Use and Limits of (1-3)- β -D-Glucan Assay (Fungitell), Compared to Galactomannan Determination (Platelia *Aspergillus*), for Diagnosis of Invasive Aspergillosis

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This study was undertaken to examine the performance of the Fungitell β -glucan (BG) assay, to compare it with that of the galactomannan (GM) test for the diagnosis of invasive aspergillosis (IA) in patients with hematological malignancies, and to examine the rates of false-positive BG and GM test results due to β -lactam antibiotics among sera of patients with Gram-positive or Gram-negative bacteremia and selected sera with false-positive results from the GM test. Serum samples from 105 patients with proven ($n = 14$) or probable ($n = 91$) IA, 97 hematology patients at risk for invasive fungal infections, 50 healthy blood donors, and 60 patients with bacteremia were used to study the sensitivities and specificities of the assays. The GM test was more specific than the BG assay (97% versus 82%, respectively; $P = 0.0001$) and the BG assay was more sensitive than the GM test (81% versus 49%, respectively; $P < 0.0001$) for IA diagnosis. The study of 49 separate batches of β -lactam antibiotics showed high and very similar rates of false-positive results for the GM and BG assays (29 and 33%, respectively; $P = 0.82$) but with an almost complete lack of concordance between the 2 assays. For patients with bacteremia, the rate of false-positive results was much higher with the BG test than with the GM test (37% versus 2%, respectively; $P < 0.0001$), with no significant difference between Gram-positive and Gram-negative bacteremia. In conclusion, the BG test may be useful for the diagnosis of IA because of its high sensitivity in comparison with the GM test, but the overall benefit of this assay remains limited because of its inadequate specificity and its cost.

Invasive aspergillosis (IA) remains a significant cause of morbidity and mortality in immunocompromised patients. Despite the availability of new antifungal drugs, the mortality rate for IA remains high, partly due to the difficulty and delay of diagnosis based on clinical, radiological, and mycological methods (1). Because culture-based diagnostic methods have limited performance, non-culture-based methods are increasingly being used. Detection of circulating galactomannan (GM) with a sandwich enzyme-linked immunosorbent assay (ELISA) (Platelia *Aspergillus*; Bio-Rad, Marnes-la-Coquette, France) has been widely used (2, 3). Although the Platelia *Aspergillus* assay has become an important tool for the diagnosis of IA, false-positive (4–6) and false-negative (3, 7) results with this test have been reported. The occurrence of false-positive and false-negative results with the Platelia *Aspergillus* assay is a major drawback of this technique, as such results may lead to unjustified invasive or costly investigations or antifungal therapy in cases of false-positive results and underdiagnosis or delayed diagnosis in cases of false-negative results.

Another serum marker for the presence of invasive fungal infections (IFIs) is (1-3)- β -D-glucan (BG), which has been included in the relevant European Organization for Research and Treatment of Cancer (EORTC)/Mycoses Study Group (MSG) diagnostic criteria (8). BG is a component of the cell walls of most fungi. The main exceptions are *Mucorales* and cryptococci, which release no or little BG to be detected in human serum (9). The measurement of BG levels is based on the *Limulus* test (10). BG activates factor G, a serine protease zymogen of *Limulus* amoebocyte lysates,

which are extracted from amoebocytes of horseshoe crab species. This in turn activates a coagulation cascade. The activity of this reaction can be measured with the use of colorimetric or turbidimetric methods. The Fungitell assay (Associates of Cape Cod, Inc.) is a chromogenic kinetic test that was approved by the Food and Drug Administration in 2003 and carries the European CE label for the presumptive diagnosis of IFIs. However, the major limitations of BG testing for routine use are false-positive reactions (11–16) with interfering substances (17).

The main purpose of this study was to assess the utility of the Fungitell BG assay for the diagnosis of IA. This involved (i) a study of the performance of the BG test, (ii) a comparison of the performance of the BG and GM tests, and (iii) a study of the rates of false-positive BG and GM test results due to antibiotics and in sera of patients with Gram-positive and Gram-negative bacterial infections.

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TABLE 1 Underlying hematological malignancies of patients with proven or probable invasive aspergillosis^a

Underlying disease ^b	No. of patients (no. with allogeneic SCT)	
	All patients (n = 105)	Subset for GM test/BG test comparison (n = 69)
AML/MDS	34 (7)	21 (5)
CLD	30 (12)	20 (8)
ALL	21 (11)	13 (8)
CML	3 (3)	3 (3)
AA	11 (8)	6 (5)
Others	6 (2)	6 (2)

^a Data from all 105 patients were used to analyze β -glucan (BG) test performance; a subset of 69 patients whose laboratory diagnosis of IA was based on direct mycological tests only was used for comparisons of the performance of BG and galactomannan (GM) tests. For each underlying disease, the numbers of patients who had received an allogeneic stem cell transplant (SCT) are specified in parentheses.

^b AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; CLD, chronic lymphoproliferative disorder; ALL, acute lymphoid leukemia; CML, chronic myeloid leukemia; AA, aplastic anemia.

MATERIALS AND METHODS

Patients. Sera from 312 patients and control subjects were studied and allocated into four separate groups. The first group comprised 105 patients with hematological malignancies with proven ($n = 14$) or probable ($n = 91$) IA, according to EORTC/MSG diagnostic criteria with the exclusion of BG testing (8); this group was used to analyze the performance of the BG test. Within this group, a subset of 69 patients (the second group) whose laboratory diagnosis of IA was based on direct tests only (positive cytological, direct microscopic, and/or culture results) was selected; this subset was used to compare the BG and GM tests. The underlying diseases of the patients in these two first groups and whether they had received an allogeneic stem cell transplant are reported in Table 1.

A control group (the third group) comprised 97 consecutive patients from hematology wards who were at risk for an IFI but without any symptoms of an IFI, were routinely screened for the presence of GM, and did not develop any sign of an IFI during the follow-up period and 50 healthy blood donors from a local transfusion center. The fourth group comprised 60 patients with positive blood culture results for either Gram-positive or Gram-negative bacteria.

For each patient, one blood sample was studied, either selected randomly from among those collected on a routine basis (for hematology patients without an IFI) or collected close to the time of diagnosis (for patients with IA or bacteremia). In addition, 48 sera that yielded unexplained false-positive GM test results, which had been selected over 5 years of routine practice, were analyzed for BG.

Antibiotics. A total of 49 separate batches of β -lactam antibiotics were tested, including 33 batches of piperacillin-tazobactam (Wyeth, Mylan, France) (1 g/vial), 9 of amoxicillin (GlaxoSmithKline, France) (1 g/vial), and 7 of amoxicillin-clavulanic acid (GlaxoSmithKline, France) (1 g/vial). The vials were reconstituted at the concentrations used for intravenous administration, according to the manufacturers' instructions, and were tested. For each batch, an aliquot was processed by the same procedure as that applied to serum. Batches that yielded GM indices of >0.5 or BG levels of >80 pg/ml when undiluted were retested, to estimate the true level of GM or BG and to avoid a prozone effect in the GM test, as observed previously with antibiotics (5). Dilutions were prepared in sterile 0.9% NaCl (for GM) or pyrogen-free water (for BG) and then were tested for GM and BG.

Antigen detection techniques. Blood samples were collected in sterile BG-free vacuum tubes. Serum was separated, divided into aliquots, and stored in BG-free tubes at 2°C to 8°C for up to 48 h or frozen at -20°C until testing.

GM antigen was detected using a sandwich immunocapture ELISA

(Platelia *Aspergillus*; Bio-Rad, Marnes-la-Coquette, France), according to the manufacturer's recommendations. Positive and negative controls were included in each assay. A result was considered positive with index values of ≥ 0.5 in duplicate tests.

BG was detected with the Fungitell test kit, essentially as recommended by the manufacturer (Associates of Cape Cod). Briefly, serum samples were treated for 10 min at 37°C with a solution containing 0.6 M KCl and 0.125 M KOH. A kinetic colorimetric assay was performed at 37°C and monitored at 405 nm for 25 min. The concentration of BG in each sample was automatically calculated using a calibration curve prepared with standard solutions ranging from 6.23 to 100 pg/ml. BG levels of ≥ 80 pg/ml were considered positive results. Serum assays were performed in duplicate. BG tests were performed without knowledge of the results of GM determinations.

Statistical analysis. The diagnostic performance of the GM and BG tests was evaluated as sensitivity and specificity, with 95% confidence intervals (CIs). Additionally, positive and negative likelihood ratios (LRs) were computed for the GM and BG tests separately and for the combination of the two. Briefly, the positive LR is the true-positive rate divided by the false-positive rate, and the negative LR is the false-negative rate divided by the true-negative rate. The LR summarizes how much more likely patients with IA are to have that particular result (positive or negative for a binary result, or a given result for a multiple-value test) than are patients without IA (controls) (18). It is generally considered that LRs of >10 or <0.1 have large effects on diagnosis and LRs of 5 to 10 or 0.1 to 0.2 have moderate effects. The impact on diagnosis is considered small for LRs of 2 to 5 or 0.2 to 0.5 and negligible for LRs of 0.5 to 2, with a LR of 1 indicating no diagnostic accuracy at all (19). Test results in the same group of patients were compared using McNemar's test for paired proportions. Between-group comparisons were performed with Fisher's exact tests or Wilcoxon rank-sum tests. Agreement between tests was assessed using the kappa statistic, and the overall diagnostic performance was summarized by Youden's index, defined as sensitivity + specificity $- 1$.

RESULTS

In a first analysis, data obtained for 105 subjects with IA and 147 controls (50 donors at a local transfusion center and 97 subjects from hematology wards) were analyzed for determination of BG test performance. The sensitivity and specificity were 78% and 82%, respectively. Likelihood ratios of 4.25 for a positive result and 0.27 for a negative result suggested weak effects on diagnosis for both positive and negative results.

In a second analysis, the performance characteristics of BG and GM tests were compared using the subset of 69 patients with IA whose laboratory diagnoses were established independently of the results of GM or BG tests (if performed). For BG testing, restricting the analysis to those patients yielded results close to those of the first analysis, i.e., sensitivity of 81%, positive LR of 4.42, and negative LR of 0.23; the specificity was unchanged since the definition of control was unchanged.

The distributions of GM index values and BG levels according to the type of IA diagnosis are presented in Fig. 1, and the diagnostic performance characteristics of the two tests are presented in Table 2. The GM assay was found to be more specific than the BG assay (97% versus 82%, respectively; $P = 0.0001$) and the BG assay more sensitive than the GM assay (81% versus 49%, respectively; $P = 0.0003$) for IA diagnosis. Overall, based on Youden's index ($P = 0.020$), the BG assay was found to perform better than the GM assay.

Analysis of likelihood ratios (LRs) showed a better diagnostic value for a positive GM test result (positive LR, >10 [lower 95% confidence limit, >5]) than for a positive BG test result. Symmetrically, a negative BG assay result had a better diagnostic value

FIG 1 Distribution of galactomannan (GM) index values and (1-3)- β -D-glucan (BG) levels according to the IA diagnosis. The box-and-whisker plots display the medians (thick solid lines) and 25th and 75th percentiles (boxes) of the distribution and the most extreme data points that are ≤ 1.5 times the interquartile range from the box (dashed whiskers). Horizontal dashed lines, positivity thresholds.

than a negative GM assay result, although with only a small diagnostic impact in absolute terms (negative LR, 0.23 [95% confidence interval, 0.1 to 0.4]). Combining GM and BG assays resulted in a very high diagnostic value for two positive results (positive LR, >60 [lower 95% confidence limit, 9.5]), with only 1 control specimen of 147 being falsely positive by both assays. Conversely, negative results from both assays added only limited information to rule out IA (positive LR, 0.20 [95% confidence interval, 0.12 to 0.35]). Sixteen percent of patients with probable or proven IA had negative results for both assays. Finally, discordant GM and BG test results were mainly uninformative for IA diagnosis (positive and negative LRs between 0.79 and 1.97).

The analysis of GM and BG test results for the sera of patients with bacteremia is presented in Table 3. Positive results were observed in 22/60 cases with the BG assay, compared with 1/60 cases with the GM assay ($P < 0.0001$). No significant difference was found in the positive BG test result rates for the Gram-negative and Gram-positive groups ($P = 0.55$) or between BG levels in these two groups ($P = 0.23$). The 7 Gram-negative bacteria implicated in false-positive BG test results were *Escherichia coli* ($n = 2$), *Enterobacter faecalis* ($n = 1$), *Acinetobacter baumannii* ($n = 1$), *Klebsiella pneumoniae* ($n = 1$), *Providencia alcalifaciens* ($n = 1$), and *Pseudomonas aeruginosa* ($n = 1$). The 15 Gram-positive bacteria found in cases of false-positive BG test results were *Staphylococcus aureus* ($n = 12$), *Peptostreptococcus magnus* ($n = 1$), and a *Micrococcus* sp. ($n = 1$).

The analysis of GM and BG test results for antibiotic samples is presented in Table 4. Similar rates of false-positive results were observed for the two assays (GM test, 29%; BG test, 33%; $P = 0.82$) but there was no agreement between the two assays ($\kappa = 0.04$), with only 5 samples being positive by both tests and 20 being positive by one or the other. Testing of the panel of 48

sera with false-positive results from the GM test showed that 33 sera were BG test negative (68.5%) and 15 were BG test positive.

DISCUSSION

The diagnostic performance of the (1-3)- β -D-glucan assay for invasive fungal infections has been evaluated in several meta-analyses (20–22), and this assay is considered a useful diagnostic tool if it is used for diagnosis among immunocompromised patients, with knowledge of the limitations of the assay (17). For the diagnosis of invasive aspergillosis, BG and GM assays seem to be in the same performance range, considering the wide dispersion of the reported sensitivity and specificity values in studies. The reported sensitivity values for the GM assay have been variable, with a range of 30 to 100%, and specificity values have ranged from 38% to 98% (23, 24). Similarly, variable results have been reported for the BG assay, with sensitivity values ranging from 80% to 90% and specificity values ranging from 36% to 92%, according to the cut-off value used (25, 26). However, few studies have performed direct comparisons of the 2 assays for the diagnosis of IA in the same patients. One study favored the BG assay (27), one study favored the GM assay (28), one found no major difference between the two assays (29), and two showed moderate agreement between them (30, 31). Therefore, we undertook this study to examine the performance of the BG assay for the diagnosis of invasive aspergillosis in patients with hematological malignancies.

Encouraging results were observed for the diagnosis of IA, since the BG test was found to be more sensitive than the GM test for patients with proven or probable IA, but this higher sensitivity was associated with lower specificity versus the GM test. To estimate the benefits of BG and/or GM tests for routine practice, we paid particular attention to characterizing the risk of false-positive

TABLE 2 Performance of galactomannan and (1-3)- β -D-glucan tests for diagnosis of invasive aspergillosis

Variable	No. (%)		LR (95% CI)
	IA ^a (<i>n</i> = 69)	Control (<i>n</i> = 147)	
Galactomannan test ^b			
Negative	35 (51)	142 (97)	0.53 (0.42–0.66)
Positive	34 (49)	5 (3)	14.5 (5.92–35.4)
(1-3)-β-D-Glucan test ^c			
Negative	13 (19)	120 (82)	0.23 (0.14–0.38)
Positive	56 (81)	27 (18)	4.42 (3.09–6.33)
GM test/BG test			
Negative/negative	11 (16)	116 (79)	
Positive			0.20 (0.12–0.35)
Negative			3.99 (2.87–5.54)
Negative/positive	24 (35)	26 (18)	
Positive			1.97 (1.22–3.16)
Negative			0.79 (0.66–0.96)
Positive/negative	2 (3)	4 (3)	
Positive			1.07 (0.20–5.68)
Negative			1.00 (0.95–1.05)
Positive/positive	32 (46)	1 (1)	
Positive			68.2 (9.51–488.7)
Negative			0.54 (0.43–0.67)

^a Proven and probable diagnoses according to EORTC/MSG criteria.

^b Specificity, 97% (95% CI, 92 to 99%); sensitivity, 49% (95% CI, 37 to 62%).

^c Specificity, 82% (95% CI, 74 to 88%); sensitivity, 81% (95% CI, 70 to 90%).

TABLE 3 Galactomannan and (1-3)-β-D-glucan test results for serum samples from patients with bacteremia

Variable	All (<i>n</i> = 60)	Gram-negative (<i>n</i> = 16)	Gram-positive (<i>n</i> = 44)	<i>P</i>
Galactomannan test				>0.99
No. (%) negative	59 (98)	16 (100)	43 (98)	
No. (%) positive	1 (2)	0 (0)	1 (2)	
Median GM index value (range)	0.05 (0.00–0.68)	0.06 (0.00–0.21)	0.06 (0.05–0.68)	0.72
(1-3)-β-D-Glucan test				0.55
No. (%) negative	38 (63)	9 (56)	29 (66)	
No. (%) positive	22 (37)	7 (44)	15 (34)	
Median BG level (pg/ml [range])	54 (7–523)	66 (7–523)	45 (7–319)	0.23
GM test/BG test				0.66
No. (%) negative/negative	38 (63)	9 (56)	29 (66)	
No. (%) negative/positive	21 (35)	7 (44)	14 (32)	
No. (%) positive/negative	1 (2)	0 (0)	1 (2)	

results. Several factors that may increase BG levels for reasons other than IFIs have been identified (9), including hemodialysis with cellulose membranes (13), thrombocyte infusion with leukocyte-removing filters (16, 32), the administration of human blood products (immunoglobulins or albumins) (33, 34), the use of antibiotics such as amoxicillin-clavulanate or piperacillin-tazobactam (14, 27), the presence of serious bacterial infections (35), the use of surgical gauzes containing glucan (36), and severe mucositis (37). Among these, we focused on β-lactams, which also cause false-positive GM test results, and bacteremia, as a possible confounding cause of fever in hematology patients. Regarding the interacting effects of β-lactams, our results showed high and similar rates of false-positive results with the 2 assays but with an almost complete lack of concordance between the two tests, meaning that the tests were sensitive to different specific interacting factors. It was suggested previously that the release of galactomannan during the manufacturing process for some antibiotics might be responsible for positive GM assay results (38). The same explanation can be proposed for the BG assay, since (1-3)-β-D-glucan is present in some supports, such as cellulose filters, that can be used during manufacturing (16). For patients with bacteremia, our results showed that the rate of false-positive results was

much higher for the BG assay than for the GM assay ($P < 0.0001$), i.e., 30% false-positive BG test results in bacteremic patients, with no significant difference between Gram-positive and Gram-negative bacteremia. The interacting effects of bacteremia on the performance of BG assays are still debated. False-positive BG test results during bacteremia were not found in a recent prospective study (39) but were reported, at a rate similar to that in our study, for patients with positive blood culture results for Gram-positive bacteria (35). We have no explanations for these discrepant results.

Based on the individual performance of each test and their discordances, the use of the two tests to complement each other can be proposed. As in another study (29), we found that combining the two tests had some benefit in terms of specificity, since the association of positive BG assay results and positive GM assay results was almost 100% specific, but with lower sensitivity, in comparison with the sensitivity of each assay alone. The study of the selected panel of sera that yielded false-positive GM test results showed that almost two-thirds of them were invalidated by negative BG test results, as observed previously by Persat et al. for false-positive GM test results due to cross-reactivity with β-lactam antibiotics (30). Therefore, combining the two assays can be proposed when an IA diagnosis is difficult to establish, provided that the results of the two tests are positive.

In conclusion, we think that the BG test alone has major limitations for the diagnosis of IA, due to the high rate of false-positive results, the origins of which are not completely known. Its use in combination with the GM test may strengthen IA diagnosis, but the additional costs due to the BG test are high and the overall benefit of such a combination remains limited.

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TABLE 4 Galactomannan and (1-3)-β-D-glucan test results for antibiotic samples (*n* = 49)

Variable	Sample value
Galactomannan test	
No. (%) negative	35 (71)
No. (%) positive	14 (29)
Median GM index value (range)	0.09 (0.00 to >10)
(1-3)-β-D-Glucan test	
No. (%) negative	33 (67)
No. (%) positive	16 (33)
Median BG level (pg/ml [range])	59 (11–523)
GM test/BG test	
No. (%) negative/negative	24 (49)
No. (%) negative/positive	11 (22)
No. (%) positive/negative	9 (18)
No. (%) positive/positive	5 (10)

Pfizer and MSD, and has served on speakers' bureaus for Gilead, MSD, Pfizer, and Schering-Plough. No other conflicts are declared.

REFERENCES

- Nucci M, Nouér SA, Cappone D, Anaissie E. 2013. Early diagnosis of invasive pulmonary aspergillosis in hematologic patients: an opportunity to improve the outcome. *Haematologica* 98:1657–1660. <http://dx.doi.org/10.3324/haematol.2013.094359>.
- Maertens J, Verhagen J, Demuyneck H, Brock P, Verhoef G, Vandenbergh P, Van Eldere J, Verbist L, Boogaerts M. 1999. Autopsy-controlled prospective evaluation of serial screening for circulating galactomannan by a sandwich enzyme-linked immunosorbent assay for haematological patients at risk for invasive aspergillosis. *J. Clin. Microbiol.* 37:3223–3228.
- Sulahian A, Boutboul F, Ribaud P, Leblanc T, Lacroix C, Derouin F. 2001. Value of antigen detection using an enzyme immunoassay in the diagnosis and prediction of invasive aspergillosis in two adult and pediatric hematology units during a 4-year prospective study. *Cancer* 91:311–318. [http://dx.doi.org/10.1002/1097-0142\(20010115\)91:2<311::AID-CNCR1003>3.0.CO;2-3](http://dx.doi.org/10.1002/1097-0142(20010115)91:2<311::AID-CNCR1003>3.0.CO;2-3).
- Sulahian A, Touratier S, Ribaud P. 2003. False-positive test for *Aspergillus* antigenemia related to concomitant administration of piperacillin and tazobactam. *N. Engl. J. Med.* 349:2366–2367. <http://dx.doi.org/10.1056/NEJM200312113492424>.
- Aubry A, Porcher R, Bottero J, Touratier S, Leblanc T, Brethon B, Rousselot P, Raffoux E, Menotti J, Derouin F, Sulahian A. 2006. Occurrence and kinetics of false-positive *Aspergillus* galactomannan test results following treatment with β -lactam antibiotics in patients with haematological disorders. *J. Clin. Microbiol.* 44:389–394. <http://dx.doi.org/10.1128/JCM.44.2.389-394.2006>.
- Guigues N, Menotti J, Ribaud P. 2013. False positive galactomannan test after ice-pop ingestion. *N. Engl. J. Med.* 369:97–98. <http://dx.doi.org/10.1056/NEJMc1210430>.
- Herbrecht R, Letscher-Bru V, Oprea C, Liou B, Waller J, Campos F, Vilard O, Liu KL, Natarajan-Amé S, Lutz P, Dufour P, Bergerat JP, Candolfi E. 2002. *Aspergillus* galactomannan detection in the diagnosis of invasive aspergillosis in cancer patients. *J. Clin. Oncol.* 20:1898–1906. <http://dx.doi.org/10.1200/JCO.2002.07.004>.
- De Pauw B, Walsh TJ, Donnelly JP, Stevens DA, Edwards JE, Calandra T, Pappas PG, Maertens J, Lortholary O, Kauffman CA, Denning DW, Patterson TF, Maschmeyer G, Bille J, Dismukes WF, Herbrecht R, Hope WW, Kippler CC, Kullberg BJ, Marr KA, Munoz P, Odds FC, Perfect JR, Restrepo A, Ruhnke M, Segal BH, Sobel JD, Sorrel TC, Viscoli C, Wingard JR, Zaoutis T, Bennett JE. 2008. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. *Clin. Infect. Dis.* 46:1813–1821. <http://dx.doi.org/10.1086/588660>.
- Marty FM, Koo S. 2009. Role of (1 $\rightarrow$ 3)- β -D-glucan in the diagnosis of invasive aspergillosis. *Med. Mycol.* 47(Suppl):S233–S240. <http://dx.doi.org/10.1080/13693780802308454>.
- Hope WW, Walsh TJ, Denning DW. 2005. Laboratory diagnosis of invasive aspergillosis. *Lancet Infect. Dis.* 5:609–622. [http://dx.doi.org/10.1016/S1473-3099\(05\)70238-3](http://dx.doi.org/10.1016/S1473-3099(05)70238-3).
- Mennink-Kersten MA, Warris A, Verweij PE. 2006. 1 $\rightarrow$ 3- β -D-Glucan in patients receiving intravenous amoxicillin-clavulanic acid. *N. Engl. J. Med.* 354:2834–2835. <http://dx.doi.org/10.1056/NEJMc053340>.
- Kanamori H, Kanemitsu K, Miyazaki T, Ameku K, Endo S, Aoyagi T, Inden K, Hatta M, Yamamoto N, Kunishima H, Yano H, Kaku K, Hirakata Y, Kaku M. 2009. Measurement of (1 $\rightarrow$ 3)- β -D-glucan derived from different gauze types. *Tohoku J. Exp. Med.* 217:117–121. <http://dx.doi.org/10.1620/tjem.217.117>.
- Kanda H, Kubo K, Hamasak K, Kanda Y, Nakao A, Kitamura T, Fujita T, Yamamoto K, Mimura T. 2001. Influence of various hemodialysis membranes on the plasma (1 $\rightarrow$ 3)- β -D-glucan level. *Kidney Int.* 60:319–323. <http://dx.doi.org/10.1046/j.1523-1755.2001.00802.x>.
- Marty FM, Lowry CM, Lempitski SJ, Kubiak DW, Finkelman MA, Baden LR. 2006. Reactivity of (1 $\rightarrow$ 3)- β -D-glucan assay with commonly used intravenous antimicrobials. *Antimicrob. Agents Chemother.* 50:3450–3453. <http://dx.doi.org/10.1128/AAC.00658-06>.
- Racil Z, Kocmanova I, Lengrova M, Weinbergerova B, Buresova L, Toskova M, Winterova J, Timilsina S, Rodriguez I, Mayer J. 2010. Difficulties in using 1,3- β -D-glucan as the screening test for the early diagnosis of invasive fungal infections in patients with haematological malignancies: high frequency of false-positive results and their analysis. *J. Med. Microbiol.* 59:1016–1022. <http://dx.doi.org/10.1099/jmm.0.019299-0>.
- Nagasawa K, Yano T, Kitabayashi G, Morimoto H, Yamada Y, Ohata A, Usami M, Horiuchi T. 2003. Experimental proof of contamination of blood components by (1,3)- β -D-glucan caused by filtration with cellulose filters in the manufacturing process. *J. Artif. Organs* 6:49–54. <http://dx.doi.org/10.1007/s100470300008>.
- Theel ES, Doern CD. 2013. β -D-Glucan testing is important for diagnosis of invasive fungal infections. *J. Clin. Microbiol.* 51:3478–3483. <http://dx.doi.org/10.1128/JCM.01737-13>.
- Deeks JJ, Altman DG. 2004. Diagnostic tests 4: likelihood ratios. *BMJ* 329:168–169. <http://dx.doi.org/10.1136/bmj.329.7458.168>.
- Hayden SR, Brown MD. 1999. Likelihood ratio: a powerful tool for incorporating the results of a diagnostic test into clinical decision making. *Ann. Emerg. Med.* 33:575–580. [http://dx.doi.org/10.1016/S0196-0644\(99\)70346-X](http://dx.doi.org/10.1016/S0196-0644(99)70346-X).
- Koo S, Bryar JM, Page JH, Baden LR, Marty FM. 2009. Diagnostic performance of the (1 $\rightarrow$ 3)- β -D-glucan assay for invasive fungal disease. *Clin. Infect. Dis.* 49:1650–1659. <http://dx.doi.org/10.1086/647942>.
- Karageorgopoulos DE, Vouloumanou EK, Ntziora F, Michalopoulos A, Rafailidis PI, Falagas MP. 2011. β -D-Glucan assay for the diagnosis of invasive fungal infections: a meta-analysis. *Clin. Infect. Dis.* 52:750–770. <http://dx.doi.org/10.1093/cid/ciq206>.
- Lamoth F, Cruciani M, Mengoli C, Castagnola E, Lortholary O, Richardson M, Marchetti O, Third European Conference on Infections in Leukemia (ECIL-3). 2012. β -Glucan antigenemia assay for the diagnosis of invasive fungal infections in patients with hematological malignancies: a systematic review and meta-analysis of cohort studies from the Third European Conference on Infections in Leukemia (ECIL-3). *Clin. Infect. Dis.* 54:633–643. <http://dx.doi.org/10.1093/cid/cir897>.
- Pfeiffer CD, Fine JP, Safdar N. 2006. Diagnosis of invasive aspergillosis using a galactomannan assay: a meta-analysis. *Clin. Infect. Dis.* 42:1417–1427. <http://dx.doi.org/10.1086/503427>.
- Leeflang MM, Debets-Ossenkopp YJ, Visser CE, Scholten RJ, Hooft L, Bijlmer HA, Reitsma JB, Bossuyt PM, Vandenbroucke-Grauls CM. 2008. Galactomannan detection for invasive aspergillosis in immunocompromised patients. *Cochrane Database Syst. Rev.* (4):CD007394. <http://dx.doi.org/10.1002/14651858.CD007394>.
- Ostrosky-Zeichner L, Alexander BD, Kett DH, Vasquez J, Pappas PG, Saek F, Ketchum PA, Wingard J, Schiff R, Tamura H, Finkelman MA, Rees JH. 2005. Multicenter clinical evaluation of the (1 $\rightarrow$ 3)- β -D-glucan assay as an aid to diagnosis of fungal infections in humans. *Clin. Infect. Dis.* 41:654–659. <http://dx.doi.org/10.1086/432470>.
- De Vlieger G, Lagrou K, Maertens J, Verbeke E, Meersseman W, Van Wijngaerden E. 2011. β -D-Glucan detection as a diagnostic test for invasive aspergillosis in immunocompromised critically ill patients with symptoms of respiratory infection: an autopsy-based study. *J. Clin. Microbiol.* 49:3783–3787. <http://dx.doi.org/10.1128/JCM.00879-11>.
- Hachem RY, Kontouyannis DP, Chemaly RF, Jiang Y, Retzel R, Raad I. 2009. Utility of galactomannan enzyme immunoassay and (1,3) β -D-glucan in diagnosis of invasive fungal infections: low sensitivity for *Aspergillus fumigatus* infection in hematologic malignancy patients. *J. Clin. Microbiol.* 47:129–133. <http://dx.doi.org/10.1128/JCM.00506-08>.
- Kawazu M, Kanda Y, Nannya Y, Aoki K, Kurokawa M, Shiba S, Motokura T, Hirai H, Ogawa S. 2004. Prospective comparison of the diagnostic potential of real-time PCR, double-sandwich enzyme-linked immunosorbent assay for galactomannan, and a (1 $\rightarrow$ 3) β -D-glucan test in weekly screening for invasive aspergillosis in patients with hematological disorders. *J. Clin. Microbiol.* 42:2733–2741. <http://dx.doi.org/10.1128/JCM.42.6.2733-2741.2004>.
- Pazos C, Ponton J, Del Palacio A. 2005. Contribution of (1 $\rightarrow$ 3) β -D-glucan chromogenic assay to diagnosis and therapeutic monitoring of invasive aspergillosis in neutropenic patients: a comparison with serial screening for circulating galactomannan. *J. Clin. Microbiol.* 43:299–305. <http://dx.doi.org/10.1128/JCM.43.1.299-305.2005>.
- Persat F, Ranque S, Derouin F, Michel-Nguyen A, Picot S, Sulahian A. 2008. Contribution of the (1 $\rightarrow$ 3)- β -D-glucan assay for diagnosis of invasive fungal infections. *J. Clin. Microbiol.* 46:1009–1013. <http://dx.doi.org/10.1128/JCM.02091-07>.
- Fontana C, Gaziano R, Favaro M, Casalnuovo Pistoia E, Di Fran-

- cesco P. 2012. (1-3)- β -D-Glucan vs galactomannan antigen in diagnosing invasive fungal infections (IFIs). *Open Microbiol. J.* 6:70–73. <http://dx.doi.org/10.2174/1874285801206010070>.
32. Metan G, Koç AN, Kaynar LG, Atalay A, Oztürk A, Eser B, Cetin M. 2013. What is the role of the (1 $\rightarrow$ 3)- β -D-glucan assay in the screening of patients undergoing autologous haematopoietic stem-cell transplantation? *Mycoses* 56:34–38. <http://dx.doi.org/10.1111/j.1439-0507.2012.02195.x>.
33. Ikemura K, Ikegami K, Shimazu T, Yoshida T, Sugimoto T. 1989. False-positive result in *Limulus* test caused by *Limulus* amebocyte lysate-reactive material in immunoglobulin products. *J. Clin. Microbiol.* 27: 1965–1968.
34. Martín-Rabadán P, Gijón P, Alonso Fernández R, Ballesteros M, Anguita J, Bouza E. 2012. False-positive *Aspergillus* antigenemia due to blood product conditioning fluids. *Clin. Infect. Dis.* 55:e22–e27. <http://dx.doi.org/10.1093/cid/cis493>.
35. Pickering JW, Sant HW, Bowles CAP, Roberts WL, Woods GL. 2005. Evaluation of a (1 $\rightarrow$ 3)- β -D-glucan assay for diagnosis of invasive fungal infections. *J. Clin. Microbiol.* 43:5957–5962. <http://dx.doi.org/10.1128/JCM.43.12.5957-5962.2005>.
36. Nakao A, Yasui M, Kawagoe T, Tamura H, Tanaka S, Takagi H. 1997. False-positive endotoxemia derives from glucan after hepatectomy for hepatocellular carcinoma with cirrhosis. *Hepatogastroenterology* 44: 1413–1418.
37. Ellis M, al Ramdi B, Finkelman M, Hedstrom U, Kristensen J, Ali-Zadeh H, Klingspor L. 2008. Assessment of clinical utility of serial β -D-glucan concentrations in patients with persistent neutropenic fever. *J. Med. Microbiol.* 57:287–295. <http://dx.doi.org/10.1099/jmm.0.47479-0>.
38. Ansorg R, van den Boom R, Rath PM. 1997. Detection of *Aspergillus* galactomannan antigen in foods and antibiotics. *Mycoses* 40:353–357. <http://dx.doi.org/10.1111/j.1439-0507.1997.tb00249.x>.
39. Racil Z, Kocmanova I, Toskova M, Winterova J, Lengerova M, Timilsina S, Mayer J. 2013. Reactivity of the 1,3- β -D-glucan assay during bacteraemia: limited evidence from a prospective study. *Mycoses* 56:101–104. <http://dx.doi.org/10.1111/j.1439-0507.2012.02210.x>.