Μη καλλιεργητικές τεχνικές διάγνωσης ΔΜ

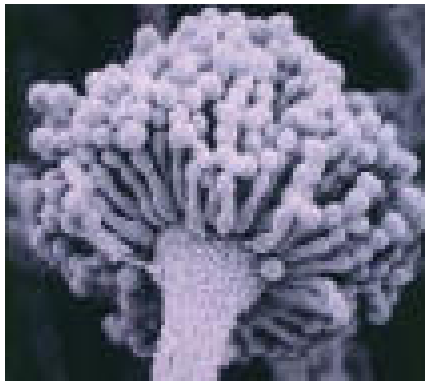
β-D-γλυκάνη: η σημασία της ως πανμυκητικός δείκτης

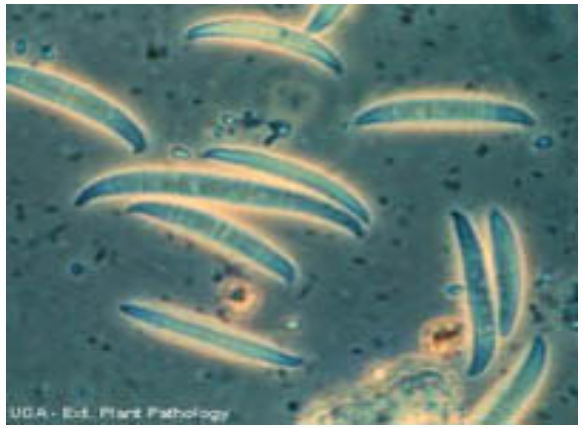
(1,3)-ß-D-glucan

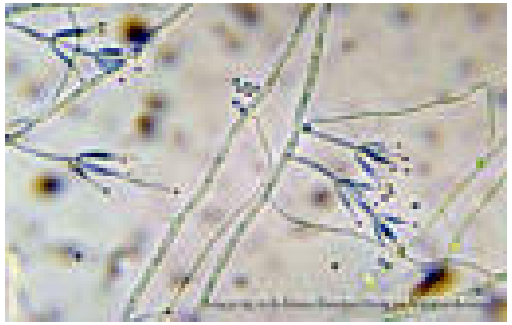
Panfungal marker

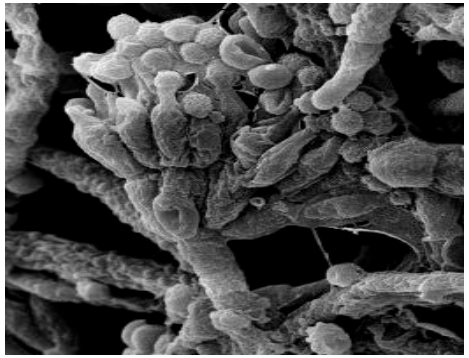
Absent in zygomycetes and most cryptococci

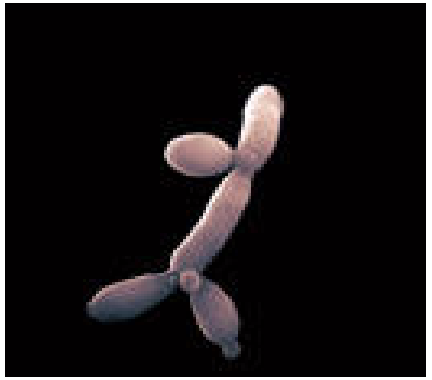
Πανμυκητικός δείκτης

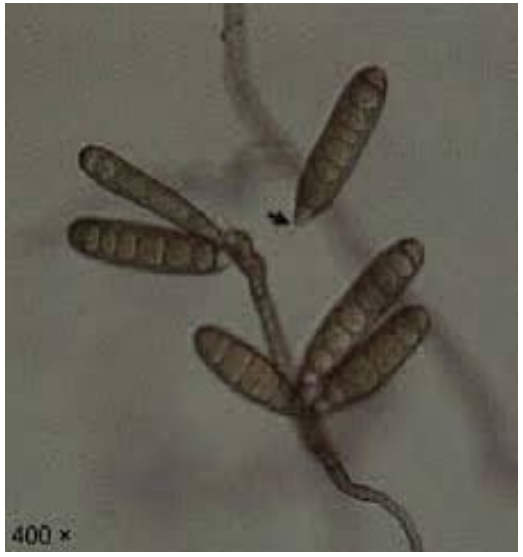


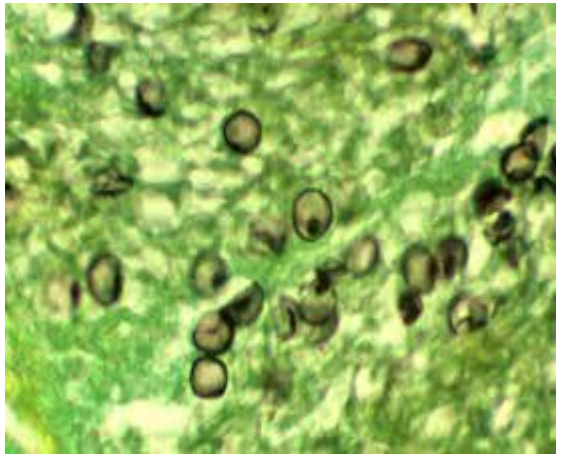


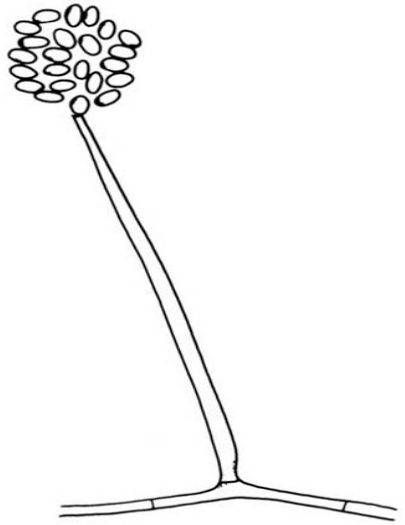












1,3 β-D glucan (BDG) assay

- Based on the detection of the polysaccharide (1,3)-ß-D-glucan.
- Component of the cell wall of many pathogenic fungi, except for Mucorales and most strains of *Cryptococcus*.

Limulus

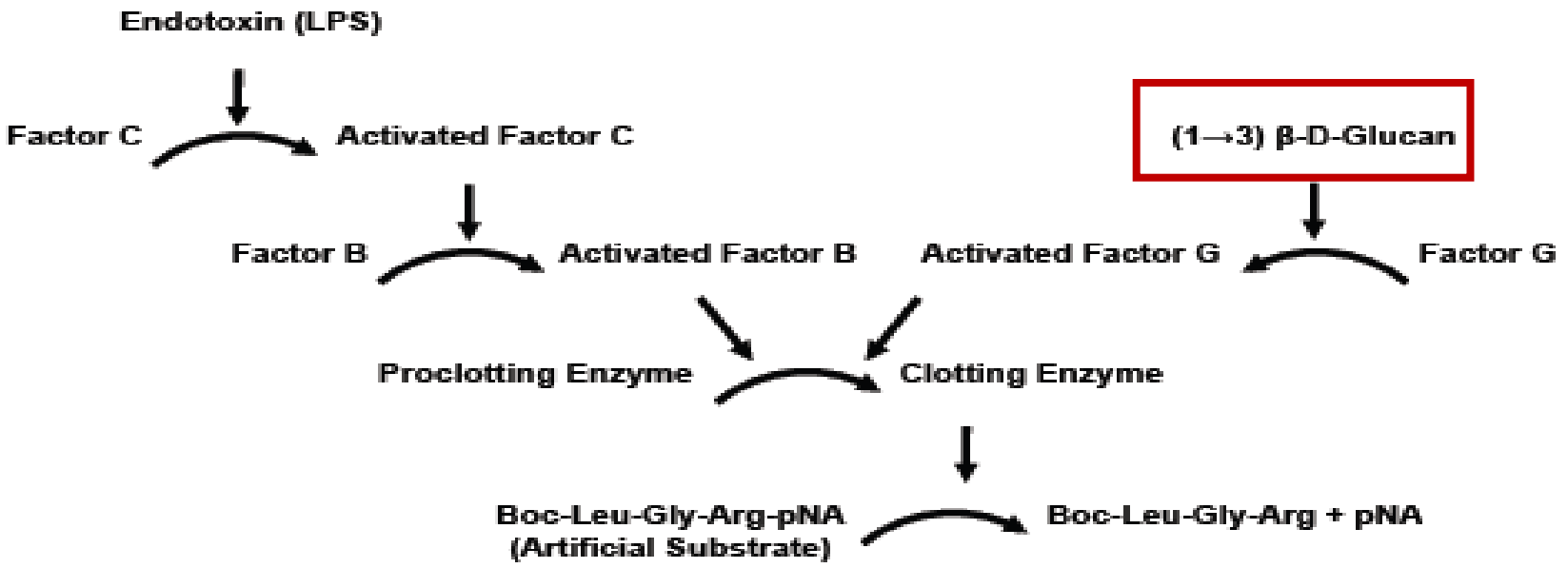


Table 1. Comparison of characteristics of different beta-D-glucan test assays.

Test Assay	Availability	Producer	Method	Cut-Off Value	Overall Sensitivity	Overall Specificity	Comments
Fungitell	Europe, US	Associates of Cape Cod, Falmouth, MA, USA	Colorimetric	Intermediate 60–79 pg/mL Positive > 80 pg/mL	27–100%	0–100%	BDG detection by Fungitell is part of EORTC/MSG criteria (positive serum BDG in combination with host factor and clinical criterion) for probable invasive candidiasis or PJP.
Fungitell STAT	Europe, US	Associates of Cape Cod, Falmouth, MA, USA		Indeterminate 0.75–1.1 Positive ≥ 1.2	ND	ND	New rapid test that can be run on one or more patient specimens (single sample testing), but initial clinical validation reported 98–99% concordance with indeterminate results excluded and 74–91% if included.

Test Assay	Availability	Producer	Method	Cut-Off Value	Overall Sensitivity	Overall Specificity	Comments
Glucatell	Europe, US	Associates of Cape Cod, Falmouth, MA, USA	Colorimetric	80 pg/mL	50–92%	41–94%	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.
Wako Wako-EU	Asia, Europe	Wako Pure Chemical Industries, Osaka, Japan	Turbidimetric method	11 pg/mL	50–86%	89–100%	

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Test Assay	Availability	Producer	Method	Cut-Off Value	Overall Sensitivity	Overall Specificity	Comments
Fungitec G test ES Fungitec G test MKII	Europe, US	Seikagaku Kogyo Corporation, Tokyo, Japan Subsidiary Associates of Cape Cod, Falmouth, MA, USA	Colorimetric method	20 pg/mL	67–88%	60–85%	
Dynamiker Fungus	Some European countries and North Africa	Dynamiker Biotechnology Ltd., Tianjin, China	Turbidimetric method	95 pg/mL	64–81%	78–80%	

ND, no data.

Recent Data on Optimized Thresholds

❑A recent prospective comparison of Fungitell and Wako resulted in:

- Proposal of optimized thresholds (higher and lower than the manufacturer's recommendations, respectively)
- 120 pg/mL (Fungitell) and 4 pg/mL (Wako).

- Sensitivity and specificity were 82% and 95% for both Fungitell and Wako.
- The study was performed in 171 patients, mainly with HM (62%), who had experienced 175 episodes of suspected IFD, with the final diagnosis of 23 infections due to BDG-producing fungi (including 12 cases of PJP).

Med. Mycol. 2021, 59, 882–889

Recent Data on Optimized Thresholds

- In a recent retrospective analysis of serum samples from pts with and without PJP based on clinical and radiological characteristics and PCR in BAL, the performance of Fungitell and Wako assays was compared.
- At the manufacturer's recommended cut-offs (80 pg/mL and 11 pg/mL, respectively), the Wako assay was found significantly more specific (0.98 vs. 0.87) and the Fungitell assay more sensitive (0.78 vs. 0.85), with similar overall performance.
- At a cut-off of 3.616 pg/mL, the Wako assay had similar sensitivity to the Fungitell assay (0.88 at a cut-off of ≥60 pg/mL), but its specificity was significantly higher (0.89 vs. 0.82).

J. Clin. Microbiol. 2019, 57, e00322-19

Recent Data on Optimized Thresholds -Conclusions

- A higher cut-off for the Fungitell and lower for the Wako assay might result in improved overall performance in patients with HM.
- However, reducing the sensitivity of Fungitell, which is the advantage of this assay, might be counterproductive in the haematology setting, especially in the diagnosis of IPA or pre-emptive antifungal therapy for febrile neutropenia, when sensitivity is crucial.
- In these cases, lower specificity of standard cut-offs might be preferable, and repeated sampling might rule out false positive results.

False Positive Results of BDG Assay

FALSE POSITIVES	Mechanism	Comments
Iatrogenic contamination		
Haemodialysis	Use of regenerated cellulose dialysis membrane	Modern dialysis membranes (non-BDG-leaching synthetic membranes) no longer release BDG, and BDG was highly specific for the diagnosis of IFD in the serum of patients receiving haemodialysis in a recent study [27].
Blood and blood derivates such as immunoglobulins and albumin	Cellulosic depth filters are generally mixtures of cellulose and diatomaceous earth and are used to provide initial clarification of blood plasma. Process solutions may also contain BDG and introduce contamination.	The risk of false positivity after receiving blood or blood components seems dependent on the product's concentrations of BDG and is not constant (for example, never observed in our hospital), while immunoglobulin preparations almost invariably contain BDG [17]. These high titres usually decline rather quickly, and such responses support suspicion of iatrogenic contamination. The depth filters flush strategy was developed to control beta-glucan leaching into the product pool [23].
Cellulose containing gauzes/surgical sponges		The release of BDG from surgery gauzes is temporary and depends on the type of gauze used [25].
Non-glucan-free laboratory equipment		Currently unlikely, since glucan-free laboratory equipment is available.
Beta-lactam antibiotics (e.g., ampicillin-sulbactam, amoxicillin-clavulanate)	BDG may be present in the original source material itself, such as products made by fungal fermentation, in excipients added to the formulation, from media used in microbial or cell culture, or from process equipment, materials, and solutions.	Possible; however, the high level of dilution generated upon injection of relatively low volumes of antibiotic make this unlikely. Further, the high negative predictive value for IFD observed for patients receiving a vast array of antibiotics suggests that this is not a significant problem [20].
Bacteriemia	Translocation as a consequence of ischemic damage to the intestinal barrier due to septic shock	Some experiences suggest that bacteraemia is a very rare source of false positivity [21].

False Positive Results of BDG Assay

FALSE POSITIVES	Mechanism	Comments
Intestinal translocation		
Severe mucositis	Possible translocation of fungal antigens through the intestinal mucosa damaged by chemotherapy	Whether or not this might truly affect specificity of BDG in adult hematologic patients remains controversial, but should be considered in patients with intestinal GvHD or severe mucositis [2].
Major abdominal surgery	Translocation as a consequence of loss of integrity of the intestinal wall	Rare in haematology.
Gut ischemia	Translocation as a consequence of is chemic damage	
Burns	Large surface area burns	Validation of alternative cut-offs in specific clinical contexts known to contribute to elevated BDG titre may provide the solution to specificity issues [24]. Unknown if applicable also to severe skin acute GvHD.
Chronic kidney disease	Uremia's metabolic toxicity	
Enterococcus spp. bacteremia	Protease-producing intestinal enterococci	
Hepatic function		
End-stage liver disease	Reduced clearance	
Bacterial infections		
Nocardia spp. infection		Although rare, needs to be considered in differential diagnosis in case of compatible clinical presentation (pulmonary, cerebral) [15].
Streptococcus pneumoniae Type 37	Producing a BDG with a (1→3)-β-backbone [15]	
Pseudomonas spp.	Producing (1→2)-β-linked glucan sequences [15]	
Interference		
Pegylated asparaginase	Drug-related alterations in heme metabolism, which in turn interfere with measurement of BDG in serum [2]	
Haemolysis	Interference with test procedure.	Possible interference, particularly for colorimetric assays [22].

False Negative Results of BDG Assay

FALSE NEGATIVES		
Antifungal prophylaxis and therapy	Low pre-test probability of IFI	Lower median BDG values were reported in breakthrough IFI episodes [16]. BDG should be used to exclude rather than for diagnosis in these patients [18].
Sanctuary sites or poorly vascularized sites of infection	BDG not released into blood	
Candida parapsilosis or *Candida auris*	Lower content of BDG component in fungal wall	Lower levels of BDG reported [19,26].
Hyperbilirubinemia	Interference with test procedure	Possible interference, particularly for colorimetric assays [22].

Screening and diagnostic testing

In patients with haematological malignancies

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BDG in haematology patients

- In the haematology setting, NPV is insufficient to exclude diagnosis of IFD.
- Sensitivity for IFD seems lower than in other populations.

➢ because of the type of IFD (lower sensitivity in case of aspergillosis compared to candidiasis and pneumocystosis) or the use of prophylaxis.

- Specificity of the test can be improved:

➢ by using two consecutive positive assays

➢ and avoiding testing in the case of the concomitant presence of factors associated with false positive results.

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Meta-analysis performed for the European Conference on Infections in Leukemia (ECIL) and published in 2012

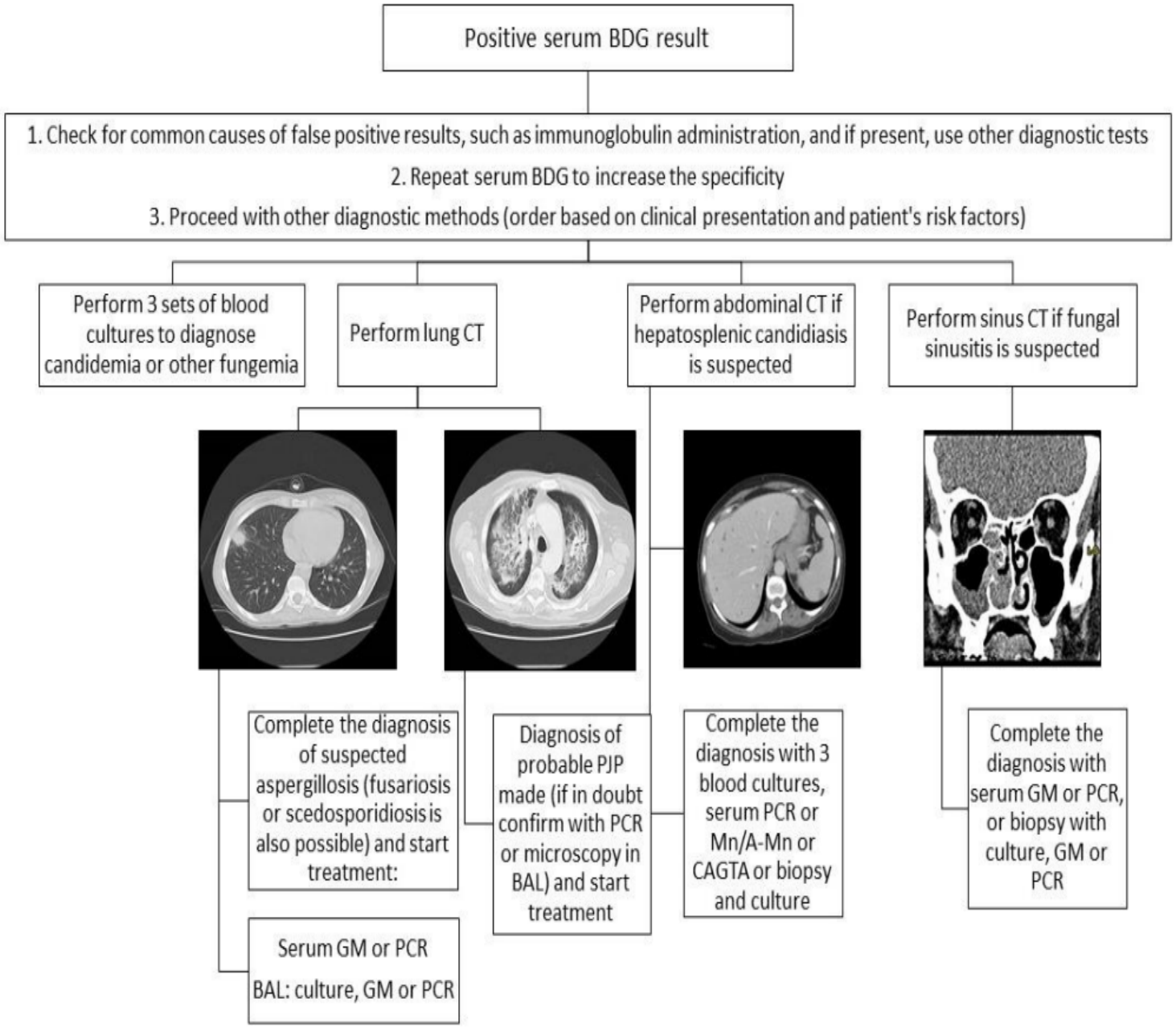
For the diagnosis of proven or probable IFD,

Positive test sensitivity and specificity were 70% and 91%, respectively,

while for two consecutive positive tests, they were 50% and 99% respectively.

Earlier diagnosis if more than one marker is used

With combination of fungal biomarkers


Positive serum BDG result
1. Check for common causes of false positive results, such as immunoglobulin administration, and if present, use other diagnostic tests
2. Repeat serum BDG to increase the specificity
3. Proceed with other diagnostic methods (order based on clinical presentation and patient's risk factors)
Perform 3 sets of blood cultures to diagnose candidemia or other fungemia
Perform lung CT
Perform abdominal CT if hepatosplenic candidiasis is suspected
Perform sinus CT if fungal sinusitis is suspected
Complete the diagnosis of suspected aspergillosis (fusariosis or scedosporidiosis is also possible) and start treatment:
Serum GM or PCR
BAL: culture, GM or PCR
Diagnosis of probable PJP made (if in doubt confirm with PCR or microscopy in BAL) and start treatment
Complete the diagnosis with 3 blood cultures, serum PCR or Mn/A-Mn or CAGTA or biopsy and culture
Complete the diagnosis with serum GM or PCR, or biopsy with culture, GM or PCR

BDG in Samples Other Than Serum

- Performance in cerebrospinal fluid (CSF) is particularly interesting.
- A systematic review of BDG in CSF included 14 studies and various fungi such as *Candida*, *Aspergillus*, *Exserohilum*, and *Histoplasma*.
- For *Histoplasma* meningitis, BDG was found to have 53% sensitivity and 87% specificity, while for *Exserohilum rostratum* it was 100% and 98%, respectively, for the cut-off of 138 pg/mL.
- Although most cryptococci are thought to not contain BDG, in an HIV-positive cohort with cryptococcal meningitis, CSF BDG sensitivity was 89% and specificity 85%.
- On the contrary, the detection of BDG in BAL has been repeatedly found to have very poor specificity and is therefore of little use.
- Its potential to be used to rule out aspergillosis, which has been proposed for the ICU setting, should be confirmed in other studies.

Open Forum Infect. Dis. 2014, 1, ofu105
J. Crit. Care 2018, 47, 198–203

BDG in Children with Haematological Malignancies Undergoing Antineoplastic Chemotherapy or Stem Cell Transplantation

- Poor performance of BDG test in paediatrics, unless using a cut-off much higher than the 80 pg/mL, indicated as a threshold for a positive test.
- Discouraged for prospective monitoring or diagnostic use in paediatric patients at high risk of invasive fungal disease in the most recent guideline on management of IFD in children receiving chemotherapy or SCT (grade D recommendation, level of evidence II).
- Recently, BDG has been proposed in combination with GM and *Aspergillus* PCR for the diagnosis of invasive aspergillosis in paediatric patients.
- The performance of the test was improved using a higher cut-off (>300 pg/mL) and using the test only after the acute post-transplant phase, where a high proportion of false positive results were observed.

Lancet Oncol. 2021, 22, e254–e269.
J. Fungi 2021, 7, 238.

BDG for Monitoring of Response in Invasive Fungal Diseases

- Limited data on the kinetics of BDG decline in the case of IFDs.
- Might be correlated with the clinical outcome.

Serum BDG in the Diagnostic Criteria of Invasive Fungal Diseases

In the guidelines of the Third European Conference on Infections in Leukemia (ECIL-3),

The use of BDG was given a **B-II** grading of recommendation for the diagnosis of IFD (moderate evidence for use).

Clin. Infect. Dis 2012, 54, 633–643.

Serum BDG in the Diagnostic Criteria of Invasive Fungal Diseases

ESCMID guidelines recommend, with grade **C II**, the use of serum BDG

to diagnose IFD and to diagnose and screen for IA in adults with HM and after stem cell transplant

Clin. Microbiol. Infect. 2018, 24 (Suppl. S1), e1–e38.

Serum BDG in the Diagnostic Criteria of Invasive Fungal Diseases

- BDG was included in the 1st revision in **2008** of EORTC/MSG criteria for the diagnosis of IFD in the immunocompromised as a mycological criterion for the diagnosis of any probable invasive fungal disease other than cryptococcosis and mucormycosis.
- In the 2nd revision of the EORTC/MSG diagnostic criteria in **2019**, serum BDG (two consecutive samples above 80 pg/mL for Fungitell assay) is considered as a mycological criterion only of probable invasive candidiasis or probable pneumocystosis, but not among the criteria for the diagnosis of invasive aspergillosis or other invasive mould infections.
- This is the first time that these criteria include also pneumocystis, and in case of this IFD, both positive serum BDG and molecular detection of Pneumocystis-DNA in BAL are considered as criteria of probable infection, while microscopic detection is required for the diagnosis of proven PJP.

Clin. Infect. Dis. 2020, 71, 1367–1376.
Clin. Infect. Dis. 2021, 72, S114–S120.

Strengths and Limitations of BDG Assay

Strengths

- Almost panfungal assay
- Rapid turnaround time (approx. 1 h)
- Several assays available, including a single sample format
- Used both for screening and targeted testing
- In haematological patients, high specificity of two consecutive positive tests
- More sensitive than blood cultures for deep-seated candidiasis
- High sensitivity for PJP
- The only non-invasive test to support the diagnosis of PJP, especially in situations where critical illness precludes invasive diagnostic procedures such as BAL
- Possibility of use in other sterile fluids such as cerebrospinal fluid for fungal central nervous system infections

Limitations

- Not applicable to Mucorales, *Blastomyces*, and most cryptococci
- Batch testing required with most assays
- Not specific for any fungus and thus needs to be used in combination with other diagnostic methods for identification of species (GM, PCR, radiology, etc.)
- Cut-off provided by manufacturers might need optimizing for better performance
- The need to use glucan-free laboratory materials
- Possibility of false positive results
- Lower sensitivity in patients with haematologic malignancies and IFD compared to other patient groups
- Of limited use in paediatric population
- In case of invasive candidiasis, lower sensitivity in case of certain species, such as *C. parapsilosis* or *C. auris*
- Not applicable for use in BAL due to high rate of false positive results
- Unpredictable rate of decline, unsuitable for rapid evaluation of treatment response