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(1→3)-β-D-glucan testing for the detection of invasive fungal infections in immunocompromised or critically ill people (Review)

White SK, Schmidt RL, Walker BS, Hanson KE

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[Diagnostic Test Accuracy Review]

(1→3)-β-D-glucan testing for the detection of invasive fungal infections in immunocompromised or critically ill people

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ABSTRACT

Background

Invasive fungal infections (IFIs) are life-threatening opportunistic infections that occur in immunocompromised or critically ill people. Early detection and treatment of IFIs is essential to reduce morbidity and mortality in these populations. (1→3)-β-D-glucan (BDG) is a component of the fungal cell wall that can be detected in the serum of infected individuals. The serum BDG test is a way to quickly detect these infections and initiate treatment before they become life-threatening. Five different versions of the BDG test are commercially available: Fungitell, Glucatell, Wako, Fungitec-G, and Dynamiker Fungus.

Objectives

To compare the diagnostic accuracy of commercially available tests for serum BDG to detect selected invasive fungal infections (IFIs) among immunocompromised or critically ill people.

Search methods

We searched MEDLINE (via Ovid) and Embase (via Ovid) up to 26 June 2019. We used SCOPUS to perform a forward and backward citation search of relevant articles. We placed no restriction on language or study design.

Selection criteria

We included all references published on or after 1995, which is when the first commercial BDG assays became available. We considered published, peer-reviewed studies on the diagnostic test accuracy of BDG for diagnosis of fungal infections in immunocompromised people or people in intensive care that used the European Organization for Research and Treatment of Cancer (EORTC) criteria or equivalent as a reference standard. We considered all study designs (case-control, prospective consecutive cohort, and retrospective cohort studies). We excluded case studies and studies with fewer than ten participants. We also excluded animal and laboratory studies. We excluded meeting abstracts because they provided insufficient information.

Data collection and analysis

We followed the standard procedures outlined in the *Cochrane Handbook for Diagnostic Test Accuracy Reviews*. Two review authors independently screened studies, extracted data, and performed a quality assessment for each study. For each study, we created a 2 × 2 matrix and calculated sensitivity and specificity, as well as a 95% confidence interval (CI). We evaluated the quality of included studies using the Quality Assessment of Studies of Diagnostic Accuracy-Revised (QUADAS-2). We were unable to perform a meta-analysis due to

considerable variation between studies, with the exception of *Candida*, so we have provided descriptive statistics such as receiver operating characteristics (ROCs) and forest plots by test brand to show variation in study results.

Main results

We included in the review 49 studies with a total of 6244 participants. About half of these studies (24/49; 49%) were conducted with people who had cancer or hematologic malignancies. Most studies (36/49; 73%) focused on the Fungitell BDG test. This was followed by Glucatell (5 studies; 10%), Wako (3 studies; 6%), Fungitec-G (3 studies; 6%), and Dynamiker (2 studies; 4%). About three-quarters of studies (79%) utilized either a prospective or a retrospective consecutive study design; the remainder used a case-control design.

Based on the manufacturer's recommended cut-off levels for the Fungitell test, sensitivity ranged from 27% to 100%, and specificity from 0% to 100%. For the Glucatell assay, sensitivity ranged from 50% to 92%, and specificity ranged from 41% to 94%. Limited studies have used the Dynamiker, Wako, and Fungitec-G assays, but individual sensitivities and specificities ranged from 50% to 88%, and from 60% to 100%, respectively. Results show considerable differences between studies, even by manufacturer, which prevented a formal meta-analysis. Most studies (32/49; 65%) had no reported high risk of bias in any of the QUADAS-2 domains. The QUADAS-2 domains that had higher risk of bias included participant selection and flow and timing.

Authors' conclusions

We noted considerable heterogeneity between studies, and these differences precluded a formal meta-analysis. Because of wide variation in the results, it is not possible to estimate the diagnostic accuracy of the BDG test in specific settings. Future studies estimating the accuracy of BDG tests should be linked to the way the test is used in clinical practice and should clearly describe the sampling protocol and the relationship of time of testing to time of diagnosis.

PLAIN LANGUAGE SUMMARY

Measurement of β-D-glucans to detect invasive fungal infection in immunocompromised people

Why is improving the diagnosis of invasive fungal infections important?
Fungal infections occur in people who are unable to fight infection, and these infections can be life-threatening in this group of people. Fungal infections are difficult to diagnose. Failure to recognize a fungal infection when it is present (a false-negative test result) leads to delayed treatment and poorer outcomes. An incorrect diagnosis of infection (a false-positive result) may result in wasted resources and unnecessary investigation and treatment.

What is the aim of this review?
The aim of this review is to find out how accurate a blood test is for diagnosis of fungal infections in people who are unable to fight infection. Review authors included 49 studies to answer this question.

What was studied in this review?
Five kinds of blood tests were compared. All of these tests use similar biochemical methods to detect the presence of a sugar molecule (β-D-glucan) that is a component of the fungal cell wall. This molecule does not normally occur in blood, so its detection indicates that fungi are present. The tests require a blood sample, which is then sent to a laboratory for analysis. Diagnosis of fungal infections is difficult, and the diagnosis is often made only after the disease has advanced. Blood tests can provide an earlier diagnosis, so they would offer an advantage over current methods.

What are the main results of the review?
This review included studies of 6244 people who were at risk of getting fungal infections. Study results show that accuracy varied widely across studies. The variation was so great that it was not possible to obtain a reliable estimate of the accuracy of the various tests.

How reliable are results of the studies in this review?
In the included studies, the diagnosis of invasive fungal infection was made using criteria developed by the European Organization for Research and Treatment of Cancer (EORTC)*. The EORTC criteria are considered reliable and the studies were generally well conducted, so it is likely that the reference diagnoses were accurate. Accuracy of blood tests for invasive fungal infections varied widely. Some studies found that the blood test was accurate, but others found that the blood test was not very accurate. The reason for this variation is not understood.

*The EORTC criteria provide the reference diagnosis. Results of the blood test are compared to the reference diagnosis.

Who do the results of this review apply to?
Most included studies were performed at academic medical centers or public hospitals in the United States, Germany, and Italy. The most common underlying conditions were cancer (47%) and admission to intensive care (33%). A majority of participants were adults. The overall prevalence of invasive fungal infection was 28%.

What are the implications of this review?

Accuracy of the diagnosis varied widely across studies. It is not clear whether testing can accurately detect invasive fungal infections. Testing accurately detects disease in some studies, but in others it does not. The reasons for the variation in accuracy are not understood.

How up-to-date is this review?
The review authors searched for and reviewed studies published up to June 2019.

SUMMARY OF FINDINGS

Summary of findings 1. Summary of findings

Participants/Populations: immunocompromised people at risk for invasive fungal infections

Prior testing: none

Settings: hospital setting

Index test: commercially available serum BDG test

Importance: test needed to accurately detect fungal infections in susceptible people at an early enough stage to facilitate antifungal treatment

Reference standard: EORTC/MSG criteria, or by microscopy or autopsy

Studies: 49 studies with 6244 participants

1. Test assay

Test/Subgroup	No. of participants (studies)	Overall sensitivity (range)	Overall specificity (range)	Implications	Quality and comments
Fungitell	4316 (36)	27% to 100%	0 to 100%	Wide variation in sensitivity and specificity. Summary estimates would not be meaningful	
Glucatell	957 (5)	50% to 92%	41% to 94%	Wide variation in sensitivity and specificity. Summary estimates would not be meaningful	
Wako	420 (3)	50% to 86%	89% to 100%		Insufficient number of studies for meta-analysis
Fungitec-G	353 (3)	67% to 88%	60% to 85%		Too few studies for meta-analysis
Dynamiker Fungus	198	64% to 81%	78% to 80%		Too few studies for meta-analysis

	(2)				
2. Fungal organism					
Test/Subgroup	**No. of participants (studies)**	**Sensitivity estimate (95% CI)**	**Specificity estimate (95% CI)**	**Implications**	**Quality and comments**
Candida	1185 (10)	81% (75% to 86%)	64% (56% to 72%)	Results are more homogeneous for *Candida* testing than for all fungi	

BDG: beta-D-glucan test; CI: confidence interval; EORTC/MSG: European Organization for Research and Treatment of Cancer Mycoses Study Group.

BACKGROUND

Target condition being diagnosed

Invasive fungal infection (IFI) is a major cause of morbidity and mortality in immunosuppressed and critically ill people (Lemonovich 2018; Person 2010). Prompt diagnosis is important because early initiation of appropriate antifungal therapy improves patient outcomes (Chamilos 2008; Garey 2006; Morrell 2005; von Eiff 1995). Diagnosis of IFI is challenging because the standard methods of clinical diagnosis (e.g. clinical signs and symptoms, host risk assessment, physical examination, radiography) are not specific to IFI. In addition, traditional microbiological methods often have limited clinical utility because cultures are frequently negative or become positive only in advanced stages of infection (Clancy 2013). Histopathologic examination of infected tissue has been the historic gold standard, but invasive testing may not be feasible in unstable participants or in those with underlying coagulopathy. Although composite definitions for IFI have been developed by the European Organization for Research and Treatment of Cancer Mycoses Study Group (EORTC/MSG), these definitions are best suited for research purposes (De Pauw 2008). The EORTC/MSG diagnostic categories of IFI include proven, probable, and possible disease.

Current strategies for prevention and management of IFI include antifungal prophylaxis, pre-emptive therapy, empiric treatment, and treatment of established infection (Leroux 2013). Universal prophylaxis is effective and logistically easy, but the medications can have toxic effects, can potentially promote antimicrobial resistance, and are expensive. Empiric therapy based on symptoms or treatment of established IFI potentially delays initiation of potentially life-saving therapy. In contrast, pre-emptive therapy is a more selective approach in which people are sequentially monitored and treatment is based on detection of laboratory biomarkers in blood, often before clinical signs or symptoms of an IFI are apparent. Pre-emptive approaches are designed to identify the highest-risk people who are most likely to benefit from early antifungal therapy. Examples of fungal biomarkers include circulating fungal DNA and cell wall components such as galactomannan (GM), glucuronoxylomannan, mannan, and (1→3)-beta-D-glucan (BDG). Tests designed to detect these markers may be deployed as part of a pre-emptive treatment strategy or may be used to facilitate selection of empiric treatment for symptomatic at-risk people.

Index test(s)

Non-invasive, non-culture-based methods for diagnosing invasive fungal disease have the potential for significant clinical utility (Powers-Fletcher 2016). BDG is a cell wall polysaccharide found in a wide variety of medically important fungi including *Candida* species (spp) (*Aspergillus* spp and *Pneumocystis jirovecii*; important exceptions are *Mucorales*, *Cryptococcus* spp, and the yeast form of *Blastomyces* (Wright 2011)). Assays designed to detect BDG in human serum have been used both as an adjunct for diagnosis of IFI and for serial surveillance during periods of risk. Commercially available assays include the Fungitell and Glucatell assays (Associates of Cape Code, Falmouth, MA, USA), which are used in America and in Europe, as well as the Fungitec-G assay (Seikagaku Kogyo Corporation, Tokyo, Japan) and the Wako test (Wako Pure Chemical Industries, Osaka, Japan), both of which are used in Japan. The Dynamiker Fungus assay (Dynamiker Biotechnology Ltd, Tianjin, China) is a new test that was recently developed in China.

These assays are based on the ability of the BDG molecule to induce clot formation in the hemolymph of horseshoe crabs. BDG activates Factor G, which is a serine protease in the horseshoe crab coagulation cascade. Activated Factor G then converts an inactive proclotting enzyme to its active form, which, in turn, cleaves an artificial substrate that can be detected. The assays differ in the substrate used for detection. The Fungitell and Glucatell assays use a chemiluminescent method. The Glucatell test differs from the Fungitell test in that the Glucatell reagent is processed to eliminate Factor C. This makes the Glucatell test more specific for BDG linkages. The Glucatell reagent does not react to other polysaccharides including beta-glucans with other glycosidic linkages. For the other assays, Dynamiker Fungus uses a spectrophotometric method, the Wako assay is a turbidometric method, and Fungitec-G is a colorimetric method. Each of these tests uses a different interpretive cut-off value. In the Fungitell and Glucatell assays, a value of 60 pg/mL or less is negative, a value of 60 to 80 pg/mL is equivocal, and a value of 80 pg/mL or more is positive. For the Fungitec-G assay, a value greater than 20 pg/mL is considered positive, whereas for Wako, it is 11 pg/mL. The Dynamiker Fungus test considers values above 95 pg/mL as positive. These differences may be due to the fact that the reagents are obtained from different genera of horseshoe crabs (Fungitell reagents are extracted from *Limulus polyphemus,* whereas Fungitec and Wako reagents are extracted from *Tachypleus tridentatus*).

Studies vary in the criteria used for BDG positivity. For example, a single positive BDG result may be sufficient to classify a person as "BDG positive" in some studies, whereas other studies may use more stringent criteria such as two consecutive positive tests, or two positive tests within a specified time period. Similarly, studies use different sampling plans, which may affect test performance. Some studies may use a single sample, whereas others may use a prolonged sampling regimen (e.g. twice-a-week sampling for several weeks).

Clinical pathway

Presentation

The fungi capable of causing invasive disease in humans are a diverse group of eukaryotic microorganisms including yeasts, molds, and dimorphic fungi. *Candida* and *Aspergillus* are the pathogens most commonly diagnosed after solid organ transplantation or critical care (Pappas 2010), and *Aspergillus* and other filamentous fungi predominate after hematopoietic stem cell transplantation or as a complication of cytotoxic chemotherapy for hematologic malignancy (Kontoyiannis 2010; Neofytos 2009). In addition, *Pneumocystis jirovecii* remains an important opportunistic pathogen that affects people with AIDS and those receiving cytotoxic or immunosuppressive therapy. Clinical signs and symptoms of IFI vary widely. The clinical presentation of IFI varies widely according to the infecting pathogen, the overall net state of immunosuppression (i.e. the host), and the site and severity of infection. Invasive candidiasis comprises a spectrum of diseases including bloodstream infection and deep-seated infection (e.g. intra-abdominal abscess), which may occur independently or concurrently. The filamentous fungi typically present with pulmonary or sino-cerebral disease. Pneumonia is the most common manifestation of *Pneumocystis*.

Standard diagnostic practice

In general, the current approach to IFI diagnosis combines a variety of complementary testing modalities. Diagnostic imaging helps clinicians to identify potential sites of infection. Cultures of blood, body fluids, and/or tissue are performed in combination with molecular tests and serum fungal biomarkers in an attempt to detect and identify fungi. Use of targeted imaging may help to guide biopsy sampling of infected tissue for histopathology.

Alternative test(s)

Classical methods of diagnosis include direct stains for fungi (i.e. calcofluor white, cytology, or histopathology) and fungal culture. Despite availability of a variety of test modalities, the clinical utility of this routine testing is often limited. For example, cultures are slow and relatively insensitive. Positive results, however, are useful for definitive organism identification and antifungal susceptibility testing. Cytology and calcofluor white stains applied to body fluid also lack sensitivity. Furthermore, deciphering colonization from invasive disease can be extremely difficult when samples are obtained from non-sterile body sites such as the respiratory tract. Visualizing fungal elements in tissue remains the diagnostic gold standard for IFI, but invasive testing may not be feasible for critically ill or coagulopathic people. Additionally, biopsy results may be affected by sampling error, and current staining techniques are neither genus- nor species-specific. This level of organism discrimination, however, is essential for selection of optimal antifungal therapy.

Detection of fungal biomarkers including nucleic acid and cell wall components helps support the diagnosis of IFI. Rapid polymerase chain reaction (PCR) techniques targeting fungi have been widely applied in clinical practice (Arvanitis 2014; Avni 2011; Fan 2013; Lu 2011a; Mengoli 2009; Sun 2011). Unfortunately, laboratory-developed PCR tests lack standardization, and commercial assays are not widely available. The Candida T2 assay (T2 Biosystems, Lexington, MA, USA) is a rapid and accurate test for the detection of *Candida* DNA directly in whole blood (Tang 2019). Unfortunately, this test targets only the five most common *Candida* species and requires expensive instrumentation/reagents. Detecting mannan antigen and anti-mannan antibodies also has potential utility for the diagnosis of invasive candidiasis, but commercial assays are mainly limited to European markets (Mikulska 2010). Last, lateral flow assays for Aspergillus GM have been developed for use with serum and bronchoalveolar lavage samples (Mercier 2019; Verdaguer 2007). A potential benefit of antigens like GM and BDG is that these polysaccharides can be detected non-invasively in blood at an early stage of infection, whereas release of fungal DNA may be negligible in initial phases of the disease (Monique 2006). Alternatively, limitations of the Aspergillus GM test include limited sensitivity in non-neutropenic patient populations and potential cross-reactivity with closely related fungi or other antigenically similar substances (Demiraslan 2017; Verweij 2006; Viscoli 2004).

Rationale

Here we perform an updated review of the BDG literature with a focus on immunocompromised or critically ill people. BDG suffers from many of the same limitations as the Aspergillus GM test. Sensitivity may vary by population and organism type, and false positives are thought to result from cross-reacting substances in certain medications or materials, or possibly in bacteria (Marty 2006; Tran 2016; Wright 2011). Thus, it is important to understand the diagnostic performance of BDG across a variety of at-risk populations and testing strategies. Our objective was to provide summary estimates of the diagnostic performance of BDG that could be used to inform future guideline updates and serve as a benchmark for emerging diagnostics tests such as PCR and the Candida T2 assay. Both BDG and the Aspergillus GM test results have been incorporated into the revised EORTC/MSG criteria for probable IFI.

OBJECTIVES

Primary objective

To compare the diagnostic accuracy of commercially available tests for serum BDG to detect selected invasive fungal infections (IFIs) among immunocompromised or critically ill people.

Secondary objectives

To assess possible sources of heterogeneity that could affect sensitivity and specificity estimates in this study (see Investigations of heterogeneity).

METHODS

Criteria for considering studies for this review

Types of studies

Published peer-reviewed studies that compared the results of BDG tests against a clearly defined reference standard (EORTC criteria or equivalent) for diagnosis of IFI were included in the analysis.

We included the following types of studies.

1. Retrospective studies in which BDG samples were collected from consecutive people at risk.
2. Prospective studies in which BDG samples were collected from consecutive people at risk.
3. Case-control studies in which controls were people at risk.

We excluded the following types of studies.

1. Case reports or case series.
2. Studies reported only as meeting abstracts.
3. Case-control studies using healthy controls, due to the high risk of spectrum bias.
4. Animal studies.

Participants

Study participants included the following categories of immunocompromised people, with results for both the index test and the reference test.

1. Those with cancer, specifically:
   a. patients with hematologic malignancies; those receiving stem cell transplants, chemotherapeutics, or other immunosuppressive drugs; and
   b. patients receiving chemotherapy.
2. Those receiving prolonged immunosuppressive therapy for:
   a. solid organ transplant; or
   b. connective tissue diseases.

3. Individuals with congenital or acquired immune disorders, including:
   a. human immunodeficiency virus (HIV) or acquired immunodeficiency syndrome (AIDS); or
   b. inherited immune disorders.
4. People receiving treatment in the intensive care unit (ICU).

There was no restriction on age or comorbidities.

Index tests

We included studies that used any commercially available BDG tests that were approved for clinical use and followed the manufacturer's recommended cut-off values.

1. Fungitell (cut-off: 80 pg/mL).
2. Glucatell (cut-off: 80 pg/mL).
3. Wako (cut-off: 11 pg/mL).
4. Fungitec-G (cut-off: 20 pg/mL).
5. Dynamiker Fungus (cut-off: 95 pg/mL).

Target conditions

The target condition included proven or probable IFI due to *Aspergillus* or *Candida*, or other IFIs as defined by EORTC/MSG criteria (De Pauw 2008). It should be noted that EORTC/MSG criteria were developed for people with malignancy and for hematopoietic stem cell transplant recipients; these criteria are not easily generalizable to all risk groups and/or fungal diseases. Therefore, *Pneumocystis jirovecii* pneumonia (PJP) and *Candida* studies outside of the cancer population were also included if proven infection was determined by microscopy (*Pneumocystis*) or by sterile site culture (*Candida*). People with colonized *Candida* were considered as non-cases.

Reference standards

We included studies that used the following reference standards for invasive fungal disease.

- Autopsy.
- EORTC/MSG criteria from either 2002 or 2008 guidelines (Ascioglu 2002; De Pauw 2008).
- Microscopy or sterile site culture for proven PJP or *Candida* infection, respectively.

The criteria for proven IFI are listed below.

Microscopic analysis of sterile material

- Molds: 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
- Yeast: histopathologic, cytopathologic, or direct microscopic examination of a specimen obtained by needle aspiration or biopsy from a normally sterile site

Culture of sterile material

- Molds: recovery of a mold or black yeast by culture obtained by 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
- Yeast: recovery of a yeast by culture of a sample obtained by a sterile procedure (including a freshly placed drain < 24 hours) from a normally sterile site showing a clinical or radiological abnormality consistent with an infectious disease process

Blood culture

- Molds: blood culture that yields a mold in the context of a compatible infectious disease process
- Yeast: blood culture that yields yeast or yeast-like fungi

The criteria for probable IFI include host factors (e.g. receipt of allogeneic stem cell transplant), clinical criteria, and mycologic criteria. As of 2008, the EORTC/MSG mycologic criteria now include biomarker tests such as BDG or GM. This creates a possible source of incorporation bias because the index test (BDG) is sometimes used as part of the reference standard for possible IFI. Therefore, we excluded studies that used BDG as part of the reference standard.

Search methods for identification of studies

Electronic searches

An initial search to identify articles related to the diagnostic accuracy of BDG using the search strategies described in Appendix 1 and Appendix 2 was completed in April 2017. The last update was performed on 26 June 2019.

- MEDLINE (R) via Ovid (1946 to June week 3, 2019).
- Embase via Ovid (1980 to week 25, 2019).

Because the commercial BDG test was not implemented until 1995, the search was restricted to articles published in 1995 or later. The search was not restricted with respect to language or study design.

We performed an additional electronic search based on the set of potentially relevant studies identified in June 2017 from MEDLINE and Embase. This search was a forward and backward citation search to identify all studies cited by or citing the set of potentially relevant studies. This citation search was performed using SCOPUS on June 6, 2017.

Data collection and analysis

Selection of studies

Two review authors (RLS, SKW) screened the titles and abstracts of all articles to identify potentially relevant studies. Disagreements were resolved by discussion.

Each study in the set of potentially relevant studies was given a full-text review. An initial abstract form (see Appendix 3) was used to retrieve preliminary information that was used to determine whether the article met the inclusion criteria. Full-text review was performed independently by two review authors (RLS, SKW). This included information on study design, participant population, sample type, and IFI category (proven, probable, or possible), and whether EORTC/MSG, autopsy, or another method was used as the reference standard. These were reviewed together, and any discrepancies were resolved by discussion between the two review authors. Foreign language articles were assessed by a native speaker with scientific training (but not screened in duplicate) or were translated using Google Translate and reviewed by two review

authors (RLS, SKW). The review authors who determined relevance were not blinded to trial authors, publishing journal, or results.

Data extraction and management

Two review authors (SKW, BSW) extracted additional information from the selected studies on the condition (cancer, ICU, organ transplant, etc.), study design (prospective consecutive, retrospective consecutive, or case-control), sample type (serum, urine, bronchoalveolar lavage fluid, or cerebrospinal fluid), fungal organism (mixed IFI, *Candida*, *Aspergillus*, or *Pneumocystis jirovecii*), and reference standard used (EORTC or study-specific), using the data abstract form provided in Appendix 4. True-positive, false-positive, false-negative, and true-negative values were obtained to calculate sensitivity and specificity estimates. Additional information extracted during the full-text review included use of antifungal agents, sampling protocol, the assay used and the cut-off value, the number of positive samples needed to constitute a positive test result, and age of the population. All data were recorded, and discrepancies were resolved through discussion or by a third review author (RLS).

Assessment of methodological quality

We assessed study quality using the Quality Assessment of Studies of Diagnostic Accuracy-Revised (QUADAS-2) tool (Whiting 2011). Bias was assessed in four domains: participant selection, index test, reference standard, and flow/timing, and applicability was assessed in the first three domains only (participant selection, index test, and reference standard). Both were independently graded as low, high, or unclear quality by two review authors (SKW, BSW), using the interpretations listed in Appendix 5. Discrepancies were then resolved by discussion or were moderated by a third review author (RLS).

Statistical analysis and data synthesis

We transferred data into 2 × 2 matrices to calculate sensitivity and specificity for each study. We used reported values of true positives/negatives and false positives/negatives to calculate sensitivity and specificity. If these values were not reported, we back-extrapolated using reported sensitivity and specificity values.

Individual study data were presented graphically as forest plots by assay type. Studies were also plotted in receiver operating characteristic (ROC) space. We used the bivariate random-effects model for meta-analysis of the pairs of sensitivity and specificity (Reitsma 2005; van Houwelingen 1993). We restricted the analysis to standard cut-off values recommended by test manufacturers. All statistical analyses were completed using Stata v.14.2 (Stata Corporation, College Station, TX, USA). However, with the exception of studies involving *Candida*, we were unable to perform a formal meta-analysis for fungal groups because of high heterogeneity within the data, which prevented estimations of summary accuracy. This diversion from the protocol is explained in the Differences between protocol and review section.

Investigations of heterogeneity

When heterogeneity is present, subgroup analysis can be performed to determine the source. Heterogeneity between studies was supposed to be assessed by meta-regression performed on pre-selected covariates. We planned to investigate whether the following covariates or patterns of covariates had contributed to this.

1. Variation across participant subgroups (people with cancer or in the ICU compared to other groups; pediatric versus adult studies).
2. Variability in the number of positive results used to define a positive test (single positive result versus two consecutive positive samples).
3. Differences due to sampling strategies (single sample taken versus multiple samples collected over the length of stay).
4. Study design factors, including prospective versus retrospective and consecutive versus case-control.
5. Test interference (antifungal prophylaxis, pre-emptive therapy, etc.).
6. Definition of IFI: using proven and probable IFI (as defined above) as the definition of the target condition, and comparing it only to proven IFI when compared to all other categories and using proven, probable, and possible IFI compared to no IFI.

Because we were unable to perform a formal meta-analysis, we used ROC plots to visually investigate these potential sources of heterogeneity. This diversion from the protocol is explained in the Differences between protocol and review section.

Sensitivity analyses

We planned to compare pooled sensitivity and specificity estimates for studies that had low overall risk of bias versus those with at least one high risk of bias. However, we were unable to do this because a formal meta-analysis was not performed. This diversion from the protocol is explained in the Differences between protocol and review section.

RESULTS

Results of the search

Through the literature search in MEDLINE and Embase, we identified 10,354 references. Duplicate references were identified and removed (N = 1671), resulting in 8683 articles. The initial review of titles and abstracts yielded 211 potentially relevant articles (Figure 1).

Figure 1. Study flow diagram.

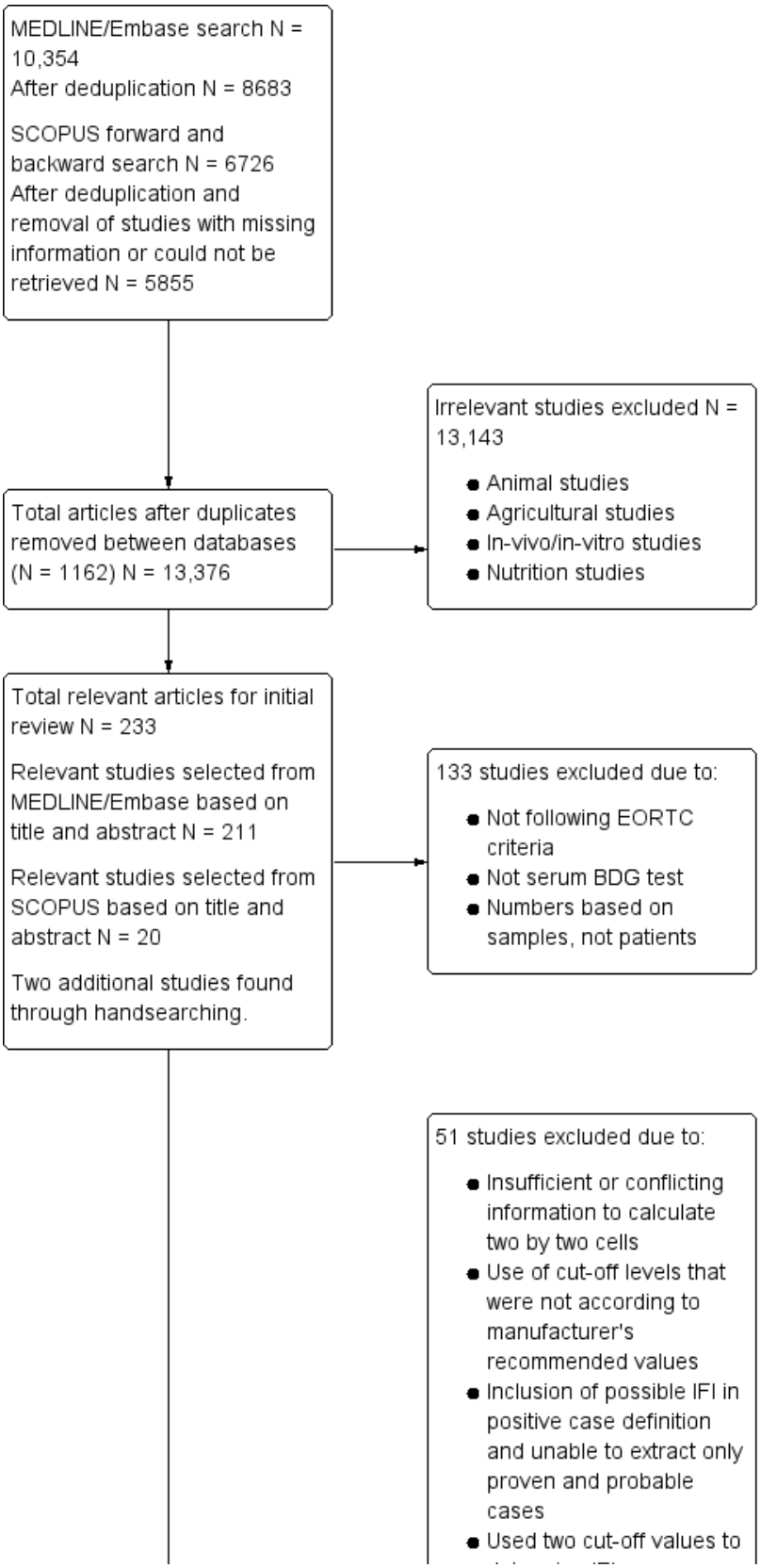
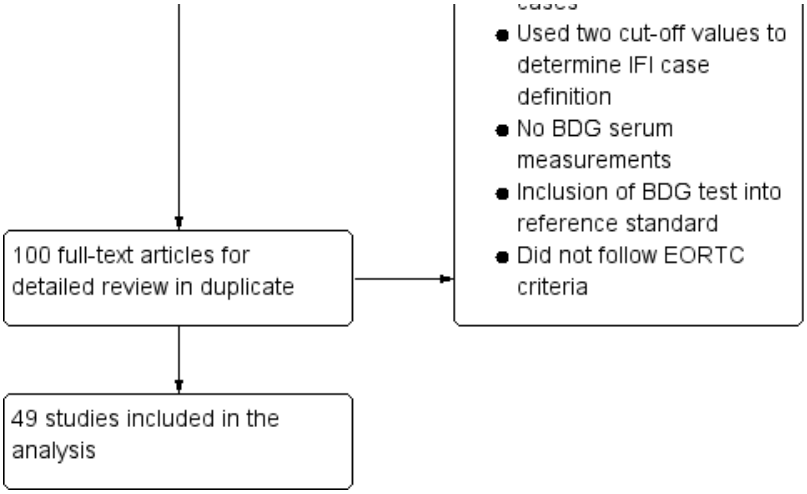


Figure 1. (Continued)



The citation search in SCOPUS (forward and backward search based on potentially relevant references) identified 6726 references. Of these, 747 were duplicates. Also, 124 references had extensive missing information and could not be retrieved, yielding a final number of 5855 references.

We compared results of the citation search (SCOPUS) with results of the initial search (MEDLINE and Embase) and identified 1162 references that had already been included in the initial search. We reviewed titles and abstracts of the remaining 13,376 references. In total, we identified 233 potentially relevant studies (211 from the MEDLINE/Embase searches, 20 from the citation search, and 2 additional articles through handsearching) that were initially reviewed for inclusion using the abstract form in Appendix 3.

After reviewing the 233 potentially relevant studies, we identified 100 studies that met study criteria in which we conducted a full-text review (Appendix 4). We contacted two study authors to receive clarification on possible IFI results and study design, which we received. At the conclusion of the full-text review, we identified 49 studies to be included in the systematic review (Table 1; Characteristics of included studies). A flow diagram of the selection process is shown in Figure 1.

Basic features of included studies

Details of the included studies are presented in Table 2, 'Overall characteristics of included studies'. We included 49 studies with a total of 6244 participants.

1. Participants: nearly half of the studies involved people with hematologic and oncologic diseases (N = 23; 47%), followed by people in the ICU (N = 16) and mixed at-risk cases (N = 7) (see Table 2). A majority (N = 26; 53%) were focused on adult populations, five had both adult and pediatric cases, and six focused solely on pediatrics (N = 3) or neonates (N = 3).
2. Study design: of the 49 studies, a little more than half (N = 26; 53%) were prospective in design. Of the 23 retrospective studies, 12 were consecutive, with the remaining employing a case-control design.
3. Assay characteristics: a majority (N = 36) used the Fungitell assay, followed by Glucatell (N = 5), Fungitec-G (N = 3), Wako (N = 3), and Dynamiker Fungus (N = 2).
4. Sampling: almost all studies reported only estimates based on a single positive test, although two studies did provide results based on two consecutive positive samples. Sampling design varied greatly between studies, ranging from a single sample collection (41%) to multiple samples collected over several weeks. Studies that collected multiple samples reported differing criteria for the classification of a positive BDG result, such as using the first sample collected or the highest BDG value recorded.
5. Organisms: studies that included all types of organisms were most common (N = 24), although several focused exclusively on *Candida* (N =10) or *Aspergillus* (N = 12). Per the selection criteria, all studies either used the EORTC/MSG criteria (N = 36) or followed the criteria used in the diagnosis of proven PJP or candidiasis.
6. Language: almost all studies (N = 47) were published in English, with the two remaining articles published in Chinese and Japanese.

Excluded studies

From our full-text review, we identified 51 studies to be excluded (see Characteristics of excluded studies) for the following reasons.

- Unable to determine 2 × 2 cell counts for overall sensitivity and specificity estimates (N = 17).
- Used cut-off values that did not follow the manufacturer's recommended level or utilized two cut-off values to determine a positive test (N = 11).
- Included BDG tests as part of the reference standard or did not follow EORTC/MSG guidelines (N = 7).
- Did not meet inclusion criteria for the study population or inclusion criteria were unknown (N = 6).
- Included probable (PJP or *Candida*) or possible IFI cases in the IFI definition, which could not be separated (N = 5).
- Other reasons (N = 4).

Methodological quality of included studies

Thirty-two studies had no concerns regarding risk of bias or applicability among the four QUADAS-2 domains (Figure 2 and Figure 3). Details on bias for individual studies are provided in the

Characteristics of included studies table. For studies that had high risk of bias or concerns regarding applicability, this was due mainly to (1) case-control design (Cornu 2018; De Vlieger 2011; Dichtl 2018; Fontana 2012; Metan 2012; Persat 2008; Pini 2019; Verduyn Lunel 2009; White 2017), and (2) exclusion of possible IFI cases from the findings (Hammarstrom 2015; Hammarstrom 2018; Jin 2013; Theel 2013).

Figure 2. Risk of bias and applicability concerns summary: review authors' judgements about each domain for each included study.

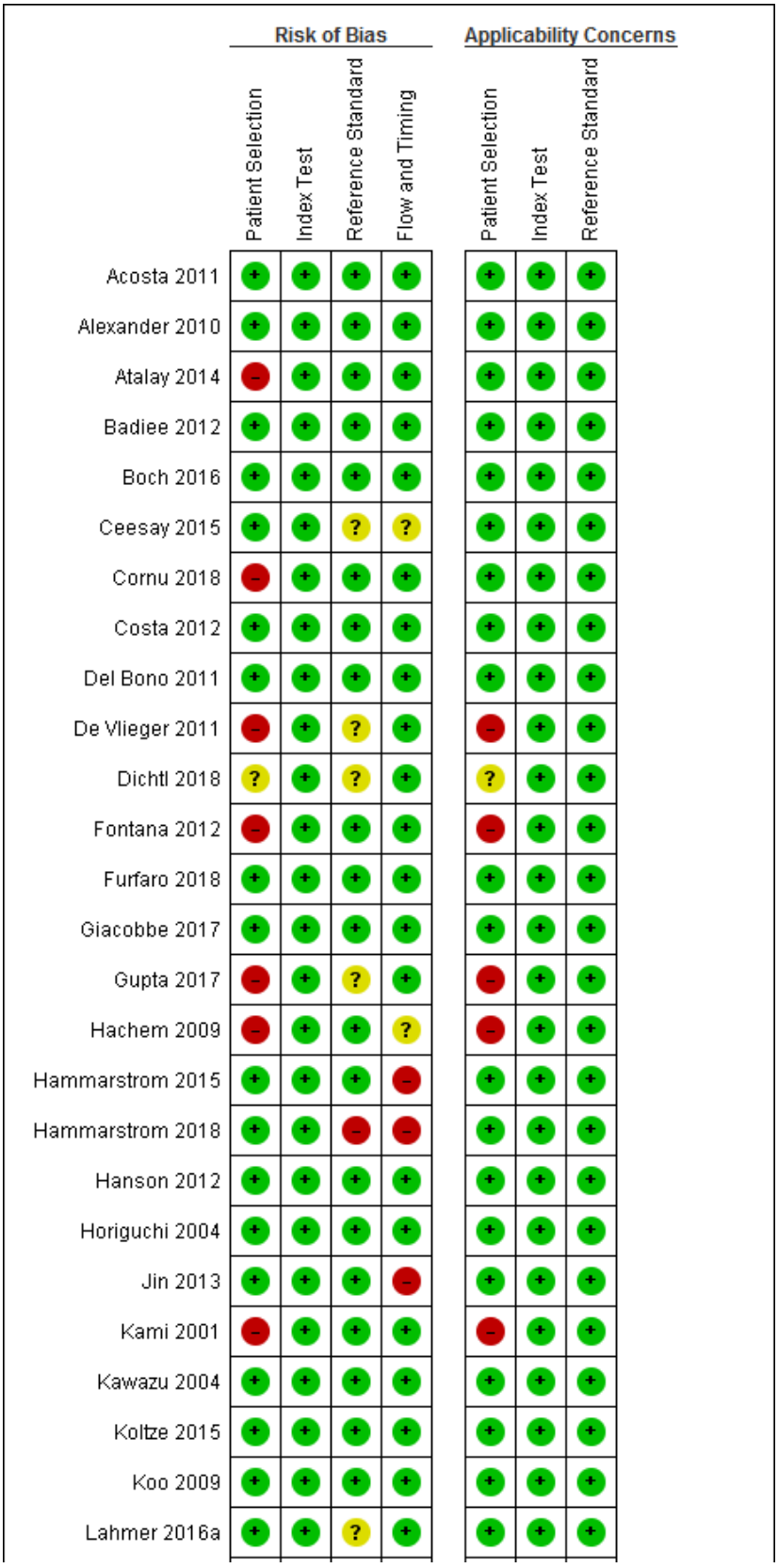


Figure 2. (Continued)

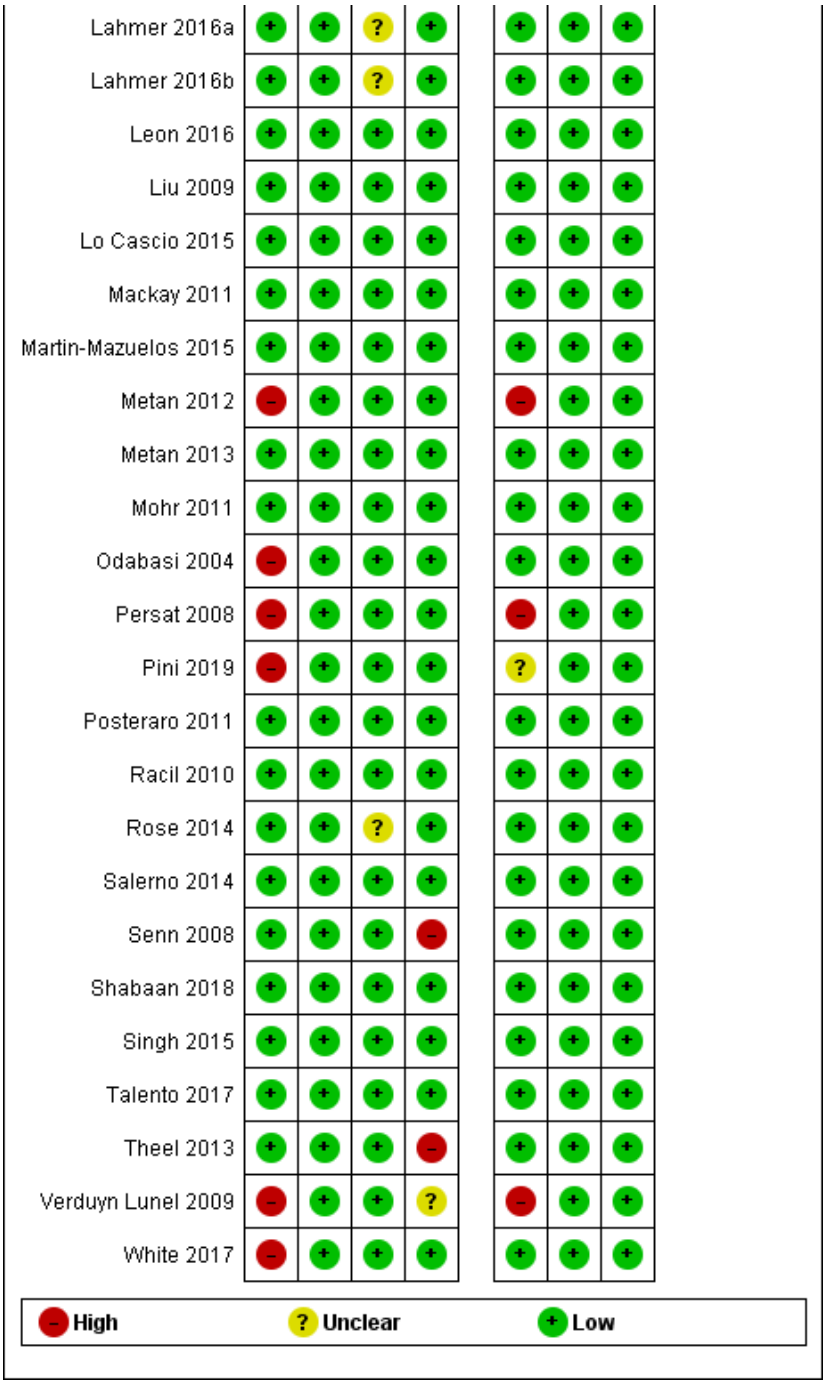
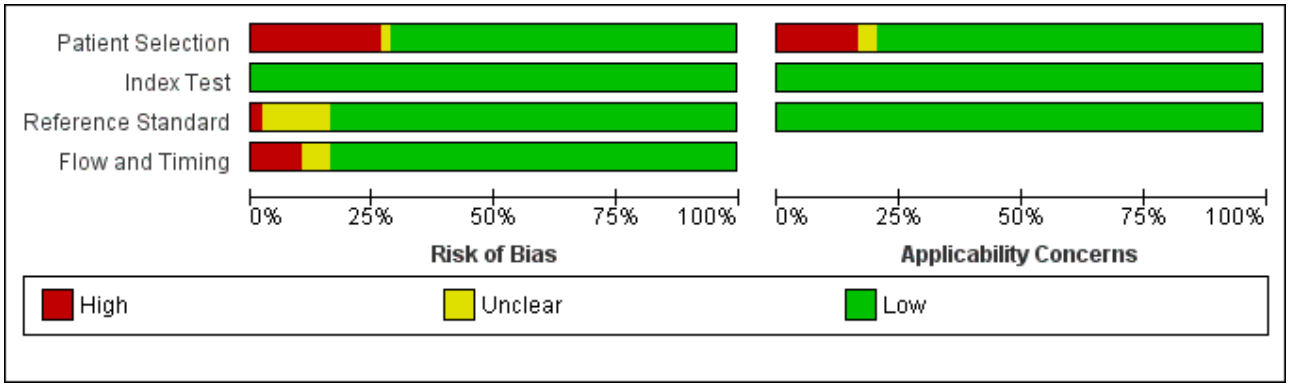


Figure 3. Risk of bias and applicability concerns graph: review authors' judgements about each domain presented as percentages across included studies.



Thirty-six of the studies failed to report the time interval between BDG testing and the reference standard. Only 13 studies provided a time frame; however, we still judged all other studies as low bias if other criteria were met (Figure 2).

Due to study design criteria, all studies pre-specified cut-off values or reported values that met the manufacturer's recommendations. BDG is an objective quantitative test that is generally performed without knowledge of the participant's true infection status. Therefore, failure to blind investigators to the reference test poses little risk of bias with respect to interpretation of the BDG test result. Thus, even if the study did not report blinding, we considered both the index test and reference standard domains to still be at low bias (Figure 2; Figure 3).

The reference standard was likely to classify IFIs correctly by using either EORTC/MSG criteria or confirmation by culture or microscopy. The EORTC/MSG criteria were revised in 2008. One of the important changes was that BDG was added as a criterion for IFI. Thus, to avoid incorporation bias, studies had to exclude BDG from the diagnostic criteria. Forty-two studies reported that they did not incorporate BDG testing in the reference standard, and we excluded two studies that included BDG as part of the reference test. It is unclear in seven studies whether BDG testing had been excluded (Ceesay 2015; De Vlieger 2011; Fontana 2012; Gupta 2017; Lahmer 2016a; Lahmer 2016b; White 2017). We elected to include these studies. Most studies were careful not to incorporate BDG testing, and we assumed that these studies most likely would have done so as well.

Findings

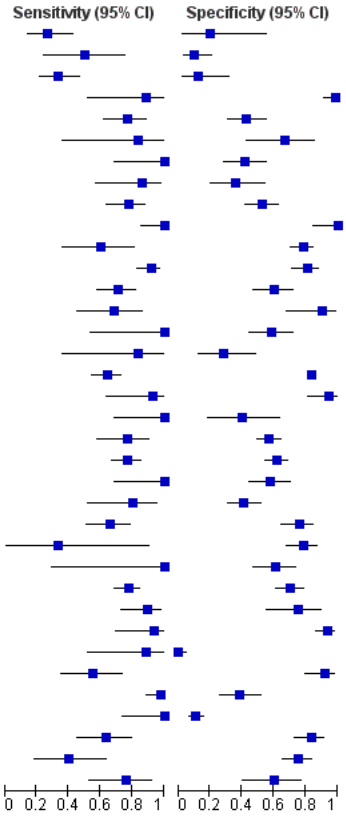
The prevalence of IFI ranged from 4% to 59% among all studies (mean 23%, 95% confidence interval (CI) 18% to 28%). In addition, estimates of sensitivity and specificity varied widely. Due to the high degree of heterogeneity between studies, we did not perform a formal meta-analysis, with the exception of *Candida*.

- Fungitell (36 studies): sensitivity for individual studies ranged between 27% and 100% and specificity range between 0% and 100% (Figure 4; Figure 5). A large amount of uncertainty was noted in study estimates, as evidenced by wide confidence intervals in the forest plot (Figure 4). Because IFI is relatively rare, many studies had a small number of positive cases. Koo had the largest study, with a study population of 871 (Koo 2009).
- Glucatell (5 studies): study estimates for sensitivity and specificity for Glucatell also ranged widely. Sensitivity ranged from 50% to 92%, and specificity ranged from 41% to 94%, among the 5 studies (Figure 4; Figure 5).
- Wako (3 studies): only 3 studies used the Wako assay at the manufacturer's specified cut-off level. Two studies reported lower sensitivities (55% and 50%, respectively) but higher specificities (98% and 89%) (Figure 4; Figure 5) (Kawazu 2004; Senn 2008). Dichtl 2018 reported fairly high sensitivity (86%) and specificity (100%) among a group of 98 people.
- Fungitec-G (3 studies): estimates for the 3 studies using Fungitec ranged from 67% to 88% for sensitivity and from 60% to 85% for specificity (Figure 4; Figure 5).
- Dynamiker (2 studies): only 2 recent studies had published results regarding Dynamiker Fungus (Figure 4; Figure 5). White 2018 reported sensitivity of 81% and specificity of 78%, and Shabaan 2018 reported sensitivity and specificity of 64% and 80%, respectively.

Figure 4. Forest plot of tests: Fungitell, Glucatell, Wako, Fungitec, Dynamiker.

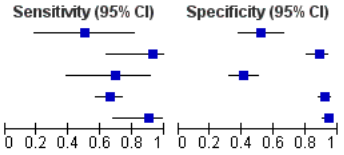
Fungitell

Study	TP	FP	FN	TN	Underlying condition	Fungi type	Sensitivity (95% CI)	Specificity (95% CI)
Acosta 2011	11	8	30	2	ICU	Mixed	0.27 [0.14, 0.43]	0.20 [0.03, 0.56]
Alexander 2010	8	51	8	6	Organ transplant	Mixed	0.50 [0.25, 0.75]	0.11 [0.04, 0.22]
Atalay 2014	20	21	39	3	Cancer	Mixed	0.34 [0.22, 0.47]	0.13 [0.03, 0.32]
Boch 2016	8	1	1	61	Cancer	Mixed	0.89 [0.52, 1.00]	0.98 [0.91, 1.00]
Ceesay 2015	34	41	10	31	Mixed at-risk	Mixed	0.77 [0.62, 0.89]	0.43 [0.31, 0.55]
Cornu 2018	5	7	1	14	ICU	Candida	0.83 [0.36, 1.00]	0.67 [0.43, 0.85]
Costa 2012	10	35	0	25	Mixed at-risk	PCP	1.00 [0.69, 1.00]	0.42 [0.29, 0.55]
De Vlieger 2011	12	21	2	12	ICU	Aspergillosis	0.86 [0.57, 0.98]	0.36 [0.20, 0.55]
Del Bono 2011	41	47	12	52	ICU	Candida	0.77 [0.64, 0.88]	0.53 [0.42, 0.63]
Fontana 2012	24	0	0	22	Cancer	Mixed	1.00 [0.86, 1.00]	1.00 [0.85, 1.00]
Furfaro 2018	12	32	8	115	Cancer	Aspergillosis	0.60 [0.36, 0.81]	0.78 [0.71, 0.85]
Giacobbe 2017	67	18	6	75	ICU	Candida	0.92 [0.83, 0.97]	0.81 [0.71, 0.88]
Gupta 2017	44	25	18	38	Cancer	Mixed	0.71 [0.58, 0.82]	0.60 [0.47, 0.72]
Hachem 2009	15	2	7	18	Cancer	Aspergillosis	0.68 [0.45, 0.86]	0.90 [0.68, 0.99]
Hanson 2012	6	23	0	33	ICU	Mixed	1.00 [0.54, 1.00]	0.59 [0.45, 0.72]
Koltze 2015	5	20	1	8	Cancer	Mixed	0.83 [0.36, 1.00]	0.29 [0.13, 0.49]
Koo 2009	72	124	40	635	Cancer	Mixed	0.64 [0.55, 0.73]	0.84 [0.81, 0.86]
Lahmer 2016a	12	2	1	34	ICU	Aspergillosis	0.92 [0.64, 1.00]	0.94 [0.81, 0.99]
Lahmer 2016b	10	12	0	8	ICU	Aspergillosis	1.00 [0.69, 1.00]	0.40 [0.19, 0.64]
Leon 2016	23	83	7	111	ICU	Candida	0.77 [0.58, 0.90]	0.57 [0.50, 0.64]
Lo Cascio 2015	70	67	21	109	ICU	Candida	0.77 [0.67, 0.85]	0.62 [0.54, 0.69]
Mackay 2011	10	26	0	36	ICU	Mixed	1.00 [0.69, 1.00]	0.58 [0.45, 0.70]
Martin-Mazuelos 2015	12	54	3	38	ICU	Candida	0.80 [0.52, 0.96]	0.41 [0.31, 0.52]
Metan 2012	33	19	17	59	Cancer	Aspergillosis	0.66 [0.51, 0.79]	0.76 [0.65, 0.85]
Metan 2013	1	16	2	60	Cancer	Aspergillosis	0.33 [0.01, 0.91]	0.79 [0.68, 0.87]
Mohr 2011	3	21	0	33	ICU	Candida	1.00 [0.29, 1.00]	0.61 [0.47, 0.74]
Persat 2008	91	36	26	86	Cancer	Mixed	0.78 [0.69, 0.85]	0.70 [0.62, 0.78]
Pini 2019	26	7	3	21	Mixed at-risk	Candida	0.90 [0.73, 0.98]	0.75 [0.55, 0.89]
Posteraro 2011	15	5	1	74	ICU	Mixed	0.94 [0.70, 1.00]	0.94 [0.86, 0.98]
Racil 2010	8	64	1	0	Cancer	Mixed	0.89 [0.52, 1.00]	0.00 [0.00, 0.06]
Rose 2014	16	4	13	43	Mixed at-risk	Mixed	0.55 [0.36, 0.74]	0.91 [0.80, 0.98]
Salerno 2014	45	38	1	24	HIV	PCP	0.98 [0.88, 1.00]	0.39 [0.27, 0.52]
Singh 2015	12	160	0	20	Organ transplant	Mixed	1.00 [0.74, 1.00]	0.11 [0.07, 0.17]
Talento 2017	21	11	12	56	ICU	Mixed	0.64 [0.45, 0.80]	0.84 [0.73, 0.92]
Theel 2013	8	22	12	67	Mixed at-risk	Mixed	0.40 [0.19, 0.64]	0.75 [0.65, 0.84]
Verduyn Lunel 2009	16	12	5	18	Cancer	Candida	0.76 [0.53, 0.92]	0.60 [0.41, 0.77]



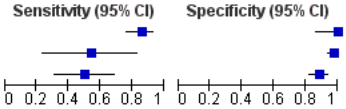
Glucatell

Study	TP	FP	FN	TN	Underlying condition	Fungi type	Sensitivity (95% CI)	Specificity (95% CI)
Badiee 2012	5	25	5	27	Cancer	Aspergillosis	0.50 [0.19, 0.81]	0.52 [0.38, 0.66]
Hammarstrom 2015	12	11	1	85	Cancer	Mixed	0.92 [0.64, 1.00]	0.89 [0.80, 0.94]
Hammarstrom 2018	9	66	4	46	Cancer	Mixed	0.69 [0.39, 0.91]	0.41 [0.32, 0.51]
Jin 2013	94	19	49	216	Cancer	Aspergillosis	0.66 [0.57, 0.73]	0.92 [0.88, 0.95]
Odabasi 2004	18	15	2	248	Cancer	Mixed	0.90 [0.68, 0.99]	0.94 [0.91, 0.97]



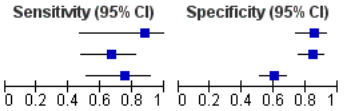
Wako

Study	TP	FP	FN	TN	Underlying condition	Fungi type	Sensitivity (95% CI)	Specificity (95% CI)
Dichtl 2018	63	0	10	25	Mixed at-risk	PCP	0.86 [0.76, 0.93]	1.00 [0.86, 1.00]
Kawazu 2004	6	3	5	135	Cancer	Aspergillosis	0.55 [0.23, 0.83]	0.98 [0.94, 1.00]
Senn 2008	15	16	15	127	Cancer	Mixed	0.50 [0.31, 0.69]	0.89 [0.82, 0.93]



Fungitec

Study	TP	FP	FN	TN	Underlying condition	Fungi type	Sensitivity (95% CI)	Specificity (95% CI)
Horiguchi 2004	7	9	1	52	Cancer	Aspergillosis	0.88 [0.47, 1.00]	0.85 [0.74, 0.93]
Kami 2001	22	14	11	75	Cancer	Aspergillosis	0.67 [0.48, 0.82]	0.84 [0.75, 0.91]
Liu 2009	15	57	5	85	Cancer	Mixed	0.75 [0.51, 0.91]	0.60 [0.51, 0.68]



Dynamiker

Study	TP	FP	FN	TN	Underlying condition	Fungi type	Sensitivity (95% CI)	Specificity (95% CI)
Shabaan 2018	7	13	4	53	ICU	Candida	0.64 [0.31, 0.89]	0.80 [0.69, 0.89]
White 2017	35	17	8	61	Mixed at-risk	Mixed	0.81 [0.67, 0.92]	0.78 [0.67, 0.87]

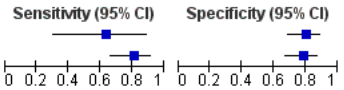
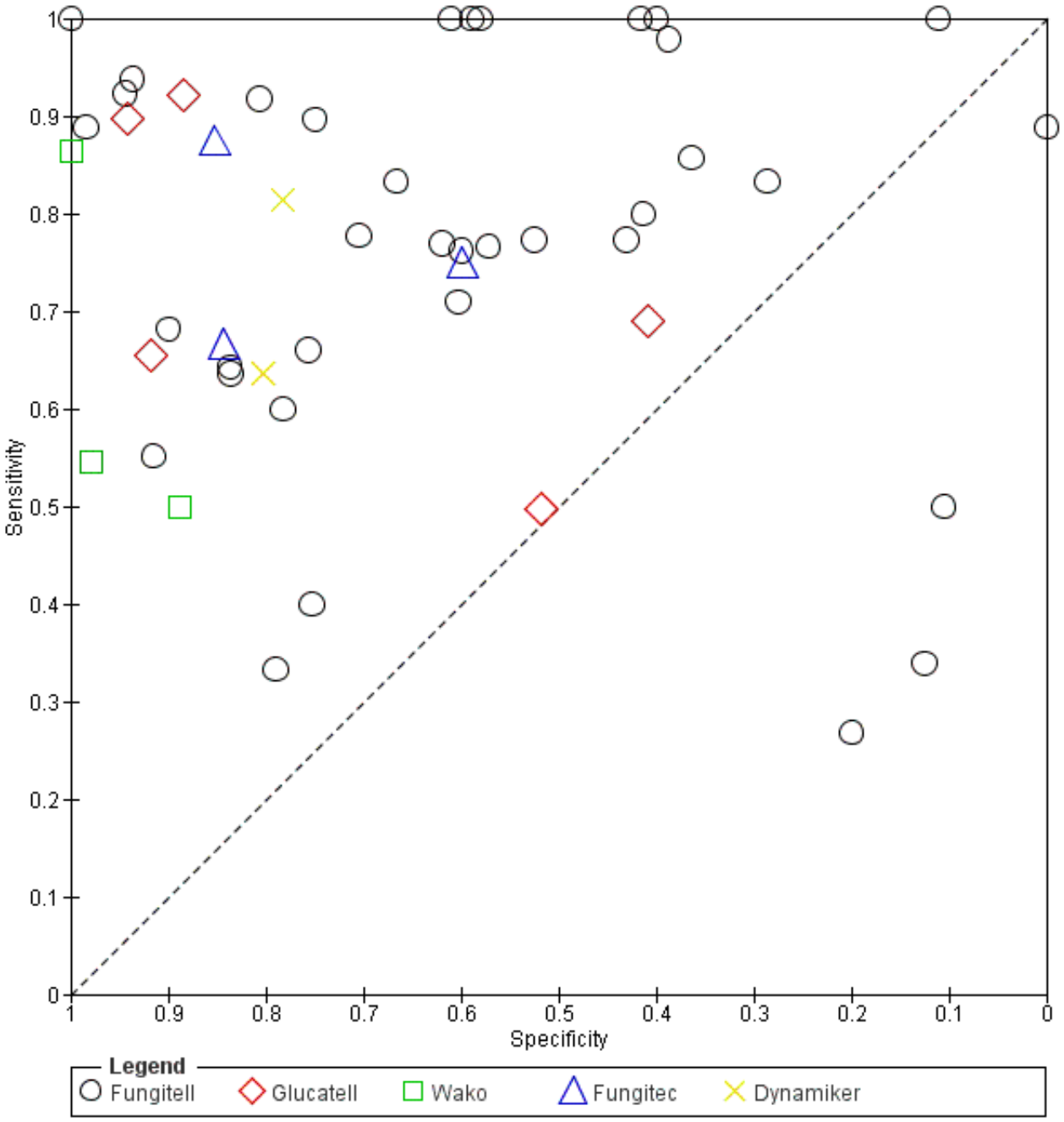


Figure 5. Summary ROC plot of tests: 1 Fungitell, 2 Glucatell, 3 Wako, 4 Fungitec, 5 Dynamiker.



We included 10 studies in the meta-analysis, from which an estimate for *Candida* could be obtained. Estimated sensitivity and specificity for these studies was 81.3% (95% CI 75.3% to 86.0%) and 64.1% (95% CI 55.6% to 71.8%), respectively. Almost all (N = 9; 90%) used Fungitell, with 8 of the 10 studies involving people in ICU settings. Forty per cent utilized multiple samples, and the remainder relied on a single test.

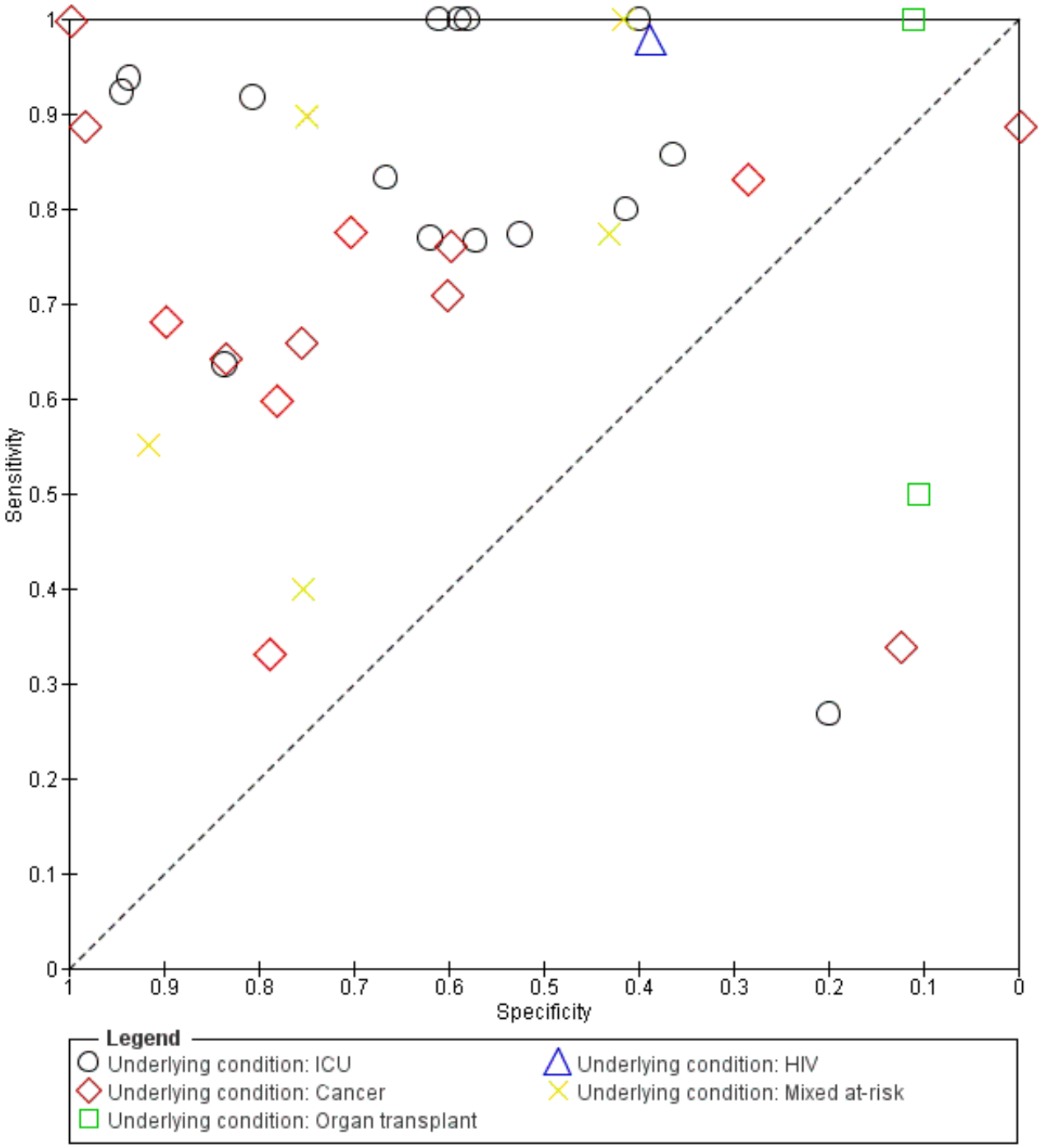
Investigations of heterogeneity

Heterogeneity was assessed by ROC plots that examined differences in individual sensitivity and specificity estimates by participant population, fungal organism, reference standard, and single versus multiple testing.

Heterogeneity could not be explained by the participant population (Figure 6). We restricted this analysis to a single test platform to limit a potential source of variation. We selected the Fungitell assay because it was the most commonly used test platform (36 of 49

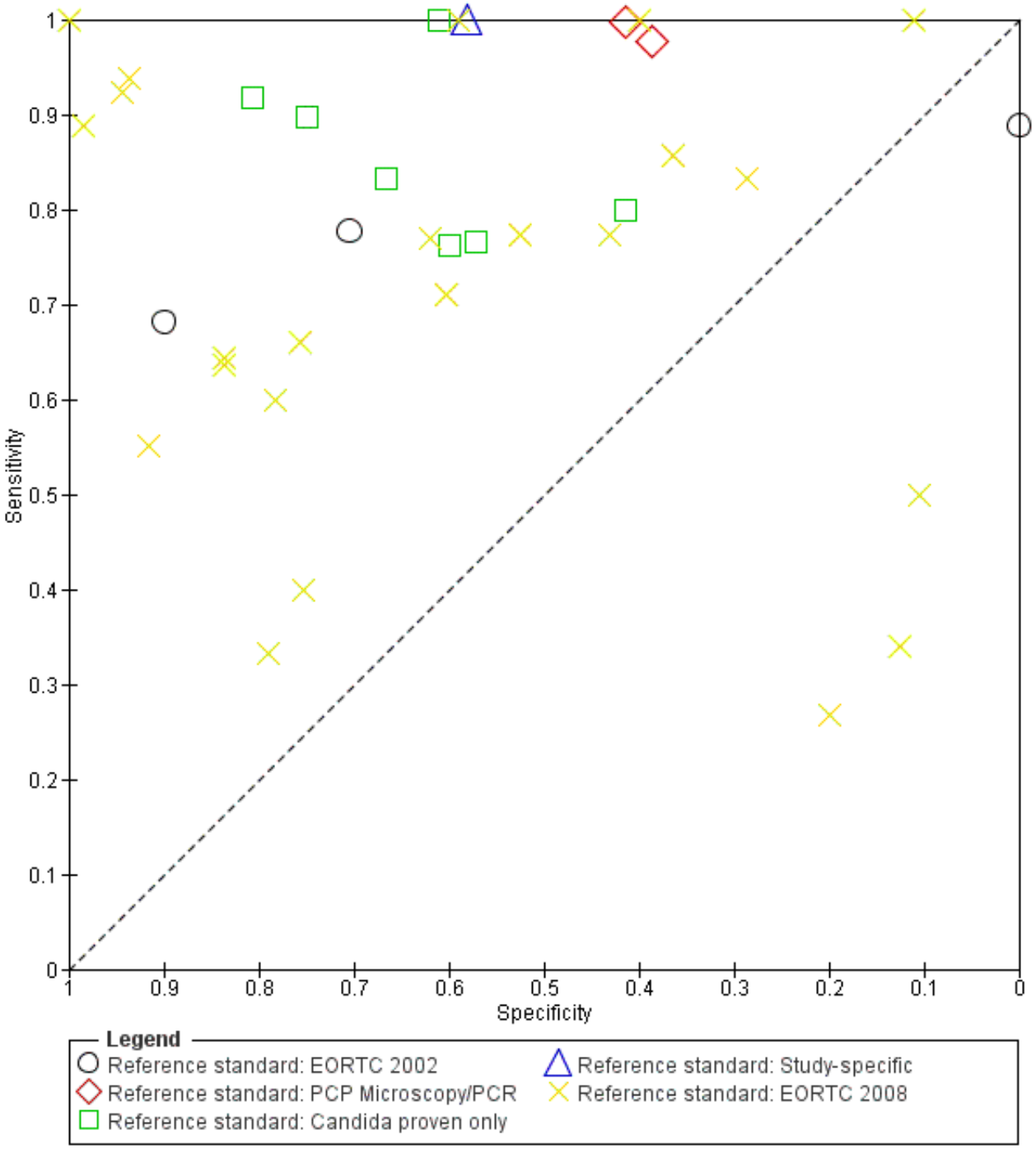
studies). In 13 studies involving participants with cancer, sensitivity ranged from 33% to 100% and specificity ranged from 0% to 100%. In 15 studies involving participants who had been admitted to the ICU, sensitivity ranged from 27% to 100% and specificity ranged from 20% to 94%. Finally, in 5 studies with a mixture of participants, sensitivity ranged from 40% to 100% and specificity ranged from 42% to 91%. All participant groups had a wide range of sensitivity and specificity. Considerable overlap could be seen in the ranges of sensitivity and specificity for each group. It was not possible to identify an underlying condition that was associated with higher or lower levels of sensitivity or specificity.

Figure 6. Summary ROC plot of underlying medical conditions for Fungitell studies.



Heterogeneity could not be explained by the reference standard (Figure 7). This analysis was also restricted to studies performed with the Fungitell assay. In 26 tests using EORTC criteria, sensitivity ranged from 27% to 100% and specificity ranged from 0% to 100%. In seven studies testing for *Candida*, sensitivity ranged from 76% to 100% and specificity ranged from 41% to 81%.

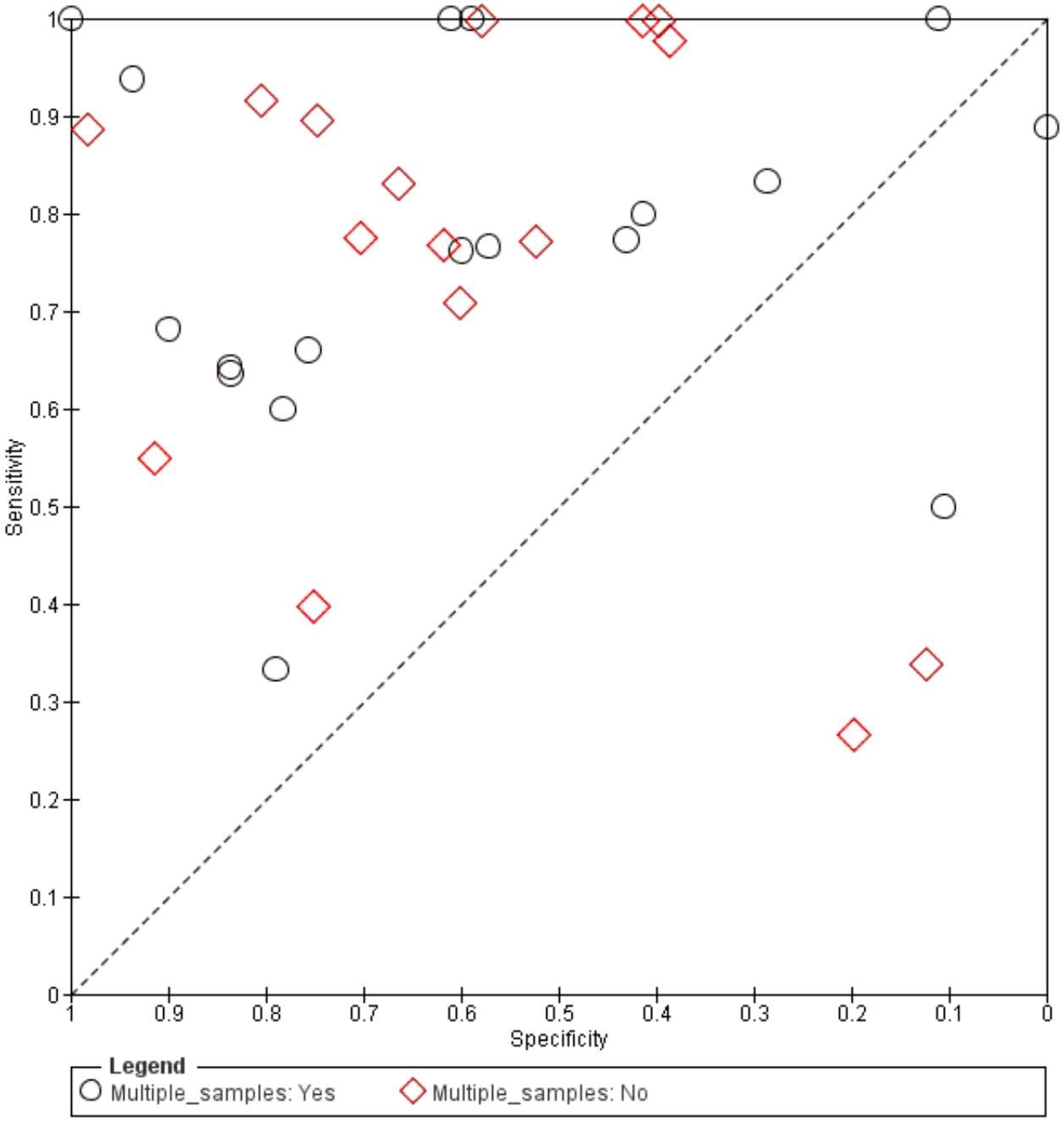
Figure 7. Summary ROC plot of reference standard for Fungitell studies.



In some studies, the BDG test was performed once, and in other studies, BDG testing was performed multiple times (e.g. twice a week). Heterogeneity could not be explained by the number of tests (Figure 8). This analysis was restricted to studies performed with the Fungitell test. In 16 studies that used a single sample, sensitivity ranged from 27% to 100% and specificity ranged from 12% to 98%. In 18 studies that used multiple tests per person, sensitivity ranged from 33% to 100% and specificity ranged from 0%

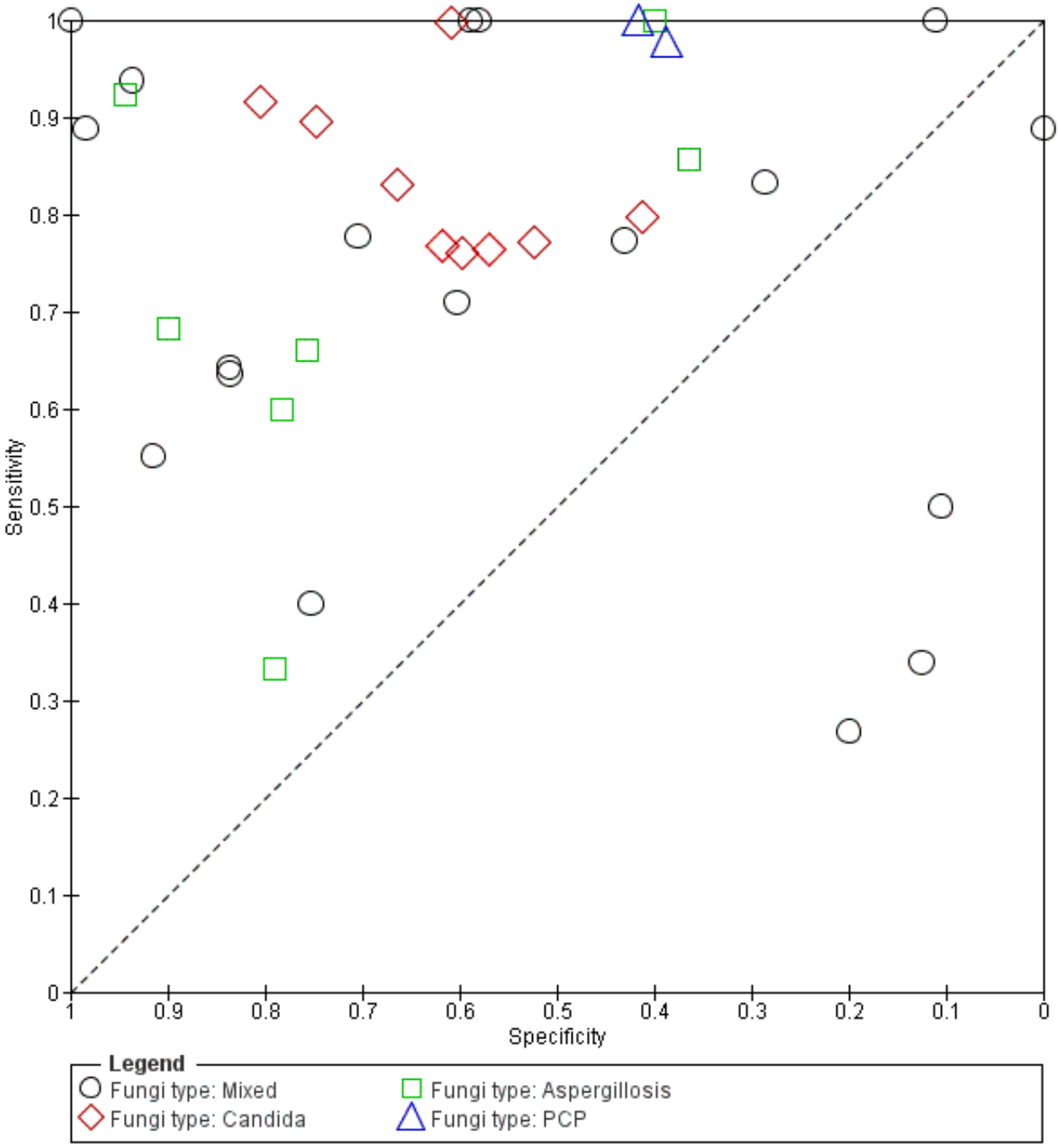
to 100%. Both groups had a wide range of sensitivity and specificity with substantial overlap. It was not possible to identify a sampling policy that was associated with higher or lower levels of sensitivity or specificity.

Figure 8. Summary ROC plot of single versus multiple sampling for Fungitell.



Studies that focused on *Candida* infection did appear to be more homogeneous than those focused on other fungal organisms (Figure 9); therefore, we obtained a summary estimate for these studies.

Figure 9. Summary ROC plot of fungal organisms for Fungitell studies.



DISCUSSION

Summary of main results

Literature on the use of (1→3)-β-D-glucan (BDG) for diagnosis of invasive fungal infection (IFI) shows wide variation in diagnostic accuracy. Sensitivity ranged from 27% to 100%, and specificity ranged from 0% to 100%. Because of this variation, we did not perform a formal meta-analysis, with the exception of *Candida* studies.

There were many potential sources of heterogeneity. These include study design (case-control retrospective, prospective), differences in populations (immunodeficient versus critically ill), sampling (single sample, multiple samples, monitoring with two samples per week), assays (Fungitell, Glucatell, etc.), target organisms (all IFI, *Candida*, *Aspergillus*, etc), and threshold for positivity (one positive BDG test, two consecutive positive BDG tests). Application of European Organization for Research and Treatment of Cancer (EORTC) criteria is another potential source of heterogeneity. The accuracy with which physicians perform this task may vary, and

because the number of physicians in any study is low, differences in classification accuracy are unlikely to average out. We are not aware of any agreement of studies on EORTC criteria. This variation made it difficult to obtain meaningful estimates of sensitivity and specificity. Thus, it is not possible to predict how the BDG test will perform in a particular context.

Going forward, it would be helpful if studies limited variation in these factors. Prospective studies should be preferred over case-control and retrospective studies. Prospective studies are more closely aligned with the clinical context and allow various sampling policies to be compared in a single study. For example, one could perform twice-weekly sampling and compare the diagnostic accuracy of the first positive BDG result, two consecutive BDG-positive results, positive BDG when a person is first symptomatic, etc. It is not clear whether studies on individual organisms are helpful. Several studies focused on infections in a single organism. Although such studies provide useful knowledge regarding test performance, they do not address a clinically relevant question. The clinical question that is addressed by BDG testing is whether a person has an IFI rather than whether a person is infected with a particular organism. It might be better to conduct instrument comparisons in laboratory studies rather than in clinical studies.

We found that the quality of studies was generally good. Risk of bias was generally low. We did exclude a number of case-control studies that included healthy controls. These study designs produce inflated estimates of sensitivity and specificity due to spectrum bias (White 2019). There is some room for improvement in reporting. Studies should not include BDG as part of the reference test and should explicitly state this. Also, it would be helpful if studies reported results for all four EORTC categories (proven, probable, possible, none). One must aggregate categories to calculate sensitivity and specify; however, to facilitate meta-analysis, results should be available as individual categories. Studies should report timing of the BDG test relative to the reference test, and whether the reference test was blinded to the BDG test result.

Comparison of our results with other meta-analyses

Four meta-analyses on the diagnostic accuracy of BDG have been previously published (He 2015; Karageorgopoulos 2011; Lu 2011b; White 2019). These meta-analyses studied the use of BDG in similar populations of people (immunocompromised) and included between 13 and 28 studies. Our analysis summarized 49 studies, which reflects the large number of studies conducted in the past five years. Previous reviews have also reported high levels of heterogeneity.

Strengths and weaknesses of the review

This review represents the most up-to-date systematic assessment of BDG test performance. The high level of heterogeneity is a significant limitation. Current BDG diagnostic literature remains impacted by variability in study design, heterogenous populations, limited information on baseline use of antifungal therapy or other potential assay interferences, and lack of consistently robust adjudication of potential colonization versus invasive disease. In addition, microscopy for PJP and culture confirmation of *Candida* are imperfect reference standards that may miss true cases of invasive disease and may impact calculations of test specificity.

There was considerable variation in the prevalence of probable/proven IFI (range 4% to 59%). This could reflect differences in populations or differences in interpretation of the reference standard. Variation in interpretation of the reference standard is a potential source of heterogeneity. We used simple descriptive methods to investigate potential sources of heterogeneity; however, this was largely unsuccessful. Future work might benefit from the application of a latent-class meta-analysis, which could potentially address the issue of variable, imperfect reference standards across studies.

Applicability of findings to the review question

We summarized the diagnostic accuracy of several BDG tests. We found significant heterogeneity between study estimates. Given this variability, a summary estimate is unlikely to be applicable at any given location. We were unable to make a meaningful comparison between different commercial tests, and we were unable to determine factors that affect diagnostic accuracy (e.g. population, positivity criteria, sampling).

AUTHORS' CONCLUSIONS

Implications for practice

The potential value of BDG testing relies on detecting infection at an early stage. Based on this review, it is unclear whether this occurs. It is also unclear whether a pre-emptive strategy (supported by BDG testing) leads to earlier diagnosis and better outcomes when compared to prophylaxis or empiric therapy.

Implications for research

This review was limited by wide variation in outcomes. This, in turn, was driven by wide variation in study designs, positivity criteria, sampling protocols, and tests. It seems it will be necessary to reduce the variation in study design to reduce variation in outcomes. To that end, it would be beneficial if future studies were designed in a way that is most closely aligned with clinical practice, for example, continuous monitoring (e.g. twice weekly) during periods of risk versus testing at a single time point for people with clinical signs or symptoms of invasive fungal infection. Studies could easily compare positivity criteria (one positive sample versus two consecutive positive samples). It is unclear whether additional case-control and retrospective studies would be informative. Such studies may have been informative in the early development of BDG tests, but they do not reflect the way that BDG tests are used in practice. Timing of the reference test relative to the BDG test result needs to be accurately reported. Studies also need to avoid incorporation bias by insuring that the reference test is blinded from the BDG test result. We are unaware of any study on inter-rater agreement of the EORTC criteria for IFI. Such a study may be useful.

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expressed therein are those of the review authors and do not necessarily reflect those of the Systematic Reviews Programme, NIHR, NHS, or the Department of Health.

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CHARACTERISTICS OF STUDIES

Characteristics of included studies *[ordered by study ID]*

Acosta 2011

Study characteristics	
Patient Sampling	Serum BDG collected once per patient as part of a prospective study
Patient characteristics and setting	847 patients were admitted to the ICU over a 21-month period. Of these, 51 patients met the study inclusion criteria of having a clinical syndrome compatible with pneumonia and 1 host factor. Two-thirds (34/51) were male; no information was provided on age range. Thirteen met the criteria for proven or probable IFI
Index tests	Fungitell test using 80 pg/mL as cut-off for positivity
Target condition and reference standard(s)	Invasive aspergillosis or PJP determined by the 2008 EORTC criteria with BDG excluded
Flow and timing	Unclear time frame between sample and reference standard; all received index test and reference standard and were included in the analysis
Comparative	

Acosta 2011 *(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		
Could the selection of patients have introduced bias?		Low risk	
Are there concerns that the included patients and setting do not match the review question?			Low concern
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		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
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		
Could the patient flow have introduced bias?		Low risk	

Alexander 2010

Study characteristics	
Patient Sampling	Serum BDG collected twice weekly as part of a prospective study
Patient characteristics and setting	Between August 2004 and March 2006, 79 liver transplant patients were enrolled in the study before transplantation. Six patients were excluded from the analysis. More than half (40/73) were

Alexander 2010 *(Continued)*

	male, with a median age of 52 years. Fourteen patients met the criteria for IFI
Index tests	Fungitell test using 80 pg/mL as cut-off for positivity
Target condition and reference standard(s)	Mixed IFI determined by the 2008 EORTC criteria with BDG excluded
Flow and timing	BDG test was done within 14 days of the reference standard; all received index test and reference standard and were included in the analysis
Comparative	
Notes	BDG tests and equipment were provided by Associates of Cape Cod

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		
Could the selection of patients have introduced bias?		Low risk	
Are there concerns that the included patients and setting do not match the review question?			Low concern
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		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Yes		
Did all patients receive the same reference standard?	Yes		
Were all patients included in the analysis?	Yes		

Alexander 2010 *(Continued)*

Could the patient flow have introduced bias?	Low risk

Atalay 2014

Study characteristics			
Patient Sampling	Retrospective study of patients with at least 1 BDG test. Samples were collected twice weekly		
Patient characteristics and setting	Records of all inpatients from August 2009 to August 2011 were reviewed. Forty-three pediatric and adult patients, most with hematologic or solid tumor malignancies, were selected and had at least 1 BDG test and a diagnosis of proven, probable, or possible IFI. A control group of 40 patients from hematology or oncology wards with no IFI was used for comparison		
Index tests	Fungitell test using 80 pg/mL as cut-off for positivity		
Target condition and reference standard(s)	Mixed IFI determined by the 2008 EORTC criteria with BDG excluded		
Flow and timing	Sample taken with 10 days of reference standard; all received index test and reference standard and were included in the analysis		
Comparative			
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?	No		
Did the study avoid inappropriate exclusions?	Yes		
Could the selection of patients have introduced bias?		High risk	
Are there concerns that the included patients and setting do not match the review question?			Low concern
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		

Atalay 2014 *(Continued)*

Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Yes		
Did all patients receive the same reference standard?	Yes		
Were all patients included in the analysis?	Yes		
Could the patient flow have introduced bias?		Low risk	

Badiee 2012

Study characteristics			
Patient Sampling	Serum BDG collected twice weekly in a prospective study		
Patient characteristics and setting	Between November 2008 and November 2009, all pediatric hematology patients at risk for IFI were enrolled. Sixty-two patients aged 1 to 14 years (median 9 years old) were analyzed. Ten of these patients had proven or probable IFI		
Index tests	Glucatell test using 80 pg/mL as cut-off for positivity		
Target condition and reference standard(s)	Invasive aspergillosis determined by the 2008 EORTC criteria with BDG excluded		
Flow and timing	Unclear time frame between sample and reference standard; all received index test and reference standard and were included in the analysis		
Comparative			
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		

Badiee 2012 *(Continued)*

Could the selection of patients have introduced bias?		Low risk	
Are there concerns that the included patients and setting do not match the review question?			Low concern
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		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
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		
Could the patient flow have introduced bias?		Low risk	

Boch 2016

Study characteristics	
Patient Sampling	Serum BDG collected once per patient as part of a prospective study
Patient characteristics and setting	From 2012 to 2015, 99 hematologic patients at high risk for IFI were included. Thirty-seven of these patients had proven or probable IFI. No information was provided on age or sex of patients
Index tests	Fungitell test using 80 pg/mL as cut-off for positivity
Target condition and reference standard(s)	Mixed IFI determined by the 2008 EORTC criteria without BDG or GM
Flow and timing	Unclear time frame between sample and reference standard; all received index test and reference standard and were included in the analysis
Comparative	
Notes	
Methodological quality	

Boch 2016 *(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?	Yes		
Could the selection of patients have introduced bias?		Low risk	
Are there concerns that the included patients and setting do not match the review question?			Low concern
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		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
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		
Could the patient flow have introduced bias?		Low risk	

Ceesay 2015

Study characteristics	
Patient Sampling	Serum BDG collected twice weekly as part of a prospective study
Patient characteristics and setting	From December 2008 to May 2010, 203 adult patients undergoing HSCT, immunosuppressive therapy, or intensive chemotherapy were included. The median age of patients was 54 years, and 61% were male. During the study period, 40 patients were diagnosed with proven or probable IFI
Index tests	Fungitell test using 80 pg/mL as cut-off for positivity

Ceesay 2015 *(Continued)*

Target condition and reference standard(s)	Mixed IFI determined by the 2008 EORTC/MSG criteria; unclear whether BDG was excluded
Flow and timing	Unclear time frame between sample and reference standard; BDG was not performed on 26 patients but reason for exclusion unclear
Comparative	
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		
Could the selection of patients have introduced bias?		Low risk	
Are there concerns that the included patients and setting do not match the review question?			Low concern
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		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Unclear risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
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		
Could the patient flow have introduced bias?		Unclear risk	

Cornu 2018

Study characteristics	
Patient Sampling	Retrospective case-control study of patients with BDG testing
Patient characteristics and setting	Between February 2012 and February 2014, 47 neonates in the NICU were selected as cases and controls. The median gestational age was 30 weeks, and 70% were male
Index tests	Fungitell test using 80 pg/mL as cut-off for positivity
Target condition and reference standard(s)	Invasive candidiasis was diagnosed by culture
Flow and timing	Unclear time frame between sample and reference standard; all received index test and reference standard and were included in the analysis
Comparative	
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		
Was a case-control design avoided?	No		
Did the study avoid inappropriate exclusions?	Yes		
Could the selection of patients have introduced bias?		High risk	
Are there concerns that the included patients and setting do not match the review question?			Low concern
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		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Unclear		

Cornu 2018 *(Continued)*

Did all patients receive the same reference standard?	Yes	
Were all patients included in the analysis?	Yes	
Could the patient flow have introduced bias?		Low risk

Costa 2012

Study characteristics			
Patient Sampling	Retrospective study of patients with BDG collected once or twice per patient		
Patient characteristics and setting	All immunocompromised patients who had undergone bronchoalveolar lavage (BAL) for diagnosis of PJP were screened. Comorbid conditions included AIDS, cancers, organ transplantations, and systemic inflammatory diseases. Sixty-three patients were included in the analyses. No information was provided on age or sex of patients		
Index tests	Fungitell test using 80 pg/mL as cut-off for positivity		
Target condition and reference standard(s)	PJP diagnosed by microscopy		
Flow and timing	Unclear time frame between sample and reference standard; all received index test and reference standard and were included in the analysis		
Comparative			
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		
Could the selection of patients have introduced bias?		Low risk	
Are there concerns that the included patients and setting do not match the review question?			Low concern
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	Yes		

Costa 2012 *(Continued)*

Were the reference standard results interpreted without knowledge of the results of the index tests?	Unclear		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
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		
Could the patient flow have introduced bias?		Low risk	

De Vlieger 2011

Study characteristics			
Patient Sampling	Retrospective study of patients with 1 BDG test		
Patient characteristics and setting	From July 2005 to December 2006, 110 immunocompromised ICU patients with clinical signs of fungal infection were selected for inclusion and were classified as having proven, probable, or possible IFI. Of these, 14 patients with proven IFI and 33 with no IFI according to the revised EORTC/MSG guidelines were chosen for this study		
Index tests	Fungitell test using 80 pg/mL as cut-off for positivity		
Target condition and reference standard(s)	Invasive aspergillosis determined by the 2008 EORTC criteria; unclear whether BDG was included		
Flow and timing	Unclear time frame between sample and reference standard; all received index test and reference standard and were included in the analysis		
Comparative			
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		

De Vlieger 2011 *(Continued)*

Was a case-control design avoided?	No		
Did the study avoid inappropriate exclusions?	Yes		
Could the selection of patients have introduced bias?		High risk	
Are there concerns that the included patients and setting do not match the review question?			High
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		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Unclear risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
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		
Could the patient flow have introduced bias?		Low risk	

Del Bono 2011

Study characteristics	
Patient Sampling	Serum BDG collected once per patient as part of a prospective study
Patient characteristics and setting	Adult ICU patients admitted between July 2008 and October 2010 who were at risk for invasive candidiasis. A total of 152 patients were included, of whom 53 were diagnosed with proven candidemia. More than half (87/152) were male, and the median age was significantly higher in the proven candidemia group (72 years) compared to the possible (47 years) or no candidemia (52 years) group.
Index tests	Fungitell test using 80 pg/mL as cut-off for positivity
Target condition and reference standard(s)	Invasive candidiasis as determined by the 2008 EORTC criteria with BDG excluded

Del Bono 2011 *(Continued)*

Flow and timing	36 of the 41 patients with proven invasive candidiasis were sampled within 48 hours of the reference standard; all received index test and reference standard and were included in the analysis
Comparative	
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		
Could the selection of patients have introduced bias?		Low risk	
Are there concerns that the included patients and setting do not match the review question?			Low concern
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		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Yes		
Did all patients receive the same reference standard?	Yes		
Were all patients included in the analysis?	Yes		
Could the patient flow have introduced bias?		Low risk	

Dichtl 2018

Study characteristics

Dichtl 2018 *(Continued)*

Patient Sampling	Retrospective case-control study of patients with BDG testing
Patient characteristics and setting	73 patients with confirmed PJP were selected as cases, and 25 controls had clinical signs of PJP but tested negative. Both groups were at risk for IFI, including hematologic malignancies, HIV, and immunosuppressive therapy
Index tests	Wako test using 11 pg/mL as cut-off for positivity
Target condition and reference standard(s)	PJP was diagnosed by PCR using respiratory tract specimens
Flow and timing	All received index test and reference standard; most received BDG test within a week of reference standard, although timing could range from 1 week before to 4 weeks after
Comparative	
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		
Was a case-control design avoided?	No		
Did the study avoid inappropriate exclusions?	Yes		
Could the selection of patients have introduced bias?		Unclear risk	
Are there concerns that the included patients and setting do not match the review question?			Unclear
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		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Unclear risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Yes		
Did all patients receive the same reference standard?	Yes		

Dichtl 2018 *(Continued)*

Were all patients included in the analysis?	Yes		
Could the patient flow have introduced bias?		Low risk	

Fontana 2012

Study characteristics			
Patient Sampling	Retrospective case-control study of patients with at least 1 BDG test. Serum BDG collected weekly		
Patient characteristics and setting	Sera from 46 patients undergoing follow-up in a hematology clinic between January 2010 and December 2011. Twenty-four patients had proven or probable IFI. No information on age or sex of patients was provided		
Index tests	Fungitell test using 80 pg/mL as cut-off for positivity		
Target condition and reference standard(s)	Mixed IFI determined by the 2008 EORTC criteria with BDG excluded		
Flow and timing	Unclear time frame between sample and reference standard; all received index test and reference standard and were included in the analysis		
Comparative			
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		
Was a case-control design avoided?	No		
Did the study avoid inappropriate exclusions?	Yes		
Could the selection of patients have introduced bias?		High risk	
Are there concerns that the included patients and setting do not match the review question?			High
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		

Fontana 2012 *(Continued)*

Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
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		
Could the patient flow have introduced bias?		Low risk	

Furfaro 2018

Study characteristics			
Patient Sampling	Retrospective study of patients with BDG testing. Samples were collected twice weekly		
Patient characteristics and setting	Records of all adult neutropenic patients with hematological malignancies with GM and BDG testing from January 2011 to December 2013 were included. The median age was 58 years, and 60% were male. There were no proven cases of invasive aspergillosis and 20 probable cases		
Index tests	Fungitell test using 80 pg/mL as cut-off for positivity		
Target condition and reference standard(s)	Invasive aspergillosis determined by the 2008 EORTC criteria with BDG excluded		
Flow and timing	Sample taken within 7 days of reference standard; all received index test and reference standard and were included in the analysis		
Comparative			
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		

Furfaro 2018 *(Continued)*

Could the selection of patients have introduced bias?		Low risk	
Are there concerns that the included patients and setting do not match the review question?			Low concern
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		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Yes		
Did all patients receive the same reference standard?	Yes		
Were all patients included in the analysis?	Yes		
Could the patient flow have introduced bias?		Low risk	

Giacobbe 2017

Study characteristics	
Patient Sampling	Retrospective study of patients with 1 BDG test
Patient characteristics and setting	Adult ICU patients with candidemia (n = 73) or bacteremia (n = 93) were included. Among candidemia patients, 59% were male and the median age was 64 years
Index tests	Fungitell test using 80 pg/mL as cut-off for positivity
Target condition and reference standard(s)	Candidemia diagnosed by a positive blood culture
Flow and timing	BDG test was done within 48 hours of the reference standard; all received index test and reference standard and were included in the analysis
Comparative	
Notes	
Methodological quality	

Giacobbe 2017 *(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		
Could the selection of patients have introduced bias?		Low risk	
Are there concerns that the included patients and setting do not match the review question?			Low concern
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		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Yes		
Did all patients receive the same reference standard?	Yes		
Were all patients included in the analysis?	Yes		
Could the patient flow have introduced bias?		Low risk	

Gupta 2017

Study characteristics	
Patient Sampling	Serum BDG collected once per patient as part of a prospective study
Patient characteristics and setting	Included 125 pediatric patients with hematologic or solid tumor malignancies with and without IFI. The age of patients ranged from 1 to 15 years, and 58% were male. Two patients had proven IFI, and 60 had probable IFI
Index tests	Fungitell test using 80 pg/mL as cut-off for positivity

Gupta 2017 *(Continued)*

Target condition and reference standard(s)	Mixed IFI determined by the 2008 EORTC criteria; unclear if BDG was excluded
Flow and timing	Unclear time frame between sample and reference standard; all received index test and reference standard and were included in the analysis
Comparative	
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?	No		
Did the study avoid inappropriate exclusions?	No		
Could the selection of patients have introduced bias?		High risk	
Are there concerns that the included patients and setting do not match the review question?			High
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		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Unclear risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
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		
Could the patient flow have introduced bias?		Low risk	

Hachem 2009

Study characteristics	
Patient Sampling	Serum BDG collected twice the first week, once every other week after that, as part of a prospective study
Patient characteristics and setting	Patients with hematologic malignancies or solid tumor malignancies. Patients with proven or probable IA were cases, and those with solid tumor were controls. The age range of patients was 10 to 81 years, and 60% were male
Index tests	Fungitell test using 80 pg/mL as cut-off for positivity
Target condition and reference standard(s)	Invasive aspergillosis determined by the 2002 EORTC criteria
Flow and timing	Unclear time frame between sample and reference standard; all received index test and reference standard and were included in the analysis
Comparative	
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		
Was a case-control design avoided?	No		
Did the study avoid inappropriate exclusions?	No		
Could the selection of patients have introduced bias?		High risk	
Are there concerns that the included patients and setting do not match the review question?			High
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		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
DOMAIN 4: Flow and Timing			

Hachem 2009 *(Continued)*

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		
Could the patient flow have introduced bias?		Unclear risk	

Hammarstrom 2015

Study characteristics			
Patient Sampling	Retrospective study of patients for whom serum BDG was collected twice weekly		
Patient characteristics and setting	Adult hematology or hematopoietic allogenic stem cell transplantation patients with 2 consecutive BDG tests. Thirteen patients had proven or probable IFI. The median age was 53 years (range 17 to 79 years), and 61% were male		
Index tests	Glucatell test using 80 pg/mL as cut-off for positivity		
Target condition and reference standard(s)	Mixed IFI determined by the 2008 EORTC criteria with BDG excluded		
Flow and timing	BDG test was done with 1 week of reference standard. All received index test and reference standard; however, possible IFI cases were excluded from the analysis		
Comparative			
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		
Could the selection of patients have introduced bias?		Low risk	
Are there concerns that the included patients and setting do not match the review question?			Low concern
DOMAIN 3: Reference Standard			

Hammarstrom 2015 *(Continued)*

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		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Yes		
Did all patients receive the same reference standard?	Yes		
Were all patients included in the analysis?	No		
Could the patient flow have introduced bias?		High risk	

Hammarstrom 2018

Study characteristics			
Patient Sampling	Serum BDG collected once to twice weekly as part of a prospective study.		
Patient characteristics and setting	Between September 2011 and December 2012, 135 adult hematology patients were enrolled in the study. More than half (56%) were male, with a median age of 55 years. Thirteen patients met the criteria for IFI		
Index tests	Glucatell test using 80 pg/mL as cut-off for positivity		
Target condition and reference standard(s)	Mixed IFI determined by the 2008 EORTC criteria with BDG excluded		
Flow and timing	All received index test and reference standard; 10 possible IFI cases were excluded from the analysis		
Comparative			
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		

Hammarstrom 2018 *(Continued)*

Was a case-control design avoided?	Yes		
Did the study avoid inappropriate exclusions?	Yes		
Could the selection of patients have introduced bias?		Low risk	
Are there concerns that the included patients and setting do not match the review question?			Low concern
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		
Could the reference standard, its conduct, or its interpretation have introduced bias?		High risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Yes		
Did all patients receive the same reference standard?	Yes		
Were all patients included in the analysis?	No		
Could the patient flow have introduced bias?		High risk	

Hanson 2012

Study characteristics	
Patient Sampling	Serum BDG collected twice weekly as part of a prospective study
Patient characteristics and setting	64 adult ICU patients with a stay of at least 3 days. Included 1 proven and 5 probable cases of invasive candidiasis. The median age of patients was 60 years (range 19 to 82 years), and 69% were male
Index tests	Fungitell test using 80 pg/mL as cut-off for positivity
Target condition and reference standard(s)	Invasive candidiasis determined by the 2008 EORTC criteria with BDG excluded
Flow and timing	Unclear time frame between sample and reference standard; all received index test and reference standard and were included in the analysis
Comparative	

Hanson 2012 *(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		
Could the selection of patients have introduced bias?		Low risk	
Are there concerns that the included patients and setting do not match the review question?			Low concern
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		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
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		
Could the patient flow have introduced bias?		Low risk	

Horiguchi 2004

Study characteristics	
Patient Sampling	Serum BDG collected as part of a prospective consecutive study
Patient characteristics and setting	Sixty-nine adult patients with hematological malignancies were enrolled. Eight met the criteria for proven or probable IFI

Horiguchi 2004 *(Continued)*

Index tests	Fungitec-G test using 20 pg/mL as cut-off for positivity
Target condition and reference standard(s)	Invasive aspergillosis as determined by the 2002 EORTC criteria
Flow and timing	Unclear time frame between sample and reference standard; all received index test and reference standard and were included in the analysis
Comparative	
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		
Could the selection of patients have introduced bias?		Low risk	
Are there concerns that the included patients and setting do not match the review question?			Low concern
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		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
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		
Could the patient flow have introduced bias?		Low risk	

Jin 2013

Study characteristics	
Patient Sampling	Serum BDG collected twice weekly as part of a prospective study
Patient characteristics and setting	Adult patients with hematological malignancies who had been admitted to the hospital between 2005 and 2010. Of the 378 patients in the study, 143 had proven or probable invasive aspergillosis. The age range of patients was between 20 and 76 years, and most were male (78%)
Index tests	Glucatell test using 80 pg/mL as cut-off for positivity
Target condition and reference standard(s)	Invasive aspergillosis determined by the 2002 EORTC criteria
Flow and timing	Unclear time frame between sample and reference standard; possible IA cases were excluded from the analysis
Comparative	
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		
Could the selection of patients have introduced bias?		Low risk	
Are there concerns that the included patients and setting do not match the review question?			Low concern
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		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
DOMAIN 4: Flow and Timing			

Jin 2013 *(Continued)*

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		
Could the patient flow have introduced bias?		High risk	

Kami 2001

Study characteristics			
Patient Sampling	Both retrospective and prospective BDG samples were collected from patients		
Patient characteristics and setting	All BMT patients were included in this study, as well as some high-dose chemotherapy patients. Of the 122 patients, 33 had proven invasive aspergillosis. A majority (76%) were male, and patients ranged in age from 17 to 80 years		
Index tests	Fungitec-G test using 20 pg/mL as cut-off for positivity		
Target condition and reference standard(s)	Invasive aspergillosis diagnosed by histologic evidence and positive for *Aspergillus* in sputum, biopsy, or autopsy		
Flow and timing	Unclear time frame between sample and reference standard; all received index test and reference standard and were included in the analysis		
Comparative			
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?	No		
Did the study avoid inappropriate exclusions?	No		
Could the selection of patients have introduced bias?		High risk	
Are there concerns that the included patients and setting do not match the review question?			High
DOMAIN 3: Reference Standard			

Kami 2001 *(Continued)*

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		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
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		
Could the patient flow have introduced bias?		Low risk	

Kawazu 2004

Study characteristics			
Patient Sampling	Serum BDG collected weekly as part of a prospective study		
Patient characteristics and setting	Adult hematological patients at high risk for invasive aspergillosis. There were 149 episodes occurring among 96 consecutive patients; of these, 11 were cases of proven or probable invasive aspergillosis. The mean age of patients was 45 years, and 70% were male		
Index tests	Wako test using multiple cut-offs for positivity, including 2, 3, 5, and 11 pg/mL		
Target condition and reference standard(s)	Invasive aspergillosis determined by the 2002 EORTC criteria		
Flow and timing	Unclear time frame between sample and reference standard; all received index test and reference standard and were included in the analysis		
Comparative			
Notes			
Methodological quality			
Item	**Authors' judgement**	**Risk of bias**	**Applicability concerns**
DOMAIN 1: Patient Selection			

Kawazu 2004 *(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		
Could the selection of patients have introduced bias?		Low risk	
Are there concerns that the included patients and setting do not match the review question?			Low concern
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		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
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		
Could the patient flow have introduced bias?		Low risk	

Koltze 2015

Study characteristics	
Patient Sampling	Serum BDG collected weekly as part of a prospective study
Patient characteristics and setting	34 pediatric patients who had undergone HSCT transplants. Two patients were diagnosed with a proven IFI, and 4 patients had probable invasive aspergillosis. The age of patients ranged from 0 to 16 years, and 56% were male
Index tests	Fungitell test using 80 pg/mL as cut-off for positivity
Target condition and reference standard(s)	Mixed IFI determined by the 2008 EORTC criteria with BDG excluded
Flow and timing	Unclear time frame between sample and reference standard; all received index test and reference standard and were included in the analysis

Koltze 2015 *(Continued)*

Comparative			
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		
Could the selection of patients have introduced bias?		Low risk	
Are there concerns that the included patients and setting do not match the review question?			Low concern
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		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
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		
Could the patient flow have introduced bias?		Low risk	

Koo 2009

Study characteristics	
Patient Sampling	Retrospective study of patients with at least 1 BDG test
Patient characteristics and setting	871 adult patients at risk for IFI who had a BDG test, mostly those with hematologic malignancies. There were 116 proven or prob-

Koo 2009 *(Continued)*

	able IFI cases. The median age of patients was 54 years, and 44% were female
Index tests	Fungitell test using 80 pg/mL as cut-off for positivity
Target condition and reference standard(s)	Mixed IFI determined by the 2008 EORTC criteria with BDG excluded
Flow and timing	Sample taken within 1 week of reference standard; all received index test and reference standard and were included in the analysis
Comparative	
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		
Could the selection of patients have introduced bias?		Low risk	
Are there concerns that the included patients and setting do not match the review question?			Low concern
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		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Yes		
Did all patients receive the same reference standard?	Yes		
Were all patients included in the analysis?	Yes		
Could the patient flow have introduced bias?		Low risk	

Lahmer 2016a

Study characteristics	
Patient Sampling	Retrospective study; sampling strategy uncertain
Patient characteristics and setting	49 immunosuppressed patients who were in the ICU between December 2014 and December 2015. Of these, 13 had probable invasive aspergillosis. The mean age of patients was 59 years (range 18 to 84 years), and 57% were male
Index tests	Fungitell test using 80 pg/mL as cut-off for positivity
Target condition and reference standard(s)	Invasive aspergillosis as determined by the 2008 EORTC criteria; unclear whether BDG was excluded
Flow and timing	BDG sampled within 6 days (± 2 days) of the reference standard; all received index test and reference standard and were included in the analysis
Comparative	
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		
Could the selection of patients have introduced bias?		Low risk	
Are there concerns that the included patients and setting do not match the review question?			Low concern
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		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Unclear risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
DOMAIN 4: Flow and Timing			

Lahmer 2016a *(Continued)*

Was there an appropriate interval between index test and reference standard?	Yes		
Did all patients receive the same reference standard?	Yes		
Were all patients included in the analysis?	Yes		
Could the patient flow have introduced bias?		Low risk	

Lahmer 2016b

Study characteristics			
Patient Sampling	Retrospective study of patients with 1 BDG test		
Patient characteristics and setting	30 adult hematologic patients in ICU for septic shock. Of these, 10 had proven or probable invasive aspergillosis. The mean age of patients was 59 years, and about half (56%) were male		
Index tests	Fungitell test using 80 pg/mL as cut-off for positivity		
Target condition and reference standard(s)	Invasive aspergillosis determined by the 2008 EORTC criteria; unclear whether BDG was excluded		
Flow and timing	Unclear time frame between sample and reference standard; all received index test and reference standard and were included in the analysis		
Comparative			
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		
Could the selection of patients have introduced bias?		Low risk	
Are there concerns that the included patients and setting do not match the review question?			Low concern
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	Yes		

Lahmer 2016b *(Continued)*

Were the reference standard results interpreted without knowledge of the results of the index tests?	Unclear		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Unclear risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
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		
Could the patient flow have introduced bias?		Low risk	

Leon 2016

Study characteristics			
Patient Sampling	Serum BDG collected twice weekly as part of a prospective study		
Patient characteristics and setting	233 ICU patients with a hospital stay longer than 7 days. Included 31 cases of invasive candidiasis, 154 colonized, and 48 with neither. The mean age of patients was 66.7 years, and two-thirds (67%) were male		
Index tests	Fungitell test using 80 pg/mL as cut-off for positivity; 2 sequential positives required		
Target condition and reference standard(s)	Invasive candidiasis diagnosed by a positive blood culture		
Flow and timing	Unclear time frame between sample and reference standard; all received index test and reference standard and were included in the analysis		
Comparative			
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		

Leon 2016 *(Continued)*

Did the study avoid inappropriate exclusions?	Yes		
Could the selection of patients have introduced bias?		Low risk	
Are there concerns that the included patients and setting do not match the review question?			Low concern
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		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
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		
Could the patient flow have introduced bias?		Low risk	

Liu 2009

Study characteristics	
Patient Sampling	Retrospective study of patients with 1 BDG test
Patient characteristics and setting	162 hematologic or BMT patients ranging in age from 6 to 85 years. Twenty had proven or probable IFI
Index tests	Fungitec-G using 20 pg/mL as cut-off for positivity
Target condition and reference standard(s)	Mixed IFI as determined by the 2008 EORTC criteria with BDG excluded
Flow and timing	Unknown time frame between sample and reference standard; all received index test and reference standard and were included in the analysis
Comparative	
Notes	

Liu 2009 *(Continued)*

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		
Could the selection of patients have introduced bias?		Low risk	
Are there concerns that the included patients and setting do not match the review question?			Low concern
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		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
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		
Could the patient flow have introduced bias?		Low risk	

Lo Cascio 2015

Study characteristics	
Patient Sampling	Retrospective study of patients with 1 BDG test
Patient characteristics and setting	267 ICU patients with high risk factors for invasive candidiasis. Included 91 patients with proven or probable IFIs. The median age was 61.5 years, and 34% were female
Index tests	Fungitell test using 80 pg/mL as cut-off for positivity

Lo Cascio 2015 *(Continued)*

Target condition and reference standard(s)	Proven invasive candidiasis determined by the 2008 EORTC criteria as positive blood culture; unclear whether BDG was included for probable invasive candidiasis
Flow and timing	Unclear time frame between sample and reference standard; all received index test and reference standard and were included in the analysis
Comparative	
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		
Could the selection of patients have introduced bias?		Low risk	
Are there concerns that the included patients and setting do not match the review question?			Low concern
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		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
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		
Could the patient flow have introduced bias?		Low risk	

Mackay 2011

Study characteristics	
Patient Sampling	Serum BDG collected once per patient as part of a prospective study
Patient characteristics and setting	72 ICU neonates with clinically suspected late-onset sepsis. Of these, 19 had proven or probable IFI. The median gestational age was 31 weeks, and 63% were male
Index tests	Fungitell test using 60 and 80 pg/mL as cut-offs for positivity
Target condition and reference standard(s)	Mixed IFI diagnosed by a positive culture from a sterile site
Flow and timing	Unclear on time frame between sample and reference standard; all received index test and reference standard and were included in the analysis
Comparative	
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		
Could the selection of patients have introduced bias?		Low risk	
Are there concerns that the included patients and setting do not match the review question?			Low concern
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		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Unclear		

Mackay 2011 *(Continued)*

Did all patients receive the same reference standard?	Yes		
Were all patients included in the analysis?	Yes		
Could the patient flow have introduced bias?		Low risk	

Martin-Mazuelos 2015

Study characteristics			
Patient Sampling	Serum BDG collected twice weekly as part of a prospective study		
Patient characteristics and setting	107 ICU patients with a stay of at least 7 days. There were 15 cases of invasive candidiasis. The mean age of patients was 62.7 years, and 66% were male.		
Index tests	Fungitell test using 80 pg/mL as cut-off for positivity		
Target condition and reference standard(s)	Invasive candidiasis diagnosed by a positive culture		
Flow and timing	Unclear time frame between sample and reference standard; all received index test and reference standard and were included in the analysis		
Comparative			
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		
Could the selection of patients have introduced bias?		Low risk	
Are there concerns that the included patients and setting do not match the review question?			Low concern
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		

Martin-Mazuelos 2015 *(Continued)*

Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
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		
Could the patient flow have introduced bias?		Low risk	

Metan 2012

Study characteristics			
Patient Sampling	Retrospective study of patients with at least 1 BDG test. The number of samples collected varied from 1 to 23 per patient		
Patient characteristics and setting	128 hematologic patients with proven or probable IFI selected, as well as patients with no IFI; possible IFI cases were excluded. The median age was 47 years (range 17 to 77 years), and 57% were male		
Index tests	Fungitell test using 80 pg/mL as cut-off for positivity		
Target condition and reference standard(s)	Invasive aspergillosis determined by the 2008 EORTC criteria with BDG excluded		
Flow and timing	Sample taken within 10 days of reference standard; all patients selected for study received index test and reference standard for this analysis		
Comparative			
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		

Metan 2012 *(Continued)*

Could the selection of patients have introduced bias?		High risk	
Are there concerns that the included patients and setting do not match the review question?			High
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		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Yes		
Did all patients receive the same reference standard?	Yes		
Were all patients included in the analysis?	Yes		
Could the patient flow have introduced bias?		Low risk	

Metan 2013

Study characteristics	
Patient Sampling	Retrospective study of patients for whom serum BDG was collected twice weekly
Patient characteristics and setting	84 patients who underwent an autologous HSCT between April 2009 and December 2010. There were 3 cases of probable invasive aspergillosis. The median age was 47 years (range 19 to 71 years), and 70% were male
Index tests	Fungitell test using 80 pg/mL as cut-off for positivity
Target condition and reference standard(s)	Invasive aspergillosis as determined by the 2008 EORTC criteria with BDG excluded
Flow and timing	BDG sampled within 10 days of reference standard; all received index test and reference standard and were included in the analysis
Comparative	
Notes	Original article excluded from the analysis 5 possible cases, which we included

Metan 2013 *(Continued)*

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		
Could the selection of patients have introduced bias?		Low risk	
Are there concerns that the included patients and setting do not match the review question?			Low concern
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		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Yes		
Did all patients receive the same reference standard?	Yes		
Were all patients included in the analysis?	Yes		
Could the patient flow have introduced bias?		Low risk	

Mohr 2011

Study characteristics	
Patient Sampling	Serum BDG samples taken twice weekly as part of a prospective sequential study
Patient characteristics and setting	57 ICU patients with a stay of 5 days or longer. Most were male (70%), and the median age was 39 years (range 18 to 76 years). There were 3 proven cases of invasive candidiasis

Mohr 2011 *(Continued)*

Index tests	Fungitell test using 80 pg/mL as cut-off for positivity		
Target condition and reference standard(s)	Invasive candidiasis diagnosed by a positive culture from a sterile site		
Flow and timing	Sample taken the same day of reference standard; all received index test and reference standard and were included in the analysis		
Comparative			
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		
Could the selection of patients have introduced bias?		Low risk	
Are there concerns that the included patients and setting do not match the review question?			Low concern
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		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Yes		
Did all patients receive the same reference standard?	Yes		
Were all patients included in the analysis?	Yes		
Could the patient flow have introduced bias?		Low risk	

Odabasi 2004

Study characteristics	
Patient Sampling	Serum BDG collected twice weekly
Patient characteristics and setting	283 adult hematologic patients, of whom 20 had proven or probable IFI. No information was provided on age or sex of patients
Index tests	Glucatell test using 60 pg/mL as cut-off for positivity; actual measurements were also reported
Target condition and reference standard(s)	Mixed IFI determined by the 2002 EORTC criteria
Flow and timing	Test within 10 days of reference standard; all received index test and reference standard and were included in the analysis
Comparative	
Notes	Grant from the Associates of Cape Cod was provided

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?	No		
Did the study avoid inappropriate exclusions?	Unclear		
Could the selection of patients have introduced bias?		High risk	
Are there concerns that the included patients and setting do not match the review question?			Low concern
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		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Yes		

Odabasi 2004 *(Continued)*

Did all patients receive the same reference standard?	Yes		
Were all patients included in the analysis?	Yes		
Could the patient flow have introduced bias?		Low risk	

Persat 2008

Study characteristics			
Patient Sampling	Retrospective case-control study of patients with 1 BDG test		
Patient characteristics and setting	117 hematologic or ICU patients with proven or probable IFI, as well as 122 at-risk controls. No information on age or sex of patients was provided		
Index tests	Fungitell test using 80 pg/mL as cut-off for positivity		
Target condition and reference standard(s)	Mixed IFI determined by the 2002 EORTC criteria		
Flow and timing	BDG sample that was closest to the reference standard was used, but no time frame was specified; all received index test and reference standard and were included in the analysis		
Comparative			
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?	No		
Did the study avoid inappropriate exclusions?	Yes		
Could the selection of patients have introduced bias?		High risk	
Are there concerns that the included patients and setting do not match the review question?			High
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		

Persat 2008 *(Continued)*

Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
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		
Could the patient flow have introduced bias?		Low risk	

Pini 2019

Study characteristics			
Patient Sampling	Retrospective case-control study of patients with BDG testing		
Patient characteristics and setting	29 patients with confirmed *Candida* were selected as cases, and 28 controls were at risk. Cases and controls were selected from adult non-neutropenic patients hospitalized between November 2011 and January 2015; most (77%) were from the ICU		
Index tests	Fungitell test using 80 pg/mL as cut-off for positivity		
Target condition and reference standard(s)	Invasive candidiasis was diagnosed by either culture or histopathology		
Flow and timing	Unclear time frame between sample and reference standard; all received index test and reference standard and were included in the analysis		
Comparative			
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		
Was a case-control design avoided?	No		
Did the study avoid inappropriate exclusions?	Yes		

Pini 2019 *(Continued)*

Could the selection of patients have introduced bias?		High risk	
Are there concerns that the included patients and setting do not match the review question?			Unclear
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		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
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		
Could the patient flow have introduced bias?		Low risk	

Posteraro 2011

Study characteristics	
Patient Sampling	Serum BDG collected once or more often per patient as part of a prospective study
Patient characteristics and setting	95 adult ICU patients with sepsis and a stay of 5 or more days. Of these, 16 had proven IFI. The median age of patients was 69 years (range 18 to 93 years), and 68% were male
Index tests	Fungitell test using 80 pg/mL as cut-off for positivity
Target condition and reference standard(s)	Mixed IFI determined by the 2008 EORTC criteria with BDG excluded
Flow and timing	Test within 24 to 72 hours of reference standard; all received index test and reference standard and were included in the analysis
Comparative	
Notes	
Methodological quality	

Posteraro 2011 *(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		
Could the selection of patients have introduced bias?		Low risk	
Are there concerns that the included patients and setting do not match the review question?			Low concern
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		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Yes		
Did all patients receive the same reference standard?	Yes		
Were all patients included in the analysis?	Yes		
Could the patient flow have introduced bias?		Low risk	

Racil 2010

Study characteristics	
Patient Sampling	Serum BDG collected twice weekly in a prospective study
Patient characteristics and setting	91 patients with hematologic malignancies; patients could be enrolled more than once. A total of 104 patient cycles were included, of which 3 were proven and 9 were probable IFIs. No information on age or sex of patients was provided
Index tests	Fungitell test using 60 and 80 pg/mL as cut-offs for positivity

Racil 2010 *(Continued)*

Target condition and reference standard(s)	Mixed IFI determined by the 2008 EORTC criteria with BDG excluded
Flow and timing	Unclear time frame between sample and reference standard; all received index test and reference standard and were included in the analysis
Comparative	
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		
Could the selection of patients have introduced bias?		Low risk	
Are there concerns that the included patients and setting do not match the review question?			Low concern
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		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
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		
Could the patient flow have introduced bias?		Low risk	

Rose 2014

Study characteristics	
Patient Sampling	Retrospective study of consecutive patients with 1 BDG test
Patient characteristics and setting	132 patients who were seen for suspected fungal pneumonia were included. Of these, 34 had proven or probable IFI. A little more than half (55%) were male and of Caucasian race (55%). Most had either a hematologic malignancy (44%) or an allogenic stem cell transplantation (30%)
Index tests	Fungitell test using 80 pg/mL as cut-off for positivity
Target condition and reference standard(s)	Mixed IFI determined by the 2008 EORTC criteria; unclear if BDG was included
Flow and timing	Unclear time frame between sample and reference standard; all received index test and reference standard and were included in the analysis
Comparative	
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		
Could the selection of patients have introduced bias?		Low risk	
Are there concerns that the included patients and setting do not match the review question?			Low concern
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		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Unclear risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
DOMAIN 4: Flow and Timing			

Rose 2014 *(Continued)*

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		
Could the patient flow have introduced bias?		Low risk	

Salerno 2014

Study characteristics			
Patient Sampling	Retrospective study of consecutive patients with 1 BDG test		
Patient characteristics and setting	108 adult HIV patients with serum samples analyzed for BDG. Of these, 46 had proven PJP. The mean age of PJP cases was 42.9 years; the mean age of non-cases was 45.0 years; about two-thirds (69%) of patients were male		
Index tests	Fungitell test using 80 pg/mL as cut-off for positivity		
Target condition and reference standard(s)	*Pneumocystis jirovecii* pneumonia (PJP) diagnosed by microbiological confirmation		
Flow and timing	Unclear time frame between sample and reference standard; all received index test and reference standard and were included in the analysis		
Comparative			
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		
Could the selection of patients have introduced bias?		Low risk	
Are there concerns that the included patients and setting do not match the review question?			Low concern
DOMAIN 3: Reference Standard			

Salerno 2014 *(Continued)*

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		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
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		
Could the patient flow have introduced bias?		Low risk	

Senn 2008

Study characteristics			
Patient Sampling	Serum BDG collected twice weekly in the absence of fever, and daily if fever was present, as part of a prospective study		
Patient characteristics and setting	95 adult hematologic patients hospitalized for myeloablative chemotherapy between 2002 and 2006. There were 30 cases of proven or probable IFI. The average age of patients was 57 years (range 19 to 77 years), and 61% were male		
Index tests	Wako test using 11 pg/mL as cut-off for positivity		
Target condition and reference standard(s)	Mixed IFI as determined by the 2002 EORTC criteria		
Flow and timing	Median time interval between sample and reference standard was 7.5 days, with a range of 0 to 51 days; all received index test and reference standard and were included in the analysis		
Comparative			
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		

Senn 2008 *(Continued)*

Was a case-control design avoided?	Yes		
Did the study avoid inappropriate exclusions?	Yes		
Could the selection of patients have introduced bias?		Low risk	
Are there concerns that the included patients and setting do not match the review question?			Low concern
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		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	No		
Did all patients receive the same reference standard?	Yes		
Were all patients included in the analysis?	Yes		
Could the patient flow have introduced bias?		High risk	

Shabaan 2018

Study characteristics	
Patient Sampling	Serum BDG collected once within 24 hours of prospective study enrollment
Patient characteristics and setting	77 ICU neonates at high risk for IFI. There were 11 definite and 25 suspected cases of IFI. Two-thirds (68%) were male
Index tests	Dynamiker Fungus using 95 pg/mL as cut-off for positivity
Target condition and reference standard(s)	Invasive candidiasis diagnosed by a positive blood culture
Flow and timing	Unclear time frame between sample and reference standard; all received index test and reference standard and were included in the analysis
Comparative	

Shabaan 2018 *(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		
Could the selection of patients have introduced bias?		Low risk	
Are there concerns that the included patients and setting do not match the review question?			Low concern
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		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
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		
Could the patient flow have introduced bias?		Low risk	

Singh 2015

Study characteristics

Patient Sampling	Serum BDG collected once per patient as part of a prospective study

Singh 2015 *(Continued)*

Patient characteristics and setting	199 adult liver transplant recipients at high risk for IFI. Of these, 12 were diagnosed with proven IFIs. The median age of patients was 58 years (range 19 to 75 years), and 70% were male
Index tests	Fungitell test using 80 pg/mL as cut-off for positivity
Target condition and reference standard(s)	Mixed IFI as determined by the 2008 EORTC criteria, with BDG excluded
Flow and timing	Unknown time frame between sample and reference standard; all received index test and reference standard and were included in the analysis
Comparative	
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		
Could the selection of patients have introduced bias?		Low risk	
Are there concerns that the included patients and setting do not match the review question?			Low concern
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		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
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		

Singh 2015 *(Continued)*

Could the patient flow have introduced bias?	Low risk

Talento 2017

Study characteristics			
Patient Sampling	Serum BDG collected twice weekly as part of a prospective study		
Patient characteristics and setting	100 adult ICU patients with a hospital stay of 7 days or longer. There were 33 proven and probable cases of IFI. The mean age at enrollment was 64.4 years (range 20 to 85 years), and 67% were male		
Index tests	Fungitell test using 80 pg/mL as cut-off for positivity		
Target condition and reference standard(s)	Mixed IFI determined by the 2008 EORTC criteria with BDG excluded		
Flow and timing	Unclear time frame between sample and reference standard; all received index test and reference standard and were included in the analysis		
Comparative			
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		
Could the selection of patients have introduced bias?		Low risk	
Are there concerns that the included patients and setting do not match the review question?			Low concern
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		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk	

Talento 2017 *(Continued)*

Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
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		
Could the patient flow have introduced bias?		Low risk	

Theel 2013

Study characteristics			
Patient Sampling	Serum BDG collected once per patient as part of a prospective study		
Patient characteristics and setting	123 immunocompromised patients at risk for IFI. There were 4 cases of proven IFI and 16 probable cases. No information was provided on age or sex of patients		
Index tests	Fungitell test using 80 pg/mL as cut-off for positivity		
Target condition and reference standard(s)	Mixed IFI determined by the 2008 EORTC criteria with BDG excluded		
Flow and timing	Unclear time frame between sample and reference standard; 14 possible cases were excluded from the analysis		
Comparative			
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		
Could the selection of patients have introduced bias?		Low risk	
Are there concerns that the included patients and setting do not match the review question?			Low concern

Theel 2013 *(Continued)*

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		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
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		
Could the patient flow have introduced bias?		High risk	

Verduyn Lunel 2009

Study characteristics	
Patient Sampling	Retrospective case-control study for which serum BDG was collected twice weekly
Patient characteristics and setting	Records of hematology/oncology patients admitted to hospital intensive cytotoxic chemotherapy or to undergo HSCT between January 1999 and December 2005. Of these, 21 patients had proven invasive candidiasis. Thirty hematology controls were selected as a comparison group. Almost two-thirds (32/51) were male, with age range of 3 to 65 years
Index tests	Fungitell test with < 60 pg/mL considered negative
Target condition and reference standard(s)	Invasive candidiasis isolated in blood culture or from sterile site
Flow and timing	Unclear time frame between sample and reference standard, but median was 3 days (range -104 to 190 days); all received index test and reference standard and were included in the analysis
Comparative	
Notes	

Methodological quality

Item	Authors' judgement	Risk of bias	Applicability concerns

Verduyn Lunel 2009 *(Continued)*

DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	No		
Was a case-control design avoided?	No		
Did the study avoid inappropriate exclusions?	Yes		
Could the selection of patients have introduced bias?		High risk	
Are there concerns that the included patients and setting do not match the review question?			High
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		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
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		
Could the patient flow have introduced bias?		Unclear risk	

White 2017

Study characteristics	
Patient Sampling	Retrospective case-control study of patients with BDG testing
Patient characteristics and setting	Cases and controls were both at risk for invasive fungal conditions. Patients had the following underlying conditions: HIV, organ transplant, and hematology malignancies
Index tests	Dynamiker Fungus test using 95 pg/mL as cut-off for positivity
Target condition and reference standard(s)	Mixed IFI determined by the 2008 EORTC criteria; unclear if BDG was excluded. PJP diagnosed by PCR and/or radiologic evidence

White 2017 *(Continued)*

Flow and timing	Unclear time frame between sample and reference standard; all received index test and reference standard and were included in the analysis
Comparative	
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		
Was a case-control design avoided?	No		
Did the study avoid inappropriate exclusions?	Yes		
Could the selection of patients have introduced bias?		High risk	
Are there concerns that the included patients and setting do not match the review question?			Low concern
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		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
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		
Could the patient flow have introduced bias?		Low risk	

BAL: bronchoalveolar lavage; BDG: beta-D-glucan test; BMT: bone marrow transplant; EORTC: European Organization for Research and Treatment of Cancer; IA: inflammatory arthritis; IFI: Invasive fungal infection; GM: galactomannan; HSCT: hematopoietic stem cell transplantation; ICU: Intensive care unit; NICU: neonatal intensive care unit; PJP: *Pneumocystis jirovecii* pneumonia; PCR: polymerase chain reaction.

Characteristics of excluded studies *[ordered by study ID]*

Study	Reason for exclusion
Acosta 2012	Unable to determine cell counts
Akamatsu 2007	Assay cut-off did not follow manufacturer's recommendations
Azoulay 2016	Possible IFI included in case definition; unable to separate
Badiee 2016	Assay cut-off did not follow manufacturer's recommendations
Bellanger 2011	Study utilizes 2 cut-off values for determining positivity of BDG
Bhaskaran 2017	Only bronchoalveolar lavage fluid results given
Boluk 2016	Included BDG test as part of reference standard
Brasier 2015	Unable to determine cell counts
Calitri 2017	Unable to determine cell counts
Dobias 2018	Study population did not match inclusion guidelines
Donato 2017	Study population did not match inclusion guidelines
Ellis 2008	Possible IFI included in case definition; unable to separate
Goudijl 2013	Assay cut-off did not follow manufacturer's recommendations
Guitard 2016	Unable to determine cell counts
Han 2015	Unable to determine cell counts
Hartl 2018	Possible IFI included in case definition; unable to separate
Heyland 2011	Did not follow EORTC guidelines; questionable patient population
Hoenigl 2014	Only bronchoalveolar lavage fluid results given
Ji 2008	Unable to determine cell counts
Kato 2010	Included BDG as part of reference standard
Kishimoto 2019	Study population did not match inclusion guidelines
Kumar 2018	Study population did not match inclusion guidelines
Lahmer 2016c	Unable to determine cell counts
Lahmer 2017	Study population did not match inclusion guidelines
Leon 2009	Assay cut-off did not follow manufacturer's recommendations
Leon 2012	Unable to determine cell counts

Study	Reason for exclusion
Levesque 2015	Unable to determine cell counts
Levesque 2017	Unable to determine cell counts
Matsumara 2011	Study utilizes 2 cut-off values for determining positivity of BDG
McKeating 2018	Unable to determine patient population
Metan 2016	Unable to determine cell counts
Montagna 2012	Unable to determine cell counts
Mutschlechner 2015	Unable to determine cell counts
Oz 2014	Possible IFI included in case definition; unable to separate
Pazos 2005	Assay cut-off did not follow manufacturer's recommendations
Pazos 2006	Assay cut-off did not follow manufacturer's recommendations
Picardi 2019	Not a diagnostic accuracy study
Presterl 2009	Assay cut-off did not follow manufacturer's recommendations
Ramos 2017	Cannot separate confirmed *Candida* cases from probable *Candida* cases
Rhein 2014	Only cerebrospinal fluid results given
Sax 2011	Did not follow EORTC guidelines
Shi 2015	Did not follow EORTC guidelines
Su 2017	Did not follow EORTC guidelines
Sulahian 2014	Unable to determine cell counts
Tasaka 2007	Unable to determine cell counts
Watanabe 2009	Assay cut-off did not follow manufacturer's recommendations
White 2018	Unable to determine cell counts
Wood 2013	Cannot separate confirmed PJP cases from probable cases
Yang 2012	Did not follow EORTC guidelines
Yu 2010	Unable to determine cell counts
Zheng 2017	Assay cut-off did not follow manufacturer's recommendations

BDG: beta-D-glucan test; EORTC: European Organization for Research and Treatment of Cancer; IFI: invasive fungal infection; PJP: *Pneumocystis jirovecii* pneumonia.

DATA

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

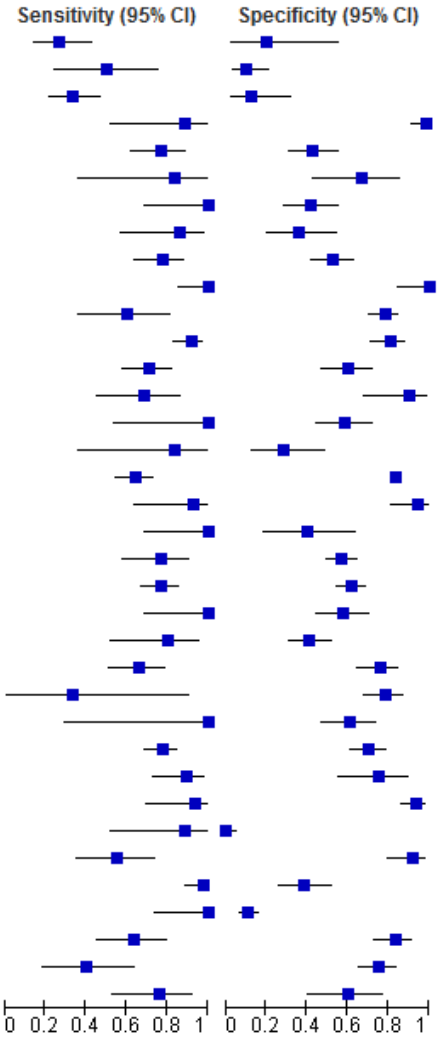
Table Tests. Data tables by test

Test	No. of studies	No. of participants
1 Fungitell	36	4316
2 Glucatell	5	957
3 Wako	3	420
4 Fungitec	3	353
5 Dynamiker	2	198

Test 1. Fungitell

Fungitell

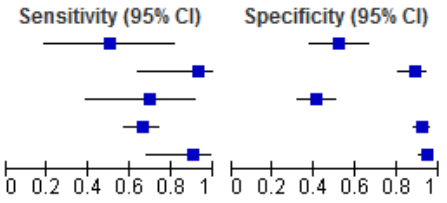
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Acosta 2011	11	8	30	2	0.27 [0.14, 0.43]	0.20 [0.03, 0.56]
Alexander 2010	8	51	8	6	0.50 [0.25, 0.75]	0.11 [0.04, 0.22]
Atalay 2014	20	21	39	3	0.34 [0.22, 0.47]	0.13 [0.03, 0.32]
Boch 2016	8	1	1	61	0.89 [0.52, 1.00]	0.98 [0.91, 1.00]
Ceesay 2015	34	41	10	31	0.77 [0.62, 0.89]	0.43 [0.31, 0.55]
Cornu 2018	5	7	1	14	0.83 [0.36, 1.00]	0.67 [0.43, 0.85]
Costa 2012	10	35	0	25	1.00 [0.69, 1.00]	0.42 [0.29, 0.55]
De Vlieger 2011	12	21	2	12	0.86 [0.57, 0.98]	0.36 [0.20, 0.55]
Del Bono 2011	41	47	12	52	0.77 [0.64, 0.88]	0.53 [0.42, 0.63]
Fontana 2012	24	0	0	22	1.00 [0.86, 1.00]	1.00 [0.85, 1.00]
Furfaro 2018	12	32	8	115	0.60 [0.36, 0.81]	0.78 [0.71, 0.85]
Giacobbe 2017	67	18	6	75	0.92 [0.83, 0.97]	0.81 [0.71, 0.88]
Gupta 2017	44	25	18	38	0.71 [0.58, 0.82]	0.60 [0.47, 0.72]
Hachem 2009	15	2	7	18	0.68 [0.45, 0.86]	0.90 [0.68, 0.99]
Hanson 2012	6	23	0	33	1.00 [0.54, 1.00]	0.59 [0.45, 0.72]
Koltze 2015	5	20	1	8	0.83 [0.36, 1.00]	0.29 [0.13, 0.49]
Koo 2009	72	124	40	635	0.64 [0.55, 0.73]	0.84 [0.81, 0.86]
Lahmer 2016a	12	2	1	34	0.92 [0.64, 1.00]	0.94 [0.81, 0.99]
Lahmer 2016b	10	12	0	8	1.00 [0.69, 1.00]	0.40 [0.19, 0.64]
Leon 2016	23	83	7	111	0.77 [0.58, 0.90]	0.57 [0.50, 0.64]
Lo Cascio 2015	70	67	21	109	0.77 [0.67, 0.85]	0.62 [0.54, 0.69]
Mackay 2011	10	26	0	36	1.00 [0.69, 1.00]	0.58 [0.45, 0.70]
Martin-Mazuelos 2015	12	54	3	38	0.80 [0.52, 0.96]	0.41 [0.31, 0.52]
Metan 2012	33	19	17	59	0.66 [0.51, 0.79]	0.76 [0.65, 0.85]
Metan 2013	1	16	2	60	0.33 [0.01, 0.91]	0.79 [0.68, 0.87]
Mohr 2011	3	21	0	33	1.00 [0.29, 1.00]	0.61 [0.47, 0.74]
Persat 2008	91	36	26	86	0.78 [0.69, 0.85]	0.70 [0.62, 0.78]
Pini 2019	26	7	3	21	0.90 [0.73, 0.98]	0.75 [0.55, 0.89]
Posteraro 2011	15	5	1	74	0.94 [0.70, 1.00]	0.94 [0.86, 0.98]
Racil 2010	8	64	1	0	0.89 [0.52, 1.00]	0.00 [0.00, 0.06]
Rose 2014	16	4	13	43	0.55 [0.36, 0.74]	0.91 [0.80, 0.98]
Salerno 2014	45	38	1	24	0.98 [0.88, 1.00]	0.39 [0.27, 0.52]
Singh 2015	12	160	0	20	1.00 [0.74, 1.00]	0.11 [0.07, 0.17]
Talento 2017	21	11	12	56	0.64 [0.45, 0.80]	0.84 [0.73, 0.92]
Theel 2013	8	22	12	67	0.40 [0.19, 0.64]	0.75 [0.65, 0.84]
Verduyn Lunel 2009	16	12	5	18	0.76 [0.53, 0.92]	0.60 [0.41, 0.77]



Test 2. Glucatell

Glucatell

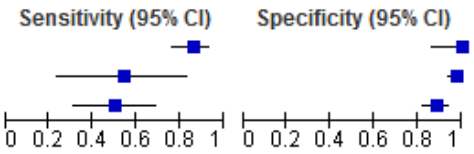
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Badiee 2012	5	25	5	27	0.50 [0.19, 0.81]	0.52 [0.38, 0.66]
Hammarstrom 2015	12	11	1	85	0.92 [0.64, 1.00]	0.89 [0.80, 0.94]
Hammarstrom 2018	9	66	4	46	0.69 [0.39, 0.91]	0.41 [0.32, 0.51]
Jin 2013	94	19	49	216	0.66 [0.57, 0.73]	0.92 [0.88, 0.95]
Odabasi 2004	18	15	2	248	0.90 [0.68, 0.99]	0.94 [0.91, 0.97]



Test 3. Wako

Wako

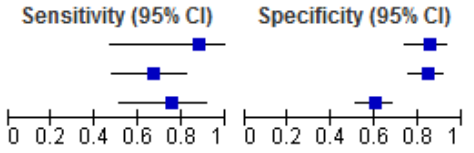
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Dichtl 2018	63	0	10	25	0.86 [0.76, 0.93]	1.00 [0.86, 1.00]
Kawazu 2004	6	3	5	135	0.55 [0.23, 0.83]	0.98 [0.94, 1.00]
Senn 2008	15	16	15	127	0.50 [0.31, 0.69]	0.89 [0.82, 0.93]



Test 4. Fungitec

Fungitec

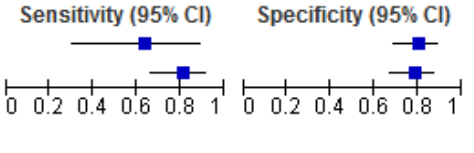
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Horiguchi 2004	7	9	1	52	0.88 [0.47, 1.00]	0.85 [0.74, 0.93]
Kami 2001	22	14	11	75	0.67 [0.48, 0.82]	0.84 [0.75, 0.91]
Liu 2009	15	57	5	85	0.75 [0.51, 0.91]	0.60 [0.51, 0.68]



Test 5. Dynamiker

Dynamiker

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Shabaan 2018	7	13	4	53	0.64 [0.31, 0.89]	0.80 [0.69, 0.89]
White 2017	35	17	8	61	0.81 [0.67, 0.92]	0.78 [0.67, 0.87]



ADDITIONAL TABLES

Table 1. Characteristics of included studies

Study name	Study design	Underlying condition	Fungal type	Test brand	Samples taken	Reference standard
Acosta 2011	Prospective consecutive	ICU	Mixed	Fungitell	Single	EORTC 2008
Alexander 2010	Prospective consecutive	Organ transplant	Mixed	Fungitell	Multiple	EORTC 2008
Atalay 2014	Retrospective consecutive	Cancer	Mixed	Fungitell	Single	EORTC 2008
Badiee 2012	Prospective consecutive	Cancer	Aspergillosis	Glucatell	Multiple	EORTC 2008
Boch 2016	Prospective consecutive	Cancer	Mixed	Fungitell	Single	EORTC 2008
Ceesay 2015	Prospective consecutive	Mixed at-risk	Mixed	Fungitell	Multiple	EORTC 2008

Table 1. Characteristics of included studies *(Continued)*

Cornu 2018	Retrospective case-control	ICU	*Candida*	Fungitell	Single	Culture from blood or sterile site
Costa 2012	Retrospective consecutive	Mixed at-risk	PJP	Fungitell	Single	Microscopy
De Vlieger 2011	Retrospective case-control	ICU	Aspergillosis	Fungitell	Unknown	EORTC 2008
Del Bono 2011	Prospective consecutive	ICU	*Candida*	Fungitell	Single	EORTC 2008
Dichtl 2018	Retrospective case-control	Mixed at-risk	PJP	Wako	Single	PCR
Fontana 2012	Retrospective case-control	Cancer	Mixed	Fungitell	Multiple	EORTC 2008
Furfaro 2018	Prospective consecutive	Cancer	Aspergillosis	Fungitell	Multiple	EORTC 2008
Giacobbe 2017	Retrospective consecutive	ICU	*Candida*	Fungitell	Single	Culture from blood or sterile site
Gupta 2017	Prospective consecutive	Cancer	Mixed	Fungitell	Single	EORTC 2008
Hachem 2009	Prospective consecutive	Cancer	Aspergillosis	Fungitell	Multiple	EORTC 2002
Hammarstrom 2015	Retrospective consecutive	Cancer	Mixed	Glucatell	Multiple	EORTC 2008
Hammarstrom 2018	Prospective consecutive	Cancer	Mixed	Glucatell	Multiple	EORTC 2008
Hanson 2012	Prospective consecutive	ICU	Mixed	Fungitell	Multiple	EORTC 2008
Horiguchi 2004	Prospective consecutive	Cancer	Aspergillosis	Fungitec	Unknown	EORTC 2002
Jin 2013	Prospective consecutive	Cancer	Aspergillosis	Glucatell	Multiple	EORTC 2002
Kami 2001	Unknown	Cancer	Aspergillosis	Fungitec	Multiple	Study-specific, comparable to EORTC
Kawazu 2004	Prospective consecutive	Cancer	Aspergillosis	Wako	Multiple	EORTC 2002
Koltze 2015	Prospective consecutive	Cancer	Mixed	Fungitell	Multiple	EORTC 2008
Koo 2009	Retrospective consecutive	Cancer	Mixed	Fungitell	Multiple	EORTC 2008
Lahmer 2016a	Retrospective consecutive	ICU	Aspergillosis	Fungitell	Unknown	EORTC 2008

Table 1. Characteristics of included studies *(Continued)*

Lahmer 2016b	Retrospective consecutive	ICU	Aspergillosis	Fungitell	Single	EORTC 2008
Leon 2016	Prospective consecutive	ICU	*Candida*	Fungitell	Multiple	Culture from blood or sterile site
Liu 2009	Retrospective consecutive	Cancer	Mixed	Fungitec	Single	EORTC 2008
Lo Cascio 2015	Retrospective consecutive	ICU	*Candida*	Fungitell	Single	EORTC
Mackay 2011	Prospective consecutive	ICU	Mixed	Fungitell	Single	Study-specific, comparable to EORTC
Martin-Mazuelos 2015	Prospective consecutive	ICU	*Candida*	Fungitell	Multiple	Culture
Metan 2012	Retrospective case-control	Cancer	Aspergillosis	Fungitell	Multiple	EORTC 2008
Metan 2013	Retrospective consecutive	Cancer	Aspergillosis	Fungitell	Multiple	EORTC 2008
Mohr 2011	Prospective consecutive	ICU	*Candida*	Fungitell	Multiple	Culture
Odabasi 2004	Unknown	Cancer	Mixed	Glucatell	Multiple	EORTC 2002
Persat 2008	Retrospective case-control	Cancer	Mixed	Fungitell	Single	EORTC 2002
Pini 2019	Retrospective case-control	Mixed	*Candida*	Fungitell	Single	Culture from blood or sterile site or pathology
Posteraro 2011	Prospective consecutive	ICU	Mixed	Fungitell	Multiple	EORTC 2008
Racil 2010	Prospective consecutive	Cancer	Mixed	Fungitell	Multiple	EORTC 2002
Rose 2014	Retrospective consecutive	Mixed at-risk	Mixed	Fungitell	Single	EORTC 2008
Salerno 2014	Retrospective consecutive	HIV	PJP	Fungitell	Single	Microscopy/PCR
Senn 2008	Prospective consecutive	Cancer	Mixed	Wako	Multiple	EORTC 2002
Shabaan 2018	Prospective consecutive	ICU	*Candida*	Dynamiker	Single	Culture from blood or sterile site
Singh 2015	Prospective consecutive	Organ transplant	Mixed	Fungitell	Multiple	EORTC 2008
Talento 2017	Prospective consecutive	ICU	Mixed	Fungitell	Multiple	EORTC 2008
Theel 2013	Prospective consecutive	Mixed at-risk	Mixed	Fungitell	Single	EORTC 2008

Table 1. Characteristics of included studies *(Continued)*

Verduyn Lunel 2009	Retrospective case-control	Cancer	*Candida*	Fungitell	Multiple	Culture from blood or sterile site
White 2017	Retrospective case-control	Mixed at-risk	Mixed	Dynamiker	Single	EORTC 2008

EORTC: European Organization for Research and Treatment of Cancer; ICU: intensive care unit; PCR: polymerase chain reaction; PJP: *Pneumocystis jirovecii* pneumonia.

Table 2. Summary of included studies

Characteristic	n	Percentage
Underlying condition		
Cancer	23	46.9%
HIV/AIDS	1	2.0%
ICU	16	32.7%
Mixed at-risk	7	14.3%
Organ transplant	2	4.1%
Age of patients		
Adult	26	53.1%
Neonate	3	6.1%
Pediatric	3	6.1%
Mixed	5	10.2%
Unknown	12	24.5%
Study design		
Prospective consecutive	26	53.1%
Retrospective consecutive	12	24.5%
Retrospective case-control	11	22.4%
Fungal type		
Aspergillus only	12	24.5%
Candida only	10	20.4%
PJP only	3	6.1%
Mixed fungal types	24	49.0%
Test brand		
Fungitell	36	73.5%

Table 2. Summary of included studies *(Continued)*

Glucatell	5	10.2%
Fungitec-G	3	6.1%
Wako	3	6.1%
Dynamiker Fungus	2	4.1%
Sampling strategy		
Single sample	20	40.8%
Multiple samples	26	53.1%
Unknown	3	6.1%
Reference standard used		
EORTC	36	73.5%
Proven *Candida*	8	16.3%
PJP microscopy/PCR	3	6.1%
Study-specific	2	4.1%
Low risk of bias		
Participant selection	35	71.4%
Index test	49	100.0%
Reference standard	41	83.7%
Flow and timing	41	83.7%

EORTC: European Organization for Research and Treatment of Cancer; ICU: intensive care unit; PCR: polymerase chain reaction; PJP: *Pneumocystis jirovecii* pneumonia.

APPENDICES

Appendix 1. MEDLINE (Ovid) strategies

MEDLINE Ovid

1 BDG*.tw.
2 Fungitel*.tw.
3 Cape Cod.tw.
4 Fungitec*.tw.
5 Seikagaku.tw.
6 Wake Test.tw.
7 (Wako* or Waco*).tw.
8 1 or 2 or 3 or 4 or 5 or 6 or 7
9 exp beta-Glucans/
10 Glucans/
11 D-glucan*.tw.
12 9 or 10 or 11
13 exp "Sensitivity and Specificity"/
14 (sensitivit* or specificit*).tw.
15 predictive value*.tw.

16 diagnosis.fs.
17 analysis.fs.
18 Reagent Kits, Diagnostic/
19 13 or 14 or 15 or 16 or 17 or 18
20 12 and 19
21 exp Mycoses/
22 exp Fungi/
23 fungal.tw.
24 fungus.tw.
25 mycos*.tw.
26 mycot*.tw.
27 aspergill*.tw.
28 candid*.tw.
29 pneumocystis.tw.
30 histoplasmosis.tw.
31 blastomycosis.tw.
32 fusarium.tw.
33 trichosporon.tw.
34 saccharomyces.tw.
35 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 or 33 or 34
36 12 and 35
37 8 or 20 or 36
38 exp animals/ not humans.sh.
39 37 not 38
40 limit 39 to yr="1995 -Current"

key:, tw = textword, fs = floating subheading

Appendix 2. Embase (Ovid) search strategy

1 BDG*.tw.
2 Fungitel*.tw.
3 Cape Cod.tw.
4 Fungitec*.tw.
5 Seikagaku.tw.
6 Wake Test.tw.
7 (Wako* or Waco*).tw.
8 1 or 2 or 3 or 4 or 5 or 6 or 7
9 exp beta-Glucans/
10 Glucans/
11 D-glucan*.tw.
12 9 or 10 or 11
13 "sensitivity and specificity"/
14 (sensitivit* or specificit*).tw.
15 predictive value*.tw.
16 di.fs.
17 diagnostic kit/
18 exp diagnostic procedure/
19 13 or 14 or 15 or 16 or 17 or 18
20 12 and 19
21 exp mycosis/
22 exp fungus/
23 fungal.tw.
24 fungus.tw.
25 mycos*.tw.
26 mycot*.tw.
27 aspergill*.tw.
28 candid*.tw.
29 pneumocystis.tw.
30 histoplasmosis.tw.
31 blastomycosis.tw.
32 fusarium.tw.

33 trichosporon.tw.
34 saccharomyces.tw.
35 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 or 33 or 34
36 12 and 35
37 7 or 20 or 36
38 (exp Animal/ or Nonhuman/ or exp Animal Experiment/) not Human/
39 37 not 38
40 limit 39 to yr="1995 - Current"

key:, tw = textword, fs = floating subheading

Appendix 3. Initial abstract form

Reviewer: _____RS _____SW

First author (last name, first initial)_____

Title_____

Year_____

Study design

_____Prospective consecutive cohort

_____Retrospective consecutive cohort

_____Retrospective case/control (does not include healthy controls)

_____None of the above (specify:_____)

Patient population

_____Cancer patients

_____Prolonged immunosuppressive therapy patients

_____Patients with congenital or acquired immune disorder

_____ICU patients

_____None of the above (specify:_____)

Type of sample

_____Serum

_____Urine

_____BAL

Invasive fungal infection (check all that apply)

_____Proven IFI

_____Probable IFI

_____Possible IFI

_____None of the above (specify:_____)

EORTC/MSG or autopsy used as a reference standard:

_____Yes

_____No (exclude)

_____Other (specify:_____)

Appendix 4. Full abstract form

Reviewer: _____SW _____RS _____BW

Author, date and language of publication: _____

1) Able to construct 2 ×2 table? If not, stop here!

	Proven IFI	Probable IFI	Possible IFI	No IFI	Totals
BDG Positive					
BDG Negative					
Totals					

a) Prevalence of IFI in the population (if reported): _____%

b) IFI cases defined as (check all that apply):

____ Proven

____ Probable

____ Possible

____ Proven/Probable

____ Proven/Probable/Possible

2) Study design:

____ Retrospective case-control

____ Prospective consecutive cohort

____ Other (describe): _____

a) Controls (check all that apply)

____ At-risk

____ Other diseases

____ Colonized

____ Healthy

b) Patient population (check all that apply)

Patient age

___ Adult

___ Pediatric

___ Neonate only

___ Mixed (specify age range): _____

Underlying condition

___ ICU

___ Heme malignancy

___ BMT

___ Solid tumor malignancy

___ SOT (specify organ): _____

___ HIV

___ Autoimmune/Connective tissue

___ Mixed at-risk

a) Invasive fungal infection (IFI)

___ Mixed IFIs

___ *Candida* only

___ *Aspergillus* only

___ *Pneumocystis* (PCP) only

b) Reference standard used to diagnose IFI

___ EORTC

___ Autopsy

___ Study-specific composite definition

___ PCP only (check all that apply)

___ Microscopy

___ PCR

___ *Candida* only (check all that apply)

___ Culture

___ Histopathology

___ Other (explain): _____

c) Assay

___ Glucatell/Funitell

___ Fungitec G

___ Wako-WB003

___ Difference in titers between Endotoxin Test-D and Endospecy

___ Other (explain): _____

d) Cut-off used to define a positive test

___ ≥ 80 pg/mL Glucatell/Fungitell

___ ≥ 20 pg/mL Fungitec G

___ ≥ 11 pg/mL Wako

___ Other (explain): _____

e) Number of positive specimens at cut-off used to define positive test

___ Single positive

___ Two sequential positives

___ Other (explain): _____

f) Specimen sampling strategy

___ Once

___ Weekly

___ Twice weekly

___ Other (explain): _____

g) Study subject receiving systemic antifungal therapy at the time of testing (include % if known)

___ Antifungal prophylaxis: _____%

___ Empiric therapy: _____%

___ Not reported: _____%

Appendix 5. QUADAS criteria (QUADAS version 2)

Domain 1: patient selection

Risk of bias

1. Was a consecutive or random sample of patients enrolled?

- YES if the study specifically states that consecutive patients or a random sample was selected.
- NO if the study clearly states that the selection of patients was not consecutive or random, or if this can be easily inferred from the design.
- UNCLEAR if not reported or cannot be determined.

2. Was a case-control design avoided?

- YES if the study is cross-sectional, prospective, or retrospective using consecutive patients or a random selection.
- NO if the study specifically states that it used a case-control design, or if this can be easily inferred from the description.
- UNCLEAR if not reported or cannot be determined.

3. Did the study avoid inappropriate conclusions?

- YES. If the study population was selected from hospitalized patients who are in one of the disease groups included in the study AND if patients were not excluded based on any criteria related to potential diagnosis of IFI by EORTC criteria.
- NO. If the study population was selected from hospitalized patients who are not in one of the disease groups included in the study OR if patients were excluded based on any criteria related to potential diagnosis of IFI by EORTC criteria.
- UNCLEAR if not reported or cannot be determined.

Could the study have introduced bias? (no criteria provided)

Concerns regarding applicability

1. Minor concern: study conducted in community or outpatient setting
2. Major concern: patients on prophylactic therapy at the time of index test
3. Major concern: patients on antifungal therapy at the time of index test
4. Major concern: patients who received BDG-positive antibiotics prior to index test
5. Major concern: patients who received IVIG prior to the index test

Overall assessment of level of concern regarding applicability (to be determined).

Domain 2: index test

Risk of bias

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

- YES if the study states that the index test was performed prior to assessment of EORTC criteria, or if the study specifically states that interpretation of the index test was blinded to the reference test.
- NO if the study clearly states that interpretation of the index test was blinded to the reference test.
- UNCLEAR if not reported or cannot be determined.

2. If a threshold was used, was it prespecified?

- YES if the threshold was prespecified.
- NO if the thresholds were not prespecified.
- UNCLEAR if not reported or cannot be determined.

Could the conduct or interpretation of the index test have introduced bias? (to be determined)

Concerns regarding applicability

1. Major concern: the test is not approved by the FDA or similar government organization.
2. Major concern: the test is approved but has been modified relative to the package insert.
3. Major concern: the sampling protocol (sampling frequency, length of sampling) is not clearly described.
4. Major concern: the sampling protocol (sampling frequency, length of sampling) varies between patients.
5. Major concern: the sampling protocol differs significantly from other studies included in the sample.
6. Major concern: the criteria for a positive BDG diagnosis are not clearly specified.
7. Major concern: the criteria for a positive BDG diagnosis vary.
8. Major concern: the criteria for a positive BDG diagnosis differ significantly from other studies in the set of included studies.

Overall assessment for concerns regarding applicability (to be determined)

Domain 3: reference standard

Risk of bias

1. Is the reference standard likely to correctly classify the target condition?

- YES. The EORTC criteria are used as the reference test.

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

Blinding: was interpretation of the reference standard results blinded to results of the index test?

- YES if the study clearly states that the index test was not used as part of the reference criteria AND if assessment of the reference criteria was blinded to the index test result.
- NO if the study clearly states that the index test was used as part of the reference criteria OR if assessment of the reference criteria was not blinded to the index test result.
- UNCLEAR if not reported or cannot be determined.

Incorporation: was the index test not used as part of the criteria for the reference standard?

- YES the study clearly states that the index test was not used in the assessment of the reference criteria. This is independent of blinding.
- NO the study clearly states that the index test was not excluded from the reference criteria.
- UNCLEAR if not reported or cannot be determined..

Could the conduct or interpretation of the index test have introduced bias? (to be determined)

Is there any concern that the target condition as defined by the reference standard does not match the review question?

Major concern: the study does not provide a disaggregated tabulation of each category of reference test diagnosis against each category of index test diagnosis.

Domain 4: timing and flow

Risk of bias

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

- YES if the BDG test result for all patients was obtained within 2 weeks of the reference test.
- NO if the reference test for all patients was performed more than 4 weeks after the BDG test.

- UNCLEAR if not reported or cannot be determined (e.g. if the time interval varies widely).

Explanation: a survey of studies shows kinds of designs:

- Cross-sectional studies in which the index (BDG) and reference test (EORTC) were evaluated at the same time. This design avoids disease progression bias but misses one of the potential benefits of the index test. BDG is attractive because it can be used as a screen to direct therapy prior to the development of EORTC criteria. Thus, one would like to assess the correlation between an early BDG test and the development of IFI (within a reasonable period). Cross-sectional correlation between BDG and EORTC represents a minimum criterion for usefulness of the test.
- Longitudinal studies in which the reference criteria are continuously monitored. This design more closely resembles the intended use of the test but poses risk of disease progression bias or misclassification due to the development of a new independent infection in the intervening period.

Thus, there is a trade-off between disease progression bias and realistic appraisal of the test. Too short a period prevents progression bias and classification errors but is an unrealistic evaluation of the test as it is intended. Too long a period leads to progression bias or the possibility of a new infection during the interval period. We chose 2 weeks and 4 weeks as cutoffs because it is unlikely that a positive reference test would be due to a new infection that developed during a 2-week period, and it is possible that a new infection could develop over a 4-week period. The best cut-off is uncertain; for that reason, the time period between index and reference tests will be investigated as a possible source of heterogeneity.

2. Did all patients receive a reference standard?

- YES if all patients (or a random subset of patients) who received the index test were referred for evaluation by the reference test AND the withdrawal rate was low (less than 1% - a level unlikely to affect results).
- NO if a non-random subset of patients was referred for evaluation by the reference test OR if the withdrawal rate was high (greater than 10%).
- UNCLEAR if not reported or cannot be determined.

3. Did all patients receive the same reference standard?

- YES EORTC criteria are the only acceptable criteria in this study.

Could patient flow have introduced bias? (to be determined)

HISTORY

Protocol first published: Issue 5, 2012
Review first published: Issue 7, 2020

CONTRIBUTIONS OF AUTHORS

Kimberly Hanson: study design, writing of the manuscript.
Robert Schmidt: study design, evaluation of study eligibility, data extraction, quality assessment, statistical analysis, writing of the manuscript.
Brandon Walker: data extraction, quality assessment.
Sandra White: evaluation of study eligibility, data extraction, quality assessment, statistical analysis, writing of the manuscript.

DECLARATIONS OF INTEREST

Kimberly Hanson: none known.
Robert Schmidt: none known.
Brandon Walker: none known.
Sandra White: none known.

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Internal sources

- Salary Support, USA

  All investigators received salaries from ARUP Laboratories (BSW) or the University of Utah (SKW, RLS, KEH). RLS and KEH serve as medical directors at ARUP Laboratories. ARUP is wholly owned by the University of Utah and performs clinical laboratory tests including BDG testing.

External sources

- No sources of support supplied

DIFFERENCES BETWEEN PROTOCOL AND REVIEW

We made the following changes to the information published in the protocol of this review (Schmidt 2012).

- QUADAS-2 was used in place of QUADAS-1. No comparison was made with GM due to lack of data and time constraints.
- We intended to perform a meta-analysis of all BDG tests but did not do so due to the high level of heterogeneity between studies.
- We did not perform a formal analysis of heterogeneity using meta-regression because we did not perform a meta-analysis.
- We excluded studies that used BDG as part of the reference standard because these studies had high risk of incorporation bias.
- We did not include studies from which we could not extract data for a 2 × 2 table.
- We included data from the Dynamiker test, which became commercially available after the protocol was published.
- We did not explore the impact of diagnostic thresholds (cut-off values) because almost all studies used the manufacturer's recommended cut-off and because the level of heterogeneity between studies was high.

INDEX TERMS

Medical Subject Headings (MeSH)

Aspergillosis [diagnosis]; beta-Glucans [*blood]; Biomarkers [blood]; Candidiasis, Invasive [diagnosis]; Case-Control Studies; *Critical Illness; *Immunocompromised Host; Invasive Fungal Infections [*diagnosis]; Pneumocystis carinii; Pneumocystis Infections [diagnosis]; Prospective Studies; Retrospective Studies; ROC Curve; Sensitivity and Specificity

MeSH check words

Humans