual patient data (IPD) available, allowing us

to investigate every possible cut-off value. The IPD of two studies were kindly sent to us by email. In five studies the diagnostic performance of an ODI of 0.5 was reported, but not of an ODI of 1.0. Four studies only reported the 1.0 ODI data but not the 0.5 ODI data. One study reported only data at an ODI of 0.8.

Figure 4. ROC plot; all studies reporting more than 1 cut-off value.

Thus, the diagnostic performance of an ODI of 0.5 was reported in 12 studies (with 1123 patients), with sensitivity ranging between 52.2% and 100% and specificity ranging between 40.4% and 94.3%. The performance of an ODI of 1.0 could be determined in 11 studies

(with 648 patients), with sensitivity ranging between 42.9% and 100% and specificity ranging between 75.6% and 100%.

There was considerable variation in the reported sensitivity and specificity (see [Figure 5](#) and [Figure 6](#)).

Figure 5. Forest plot of tests: 1 ODI 0.5, 2 ODI 0.8, 3 ODI 0.85 and 0.87, 4 ODI 1.0, 5 ODI 1.5, 6 ODI 2.0, 7 ODI 2.5.

ODI 0.5

ODI 0.8

ODI 0.85 and 0.87

ODI 1.0

ODI 1.5

ODI 2.0

Figure 5. (Continued)

Clancy 2007	5	5	0	71	1.00 [0.48, 1.00]	0.93 [0.85, 0.98]
Prattes 2014	14	3	5	67	0.74 [0.49, 0.91]	0.96 [0.88, 0.99]
Prattes 2015	2	2	5	63	0.29 [0.04, 0.71]	0.97 [0.89, 1.00]

ODI 2.5

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Clancy 2007	4	5	1	71	0.80 [0.28, 0.99]	0.93 [0.85, 0.98]
Prattes 2014	13	3	6	67	0.68 [0.43, 0.87]	0.96 [0.88, 0.99]
Prattes 2015	1	1	6	64	0.14 [0.00, 0.58]	0.98 [0.92, 1.00]

Figure 6. ROC Plot of all data in the review; all cut-off values.

Figure 5 shows the diagnostic accuracy estimates of the individual studies in tables and Forest plots. Figure 6 shows the summary ROC plot. In Figure 4, a ROC plot is shown containing all studies that reported the diagnostic accuracy statistics of more than one cut-off value.

The variation in the reported specificity might partially be explained by chance variation, because of the small number of patients with the target condition in the various studies, on average 23.0% with a minimum of 6.2%, a maximum of 45.3% and a

standard deviation of 12.4%. Above a cut-off ODI of 1.5, the heterogeneity in specificity decreased significantly and specificity was invariably > 90%. At an ODI of 1.5 or more the index test thus seems a reliable test to rule in the index disease (see Figure 5 and Figure 4).

Meta-analysis

We performed a meta-analysis of the pairs of sensitivity and specificity using a bivariate random-effects approach, with cut-

off as a covariate, see [Summary of findings 1](#) and [Figure 7](#). One included study did not report accuracy data at a cut-off ODI of 0.5 or 1.0 ([Heng 2014](#)). We did not foresee this possibility and did not

address this situation in our protocol. We chose to exclude this study from the analyses.

Figure 7. Plain language summary test consequence figure

At a cut-off level of 0.5, this resulted in an estimated sensitivity and specificity of 0.88 (95% confidence interval (CI): 0.75 to 1.00) and 0.81 (95% CI 0.71 to 0.91), respectively. The sensitivity for cut-off 1.0 was lower: 0.78 (95% CI 0.61 to 0.95), but this was not statistically

significant ($P = 0.36$). The specificity for cut-off 1.0 was higher than for cut-off 0.5: 0.93 (95% CI 0.87 to 0.98), which was statistically significant. These summary estimates and confidence regions are graphically shown in [Figure 8](#).

Figure 8. Summary estimates and confidence regions at cut-off ODI 0.5 and 1.0.

We performed a sensitivity analysis by including from one study both the accuracy data at the ODI cut-off level of 0.5 as well as the accuracy data at the ODI cut-off level of 1.0, if both were reported, see [Table 5](#). The sensitivity and specificity were 0.84 (95% CI 0.74 to 0.94) and 0.83 (95% CI 0.76 to 0.90), respectively, at a cut-off ODI of 0.5. At a cut-off ODI of 1.0 the sensitivity was 0.80 (95% CI 0.69 to 0.92), and the specificity 0.92 (95% CI 0.89 to 0.96). The P values were 0.66 for the sensitivity and 0.01 for the specificity.

Investigations of heterogeneity

Incorporation bias

We examined the possibility of heterogeneity due to incorporation bias, see [Table 6](#). We compared two groups of studies: one with the studies that incorporated serum and/or BAL galactomannan in their reference standard (or were unclear on this matter); versus a group with the studies that specifically excluded both galactomannan tests from their reference standard. In the latter group, the sensitivity and specificity were 0.98 (95% CI 0.95 to 1.00) and 0.72 (95% CI 0.53 to 0.90), respectively. In the former group the

sensitivity was 0.74 (95% CI 0.63 to 0.86; P value for difference in sensitivity 0.01) and the specificity 0.91 (95% CI 0.86 to 0.95; P value for difference in specificity 0.02).

The effect of pre-selection based on a positive serum galactomannan test

Few studies reported this quality item. We were therefore unable to investigate this possible source of heterogeneity.

The effect of the 2008 update of the reference standard

Four studies used the 2002 version, and 13 studies the 2008 version of the reference standard. Using this as a covariate had no effect on sensitivity or specificity.

The effect of a protocol that defines the exact and explicit indication of the index test

Whether or not there was a strict and exact indication for performing the index test had no significant effect on sensitivity or specificity.

Study design

We examined the possibility of heterogeneity due to study design, see [Table 7](#). We compared the prospective with the retrospective (or on this matter unclear) studies. In the latter group the sensitivity and specificity were 0.94 (95% CI 0.86 to 1.00) and 0.78 (95% CI 0.65 to 0.91), respectively. In the former group the sensitivity was 0.74 (95% CI 0.60 to 0.88) and the specificity 0.91 (95% CI 0.86 to 0.96). The corresponding P values were 0.03 for the sensitivity and 0.03 for the specificity.

The effect of sponsoring of the study by the manufacturer of the commercially available galactomannan test

Whether or not the manufacturer of the commercially available galactomannan test sponsored a study had no significant effect on sensitivity or specificity.

Other planned investigations

Department(s) where the participants were recruited

As 10 studies were performed in haematology departments, one in a paediatric department, one in a lung transplant centre, one in various transplant departments, and in four studies the departments were not clearly specified, we were able to examine the effect of dividing the departments in two groups: haematology departments versus other or unclear departments. This division had no significant effect in our meta-analysis, when used as a covariate.

Additional investigations that proved not possible

As there was only one study that mainly included **children** a meta-analysis on this item was not possible. As 10 studies included patients with a mix of **host criteria**, and the other studies were varied with respect to the study population, an analysis of the effect of host criteria (e.g. neutropenic patients versus the rest) was not possible. As there were no studies included in which prophylaxis was specifically withheld, an investigation into the effect of **anti-mould prophylaxis** was not possible.

Sensitivity analysis

Methodological quality

We performed a sensitivity analysis per QUADAS-2 domain to determine the influence of methodological quality, leaving out the studies with a high risk of bias.

In the Index Test domain there was only one study with a low risk of bias, and in the Reference Test domain only two. A sensitivity analysis was therefore not possible for these domains. In the Patient Selection and in the Flow and Timing domains the same six studies had a low risk of bias. The sensitivity analyses were identical and can be seen in [Table 8](#). The sensitivity and specificity in the overall group were 0.83 (95% CI 0.70 to 0.92) and 0.88 (95% CI 0.80 to 0.92), respectively. In the low risk of bias group these were lower, respectively 0.75 and 0.86.

Effect of reference standard definition

Finally, we performed a sensitivity analysis on the definition of the reference standard by determining the sensitivity and specificity when following a number of pre-specified different definitions, see [Table 9](#). Unfortunately, we were unable to perform a bivariate analysis comparing 'proven' versus 'probable' / 'possible' / 'no' aspergillosis, as the variance was unstable and the model did not converge. We therefore performed two univariate analyses for this definition.

Excluding the participants with 'possible' aspergillosis had only a small effect on sensitivity and increased specificity slightly. Defining participants with 'probable' aspergillosis as not having the target condition decreased sensitivity and specificity. Considering participants with 'probable' and 'possible' aspergillosis as having the target condition lowered sensitivity significantly and increased specificity.

DISCUSSION

Summary of main results

We included 17 studies on 1634 participants in our review. Meta-analysis of their results led to an estimated sensitivity of 0.88 (95% CI 0.75 to 1.00) and an estimated specificity of 0.81 (95% CI 0.71 to 0.91) at a cut-off value of an ODI of 0.5. At a cut-off value of an ODI of 1.0 these statistics were 0.78 (95% CI 0.61 to 0.95) and 0.93 (95% CI 0.87 to 0.98), respectively.

When the Platelia galactomannan test was introduced, there was no validated threshold value for use on bronchoalveolar lavage (BAL). This is reflected by the variable primary cut-off levels used in the studies of our review. The effect of the cut-off value on performance statistics, the threshold effect, can be seen in [Summary of findings 1](#). Because of the magnitude of this effect and the fact that clinical practice requires a cut-off value, we chose not to calculate overall performance statistics but to calculate them per threshold value.

The difference in diagnostic performance of a cut-off value of 0.5 versus 1.0 can be illustrated using a hypothetical group of 1000 potential invasive aspergillosis (IA) patients. As an example, we will assume a disease incidence of 12%. In such a population, 120 patients will have 'proven' or 'probable' IA, and 880 will not. If we use the test at a cut-off value of 0.5, then based on the BAL galactomannan result we will miss 14 patient with IA (sensitivity

88%, 12% false-negative rate). And there will be 167 patients incorrectly diagnosed with invasive aspergillosis (specificity of 81%, 19% false-positive rate). If we use the test at a cut-off value of 1.0, we will miss 26 patients with IA (sensitivity 78%, 22% false-negative rate). There will be 62 patients incorrectly diagnosed with invasive aspergillosis (specificity of 93%, 7% false-positive rate). Therefore, the optimal cut-off value will depend on the local incidence of invasive aspergillosis, in an ideal situation specifically determined for the population at hand. And, it will depend on the relative importance of false-positive and false-negative results, as determined by the role of the index test within the clinical pathway.

Many quality features were poorly reported in the included studies, e.g. whether the clinicians were blinded, how uninterpretable or intermediate index and reference test results were dealt with, whether the results of the reference standard were known when the index test result was interpreted (and vice versa), the time interval between the execution of the index test and reference standard, and how the reference standard was exactly executed. Many studies did not confirm that all patients qualified as having a EORTC/MSG host criterion, or this seemed not to be the case in all participants. Many studies included patients with varying underlying pathologies and did not report extensively on subgroups. Studies often did not have a detailed clinical protocol specifying the indication and exact timing of the index test.

The sensitivity was more heterogeneous than specificity. Part of this heterogeneity can be explained by the inclusion of small studies and the on average low prevalence of IA. The cut-off value had a significant effect on specificity but not on sensitivity. This could also be due to the low incidence of IA.

Comparison with other systematic reviews

A number of systematic reviews on BAL galactomannan have been published in recent years, see [Table 10](#) ([Guo 2010](#); [Zou 2012](#); [Heng 2015](#)).

[Guo 2010](#) wrote the first review, aiming to produce an overall sensitivity and specificity. If more than one cut-off value was reported in a study, the cut-off value that offered the best test performance was used. This resulted in an overall sensitivity and specificity of 0.90 and 0.94. The authors included 12 studies (not counting Becker and colleagues twice), and did not focus on a specific population.

[Heng 2015](#) focused specifically on haematological patients and included 16 studies. At a cut-off level of 0.5, the sensitivity was found to be 0.82 and the specificity 0.92. At a cut-off of 1.0 sensitivity dropped to 0.75 and specificity rose to 0.95.

[Zou 2012](#) performed the systematic review with the largest number of studies, 30 in total (not counting Becker and colleagues twice), and included non-English studies. The review did not focus on a specific population. A striking phenomenon in this review was the fact that increasing the cut-off level from 0.5 to 1.0 increased the sensitivity from 0.78 to 0.94. This suggests large differences between the studies in the 0.5 and 1.0 group, overcompensating the threshold effect.

We excluded from our review many of the studies that were included in the other reviews. We excluded, for example, case-control studies, studies that did not report all EORTC/MSG

categories, studies that used a modified reference standard, and non-English studies. Of the 16 studies [Heng 2015](#) included, we excluded 10. Of the 30 [Zou 2012](#) included, we excluded 22. We excluded six of the 13 studies [Guo 2010](#) selected.

[Table 10](#) suggests that the overall performance statistics reported in [Guo 2010](#) may be an overestimation, as the overall performance statistics are better than the statistics at the 0.5 and 1.0 ODI cut-off level. At the 0.5 ODI cut-off level our review estimated the sensitivity to be lower and the specificity to be higher than the other reviews. The other reviews had comparable performance statistics. At the 1.0 ODI cut-off level all reviews were similar.

Comparison with serum galactomannan and polymerase chain reactions (PCRs) on blood

Comparing our review with the Cochrane Review on serum galactomannan ([Leefflang 2015](#)), our review seems to show that the diagnostic accuracy of the Platelia test on BAL is slightly better than when performed on serum. When using an ODI of 0.5 as a cut-off value, the sensitivity of the serum test was 0.78 and the specificity was 0.85. At a cut-off value of 1.0, the sensitivity was 0.71 and the specificity was 0.90. At a cut-off value of 1.5, the sensitivity was 0.63 and the specificity was 0.93. A biological explanation of better performance on BAL could be the sampling in the immediate vicinity of the infection.

It has been argued that airway invasion results in a rise of the amount of galactomannan in BAL fluid ([Hope 2007](#)). And that penetration of the endothelial cell layer by hyphae (angio-invasive growth) could lead to more galactomannan in serum ([Mennink-Kersten 2004](#)). Most likely, there is a degree of complementarity between BAL and serum galactomannan. The results of a number of studies supports this hypothesis ([Bergeron 2012a](#); [Fortún 2016](#); [Hidalgo 2009](#)).

The use of the galactomannan test in serum and BAL was validated in different studies. Both our review on serum galactomannan ([Leefflang 2015](#)) and our current review showed a large amount of heterogeneity. Therefore, the comparison of the reviews on BAL and serum galactomannan is hazardous and it is not possible to determine whether the serum or BAL galactomannan obviates the use of the other test. As stated, the tests probably complement each other. It would be highly interesting for future research to focus on the relative value of both tests.

The Cochrane Library also published a review on PCR tests on blood to diagnose invasive aspergillosis in immunocompromised patients ([Cruciani 2015](#)). The overall sensitivity and specificity of a single test (80.5% and 78.5%, respectively) are about comparable with the serum galactomannan test. For two consecutive positive test results these become 58.0% and 96.2%, respectively. An methodological challenge in research on PCRs to diagnose aspergillosis is the lack of a standardised protocol on how to perform the PCR. This has been improved on by for example the European Aspergillosis PCR Initiative (EAPCRI).

Strengths and weaknesses of the review

Strengths

The main strength of our review is the rigorous application of strict methodological criteria. This led to the exclusion of studies that were included in other reviews. For example, we excluded case-

control studies, which are often used because of the low incidence of IA. However, longitudinal cohort studies are preferable, as cases and controls are taken from the same population and the prevalence of the disease can be determined precisely. We also excluded studies that enrolled participants non-consecutively or non-randomly, studies that did not report all EORTC/MSG categories, and studies that modified the reference standard.

Weaknesses

Because of the rigorous application of methodological criteria relatively few studies were included in our review. Even these studies are characterised by a high risk of bias, and a large amount of heterogeneity in sensitivity and specificity as well as in the distribution of patients with 'proven', 'probable', 'possible' and non-IA. Therefore, we were not able to investigate many of the predetermined sources of heterogeneity. Notwithstanding these weaknesses, our results show that the central role that is usually given to the galactomannan test in clinical practice is justified.

We excluded two non-English studies from the assessment which studies were eligible for inclusion, as we are only fluent in English. In the planned update of this review in 2021, we will also include non-English studies.

Applicability of findings to the review question

We decided not to assess the concern about applicability for the patient domain as we expected to find studies with a large amount of heterogeneity and a wide range of patient populations at risk for IA. We excluded no subgroups, to be able to determine generally applicable performance statistics. Instead, we chose to carefully document the types of participants and tried to analyse heterogeneity. Such analyses require large sample sizes, which may be difficult to obtain in studies on rare infections such as invasive aspergillosis. This emphasises the need for systematic reviews.

We expected the heterogeneity in the studies to derive from five categories:

- the heterogenic manifestations of the disease,
- the heterogenic characteristics of the patients,
- heterogenic study protocols,
- the imperfect nature of the reference standard; and
- incorporation bias.

Heterogeneity in disease manifestations

As stated in the introduction, invasive aspergillosis (IA) consists of many subforms, of which invasive pulmonary aspergillosis (IPA) is the most common variant. IPA can manifest itself as an alveolar infection or a tracheobronchitis, the latter being seen more frequently in HIV/AIDS and patients with a lung transplant. The alveolar infections can be located centrally or more peripherally. This raises the question whether the BAL procedure is of influence on the diagnostic accuracy (see "Heterogeneity in study protocols" below). And whether the sensitivity of BAL might be lower in peripherally localised aspergillosis.

The *Aspergillus* species involved

The assay performance might vary with the *Aspergillus* species involved, as some might produce different quantities of galactomannan (Swanink 1997; Taghizadeh-Armaki 2017). A lower

sensitivity has been reported in *A. fumigatus* infection in comparison with other *Aspergillus* species (13% versus 49%; $P = 0.001$; Hachem 2009).

Heterogeneity in patient characteristics

The populations and the results of the studies in our review were very heterogeneous. Therefore, the interpretation and extrapolation of these results has to be performed with caution.

Preventative antifungal therapy

Preventative antifungal therapy might reduce fungal burden, leading to false-negative results (Eigl 2015; Prattes 2014; Prattes 2015; Reinwald 2012a). Some clinical studies reported this (Luong 2010; Maertens 2009; Marr 2004; Park 2010), while others reported the opposite (Musher 2004), or found no effect (Frealle 2009; Nguyen 2011).

Racil 2011 reported a higher sensitivity in patients who just started antifungal therapy, and a lower sensitivity after two to three days of therapy, indicating a need to perform BAL within days of starting antifungal therapy. However, Frealle 2009 did not find this association.

Our data did not allow quantification of the effect of mould-active antifungal agents.

Neutropenia

Neutropenia could cause an increase in fungal load, thereby decreasing the false-negative rate. Conflicting results are reported. The sensitivity of BAL galactomannan varied from being no different (Maertens 2009) to significantly higher in neutropenic patients (Racil 2011). D'Haese 2012 reported that neutropenia influences the sensitivity of galactomannan in serum but not in BAL fluid.

We were unable to test whether there was an influence of neutropenia.

Colonisation

Aspergillus species can colonise the pulmonary tract and, as they are ubiquitous in the environment, their spores are inhaled on a daily basis. Colonisation can lead to false-positive results. This becomes even more relevant in populations with an underlying condition that predisposes to colonisation, such as cystic fibrosis, bronchiectasis or after a lung transplant. In such populations the specificity can be expected to be lower. Indeed, the one study in our review on lung transplant patients had a markedly low specificity (Pasqualotto 2010).

Our data did not allow formal testing of this effect.

Cross-reactive moulds

Some non-*Aspergillus* moulds such as *Histoplasma capsulatum* (Min 2012; Narreddy 2008; Wheat 2007), *Penicillium marneffei* (Huang 2007; Rimek 1999), *Fusarium oxysporum* (Nucci 2014; Tortorano 2012) and *Alternaria* species (Kappe 1993) may cross-react with galactomannan epitopes. These potential cross-reactions could be important in the geographic areas in which these moulds are endemic. We were unable to test this hypothesis.

Cross-reactive epitopes

Betalactam antibiotics such as amoxicillin-clavulanate and piperacillin-tazobactam, which are often given to these patients, have been reported to caused false-positive results, as they are derived from natural compounds produced by the genus *Penicillium* (Bart-Delabesse 2005; Boonsarngsuk 2010).

We had insufficient data to examine this possible relation.

Heterogeneity in study protocols

The study protocol has a major impact on test performance. For example, it can define a certain period of fever unresponsive to broad spectrum antibiotics as an indication for the index test. A shorter period of fever corresponds with an earlier disease phase that will be harder to detect. Shortening the period will therefore lower the estimated sensitivity of the index test. A second example is that the study protocol defines the study population and that disease prevalence influences diagnostic test accuracy. For instance, a population with a high disease prevalence may also include more severe cases of disease (Mulherin 2002).

Interpretation of the reference standard

The composite nature of the reference standard – that states that "[e]very reasonable attempt should be made to exclude an alternative etiology" for lower respiratory signs – raises the question how far one should go to exclude other aetiologies, and whether this diagnostic work-up should be standardised in a study. For example, a patient with a pulmonary nodule and a negative BAL culture will be classified as having 'possible' aspergillosis. However, if a trans-thoracic biopsy was performed, the patient would have been classified as having 'proven' aspergillosis if it was positive. The indication for trans-thoracic biopsy as well as the extent of the microbiological work-up on BAL in case of suspected IA differs among clinics. This introduces bias. Moreover, as the reference standard calls for an evaluation of the possibility of alternative aetiologies, subjectivity is introduced.

Multiple samples per patient

The number of measurements per patient can also introduce bias. Sensitivity and specificity are either calculated on a per-patient basis or a per-test basis. This means a patient is labelled as a case patient or as a control patient depending on whether they developed IA during their treatment course (per-patient basis) or had it at the time of the index test (per-test). In case of per-patient analysis, a larger number of samples will result in a higher likelihood of a positive test due to chance (and usually, the better the performance statistics will be). Both per-patient and per-test analysis can lead to bias when different numbers of samples are obtained for each patient, or if the timing of samples is not systematically applied to each patient. The more they are clustered around the moment of infection, the better the performance statistics will become. To avoid these problems, some included studies used only one index test per patient. For example, the first one performed (Becker 2003 – part I), or the one with the highest result (de Mol 2013). Effectively, the latter strategy still uses multiple tests per patient.

Bronchoalveolar lavage (BAL) procedure

Computerised tomography (CT) scans are ubiquitously available, and should preferably be used to guide the lavage. In that setting

the probability of a misguided lavage, for example due to multifocal and multifactorial pathology, seems low. However, it is not known which fraction of the BAL is associated with the most accurate test result. Often the homogenised alveolar sample is used. Hsu 2010 tested the bronchial sample. Comparative studies found that sensitivity may be lower when testing bronchial samples (Racil 2011, Zou 2012). It could also depend on the localisation of the infection. The volume of aspirated fluid is another parameter that seems important as higher volumes have been associated with higher false-negative rates (Racil 2011). Correction of the test result using a dilution marker might be a solution, as a universal standard seems unfeasible (Rennard 1986). False-positive results could additionally result from contamination at processing, transport, or testing. This has been reported for Plasmalyte, which is used as lavage fluid and components of which are produced by *A.niger* (Hage 2007, Spriet 2016). Protracted storage has also been reported to lead to false-negative results (Dufresne 2014, Johnson 2013, Johnson 2014; Kimpton 2014; Wheat 2014).

As the included studies did not use or did not report a study protocol, or had incomparable protocols, we were unable to quantify this bias. We did exclude studies with a varying number of tests per patient that reported performance statistics on a per-test basis.

Study design

A prospective study is preferable to a retrospective study. It results in a cohort that is better defined in terms of patient characteristics. Moreover, the index test and reference standard are often performed in a more standardised fashion. This is of special importance when the timing of the index test is of influence. In our meta-analysis, the study design had a large effect on the sensitivity and specificity, perhaps corresponding with an earlier BAL in the prospective studies.

The reference standard

Although the first three sources of heterogeneity could be solved by meta-analysis of large studies that are methodologically well construed, two challenges remain that are currently unsolvable.

Misclassification bias

There is no reliable reference standard for IA. *Aspergillus* infection can be proven by the isolation and culture of the organism from a normally sterile site (De Pauw 2008). However, biopsies are associated with complications, and examination of tissue samples and BAL can be negative despite ultimately proven infection. This difficulty is reflected by the reference standard that contains grades of certainty, and the fact that it had to be revised. The reference standard contains many subjective items. It calls for the interpretation of tests by radiologists, pathologists, microbiologists and pulmonologists. This can be associated with intra- and inter-observer variability. Misclassification will result in a wrong estimation of the prevalence and can underestimate a tests' sensitivity and specificity.

A non-blinded study can lead to further underestimation as a clinician who is aware of a positive test result could start therapy. This could in turn prevent the reference standard from ever becoming positive. Because of the imperfect nature of the reference standard and the promising first study results, the index test has already become part of the current reference standard. It can be

argued that blinding a clinician from the results is unethical. In 2013, a randomised controlled trial confirmed the clinical value of galactomannan testing on serum samples (Morrissey 2013).

A relatively minor problem with the reference standard is the fact that it focuses on invasive fungal disease, not aspergillosis. If microscopy or cytology is positive as a mycological criterion but all cultures are negative, it is not clear whether a patient has aspergillosis or not. If the patient also has a host and clinical criterion, he has 'probable' invasive **mycosis**. But whether he has 'probable' invasive **aspergillosis** is unclear.

Moment of infection

Not only is there no reliable way to determine whether a patient has the target disease, but due to the insidious and protracted course of the disease it is also not possible to determine the moment a patient became infected. This makes it hard to interpret a positive index test that was performed a number of days before the reference test was performed. This is called disease progression bias. Was it an early sign of the disease, a false-positive test or a different episode of the same disease that was cured or spontaneously resolved?

The composite nature of the reference standard makes it nearly unavoidable that there will be varying time intervals between the index and reference test. Moreover, as the reference test consists of multiple tests, it is not a single point in time.

Although misclassification and disease progression bias could have played a role in the large amount of heterogeneity in our review, it is not possible to quantify the effect of these biases.

Incorporation bias

A second and related major difficulty is incorporation bias. As stated, the index test is part of the current reference standard. Following this definition in a diagnostic study leads to a form of circular reasoning, overestimating sensitivity and specificity. As an extreme example, if the index test is only performed in case of a CT scan showing a clinical criterion, a positive index test will result in a positive reference standard and the specificity will always be 100%. On the other hand, not incorporating the galactomannan test will underestimate specificity (and to a lesser extent sensitivity) as a negative reference standard does not exclude the disease.

In our review, we found a large difference between studies that did and did not avoid incorporation bias. Strangely enough, the sensitivity increased spectacularly in the group that avoided incorporation bias. This could be due to chance, because of the relatively low number of studies in this group ($n = 4$); or due to the fact that the studies were performed in populations in which false-negative results are rare. The studies were three prospective studies, including only neutropenic haematological patients, and the only study on lung transplant patients.

AUTHORS' CONCLUSIONS

Implications for practice

Institutions can use our results to decide on the optimal cut-off level, taking their local incidence and the clinical pathway into

consideration. Although the sensitivities of the two cut-off levels seems nearly equal, assuming a cohort of 1000 patients and a disease incidence of 12%, at a cut-off level of 0.5, the test will on average be falsely negative in 14 patients with invasive aspergillosis (IA), and will falsely diagnose IA in 167 patients. If we use the test at a cut-off value of 1.0, we will miss 26 patients with IA and will incorrectly diagnose IA in 62 patients (see [Summary of main results](#)).

Based on our reviews, it is not possible to determine whether the serum or bronchoalveolar lavage (BAL) galactomannan is a better test, as the diagnostic accuracies were determined in different studies with a large heterogeneity (See the [Summary of main results](#)). Probably, the tests complement each other, as the other test might be positive in cases the first test is negative in. Although the diagnostic accuracy found in our review is not as high as in other reviews, it does support its prominent role in the current reference standard.

Implications for research

Research on IA is associated with a large number of methodological difficulties, as described in [Applicability of findings to the review question](#), most importantly incorporation and misclassification bias. However, the generally low-methodological quality of the studies in our review should be improved upon. Many of the theoretically plausible forms of bias were not dealt with in the design of most studies. Although there was a significant effect of study design on the performance statistics, the current body of evidence did not allow us to determine which forms of bias did and did not play a role.

A number of questions remains unanswered. It would be interesting if future research would focus more on clinical pathways and the relative value of other tests, such as serum galactomannan and the various polymerase chain reactions (PCR's). To this end it would be helpful if studies reported all components of the reference standard clearly. More studies on homogeneous and well-described patient populations would also be welcome. Reporting incorporation is paramount in determining the effect and amount of incorporation. Reporting all cut-off values would be helpful in determining the optimal cut-off value. The quality of systematic reviews would improve if they could be performed as an individual patient data (IPD) analysis instead of a meta-analysis.

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de Heer 2016

de Heer K, Gerritsen MG, Visser CE, Leeflang MM. Galactomannan detection in broncho-alveolar lavage fluid for invasive aspergillosis in immunocompromised patients. *Cochrane Database of Systematic Reviews* 2016, Issue 10. [DOI: [10.1002/14651858.CD012399](https://doi.org/10.1002/14651858.CD012399)]

CHARACTERISTICS OF STUDIES

Characteristics of included studies [ordered by study ID]

Becker 2003 -- part I

Study characteristics

Patient sampling	Department(s) where the patients were recruited: haematology Not stated whether patients were in-patients or out-patients The underlying disease spectrum: various haematological malignancies requiring intensive chemotherapy resulting in prolonged neutropenia and/or stem cell transplantation Cause of the increased risk for invasive aspergillosis: a combination of all non-inheritable 2008 EORTC/MSG host criteria (neutropenia, stem cell transplantation, steroids, immunosuppressants) The intended use of the index test in the study was: to diagnose or exclude invasive aspergillosis in patients suspected of having this disease Were the EORTC/MSG host criteria explicitly used as an inclusion criterion? Yes Study design: prospective; cohort very likely
Patient characteristics and setting	Age of the participants: median 49 years, range 18–79 years
Index tests	Was the execution of the bronchoscopy and the collection of broncho-alveolar lavage performed according to a fixed protocol, for example, defining the number and volume of lavages, and the type of fluid that was used as lavage? Unclear Was the execution of the ELISA clearly described? Yes ODI cut-off value(s) used: 1.0
Target condition and reference standard(s)	The reference standard used: the 2002 EORTC/MSG criteria How the reference standard was executed: not stated EORTC/MSG categories described in the results: all 4 EORTC/MSG classifications Was the index test incorporated in the reference standard? No, neither GM was used in the reference standard
Flow and timing	Was the time window on which the reference standard was applied stated, and/or when (or whether) the index test was performed within this window? No How uninterpretable or intermediate results were dealt with when performing the galactomannan assay: all doubtful or positive samples were retested in parallel with recent samples in the next assay. Uninterpretable or intermediate results were not reported How uninterpretable or intermediate results were dealt with when performing the reference standard: unclear, this was not stated explicitly Patients that were excluded: patients with possible aspergillosis, but it was possible to calculate all performance statistics
Comparative	
Was sponsoring of the study by the manufacturer of the commercially available galactomannan assay reported?	No

Becker 2003 -- part I (Continued)

Notes

Methodological quality

Item	Authors' judgement	Risk of bias	Applicability concerns
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Yes		
Was a case-control design avoided?	Yes		
Did the study avoid inappropriate exclusions?	No		
Was prior galactomannan testing (for example on serum) performed and could the result be a reason for performing broncho-alveolar lavage?	No		
Was there a protocol stated that defined the exact and explicit indication for performing the test?	Yes		
Was explicitly stated that this inclusion criterion was also strictly followed in practice?	Yes		
		High	Low
DOMAIN 2: Index Test All tests			
Were the index test results interpreted without knowledge of the results of the reference standard?	Unclear		
If a threshold was used, was it pre-specified?	Yes		
		High	Low
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	Yes		
Were the reference standard results interpreted without knowledge of the results of the index tests?	Unclear		
		High	Low
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Unclear		
Did all patients receive the same reference standard?	Yes		

Becker 2003 -- part I *(Continued)*

Were all patients included in the analysis? No

High

Becker 2003 -- part II
Study characteristics

Patient sampling	<p>Department(s) where the patients were recruited: haematology Not stated whether patient were in-patients or out-patients</p> <p>The underlying disease spectrum: various haematological malignancies requiring intensive chemotherapy resulting in prolonged neutropenia and/or stem cell transplantation Cause of the increased risk for invasive aspergillosis: a combination of all non-inheritable 2008 EORTC/MSG host criteria (neutropenia, stem cell transplantation, steroids, immunosuppressants)</p> <p>The intended use of the index test in the study was: to diagnose or exclude invasive aspergillosis in patients suspected of having this disease Were the EORTC/MSG host criteria explicitly used as an inclusion criterion? Yes Study design: prospective; cohort very likely</p>
Patient characteristics and setting	Age of the participants: median 47 years, range 18-74 years
Index tests	<p>Was the execution of the bronchoscopy and the collection of broncho-alveolar lavage performed according to a fixed protocol, for example, defining the number and volume of lavages, and the type of fluid that was used as lavage? Unclear Was the execution of the ELISA clearly described? Yes ODI cut-off value(s) used: 1.0</p>
Target condition and reference standard(s)	<p>The reference standard used: the 2002 EORTC/MSG criteria How the reference standard was executed: not stated EORTC/MSG categories described in the results: all 4 EORTC/MSG classifications Was the index test incorporated in the reference standard? No, neither GM was used in the reference standard</p>
Flow and timing	<p>Was the time window on which the reference standard was applied stated, and/or when (or whether) the index test was performed within this window? No How uninterpretable or intermediate results were dealt with when performing the galactomannan assay: all doubtful or positive samples were retested in parallel with recent samples in the next assay Uninterpretable or intermediate results were not reported. How uninterpretable or intermediate results were dealt with when performing the reference standard: unclear, this was not stated explicitly Patients that were excluded: in case of a clear contra-indication for BAL</p>
Comparative	
Was sponsoring of the study by the manufacturer of the commercially available galactomannan assay reported?	No
Notes	

Methodological quality

Becker 2003 -- part II *(Continued)*

Item	Authors' judgement	Risk of bias	Applicability concerns
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Yes		
Was a case-control design avoided?	Yes		
Did the study avoid inappropriate exclusions?	Yes		
Was prior galactomannan testing (for example on serum) performed and could the result be a reason for performing broncho-alveolar lavage?	No		
Was there a protocol stated that defined the exact and explicit indication for performing the test?	Yes		
Was explicitly stated that this inclusion criterion was also strictly followed in practice?	Yes		
		Low	Low
DOMAIN 2: Index Test All tests			
Were the index test results interpreted without knowledge of the results of the reference standard?	Unclear		
If a threshold was used, was it pre-specified?	Yes		
		High	Low
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	Yes		
Were the reference standard results interpreted without knowledge of the results of the index tests?	Unclear		
		High	Low
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Unclear		
Did all patients receive the same reference standard?	Yes		
Were all patients included in the analysis?	Yes		
		Low	

Bergeron 2010

Study characteristics

Patient sampling	<p>Department(s) where the patients were recruited: haematology</p> <p>Not stated whether patients were in-patients or out-patients</p> <p>The underlying disease spectrum: patients with a haematologic malignancy</p> <p>The intended use of the index test in the study was: to diagnose or exclude invasive aspergillosis in patients suspected of having this disease</p> <p>Were the EORTC/MSG host criteria explicitly used as an inclusion criterion? No</p> <p>Study design: retrospective cohort of consecutive patients with haematologic malignancies who underwent BAL for the exploration of pulmonary infiltrates</p>
Patient characteristics and setting	<p>Age of the participants: mean 45 years $\pm$ 17 (SD)</p> <p>Cause of the increased risk for invasive aspergillosis: not stated for all patients, of the 101 patients 33 underwent stem cell transplantation, for the others it was unclear</p>
Index tests	<p>Was the execution of the bronchoscopy and the collection of broncho-alveolar lavage performed according to a fixed protocol, for example, defining the number and volume of lavages, and the type of fluid that was used as lavage? Yes</p> <p>Was the execution of the ELISA clearly described? It was explicitly stated that the ELISA was performed according to the manufacturer's instructions</p> <p>ODI cut-off value(s) used: 0.5</p>
Target condition and reference standard(s)	<p>The reference standard used: the 2008 EORTC/MSG criteria</p> <p>How the reference standard was executed: not stated</p> <p>EORTC/MSG categories described in the results: all 4 EORTC/MSG classifications</p> <p>Was the index test incorporated in the reference standard? No, but the serum GM was used in the reference standard</p>
Flow and timing	<p>Was the time window on which the reference standard was applied stated, and/or when (or whether) the index test was performed within this window? No</p> <p>How uninterpretable or intermediate results were dealt with when performing the galactomannan assay: positive and negative controls were included in each assay. All doubtful or positive samples were retested in the next assay. Uninterpretable or intermediate results were not reported</p> <p>How uninterpretable or intermediate results were dealt with when performing the reference standard: unclear, this was not stated explicitly</p> <p>Patients that were excluded: none</p>
Comparative	
Was sponsoring of the study by the manufacturer of the commercially available galactomannan assay?	No

Notes

Methodological quality

Item	Authors' judgement	Risk of bias	Applicability concerns
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Yes		

Bergeron 2010 *(Continued)*

Was a case-control design avoided?	Yes
Did the study avoid inappropriate exclusions?	Yes
Was prior galactomannan testing (for example on serum) performed and could the result be a reason for performing broncho-alveolar lavage?	Yes
Was there a protocol stated that defined the exact and explicit indication for performing the test?	No
Was explicitly stated that this inclusion criterion was also strictly followed in practice?	No
	Low
	Low

DOMAIN 2: Index Test All tests

Were the index test results interpreted without knowledge of the results of the reference standard?	Unclear
If a threshold was used, was it pre-specified?	Yes
	High
	Low

DOMAIN 3: Reference Standard

Is the reference standards likely to correctly classify the target condition?	Unclear
Were the reference standard results interpreted without knowledge of the results of the index tests?	Unclear
	High
	Low

DOMAIN 4: Flow and Timing

Was there an appropriate interval between index test and reference standard?	Unclear
Did all patients receive the same reference standard?	Yes
Were all patients included in the analysis?	Yes
	Low

Brownback 2013
Study characteristics
Galactomannan detection in broncho-alveolar lavage fluid for invasive aspergillosis in immunocompromised patients (Review)

Brownback 2013 (Continued)

Patient sampling	<p>Department(s) where the patients were recruited: not clearly stated</p> <p>Not stated whether patients were in-patients or out-patients</p> <p>The intended use of the index test in the study was: to diagnose or exclude invasive aspergillosis in patients suspected of having this disease</p> <p>Were the EORTC/MSG host criteria explicitly used as an inclusion criterion? No, the criteria that are mentioned are not all part of the 2008 EORTC/MSG criteria</p> <p>Study design: retrospective cohort of all patients who underwent FOB with BAL because of a new finding of pulmonary infiltrates, at the discretion of the attending physician</p>
Patient characteristics and setting	<p>Age of the participants: mean 50.4 years, range 19-81 years</p> <p>The underlying disease spectrum: recipients of chemotherapy, solid organ and allogeneic/autologous stem cell transplantation; patients with neutropenia and HIV</p> <p>Cause of the increased risk for invasive aspergillosis: a combination of all non-inheritable 2008 EORTC/MSG host criteria (neutropenia, stem cell transplantation, steroids, immunosuppressants)</p>
Index tests	<p>Was the execution of the bronchoscopy and the collection of broncho-alveolar lavage performed according to a fixed protocol, for example, defining the number and volume of lavages, and the type of fluid that was used as lavage? No</p> <p>Was the execution of the ELISA clearly described? It was explicitly stated that the ELISA was performed according to the manufacturer's instructions</p> <p>ODI cut-off value(s) used: 0.5</p>
Target condition and reference standard(s)	<p>The reference standard used: the 2008 EORTC/MSG criteria</p> <p>How the reference standard was executed: not stated</p> <p>EORTC/MSG categories described in the results: "NO AND POSSIBLE" versus "PROBABLE AND PROVEN"</p> <p>Was the index test incorporated in the reference standard? Yes, the BAL GM test was used in the reference standard. This incorporation was not quantified</p>
Flow and timing	<p>Was the time window on which the reference standard was applied stated, and/or when (or whether) the index test was performed within this window? No</p> <p>How uninterpretable or intermediate results were dealt with when performing the galactomannan assay: not stated</p> <p>How uninterpretable or intermediate results were dealt with when performing the reference standard: unclear, this was not stated explicitly</p> <p>Patients that were excluded: bronchoalveolar lavage galactomannan was only obtained in 143 of the 150 bronchoscopies (which we considered acceptable) and in 7 patients GM was not performed on BAL at the discretion of the attending physician</p>
Comparative	
Was sponsoring of the study by the manufacturer of the commercially available galactomannan assay reported?	No
Notes	

Methodological quality

Item	Authors' judgement	Risk of bias	Applicability concerns
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	No		

Brownback 2013 (Continued)

Was a case-control design avoided?	Yes
Did the study avoid inappropriate exclusions?	Unclear
Was prior galactomannan testing (for example on serum) performed and could the result be a reason for performing broncho-alveolar lavage?	Yes
Was there a protocol stated that defined the exact and explicit indication for performing the test?	No
Was explicitly stated that this inclusion criterion was also strictly followed in practice?	No
High Low	
DOMAIN 2: Index Test All tests	
Were the index test results interpreted without knowledge of the results of the reference standard?	Unclear
If a threshold was used, was it pre-specified?	Yes
High Low	
DOMAIN 3: Reference Standard	
Is the reference standards likely to correctly classify the target condition?	No
Were the reference standard results interpreted without knowledge of the results of the index tests?	No
High Low	
DOMAIN 4: Flow and Timing	
Was there an appropriate interval between index test and reference standard?	Unclear
Did all patients receive the same reference standard?	Yes
Were all patients included in the analysis?	No
High	

Clancy 2007

Study characteristics

Patient sampling	Department(s) where the patients were recruited: transplant department Not stated whether patients were in-patients or out-patients The intended use of the index test in the study was: not clearly stated Were the EORTC/MSG host criteria explicitly used as an inclusion criterion? Yes Study design: retrospective cohort
Patient characteristics and setting	Age of the participants: median 54 years, range 4–79 years The underlying disease spectrum: recipients of a solid organ transplant: 24 heart, 22 kidney, 19 liver, and 16 lung Cause of the increased risk for invasive aspergillosis: prolonged use of corticosteroids (excluding in patients with allergic bronchopulmonary aspergillosis) at a mean minimum dose of 0.3 mg/kg/day of prednisone equivalent for > 3 weeks, treatment with other recognised T cell immunosuppressants, such as cyclosporine, TNF- α blockers, specific monoclonal antibodies (such as alemtuzumab), or nucleoside analogues during the past 90 days
Index tests	Was the execution of the bronchoscopy and the collection of broncho-alveolar lavage performed according to a fixed protocol, for example, defining the number and volume of lavages, and the type of fluid that was used as lavage? Yes Was the execution of the ELISA clearly described? It was explicitly stated that the ELISA was performed according to the manufacturer's instructions ODI cut-off value(s) used: 0.5
Target condition and reference standard(s)	The reference standard used: modified EORTC/MSG criteria but enough information was stated to deduct the 2008 classification (http://www.doctorfungus.org/lecture/eortc_msg_rev06.htm) How the reference standard was executed: the physician investigators were blinded EORTC/MSG categories described in the results: all 4 EORTC/MSG classifications Was the index test incorporated in the reference standard? No, but the serum GM was used in the reference standard
Flow and timing	Was the time window on which the reference standard was applied stated, and/or when (or whether) the index test was performed within this window? No How uninterpretable or intermediate results were dealt with when performing the galactomannan assay: not stated How uninterpretable or intermediate results were dealt with when performing the reference standard: in case of disagreement consensus was reached Uninterpretable or indeterminate results of the reference standard were not reported Patients that were excluded: unclear
Comparative	
Was sponsoring of the study by the manufacturer of the commercially available galactomannan assay reported?	Yes

Notes

Methodological quality

Item	Authors' judgement	Risk of bias	Applicability concerns
DOMAIN 1: Patient Selection			

Clancy 2007 *(Continued)*

Was a consecutive or random sample of patients enrolled?	Unclear
Was a case-control design avoided?	Yes
Did the study avoid inappropriate exclusions?	Unclear
Was prior galactomannan testing (for example on serum) performed and could the result be a reason for performing broncho-alveolar lavage?	Yes
Was there a protocol stated that defined the exact and explicit indication for performing the test?	No
Was explicitly stated that this inclusion criterion was also strictly followed in practice?	Yes
High Low	
DOMAIN 2: Index Test All tests	
Were the index test results interpreted without knowledge of the results of the reference standard?	Unclear
If a threshold was used, was it pre-specified?	Yes
High Low	
DOMAIN 3: Reference Standard	
Is the reference standards likely to correctly classify the target condition?	Yes
Were the reference standard results interpreted without knowledge of the results of the index tests?	Yes
Low Low	
DOMAIN 4: Flow and Timing	
Was there an appropriate interval between index test and reference standard?	Unclear
Did all patients receive the same reference standard?	Yes
Were all patients included in the analysis?	Unclear
High	

de Mol 2013

Study characteristics

Patient sampling	<p>Department(s) where the patients were recruited: paediatrics</p> <p>Not stated whether patients were in-patients or out-patients</p> <p>The intended use of the index test in the study was: to diagnose or exclude invasive aspergillosis in patients suspected of having this disease</p> <p>Were the EORTC/MSG host criteria explicitly used as an inclusion criterion? Yes</p> <p>Study design: retrospective cohort</p>
Patient characteristics and setting	<p>Age of the participants: median 9.8 years, range 1.1-18.2 years</p> <p>The underlying disease spectrum: patients with a haematological malignancy and prolonged neutropenia due to intensive chemotherapy or stem cell transplantation, and patients with a kidney transplant, solid tumour or chronic granulomatous disease</p> <p>Cause of the increased risk for invasive aspergillosis: a recent history of neutropenia ($<0.5 \times 10^9$ neutrophils/L > 10 days) temporally related to the onset of fungal disease, receipt of an allogeneic stem cell transplant, prolonged use of corticosteroids (excluding in patients with allergic bronchopulmonary aspergillosis) at a mean minimum dose of 0.3 mg/kg/day of prednisone equivalent for > 3 weeks, treatment with other recognised T cell immunosuppressants, such as cyclosporine, TNF-α blockers, specific monoclonal antibodies (such as alemtuzumab), or nucleoside analogues during the past 90 days</p>
Index tests	<p>Was the execution of the bronchoscopy and the collection of broncho-alveolar lavage performed according to a fixed protocol, for example, defining the number and volume of lavages, and the type of fluid that was used as lavage? Yes</p> <p>Was the execution of the ELISA clearly described? It was explicitly stated that the ELISA was performed according to the manufacturer's instructions</p> <p>ODI cut-off value(s) used: 0.5</p>
Target condition and reference standard(s)	<p>The reference standard used: the 2008 EORTC/MSG criteria</p> <p>How the reference standard was executed: not stated</p> <p>EORTC/MSG categories described in the results: all 4 EORTC/MSG classifications</p> <p>Was the index test incorporated in the reference standard? No, but the serum GM was used in the reference standard</p>
Flow and timing	<p>Was the time window on which the reference standard was applied stated, and/or when (or whether) the index test was performed within this window? No</p> <p>How uninterpretable or intermediate results were dealt with when performing the galactomannan assay: not stated</p> <p>How uninterpretable or intermediate results were dealt with when performing the reference standard: unclear, this was not stated explicitly</p> <p>Patients that were excluded: if more than one bronchoscopy was performed within a patient, the bronchoscopy done for diagnosing IPA was taken, 14 of the remaining 61 patients were excluded since the chest CT-scan was not related to the time of bronchoscopy (Fig. 1), patients in whom GM was not performed were also excluded</p>
Comparative	
Was sponsoring of the study by the manufacturer of the commercially available galactomannan assay reported?	No
Notes	

Methodological quality

de Mol 2013 (Continued)

Item	Authors' judgement	Risk of bias	Applicability concerns
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	No		
Was a case-control design avoided?	Yes		
Did the study avoid inappropriate exclusions?	Unclear		
Was prior galactomannan testing (for example on serum) performed and could the result be a reason for performing broncho-alveolar lavage?	Yes		
Was there a protocol stated that defined the exact and explicit indication for performing the test?	Yes		
Was explicitly stated that this inclusion criterion was also strictly followed in practice?	Yes		
		High	Low
DOMAIN 2: Index Test All tests			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	Yes		
		Low	Low
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	Yes		
Were the reference standard results interpreted without knowledge of the results of the index tests?	Unclear		
		High	Low
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Unclear		

de Mol 2013 (Continued)

Did all patients receive the same reference standard? Yes

Were all patients included in the analysis? No

High

Fisher 2014
Study characteristics

Patient sampling

Department(s) where the patients were recruited: haematology
Not stated whether patients were in-patients or out-patients
The underlying disease spectrum: recipients of allogeneic stem cell transplantation
Cause of the increased risk for invasive aspergillosis: receipt of an allogeneic stem cell transplant
The intended use of the index test in the study was: to diagnose or exclude invasive aspergillosis in patients suspected of having this disease
Were the EORTC/MSG host criteria explicitly used as an inclusion criterion? Yes
Study design: retrospective cohort of patients who underwent BAL evaluation for suspected pulmonary disease within 100 days of an allogeneic HCT

Patient characteristics and setting

Age of the participants: proven/probable: median 52 years, range 23–74 years; possible/no: 50 (18–75 years)

Index tests

Was the execution of the bronchoscopy and the collection of broncho-alveolar lavage performed according to a fixed protocol, for example, defining the number and volume of lavages, and the type of fluid that was used as lavage? Unclear
Was the execution of the ELISA clearly described? It was explicitly stated that the ELISA was performed according to the manufacturer's instructions
ODI cut-off value(s) used: 0.5

Target condition and reference standard(s)

The reference standard used: the 2008 EORTC/MSG criteria
How the reference standard was executed: not stated
EORTC/MSG categories described in the results: “NO AND POSSIBLE” versus “PROBABLE” versus “PROVEN”
Was the index test incorporated in the reference standard? No, but the serum GM was used in the reference standard

Flow and timing

Was the time window on which the reference standard was applied stated, and/or when (or whether) the index test was performed within this window? No
How uninterpretable or intermediate results were dealt with when performing the galactomannan assay: not stated
How uninterpretable or intermediate results were dealt with when performing the reference standard: unclear, this was not stated explicitly
Patients that were excluded: patients were excluded from the study if they were diagnosed with IPA pre-transplantation or if evidence of disseminated aspergillosis was present at the time of diagnosis

Comparative

Was sponsoring of the study by the manufacturer of the commercially available galactomannan assay reported? No

Fisher 2014 (Continued)

Notes

Methodological quality

Item	Authors' judgement	Risk of bias	Applicability concerns
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Yes		
Was a case-control design avoided?	Yes		
Did the study avoid inappropriate exclusions?	Yes		
Was prior galactomannan testing (for example on serum) performed and could the result be a reason for performing broncho-alveolar lavage?	Yes		
Was there a protocol stated that defined the exact and explicit indication for performing the test?	Yes		
Was explicitly stated that this inclusion criterion was also strictly followed in practice?	Yes		
		Low	Low
DOMAIN 2: Index Test All tests			
Were the index test results interpreted without knowledge of the results of the reference standard?	Unclear		
If a threshold was used, was it pre-specified?	Yes		
		High	Low
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	Yes		
Were the reference standard results interpreted without knowledge of the results of the index tests?	Unclear		
		High	Low
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Unclear		

Fisher 2014 (Continued)

Did all patients receive the same reference standard? Yes

Were all patients included in the analysis? Yes

Low

Frealle 2009
Study characteristics

Patient sampling	<p>Department(s) where the patients were recruited: haematology</p> <p>Not stated whether patients were in-patients or out-patients</p> <p>The underlying disease spectrum: various haematological malignancies requiring intensive chemotherapy resulting in prolonged neutropenia and/or stem cell transplantation</p> <p>Cause of the increased risk for invasive aspergillosis: a combination of all non-inheritable 2008 EORTC/MSG host criteria (neutropenia, stem cell transplantation, steroids, immunosuppressants)</p> <p>The intended use of the index test in the study was: this was not consistently reported</p> <p>Were the EORTC/MSG host criteria explicitly used as an inclusion criterion? Yes</p> <p>Study design: retrospective; cohort likely</p>
Patient characteristics and setting	Age of the participants: mean 49 years, median 51, range 20-80 years
Index tests	<p>Was the execution of the bronchoscopy and the collection of broncho-alveolar lavage performed according to a fixed protocol, for example, defining the number and volume of lavages, and the type of fluid that was used as lavage? Yes</p> <p>Was the execution of the ELISA clearly described? No</p> <p>ODI cut-off value(s) used: 1.0</p>
Target condition and reference standard(s)	<p>The reference standard used: the 2002 EORTC/MSG criteria</p> <p>How the reference standard was executed: not stated</p> <p>EORTC/MSG categories described in the results: all 4 EORTC/MSG classifications</p> <p>Was the index test incorporated in the reference standard? Yes, the BAL GM test was used in the reference standard. This incorporation was not quantified</p>
Flow and timing	<p>Was the time window on which the reference standard was applied stated, and/or when (or whether) the index test was performed within this window? No</p> <p>How uninterpretable or intermediate results were dealt with when performing the galactomannan assay: not stated</p> <p>How uninterpretable or intermediate results were dealt with when performing the reference standard: unclear, this was not stated explicitly</p> <p>Patients that were excluded: unclear</p>
Comparative	
Was sponsoring of the study by the manufacturer of the commercially available galactomannan assay reported?	No

Notes

Methodological quality

Frealle 2009 (Continued)

Item	Authors' judgement	Risk of bias	Applicability concerns
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Unclear		
Was a case-control design avoided?	Yes		
Did the study avoid inappropriate exclusions?	Unclear		
Was prior galactomannan testing (for example on serum) performed and could the result be a reason for performing broncho-alveolar lavage?	Unclear		
Was there a protocol stated that defined the exact and explicit indication for performing the test?	No		
Was explicitly stated that this inclusion criterion was also strictly followed in practice?	Yes		
		High	Low
DOMAIN 2: Index Test All tests			
Were the index test results interpreted without knowledge of the results of the reference standard?	Unclear		
If a threshold was used, was it pre-specified?	Yes		
		High	Low
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	Yes		
Were the reference standard results interpreted without knowledge of the results of the index tests?	No		
		High	Low
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Unclear		
Did all patients receive the same reference standard?	Yes		
Were all patients included in the analysis?	Unclear		
		High	

Heng 2014

Study characteristics

Patient sampling	Department(s) where the patients were recruited: haematology Not stated whether patients were in-patients or out-patients The intended use of the index test in the study was: this was not consistently reported Were the EORTC/MSG host criteria explicitly used as an inclusion criterion? No Study design: multicentre retrospective cohort of consecutive patients who underwent diagnostic bronchoscopy for investigation of pulmonary infiltrates
Patient characteristics and setting	Age of the participants: proven/probable: median 54 years, range 19-80 years; no IA: median 59 years, range 18-72 years The underlying disease spectrum: patients with haematological malignancies (AML, CML, ALL, CLL, MM, MDS, lymphoma) and/or stem cell transplant Cause of the increased risk for invasive aspergillosis: a recent history of neutropenia ($<0.5 \times 10^9$ neutrophils/L >10 days) temporally related to the onset of fungal disease, receipt of an allogeneic stem cell transplant, not stated for all patients
Index tests	Was the execution of the bronchoscopy and the collection of broncho-alveolar lavage performed according to a fixed protocol, for example, defining the number and volume of lavages, and the type of fluid that was used as lavage? Yes Was the execution of the ELISA clearly described? It was explicitly stated that the ELISA was performed according to the manufacturer's instructions ODI cut-off value(s) used: 0.8
Target condition and reference standard(s)	The reference standard used: the 2008 EORTC/MSG criteria How the reference standard was executed: two assessors were blinded to the galactomannan and PCR results EORTC/MSG categories described in the results: "NO" versus "POSSIBLE" versus "PROBABLE AND PROVEN" Was the index test incorporated in the reference standard? No, but the serum GM was used in the reference standard
Flow and timing	Was the time window on which the reference standard was applied stated, and/or when (or whether) the index test was performed within this window? No How uninterpretable or intermediate results were dealt with when performing the galactomannan assay: not stated How uninterpretable or intermediate results were dealt with when performing the reference standard: any discrepancies in classification were resolved by consensus in a meeting between the two assessors and a 3rd member. Uninterpretable or indeterminate results of the reference standard were not reported Patients that were excluded: none
Comparative	
Was sponsoring of the study by the manufacturer of the commercially available galactomannan assay reported?	No

Notes

Methodological quality

Item	Authors' judgement	Risk of bias	Applicability concerns
DOMAIN 1: Patient Selection			

Heng 2014 (Continued)

Was a consecutive or random sample of patients enrolled?	Yes
Was a case-control design avoided?	Yes
Did the study avoid inappropriate exclusions?	Yes
Was prior galactomannan testing (for example on serum) performed and could the result be a reason for performing broncho-alveolar lavage?	Yes
Was there a protocol stated that defined the exact and explicit indication for performing the test?	Yes
Was explicitly stated that this inclusion criterion was also strictly followed in practice?	No
Low	
Low	
DOMAIN 2: Index Test All tests	
Were the index test results interpreted without knowledge of the results of the reference standard?	Unclear
If a threshold was used, was it pre-specified?	Yes
High	
Low	
DOMAIN 3: Reference Standard	
Is the reference standards likely to correctly classify the target condition?	Unclear
Were the reference standard results interpreted without knowledge of the results of the index tests?	Yes
High	
Low	
DOMAIN 4: Flow and Timing	
Was there an appropriate interval between index test and reference standard?	Unclear
Did all patients receive the same reference standard?	Yes
Were all patients included in the analysis?	Yes
Low	

Hoenigl 2014
Study characteristics

Patient sampling	Department(s) where the patients were recruited: not clearly stated Not stated whether patients were in-patients or out-patients The intended use of the index test in the study was: this was not consistently reported Were the EORTC/MSG host criteria explicitly used as an inclusion criterion? No Study design: partly prospective and partly retrospective study of routinely tested BAL fluids in immunocompromised patients
Patient characteristics and setting	Age of the participants: median 58 years, range 24-77 years The underlying disease spectrum: patients with a haematological malignancy, sarcoidosis, COPD, solid organ transplantation, AIDS, chronic auto-immune disease, or treatment on an ICU Cause of the increased risk for invasive aspergillosis: not stated for all patients
Index tests	Was the execution of the bronchoscopy and the collection of broncho-alveolar lavage performed according to a fixed protocol, for example, defining the number and volume of lavages, and the type of fluid that was used as lavage? No Was the execution of the ELISA clearly described? No ODI cut-off value(s) used: 0.5 and 1.0
Target condition and reference standard(s)	The reference standard used: the 2008 EORTC/MSG criteria How the reference standard was executed: not stated EORTC/MSG categories described in the results: "NO AND POSSIBLE" versus "PROBABLE" versus "PROVEN" Was the index test incorporated in the reference standard? Unclear
Flow and timing	Was the time window on which the reference standard was applied stated, and/or when (or whether) the index test was performed within this window? No How uninterpretable or intermediate results were dealt with when performing the galactomannan assay: not stated How uninterpretable or intermediate results were dealt with when performing the reference standard: unclear, this was not stated explicitly Patients that were excluded: unclear

Comparative

Was sponsoring of the study by the manufacturer of the commercially available galactomannan assay reported? No

Notes
Methodological quality

Item	Authors' judgement	Risk of bias	Applicability concerns
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Yes		
Was a case-control design avoided?	Yes		
Did the study avoid inappropriate exclusions?	Unclear		

Hoenigl 2014 *(Continued)*

Was prior galactomannan testing (for example on serum) performed and could the result be a reason for performing broncho-alveolar lavage?	Yes
Was there a protocol stated that defined the exact and explicit indication for performing the test?	No
Was explicitly stated that this inclusion criterion was also strictly followed in practice?	No
	High Low
DOMAIN 2: Index Test All tests	
Were the index test results interpreted without knowledge of the results of the reference standard?	Unclear
If a threshold was used, was it pre-specified?	Yes
	High Low
DOMAIN 3: Reference Standard	
Is the reference standards likely to correctly classify the target condition?	Unclear
Were the reference standard results interpreted without knowledge of the results of the index tests?	Unclear
	High Low
DOMAIN 4: Flow and Timing	
Was there an appropriate interval between index test and reference standard?	Unclear
Did all patients receive the same reference standard?	Yes
Were all patients included in the analysis?	Unclear
	High

Maertens 2009
Study characteristics

Patient sampling	Department(s) where the patients were recruited: haematology Not stated whether patients were in-patients or out-patients The underlying disease spectrum: not stated Cause of the increased risk for invasive aspergillosis: not stated
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Maertens 2009 (Continued)

The intended use of the index test in the study was: to diagnose or exclude invasive aspergillosis in patients suspected of having this disease
Were the EORTC/MSG host criteria explicitly used as an inclusion criterion? No
Study design: retrospective study of all patients with haematologic disorders who had BAL fluid tested for GM

Patient characteristics and setting	Age of the participants: IA mean age 53.6 years, no-IA 50.4 years
Index tests	Was the execution of the bronchoscopy and the collection of broncho-alveolar lavage performed according to a fixed protocol, for example, defining the number and volume of lavages, and the type of fluid that was used as lavage? Yes Was the execution of the ELISA clearly described? It was explicitly stated that the ELISA was performed according to the manufacturer's instructions ODI cut-off value(s) used: many, most data are on 1.0 cut-off
Target condition and reference standard(s)	The reference standard used: autopsy and the 2008 EORTC/MSG criteria How the reference standard was executed: by two investigators blinded for GM results EORTC/MSG categories described in the results: all 4 EORTC/MSG classifications Was the index test incorporated in the reference standard? No, but the serum GM was used in the reference standard
Flow and timing	Was the time window on which the reference standard was applied stated, and/or when (or whether) the index test was performed within this window? No How uninterpretable or intermediate results were dealt with when performing the galactomannan assay: not stated How uninterpretable or intermediate results were dealt with when performing the reference standard: unclear, this was not stated explicitly Patients that were excluded: to avoid therapeutic bias, only BAL samples obtained before the start of systemic antifungal therapy were considered for the analysis. Patients with fatal cases that showed no histopathologic signs of IA on autopsy were classified as having no IA. In addition, patients with possible cases who recovered without ever receiving mould-active antifungal therapy were also classified as having no IA, given the very low likelihood of recovering from IA without appropriate therapy. However, patients with possible cases who received mould-active antifungal treatment and survived were excluded from the analysis, because the true status of fungal disease remained uncertain. We did, however, perform an additional analysis in which it was assumed that all these cases were either true-positive or true-negative cases of IA. Patients with invasive mould disease due to another mould were not considered to have IA

Comparative

Was sponsoring of the study by the manufacturer of the commercially available galactomannan assay reported? Yes

Notes
Methodological quality

Item	Authors' judgement	Risk of bias	Applicability concerns
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Yes		
Was a case-control design avoided?	Yes		

Maertens 2009 *(Continued)*

Did the study avoid inappropriate exclusions?	No
Was prior galactomannan testing (for example on serum) performed and could the result be a reason for performing broncho-alveolar lavage?	Yes
Was there a protocol stated that defined the exact and explicit indication for performing the test?	No
Was explicitly stated that this inclusion criterion was also strictly followed in practice?	No
<div>High</div> <div>Low</div>	
DOMAIN 2: Index Test All tests	
Were the index test results interpreted without knowledge of the results of the reference standard?	Unclear
If a threshold was used, was it pre-specified?	No
<div>High</div> <div>Low</div>	
DOMAIN 3: Reference Standard	
Is the reference standards likely to correctly classify the target condition?	No
Were the reference standard results interpreted without knowledge of the results of the index tests?	Yes
<div>High</div> <div>Low</div>	
DOMAIN 4: Flow and Timing	
Was there an appropriate interval between index test and reference standard?	Unclear
Did all patients receive the same reference standard?	Yes
Were all patients included in the analysis?	No
<div>High</div>	

Nguyen 2011

Study characteristics

Patient sampling	Department(s) where the patients were recruited: haematology Patients were in-patients The intended use of the index test in the study was: to diagnose or exclude invasive aspergillosis in patients suspected of having this disease Were the EORTC/MSG host criteria explicitly used as an inclusion criterion? Yes Study design: retrospective cohort study
Patient characteristics and setting	Age of the participants: median 63 years The underlying disease spectrum: various haematological malignancies requiring intensive chemotherapy resulting in prolonged neutropenia and/or stem cell transplantation Cause of the increased risk for invasive aspergillosis: a combination of all non-inheritable 2008 EORTC/MSG host criteria (neutropenia, stem cell transplantation, steroids, immunosuppressants)
Index tests	Was the execution of the bronchoscopy and the collection of broncho-alveolar lavage performed according to a fixed protocol, for example, defining the number and volume of lavages, and the type of fluid that was used as lavage? No Was the execution of the ELISA clearly described? Yes ODI cut-off value(s) used: 0.5
Target condition and reference standard(s)	The reference standard used: the 2008 EORTC/MSG criteria How the reference standard was executed: classifications were made by 5 investigators who were blinded to BAL GM results at the time EORTC/MSG categories described in the results: all 4 EORTC/MSG classifications Was the index test incorporated in the reference standard? No, but the serum GM was used in the reference standard
Flow and timing	Was the time window on which the reference standard was applied stated, and/or when (or whether) the index test was performed within this window? No How uninterpretable or intermediate results were dealt with when performing the galactomannan assay: not stated How uninterpretable or intermediate results were dealt with when performing the reference standard: in the event of disagreements, consensus was reached among the investigators. Uninterpretable or indeterminate results of the reference standard were not reported Patients that were excluded: none
Comparative	
Was sponsoring of the study by the manufacturer of the commercially available galactomannan assay reported?	Yes
Notes	

Methodological quality

Item	Authors' judgement	Risk of bias	Applicability concerns
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Yes		
Was a case-control design avoided?	Yes		

Nguyen 2011 (Continued)

Did the study avoid inappropriate exclusions?	Yes		
Was prior galactomannan testing (for example on serum) performed and could the result be a reason for performing broncho-alveolar lavage?	Yes		
Was there a protocol stated that defined the exact and explicit indication for performing the test?	Yes		
Was explicitly stated that this inclusion criterion was also strictly followed in practice?	Yes		
		Low	Low
DOMAIN 2: Index Test All tests			
Were the index test results interpreted without knowledge of the results of the reference standard?	Unclear		
If a threshold was used, was it pre-specified?	Yes		
		High	Low
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	Yes		
Were the reference standard results interpreted without knowledge of the results of the index tests?	Yes		
		Low	Low
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Unclear		
Did all patients receive the same reference standard?	Yes		
Were all patients included in the analysis?	Yes		
		Low	

Pasqualotto 2010
Study characteristics

Patient sampling	Department(s) where the patients were recruited: lung transplant department Not stated whether patients were in-patients or out-patients
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Pasqualotto 2010 (Continued)

The underlying disease spectrum: lung transplant patients
Cause of the increased risk for invasive aspergillosis: patients received an initial immunosuppressive regimen after lung transplantation that consisted of cyclosporine, azathioprine, and prednisone. Children were treated with a tacrolimus-based regimen, whereas patients with previous episodes of rejection received mycophenolate mofetil or tacrolimus
The intended use of the index test in the study was: surveillance or suspected infection
Were the EORTC/MSG host criteria explicitly used as an inclusion criterion? Yes
Study design: prospective study

Patient characteristics and setting	Age of the participants: median 55 years, range 10-72 years
Index tests	Was the execution of the bronchoscopy and the collection of broncho-alveolar lavage performed according to a fixed protocol, for example, defining the number and volume of lavages, and the type of fluid that was used as lavage? Yes Was the execution of the ELISA clearly described? It was explicitly stated that the ELISA was performed according to the manufacturer's instructions ODI cut-off value(s) used: many different cut-offs were used
Target condition and reference standard(s)	The reference standard used: the 2008 EORTC/MSG criteria How the reference standard was executed: the final diagnosis was reviewed together with physicians from the lung transplant team, and a consensus was reached for every case EORTC/MSG categories described in the results: "no / colonization / possible" versus "proven / probable" Was the index test incorporated in the reference standard? No, neither GM was used in the reference standard
Flow and timing	Was the time window on which the reference standard was applied stated, and/or when (or whether) the index test was performed within this window? No How uninterpretable or intermediate results were dealt with when performing the galactomannan assay: not stated How uninterpretable or intermediate results were dealt with when performing the reference standard: the final diagnosis was reviewed together with physicians from the lung transplant team, and a consensus was reached for every case. Uninterpretable or indeterminate results of the reference standard were not reported Patients that were excluded: patients receiving systemic antifungal drugs for any reason were excluded

Comparative

Was sponsoring of the study by the manufacturer of the commercially available galactomannan assay reported?

No

Notes
Methodological quality

Item	Authors' judgement	Risk of bias	Applicability concerns
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Unclear		
Was a case-control design avoided?	Yes		

Pasqualotto 2010 *(Continued)*

Did the study avoid inappropriate exclusions?	No
Was prior galactomannan testing (for example on serum) performed and could the result be a reason for performing broncho-alveolar lavage?	Yes
Was there a protocol stated that defined the exact and explicit indication for performing the test?	No
Was explicitly stated that this inclusion criterion was also strictly followed in practice?	Yes
High Low	
DOMAIN 2: Index Test All tests	
Were the index test results interpreted without knowledge of the results of the reference standard?	Unclear
If a threshold was used, was it pre-specified?	No
High Low	
DOMAIN 3: Reference Standard	
Is the reference standards likely to correctly classify the target condition?	Yes
Were the reference standard results interpreted without knowledge of the results of the index tests?	Unclear
High Low	
DOMAIN 4: Flow and Timing	
Was there an appropriate interval between index test and reference standard?	Unclear
Did all patients receive the same reference standard?	Yes
Were all patients included in the analysis?	No
High	

Penack 2008
Study characteristics

Penack 2008 (Continued)

Patient sampling	Department(s) where the patients were recruited: haematology Patients were in-patients The underlying disease spectrum: various haematological malignancies requiring intensive chemotherapy resulting in prolonged neutropenia and/or stem cell transplantation Cause of the increased risk for invasive aspergillosis: a combination of all non-inheritable 2008 EORCT/MSG host criteria (neutropenia, stem cell transplantation, steroids, immunosuppressants) The intended use of the index test in the study was: to diagnose or exclude invasive aspergillosis in patients suspected of having this disease Were the EORTC/MSG host criteria explicitly used as an inclusion criterion? Yes Study design: prospective cohort study		
Patient characteristics and setting	Age of the participants: median 52 years, range 23–74 years		
Index tests	Was the execution of the bronchoscopy and the collection of broncho-alveolar lavage performed according to a fixed protocol, for example, defining the number and volume of lavages, and the type of fluid that was used as lavage? Unclear Was the execution of the ELISA clearly described? It was explicitly stated that the ELISA was performed according to the manufacturer's instructions ODI cut-off value(s) used: 0.5		
Target condition and reference standard(s)	The reference standard used: the 2002 EORTC/MSG criteria How the reference standard was executed: not stated EORTC/MSG categories described in the results: “NO” versus “POSSIBLE” versus “PROBABLE AND PROVEN” Was the index test incorporated in the reference standard? No, neither GM was used in the reference standard		
Flow and timing	Was the time window on which the reference standard was applied stated, and/or when (or whether) the index test was performed within this window? No How uninterpretable or intermediate results were dealt with when performing the galactomannan assay: not stated How uninterpretable or intermediate results were dealt with when performing the reference standard: unclear, this was not stated explicitly Patients that were excluded: evidence or history of probable or proven IA before the start of the study		
Comparative			
Was sponsoring of the study by the manufacturer of the commercially available galactomannan assay reported?	Yes		
Notes			
Methodological quality			
Item	Authors' judgement	Risk of bias	Applicability concerns
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Yes		
Was a case-control design avoided?	Yes		
Did the study avoid inappropriate exclusions?	Yes		

Penack 2008 (Continued)

Was prior galactomannan testing (for example on serum) performed and could the result be a reason for performing broncho-alveolar lavage?	Yes	
Was there a protocol stated that defined the exact and explicit indication for performing the test?	Yes	
Was explicitly stated that this inclusion criterion was also strictly followed in practice?	No	
	Low	Low
DOMAIN 2: Index Test All tests		
Were the index test results interpreted without knowledge of the results of the reference standard?	Unclear	
If a threshold was used, was it pre-specified?	Yes	
	High	Low
DOMAIN 3: Reference Standard		
Is the reference standards likely to correctly classify the target condition?	Unclear	
Were the reference standard results interpreted without knowledge of the results of the index tests?	Unclear	
	High	Low
DOMAIN 4: Flow and Timing		
Was there an appropriate interval between index test and reference standard?	Unclear	
Did all patients receive the same reference standard?	Yes	
Were all patients included in the analysis?	Yes	
	Low	

Prattes 2014
Study characteristics

Patient sampling	Department(s) where the patients were recruited: haematology Not stated whether patients were in-patients or out-patients The underlying disease spectrum: various haematological malignancies requiring intensive chemotherapy resulting in prolonged neutropenia and/or stem cell transplantation
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Prattes 2014 (Continued)

Cause of the increased risk for invasive aspergillosis: not stated for all patients
The intended use of the index test in the study was: to diagnose or exclude invasive aspergillosis in patients suspected of having this disease
Were the EORTC/MSG host criteria explicitly used as an inclusion criterion? Yes
Study design: consecutive partly prospective and partly retrospective cohort study of bronchoscopies ordered at the discretion of the treating physician

Patient characteristics and setting	Age of the participants: median 61 years, range 52–69 years
Index tests	Was the execution of the bronchoscopy and the collection of broncho-alveolar lavage performed according to a fixed protocol, for example, defining the number and volume of lavages, and the type of fluid that was used as lavage? No Was the execution of the ELISA clearly described? It was explicitly stated that the ELISA was performed according to the manufacturer's instructions ODI cut-off value(s) used: 0.5, however all data were supplied to us by the author
Target condition and reference standard(s)	The reference standard used: the 2008 EORTC/MSG criteria How the reference standard was executed: not stated EORTC/MSG categories described in the results: all 4 EORTC/MSG classifications Was the index test incorporated in the reference standard? Yes, the BAL GM test was used in the reference standard. This incorporation was not quantified
Flow and timing	Was the time window on which the reference standard was applied stated, and/or when (or whether) the index test was performed within this window? No How uninterpretable or intermediate results were dealt with when performing the galactomannan assay: not stated How uninterpretable or intermediate results were dealt with when performing the reference standard: unclear, this was not stated explicitly Patients that were excluded: 280 samples (74.4%) were excluded, as these patients had either no underlying haematological malignancy or IPA was not suspected and GM not ordered, and one case was excluded due to BALF pretreatment with dithiothreitol
Comparative	
Was sponsoring of the study by the manufacturer of the commercially available galactomannan assay reported?	No
Notes	

Methodological quality

Item	Authors' judgement	Risk of bias	Applicability concerns
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Yes		
Was a case-control design avoided?	Yes		
Did the study avoid inappropriate exclusions?	Unclear		
Was prior galactomannan testing (for example on serum) performed and could the result be a reason for performing broncho-alveolar lavage?	Unclear		

Prattes 2014 (Continued)

Was there a protocol stated that defined the exact and explicit indication for performing the test?	No		
Was explicitly stated that this inclusion criterion was also strictly followed in practice?	Yes		
		High	Low
DOMAIN 2: Index Test All tests			
Were the index test results interpreted without knowledge of the results of the reference standard?	Unclear		
If a threshold was used, was it pre-specified?	Yes		
		High	Low
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	Yes		
Were the reference standard results interpreted without knowledge of the results of the index tests?	No		
		High	Low
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Unclear		
Did all patients receive the same reference standard?	Yes		
Were all patients included in the analysis?	Unclear		
		High	

Prattes 2015
Study characteristics

Patient sampling	Department(s) where the patients were recruited: not clearly stated Not stated whether patients were in-patients or out-patients The intended use of the index test in the study was: to diagnose or exclude invasive aspergillosis in patients suspected of having this disease Were the EORTC/MSG host criteria explicitly used as an inclusion criterion? Yes, in a personal communication this was confirmed
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Prattes 2015 (Continued)

Study design: prospective cohort study in patients with underlying respiratory diseases who underwent routine bronchoscopy

Patient characteristics and setting	Age of the participants: median 64 years, range 18–92 years The underlying disease spectrum: respiratory diseases: COPD, bacterial pneumonia, rheumatoid/autoimmune diseases with lung involvement, bronchial carcinoma, viral pneumonia, chronic respiratory failure, bronchial asthma, bronchiectasis, AIDS with pneumonia, pulmonary tuberculosis Cause of the increased risk for invasive aspergillosis: prolonged systemic corticosteroids above the EORTC cut-off for e.g. CNS metastasis/autoimmune/rheumatoid diseases/ COPD or T-cell suppressants within 6-months (personal communication)
Index tests	Was the execution of the bronchoscopy and the collection of broncho-alveolar lavage performed according to a fixed protocol, for example, defining the number and volume of lavages, and the type of fluid that was used as lavage? No Was the execution of the ELISA clearly described? It was explicitly stated that the ELISA was performed according to the manufacturer's instructions ODI cut-off value(s) used: 1.0, however all data were supplied to us by the author
Target condition and reference standard(s)	The reference standard used: the 2008 EORTC/MSG criteria How the reference standard was executed: not stated EORTC/MSG categories described in the results: all 4 EORTC/MSG classifications Was the index test incorporated in the reference standard? Yes, the BAL GM test was used in the reference standard. This incorporation was not quantified
Flow and timing	Was the time window on which the reference standard was applied stated, and/or when (or whether) the index test was performed within this window? No How uninterpretable or intermediate results were dealt with when performing the galactomannan assay: not stated How uninterpretable or intermediate results were dealt with when performing the reference standard: unclear, this was not stated explicitly Patients that were excluded: unclear
Comparative	
Was sponsoring of the study by the manufacturer of the commercially available galactomannan assay reported?	No
Notes	

Methodological quality

Item	Authors' judgement	Risk of bias	Applicability concerns
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Unclear		
Was a case-control design avoided?	Yes		
Did the study avoid inappropriate exclusions?	Yes		
Was prior galactomannan testing (for example on serum) performed and could	Yes		

Prattes 2015 (Continued)

the result be a reason for performing broncho-alveolar lavage?

Was there a protocol stated that defined the exact and explicit indication for performing the test? No

Was explicitly stated that this inclusion criterion was also strictly followed in practice? Yes

High
Low
DOMAIN 2: Index Test All tests

Were the index test results interpreted without knowledge of the results of the reference standard? Unclear

If a threshold was used, was it pre-specified? Yes

High
Low
DOMAIN 3: Reference Standard

Is the reference standards likely to correctly classify the target condition? Yes

Were the reference standard results interpreted without knowledge of the results of the index tests? No

High
Low
DOMAIN 4: Flow and Timing

Was there an appropriate interval between index test and reference standard? Unclear

Did all patients receive the same reference standard? Yes

Were all patients included in the analysis? Unclear

High
Rose 2014
Study characteristics

Patient sampling
Department(s) where the patients were recruited: not clearly stated
Not stated whether patients were in-patients or out-patients
Age of the participants: not stated
The intended use of the index test in the study was: to diagnose or exclude invasive aspergillosis in patients suspected of having this disease

Rose 2014 (Continued)

Were the EORTC/MSG host criteria explicitly used as an inclusion criterion? Unclear as a haematological malignancy and HIV are not host criteria in the 2008 EORTC/MSG criteria
Study design: retrospective consecutive cohort study on beta-D-glucan (in which GM was not determined in only 3 of the 132 patients)

Patient characteristics and setting	The underlying disease spectrum: patients with haematological malignancies (recipients of allogeneic stem cell transplantation or steroids, neutropenic patients), severe aplastic anaemia, HIV, chronic granulomatous disease Cause of the increased risk for invasive aspergillosis: a recent history of neutropenia ($<0.5 \times 10^9$ neutrophils/L >10 days) temporally related to the onset of fungal disease, receipt of an allogeneic stem cell transplant, prolonged use of corticosteroids (excluding in patients with allergic bronchopulmonary aspergillosis) at a mean minimum dose of 0.3 mg/kg/day of prednisone equivalent for >3 weeks, treatment with other recognised T cell immunosuppressants, such as cyclosporine, TNF- α blockers, specific monoclonal antibodies (such as alemtuzumab), or nucleoside analogues during the past 90 days
Index tests	Was the execution of the bronchoscopy and the collection of broncho-alveolar lavage performed according to a fixed protocol, for example, defining the number and volume of lavages, and the type of fluid that was used as lavage? Unclear Was the execution of the ELISA clearly described? It was explicitly stated that the ELISA was performed according to the manufacturer's instructions ODI cut-off value(s) used: 0.5
Target condition and reference standard(s)	The reference standard used: the 2008 EORTC/MSG criteria How the reference standard was executed: not stated EORTC/MSG categories described in the results: "NO AND POSSIBLE" versus "PROBABLE AND PROVEN" Was the index test incorporated in the reference standard? Unclear
Flow and timing	Was the time window on which the reference standard was applied stated, and/or when (or whether) the index test was performed within this window? No How uninterpretable or intermediate results were dealt with when performing the galactomannan assay: not stated How uninterpretable or intermediate results were dealt with when performing the reference standard: unclear, this was not stated explicitly Patients that were excluded: patients in whom GM was not performed. A bias might be the patients in whom GM was determined but not BDG (which we chose to ignore as the article states that an unselected population was tested). Patients with PCP were also excluded but results were stated
Comparative	
Was sponsoring of the study by the manufacturer of the commercially available galactomannan assay reported?	No
Notes	

Methodological quality

Item	Authors' judgement	Risk of bias	Applicability concerns
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Yes		
Was a case-control design avoided?	Yes		

Rose 2014 (Continued)

Did the study avoid inappropriate exclusions?	Yes	
Was prior galactomannan testing (for example on serum) performed and could the result be a reason for performing broncho-alveolar lavage?	Yes	
Was there a protocol stated that defined the exact and explicit indication for performing the test?	No	
Was explicitly stated that this inclusion criterion was also strictly followed in practice?	Unclear	
	Low	Low
DOMAIN 2: Index Test All tests		
Were the index test results interpreted without knowledge of the results of the reference standard?	Unclear	
If a threshold was used, was it pre-specified?	Yes	
	High	Low
DOMAIN 3: Reference Standard		
Is the reference standards likely to correctly classify the target condition?	Unclear	
Were the reference standard results interpreted without knowledge of the results of the index tests?	Unclear	
	High	Low
DOMAIN 4: Flow and Timing		
Was there an appropriate interval between index test and reference standard?	Unclear	
Did all patients receive the same reference standard?	Yes	
Were all patients included in the analysis?	Yes	
	Low	

AML: acute myeloid leukaemia; **BAL:** bronchoalveolar lavage; **BALF:** bronchoalveolar lavage fluid; **CLL:** chronic lymphocytic leukaemia; **CML:** chronic myeloid leukaemia; **CNS:** central nervous system; **COPD:** chronic obstructive pulmonary disease; **CT:** computerised tomography; **ELISA:** enzyme-linked immunosorbent assay; **EORTC:** European Organisation for Research and Treatment of Cancer; **FOB:** fiberoptic bronchoscopy; **GM:** galactomannan; **HCT:** hematopoietic cell transplantation; **IA:** invasive aspergillosis; **ICU:** intensive care unit; **IPA:** invasive pulmonary aspergillosis; **MDS:** myelodysplastic syndromes; **MM:** multiple myeloma; **MSG:** Mycoses Study Group; **ODI:** optical density index; **PCP:** pneumocystis pneumonia; **PCR:** polymerase chain reaction; **SD:** standard deviation; **TNF:** tumour necrosis factor

Characteristics of excluded studies [ordered by study ID]

Study	Reason for exclusion
Acosta 2011	Patients with possible IA were excluded.
Affolter 2014	The statistical calculations could not be repeated.
Ali 2013	Patients with possible IA were excluded.
Ali 2014	Galactomannan was not reported for serum and BAL separately. The EORTC criteria were not used as reference standard.
Aquino 2012	The Bulpa criteria were the reference standard.
Ağca 2014	BAL galactomannan was compared to BAL culture without the other diagnostic criteria for invasive aspergillosis being reported. Thus, the EORTC criteria were not used as reference standard.
Barnes 2013	Of the 612 patients in the study, 248 had mycological evidence of IA and chest x-rays showed abnormalities in many of them, but HR-CT was not performed in 91% of these cases. This makes it hard to interpret the study. BAL GM was not performed.
Barnes 2015	Individual patient data or test performance data of BAL-GM are not reported.
Bergeron 2012b	This paper is not about the diagnosis of invasive aspergillosis, but about the use of serum galactomannan in the follow-up after the start of treatment.
Boch 2016	Patients with possible IA were excluded.
Boch 2016a	Individual patient data or test performance data of BAL-GM are not reported.
Brownback 2014	This is a review, no new data are described.
Buchheidt 2001	The study did not investigate the diagnostic value of galactomannan in BAL.
Buchheidt 2002	Reference criteria used to classify patients differed significantly from the EORTC criteria, no GM data.
Buess 2012	BAL-GM was not performed in this study.
D'Haese 2012	The EORTC criteria were not used as reference standard. Exclusion of patients with possible aspergillosis.
Danpornprasert 2010	The statistical calculations could not be repeated. The EORTC criteria were not used as reference standard.
Desai 2009	The EORTC criteria were not strictly followed.
Dinand 2016	Only data on serum galactomannan were reported.

Study	Reason for exclusion
Eigl 2015	Too few data were reported to calculate specificity: data on controls were not reported.
Eigl 2015a	Data on galactomannan were not reported.
Fisher 2013	The study evaluated the use of galactomannan in serum in the follow-up after the start of treatment.
Fortún 2016	Category “no IA” was not clearly defined, but probably patients with possible aspergillosis were excluded.
Gazzoni 2014	Only cases (BAL > 1.5) were included, no controls.
Gustot 2014	This is a study on patients with alcoholic hepatitis. GM was performed in a minority of patients. BAL was not performed systematically.
Hadrich 2011	Contains data from the period 2004 to 2007 that was also included in an article published by the same author in 2012.
Hadrich 2012	Data on patients with possible aspergillosis were not reported.
He 2012	Criteria different from the EORTC criteria were used.
Hedayati 2013	Multiple samples per patient were used as separate values in calculating diagnostic accuracy parameters. The EORTC criteria were not used as reference standard.
Hidalgo 2009	Only patients with probable and proven IA were included. Patients with possible and no IA were excluded.
Hohenthal 2005	Only enough data were reported to calculate sensitivity of BAL GM, not the specificity.
Hsu 2010	The control patients were recruited from a different study population.
Husain 2007	Multiple samples per patient were used as separate values in calculating diagnostic accuracy parameters.
Husain 2008	These data were already published as Clancy (2007) and Husain (2007).
Izumikawa 2012	This study investigated the role of galactomannan detection in chronic pulmonary aspergillosis.
Jain 2004	This is not a study on BAL GM.
Johnson 2015	The statistical calculations could not be repeated.
Jung 2015	Only cases were included, no patients without aspergillosis.
Khorvash 2014	The EORTC criteria were not used as reference standard.
Kim 2015	The reference standard was not the EORTC criteria.
Kimura 2009	It was a study on sputum. This resulted in exclusion of > 10% of the patients, in case of inadequate expectoration of sputum.
Kono 2013	Invasive pulmonary aspergillosis was not the target condition. Patients with pulmonary aspergillosis other than invasive aspergillosis were included (CNPA or ABPA).

Study	Reason for exclusion
Kotby 2008	Not enough data are reported to calculate diagnostic performance statistics for BAL-GM.
Küpelı 2015	This was not a study on BAL galactomannan.
Lahmer 2016	Category “without IA” was not clearly defined, but probably patients with possible aspergillosis were excluded.
Lopes 2010	It is not clear how patients with possible IA were classified.
Luong 2010	Multiple samples per patient were used as separate values in calculating diagnostic accuracy parameters.
Luong 2011	The exclusion of patients with previous/concurrent use of antimycotics was not quantified. Patients with possible aspergillosis were not reported on.
Luong 2014	The EORTC criteria were not used as reference standard.
Maertens 2005	Serum (and not BAL) galactomannan was the index test.
Meersseman 2008	The results of the patients with probable or possible aspergillosis were not reported.
Meletiadiş 2003	Not enough data are reported to calculate diagnostic performance statistics.
Miceli 2015	The EORTC criteria were not used as reference standard.
Mohammadi 2015	The study is a case-control study.
Musher 2004	The study is a case-control study. Only patients with probable and proven IA were included. Patients with possible and no IA were excluded. BAL fluid samples obtained from patients with other diagnoses were selected randomly as a control group.
Nguyen 2007	The EORTC criteria were not used as reference standard: immunosuppressed patients were excluded.
Nosari 2014	The patients with possible aspergillosis were not described, the aim of the study was to describe the epidemiology, clinical manifestations and outcome of invasive mould disease, not to investigate the value of BAL GM in diagnosing IA.
Orsi 2015	It is not clear how patients with possible IA were classified.
Paholcsek 2015	The study is on serum galactomannan and serum PCR, not about BAL.
Park 2010	Patients (about 10% of all participants) with “no aspergillosis” according to the EORTC criteria (defined as “inconclusive aspergillosis” in the manuscript) were excluded.
Park 2011	This was a study on patients with aspergilloma who do not meet the EORTC criteria for invasive aspergillosis.
Prasad 2014	Only patients with a positive serum galactomannan were included in the study.
Prattes 2015a	No new study data. The samples of another study are used.
Racil 2011	Patients with suspected false-positive results (due to concomitant antibiotic use) and possible IA were excluded.
Racil 2013	Only patients with probable and proven IA were included.

Study	Reason for exclusion
Reinwald 2012	Not enough data on BAL GM values were reported to calculate specificity.
Reinwald 2012a	The statistical calculations (Table 2) could not be repeated.
Rieger 2015	This is not a study on BAL GM, not enough data are reported to calculate diagnostic performance statistics for BAL-GM.
Russo 2014	Study on the predictors of mortality, not about the diagnostic value of BAL galactomannan. Only patients with probable/proven IA were reported.
Salonen 2000	The EORTC criteria were not used as reference standard.
Sampsonas 2011	Not enough data are not reported to calculate diagnostic performance statistics for BAL GM.
Sanguinetti 2003	It is not clear how patients with possible IA were classified.
Schroeder 2016	Not enough data are reported to calculate diagnostic performance statistics for BAL GM. Patients with a positive serum galactomannan or positive culture were selected.
Seo 2015	The EORTC criteria were not used as reference standard.
Seyfarth 2001	This was a study in patients with a positive Pastorex galactomannan test.
Shahid 2008	Patients with lung cancer were studied, which does not qualify as an EORTC host criterium.
Siemann 2001	Case-control study in a prospective cohort. The EORTC criteria were not used as reference standard. Diagnostic criteria contained “clinical signs” which were not specified.
Slobbe 2008	This is a study on health costs. There are not enough data on BAL galactomannan to calculate performance statistics.
Tabarsi 2012	The EORTC criteria were not used as reference standard.
Taremi 2015	Study correlating BAL and bronchial washing; individual patient data for BAL-GM or test performance data are not reported. The EORTC criteria were not the reference standard.
Theel 2013	BAL GM data were not extensively reported.
Torelli 2011	The EORTC criteria were not used as reference standard.
Verweij 1995	The EORTC criteria were not used as reference standard as they did not exist at the time. For example, CT scans were not used in the work-up of patients suspected of invasive aspergillosis.
Willinger 2014	The index-test is the LFD. Galactomannan results are only provided for probable/proven cases.
Zarrinfar 2013	BAL GM data were not extensively reported.
Zhang 2015	The EORTC criteria were not used as reference standard, “antibiotic treatment invalid, but antifungal treatment against aspergillosis valid” as a microbiological criterium does not conform.
Zhang 2016	Patients with possible IA were excluded.

ABPA: allergic bronchopulmonary aspergillosis; **BAL:** bronchoalveolar lavage; **CNPA:** chronic necrotizing pulmonary aspergillosis; **CT:** computerised tomography; **EORTC:** European Organisation for Research and Treatment of Cancer; **GM:** galactomannan; **HR-CT:** high-resolution computed tomography; **IA:** invasive aspergillosis; **LFD:** Lateral-Flow Device; **PCR:** polymerase chain reaction

Characteristics of studies awaiting classification *[ordered by study ID]*

Hu 2012

Study characteristics	
Patient sampling	According to the abstract patients suspected of pulmonary aspergillosis from August 2008 to April 2012.
Patient characteristics and setting	Not stated in the abstract.
Index tests	The abstract states that the 0.5, 0.8 and 1.0 thresholds were examined.
Target condition and reference standard(s)	The 2008 version of the EORTC criteria, and a significant number of patients was diagnosed through pathological examination. It is not clear from the abstract which patients (e.g. patients classified as having proven and probable invasive aspergillosis) were considered to have the disease, or not.
Flow and timing	No information could be extracted from the abstract.
Comparative	Not applicable.
Notes	The paper will be translated, and assessed for inclusion in the first update of this review.

Ozger 2015

Study characteristics	
Patient sampling	According to the abstract patients that were admitted at a pulmonary ICU for invasive mechanical ventilation and had undergone bronchoscopy between January and December 2013.
Patient characteristics and setting	Department(s) where the patients were recruited: pulmonary ICU. Patients were in-patients.
Index tests	The abstract states that the cut-off value was determined at 0.7. Probably, this was not predefined.
Target condition and reference standard(s)	The EORTC criteria but it is not clear from the abstract which version. It seems patients with proven and probable invasive were considered to have the disease, and patients with possible and no invasive aspergillosis were considered to not have the disease.
Flow and timing	No information could be extracted from the abstract.
Comparative	Not applicable.
Notes	The paper will be translated, and assessed for inclusion in the first update of this review.

EORTC: European Organisation for Research and Treatment of Cancer; **ICU:** intensive care unit

DATA

Presented below are all the data for all of the tests entered into the review.

Table Tests. Data tables by test

Test	No. of studies	No. of participants
1 ODI 0.5	12	1123
2 ODI 0.8	4	414
3 ODI 0.85 and 0.87	4	286
4 ODI 1.0	11	911
5 ODI 1.5	3	242
6 ODI 2.0	3	242
7 ODI 2.5	3	242

Test 1. ODI 0.5.

Test 2. ODI 0.8.

Test 3. ODI 0.85 and 0.87.

Review: Galactomannan detection in broncho-alveolar lavage fluid for invasive aspergillosis in immunocompromised patients
Test: 3 ODI 0.85 and 0.87

Test 4. ODI 1.0.

Review: Galactomannan detection in broncho-alveolar lavage fluid for invasive aspergillosis in immunocompromised patients
Test: 4 ODI 1.0

Test 5. ODI 1.5.

Review: Galactomannan detection in broncho-alveolar lavage fluid for invasive aspergillosis in immunocompromised patients
Test: 5 ODI 1.5

Test 6. ODI 2.0.

Review: Galactomannan detection in broncho-alveolar lavage fluid for invasive aspergillosis in immunocompromised patients
Test: 6 ODI 2.0

Test 7. ODI 2.5.

Review: Galactomannan detection in broncho-alveolar lavage fluid for invasive aspergillosis in immunocompromised patients
Test: 7 ODI 2.5

ADDITIONAL TABLES

Table 1. EORTC/MSG criteria, 2008 version

	Host criterion	Clinical criterion	Microbiological criterion
No IA	Not required	Not required	Not required
Possible IA	Required	Required	Not required
Probable IA	Required	Required	Required

IA: invasive aspergillosis

Table 2. EORTC/MSG criteria, 2002 version

	Host criterion	Clinical criterion (1 major or 2 minor)	Microbiological criterion
No IA	Not required	Not required	Not required
Possible IA	Required	Either one of these is required	
Probable IA	Required	Required	Required

IA: invasive aspergillosis

Table 3. Full EORTC/MSG criteria, 2008 version

Possible / probable invasive aspergillosis	
Host factors	<ul style="list-style-type: none"> Recent history of neutropenia ($< 0.5 \times 10^9$ neutrophils/L [< 500 neutrophils/mm³] for > 10 days) temporally related to the onset of fungal disease. Receipt of an allogeneic stem cell transplant Prolonged use of corticosteroids (excluding among patients with allergic bronchopulmonary aspergillosis) at a mean minimum dose of 0.3 mg/kg/day of prednisone equivalent for > 3 weeks Treatment with other recognized T cell immunosuppressants, such as cyclosporine, TNF-α blockers, specific monoclonal antibodies (such as alemtuzumab), or nucleoside analogues during the past 90 days Inherited severe immunodeficiency (such as chronic granulomatous disease or severe combined immunodeficiency)

Table 3. Full EORTC/MSG criteria, 2008 version (Continued)

Clinical criteria	<ul style="list-style-type: none"> Lower respiratory tract fungal disease (every reasonable attempt should be made to exclude an alternative etiology), with the presence of 1 of the following 3 signs on CT <ul style="list-style-type: none"> Dense, well-circumscribed lesions(s) with or without a halo sign Air-crescent sign Cavity Tracheobronchitis: tracheobronchial ulceration, nodule, pseudomembrane, plaque, or eschar seen on bronchoscopic analysis Sinonasal infection: Imaging showing sinusitis plus at least 1 of the following 3 signs: <ul style="list-style-type: none"> Acute localized pain (including pain radiating to the eye) Nasal ulcer with black eschar Extension from the paranasal sinus across bony barriers, including into the orbit CNS infection: 1 of the following 2 signs: <ul style="list-style-type: none"> Focal lesions on imaging Meningeal enhancement on MRI
Mycological criteria	<p>Direct test (cytology, direct microscopy, or culture) showing mold in sputum, bronchoalveolar lavage fluid, bronchial brush, or sinus aspirate samples, indicated by 1 of the following:</p> <ul style="list-style-type: none"> Presence of fungal elements indicating a mold Recovery by culture of a mold (e.g., <i>Aspergillus</i>, <i>Fusarium</i>, <i>Zygomycetes</i>, or <i>Scedosporium</i> species) <p>Indirect tests (detection of antigen or cell-wall constituents)</p> <ul style="list-style-type: none"> Aspergillosis: Galactomannan antigen detected in plasma, serum, bronchoalveolar lavage fluid, or CSF Invasive fungal disease other than cryptococcosis and zygomycoses: b-d-glucan detected in serum
Proven aspergillosis	
Criteria	<ul style="list-style-type: none"> Histopathologic, cytopathologic, or direct microscopic examination of a specimen obtained by needle aspiration or biopsy in which hyphae or melanized yeast-like forms are seen accompanied by evidence of associated tissue damage; or, Recovery of a mold or “black yeast” by culture of a specimen obtained by a sterile procedure from a normally sterile and clinically or radiologically abnormal site consistent with an infectious disease process, excluding bronchoalveolar lavage fluid, a cranial sinus cavity specimen, and urine.

Table 4. Full EORTC/MSG criteria, 2002 version

Possible / Probable invasive aspergillosis	
Host factors	<ul style="list-style-type: none"> Neutropenia (< 500 neutrophils/mm³ for > 10 days) Persistent fever for > 96 h refractory to appropriate broad-spectrum antibacterial treatment in high-risk patients Body temperature either $> 38^{\circ}\text{C}$ or $< 36^{\circ}\text{C}$ and any of the following predisposing conditions: prolonged neutropenia (> 10 days) in previous 60 days, recent or current use of significant immunosuppressive agents in previous 30 days, proven or probable invasive fungal infection during previous episode of neutropenia, or coexistence of symptomatic AIDS Signs and symptoms indicating graft-versus-host disease, particularly severe (grade ≥ 2) or chronic extensive disease Prolonged (> 3 weeks) use of corticosteroids in previous 60 days
Clinical criteria	<p>Must be related to site of microbiological criteria and temporally related to current episode</p> <ul style="list-style-type: none"> Lower respiratory tract infection

Table 4. Full EORTC/MSG criteria, 2002 version (Continued)

	<ul style="list-style-type: none"> Major: Any of the following new infiltrates on CT imaging: halo sign, air-crescent sign, or cavity within area of consolidation, in absence of infection by organisms that may lead to similar radiological findings including cavitation, such as <i>Mycobacterium</i>, <i>Legionella</i>, and <i>Nocardia</i> species. Minor: Symptoms of lower respiratory tract infection (cough, chest pain, haemoptysis, dyspnoea); physical finding of pleural rub; any new infiltrate not fulfilling major criterion; pleural effusion
	<ul style="list-style-type: none"> Sinonasal infection <ul style="list-style-type: none"> Major: Suggestive radiological evidence of invasive infection in sinuses (i.e., erosion of sinus walls or extension of infection to neighbouring structures, extensive skull base destruction) Minor: Upper respiratory symptoms (e.g. nasal discharge, stuffiness); nose ulceration or eschar of nasal mucosa or epistaxis; periorbital swelling; maxillary tenderness; black necrotic lesions or perforation of hard palate
	<ul style="list-style-type: none"> CNS infection <ul style="list-style-type: none"> Major: Radiological evidence suggesting CNS infection (e.g. mastoiditis or other parameningeal foci, extradural empyema, intraparenchymal brain or spinal cord mass lesion) Minor: Focal neurological symptoms and signs (including focal seizures, hemiparesis, and cranial nerve palsies); mental changes; meningeal irritation findings; abnormalities in CSF biochemistry and cell count (provided that CSF is negative for other pathogens by culture or microscopy and negative for malignant cell)
Microbiological criteria	<ul style="list-style-type: none"> Positive result of culture for mold (including <i>Aspergillus</i>, <i>Fusarium</i>, or <i>Scedosporium</i> species or a <i>Zygomycetes</i>) or <i>Cryptococcus neoformans</i> or an endemic fungal pathogen from sputum or bronchoalveolar lavage fluid samples Positive result of culture or findings of cytologic/direct microscopic evaluation for mold from sinus aspirate specimen Positive findings of cytologic/direct microscopic evaluation for mold or <i>Cryptococcus</i> species from sputum or bronchoalveolar lavage fluid samples Positive result for <i>Aspergillus</i> antigen in specimens of bronchoalveolar lavage fluid, CSF, or ≥ 2 blood samples Positive result for cryptococcal antigen in blood sample Positive findings of cytologic or direct microscopic examination for fungal elements in sterile body fluid samples (e.g. <i>Cryptococcus</i> species in CSF) Positive result for <i>Histoplasma capsulatum</i> antigen in blood, urine, or CSF specimens Two positive results of culture of urine samples for yeasts in absence of urinary catheter <i>Candida</i> casts in urine in absence of urinary catheter Positive result of blood culture for <i>Candida</i> species
Proven aspergillosis	
Criterion	Histopathologic or cytopathologic examination showing hyphae from needle aspiration or biopsy specimen with evidence of associated tissue damage (either microscopically or unequivocally by imaging); or positive culture result for a sample obtained by sterile procedure from normally sterile and clinically or radiologically abnormal site consistent with infection, excluding urine and mucous membranes.

Table 5. Meta-analysis of the pairs of sensitivity and specificity, sensitivity analysis

Meta-analysis of the pairs of sensitivity and specificity, sensitivity analysis		
	0.5 ODI (n = 12)	1.0 ODI (n = 11)
sensitivity	0.84 (95% CI 0.74 to 0.94)	0.80 (95% CI 0.69 to 0.92)

Table 5. Meta-analysis of the pairs of sensitivity and specificity, sensitivity analysis *(Continued)*

specificity	0.83 (95% CI 0.76 to 0.90)	0.92 (95% CI 0.89 to 0.96)
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CI: confidence interval; ODI: optical density index

Table 6. Quantification of the incorporation bias

	with incorporation (n=12)	without incorporation (n = 4)
sensitivity	0.74 (95% CI 0.63 to 0.86)	0.98 (95% CI 0.95 to 1.00)
specificity	0.91 (95% CI 0.86 to 0.95)	0.72 (95% CI 0.53 to 0.90)

Table 7. Quantification of the effect of the study design

	prospective (n = 6)	retrospective (n = 10)
sensitivity	0.74 (95% CI 0.60 to 0.88)	0.94 (95% CI 0.86 to 1.00)
specificity	0.91 (95% CI 0.86 to 0.96)	0.78 (95% CI 0.65 to 0.91)

CI: confidence interval

Table 8. Sensitivity analyses on methodological quality

Sensitivity analyses on methodological quality		
Patients and flow and timing domains		
	overall (all studies, n = 16)	low risk of bias (n = 6)
sensitivity	0.83 (95% CI 0.70 to 0.92)	0.75 (95% CI 0.51 to 0.89)
specificity	0.88 (95% CI 0.80 to 0.92)	0.86 (95% CI 0.78 to 0.91)

CI: confidence interval

Table 9. Effect of reference standard definition

	Definition	Studies	Sensitivity	95% CI		Specificity	95% CI	
				Lower limit	Upper limit		Lower limit	Upper limit
1	Prov and Prob vs Pos and No	16	0.83	0.70	0.92	0.88	0.80	0.92
2	Prov vs Prob and Pos and No	11	0.77	0.33	0.96	0.73	0.63	0.81
3	Prov and Prob and Pos vs No	11	0.59	0.40	0.75	0.94	0.89	0.97
4	Possibles excluded	11	0.79	0.66	0.88	0.94	0.89	0.97

CI: confidence interval; **Prob:** probable; **Prov:** proven

Table 10. Other systematic reviews

Systematic review	Number of studies	Number of studies also included in our review	Cut-off ODI	Sensitivity (95% CI)	Specificity (95% CI)
Heng 2015	16	6	0.5	0.82 (0.70–0.91)	0.92 (0.85–0.96)
			1	0.75 (0.55–0.88)	0.95 (0.87–0.98)
Guo 2010	13	7	overall	0.90 (0.79–0.96)	0.94 (0.90–0.96)
			0.5	0.86 (0.70–0.94)	0.89 (0.85–0.92)
			1	0.85 (0.72–0.93)	0.94 (0.89–0.97)
Zou 2012	30	8	0.5	0.87 (0.79–0.92)	0.89 (0.85–0.92)
			1	0.86 (0.76–0.92)	0.95 (0.91–0.97)

CI: confidence interval; ODI: optical density index

APPENDICES

Appendix 1. Electronic databases and search criteria used

1. MEDLINE (through Ovid)

1 MEDLINE (through Ovid)
1 exp lung aspergillosis/ or aspergillosis/
2 aspergill*.ti,ab.
3 exp Aspergillus/
4 (invasive adj2 fungal).ti,ab.
5 (fungal adj2 infection).mp.
6 1 or 2 or 3 or 4 or 5
7 exp Immunoassay/
8 immunoassay*.mp.
9 immunosorbent.mp.
10 ELISA.ti,ab.
11 (EIA or EIAs).ti,ab.
12 galactomannan.ti,ab.
13 exp Mannans/
14 exp Serology/
15 exp Serologic Tests/
16 (serology or serodiagnosis).ti,ab.
17 Platelia.ti,ab.
18 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17
19 6 and 18
21 limit 19 to yr="2000 -Current"

2. Embase (through Ovid)

1 Aspergillosis/
2 aspergill*.mp.
3 exp Aspergillus/
4 (fungal adj2 invasive).mp.
5 (fungal adj2 infection).mp.
6 1 or 2 or 3 or 4 or 5

7 exp Immunoassay/ or (immunoassay*).mp.
8 (ELISA or ELISAs).mp.
9 (EIA or EIAs).mp.
10 Platelia.mp.
11 exp galactomannan/
12 galactomannan.mp.
13 exp serology/
14 (serology or serodiagnosis or serologic).mp.
15 immunosorbent.mp.
16 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15
17 6 and 16
18 limit 17 to yr="2000 -Current"

3. Web of Science

1TS=(aspergill*) OR TI=(aspergill*) OR FT=(aspergill*)
2TS=(fungal) OR TI=(fungal) OR FT=(fungal)
3#1 OR #2
4TS=(immunosorbent) OR TI=(immunosorbent) OR FT=(immunosorbent)
5TS=(ELISA) OR TI=(ELISA) OR FT=(ELISA)
6TS=(EIA) OR TI=(EIA) OR FT=(EIA)
7TS=(Platelia) OR TI=(Platelia) OR FT=(Platelia)
8TS=(galactomannan) OR TI=(galactomannan) OR FT=(galactomannan)
9TS=(serology) OR TS=(serodiagnosis)
10#4 OR #5 OR #6 OR #7 OR #8 OR #9
11#3 AND #10

Appendix 2. Our application of the QUADAS-2 tool

Phase 1. Drawing a flow diagram

Drawing a flow diagram of the study may be helpful but is not mandatory. Flow charts of potential participants display how many were eligible for the study, how many were actually recruited, how many received the index test, how many received the reference standard, etc. In addition, the numbers of true- and false-positives and true- and false-negatives are displayed. If necessary, a flow diagram will be drawn in the space provided on the extraction form.

Phase 2. Signalling questions, bias risk assessment and applicability concern per QUADAS-2 domain

Domain 1. Patient selection

Was a consecutive or random sample of patients enrolled?

This will be scored 'yes' if it was clearly stated in the Methods section or if the study stated that 'all' or 'a random selection of all' eligible patients were enrolled. If it is clear that the clinician made the selection, then it will be scored as 'no'.

Was a case-control design avoided?

This will be scored 'yes' for all included studies, as case-control designs will be excluded.

Did the study avoid inappropriate exclusions?

Inappropriate exclusions are exclusions of 'possible' patients, or exclusions based on the EORTC/MSG criteria.

Judgment of Risk of Bias

We will consider the risk of bias low when patients were consecutively selected.

We will not judge the applicability, as we expect to find studies with a wide range of patients; and also patients without a EORTC/MSG host criterion. Instead, we choose to carefully document the type of participants and make an inventory of:

- whether participants were in-patients or out-patients;
- the department(s) where the patients were recruited (such as the Department of Paediatrics or Adult Haematology);
- the age of the participants;
- the study design;
- the underlying disease spectrum;
- the cause of their increased risk for invasive aspergillosis;

- a recent history of neutropenia ($< 0.5 \times 10^9$ neutrophils/L > 10 days) temporally related to the onset of fungal disease
- receipt of an allogeneic stem cell transplant
- prolonged use of corticosteroids (excluding in patients with allergic bronchopulmonary aspergillosis) at a mean minimum dose of 0.3 mg/kg/day of prednisone equivalent for > 3 weeks
- treatment with other recognised T-cell immunosuppressants, such as cyclosporine, TNF- α blockers, specific monoclonal antibodies (such as alemtuzumab), or nucleoside analogues during the past 90 days
- inherited severe immunodeficiency (such as chronic granulomatous disease or severe combined immunodeficiency)
- other...
- what the intended use of the test was;
- whether there was a protocol defining the indication for performing the test;
- whether the EORTC/MSG host criteria were used as an inclusion criterion, and whether this inclusion criterion was strictly followed;
- and whether prior galactomannan testing had been performed and whether the result could be a reason for performing broncho-alveolar lavage.

Domain 2. Conduct and interpretation of index test

We will state:

- whether the execution of the bronchoscopy and the collection of broncho-alveolar lavage was performed according to a fixed protocol; for example, defining the number and volume of lavages, and the fluid that was used as lavage;
- whether the execution of the ELISA was clearly described or whether it was explicitly stated that the ELISA was performed according to the manufacturer's instructions;
- how the test was interpreted: which cut-off value(s) were used, and whether these cut-off values were predefined, and how uninterpretable or intermediate results were dealt with;
- whether the results of the reference standard were known when the index test result was interpreted.

Was the result of the index test interpreted without knowledge of the results of the reference standard?

This will be scored 'yes' if the index test was done before the reference standard was assessed, or when the authors clearly stated that the assessment of the index test was blinded.

Was the cut-off value predefined?

If the threshold was mentioned in the methods section, or if the authors stated that they followed the manufacturer's directions (which includes guidance on the threshold), then the item will be scored as 'yes'. We will score a 'no' if the authors drew a ROC plot, or if multiple thresholds (outside the commonly reported ones of 0.5, 1.0 and 1.5) were reported, or if the authors stated that the threshold they used was the optimal threshold in their study.

We will consider the risk of bias 'high' when either of the two signalling questions was answered 'no'.

Domain 3. Conduct and interpretation of reference standard, and target condition

We will note:

- whether the EORTC/MSG criteria were used, or whether enough information was stated to deduct the classification of the patients post hoc;
- how the reference standard or test was executed;
- whether the reference standard was interpreted without knowledge of the results of the index test;
- whether the index test was incorporated in the reference standard, and whether incorporation was quantified in the study;
- which EORTC/MSG categories were described in the results, e.g. all 4 EORTC/MSG classifications or just combinations of categories such as "NO AND POSSIBLE" versus "PROBABLE AND PROVEN";
- whether uninterpretable or intermediate results were reported.

Were the EORTC/MSG criteria or autopsy (or both) explicitly used to define whether the target condition was present? And were these criteria strictly adhered to?

Were the reference standard results interpreted without knowledge of the results of the index tests?

This will be scored 'yes' if the reference standard was done before the index test was assessed, or when the authors clearly stated that the assessment of the reference standard was blinded.

We will consider the risk of bias 'high' when either of the two signalling questions was answered 'no'.

Domain 4. Flow and timing

We will describe:

- whether and which patients withdrew from analysis or were not included in the analyses, for example whether partial verification occurred.
- the time between the performance of the index and reference standard (range and median, or SD and average).
- whether there was an acceptable delay between tests? Were there any withdrawals or were patients excluded from analysis?

Was there an appropriate time-interval between index test and reference standard?

We will not score this item, because the determination of the time of diagnosis is difficult when a reference standard built up from several criteria is being used.

Did the authors avoid partial verification?

We will define partial verification as applying the (composite) reference standard in less than 100% of the patients. Partial verification could occur in studies where autopsy is used as (part of) a reference standard, because it is only done when a participant dies and his or her family gives permission.

Were all patients included in the analyses?

Risk of bias will be considered high if we have clear indication of partial verification or a significant exclusion of study participants from the analysis.

Extra Domain. Study sponsoring

An extra quality item that we will assess is sponsoring of the study by the manufacturer of the commercially available galactomannan test.

WHAT'S NEW

Date	Event	Description
11 April 2020	Amended	We corrected a calculation error in the Summary of findings and in Figure 7 .

CONTRIBUTIONS OF AUTHORS

Conception of the study: KdH.

Design: KdH, MG, CV, ML.

Selection of studies: KdH, MG, CV, ML.

Assessment of methodological quality: KdH, MG, CV, ML.

Drafting the paper: KdH.

Revising it critically: KdH, MG, CV, ML.

Final approval of the version to be published: KdH, MG, CV, ML.

Agreement to be accountable for all aspects of the work: KdH, MG, CV, ML.

The first two review authors (KdH, MG) contributed equally to this article.

Contributions of editorial team

Rebecca Fortescue (Co-ordinating Editor): edited the review; advised on methodology, interpretation and content; approved the final review prior to publication.

Chris Cates (Co-ordinating Editor) checked the data entry prior to the full write up of the review.

Kristin Carson-Chahhoud (Editor): edited the review.

Emma Dennett (Managing Editor): co-ordinated the editorial process; edited the review.

Emma Jackson (Assistant Managing Editor): conducted peer review; obtained translations; edited the Plain language summary and reference sections of the protocol and the review.

Elizabeth Stovold (Information Specialist): designed the search strategy; ran the searches; edited the search methods section.

With thanks to the DTA editorial team for constructive comments and for providing peer review comments and assistance with sign-off.

DECLARATIONS OF INTEREST

CV: Invited as speaker for Becton, Dickinson and Company on laboratory automation. Pfizer partly financed attendance to TIMM in Lisbon. None of the financial activities had any influence on the present work.

KdH, ML, MG: No known conflicts of interest.

SOURCES OF SUPPORT

Internal sources

- The authors declare that no such funding was received for this systematic review, Other.

External sources

- The authors declare that no such funding was received for this systematic review, Other.

DIFFERENCES BETWEEN PROTOCOL AND REVIEW

We stated that we would check the WHO International Clinical Trials RegistryPlatform for ongoing or unpublished trials, and would contact the manufacturer of the index test. This has not yet been done.

The first selection of studies, based on title and abstract, was also performed by MG.

All review authors (KdH, MG, CV, ML) extracted data and assessed quality.

We included only studies that were peer-reviewed and published in an English journal. This was not specified in our protocol.

We only e-mailed authors when we thought that with additional information it would be possible to include their study in the review. i.e. we only e-mailed an author when one of the last two criteria mentioned in [Selection of studies](#) was fulfilled.

We did not perform a stratified analysis for clinical subgroups. The potential sources of heterogeneity were included in the meta-analysis as a covariate.

One included study did not report accuracy data at a cut-off ODI of 0.5 or 1.0. We did not foresee this possibility and did not address this situation in our protocol. We chose to exclude this study from the analyses. This is also stated in the [Results](#) section.

We added a number of covariates to the meta-analysis.

- We examined the effect of the department(s) where the patients were recruited.
- We studied the effect of the presence of a formal indication for performing the index test in the study protocol.
- We studied the heterogeneity as an effect of study design.
- We examined the effect of sponsoring of the study by the manufacturer of the commercially available galactomannan test.

We decided not to assess the concern about applicability for the patient domain. Instead, we chose to carefully document the type of participants. We already stated this in our protocol, in our application of the QUADAS-2 tool ([Appendices](#)). For the purpose of clarity, we now also state this in our [Methods](#) section and discuss this in the [Applicability of findings to the review question](#). For the index test and reference standard domains, we classified all studies to have a low applicability concern because our inclusion criteria stipulated that we only include studies that used the galactomannan ELISA performed on BAL as the index test and the EORTC/MSG criteria as reference standard.

A Plain language summary and Figure 2 were added.

We added the definition of "immunocompromised" to fully describe the target population, and defined prospective and retrospective more clearly.

We added a table with the EORTC criteria in full detail.

We changed and added a number of sentences to improve clarity.

INDEX TERMS

Medical Subject Headings (MeSH)

*Immunocompromised Host; Aspergillosis [*diagnosis] [immunology]; Biomarkers [blood]; Bronchoalveolar Lavage Fluid [*microbiology]; Galactose [analogs & derivatives]; Invasive Fungal Infections; Mannans [*blood]; Randomized Controlled Trials as Topic; Sensitivity and Specificity

MeSH check words

Humans