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Comparative Analysis of the Wako β-Glucan Test and the Fungitell Assay for Diagnosis of Candidemia and *Pneumocystis jirovecii* Pneumonia

Ricarda Friedrich,[a] Elfriede Rappold,[a] Christian Bogdan,[b] Jürgen Held[a,b]

[a]Department für Medizinische Mikrobiologie und Hygiene, Universitätsklinikum Freiburg, Freiburg, Germany
[b]Mikrobiologisches Institut-Klinische Mikrobiologie, Immunologie und Hygiene, Universitätsklinikum Erlangen und Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany

ABSTRACT (1→3)-β-D-Glucan (BDG) is a biomarker for invasive fungal disease. Until now, all BDG data in the Western Hemisphere were obtained using the Fungitell assay (FA). How it compares to the Wako β-glucan test (GT), which was recently launched in Europe, is largely unknown. We conducted a case-control study to compare the two assays in serum samples from 120 candidemia and 63 *Pneumocystis jirovecii* pneumonia (PCP) patients. Two hundred patients with bacteremia or negative blood cultures served as candidemia control group. In patients with candidemia the median BDG values of the FA and the GT were 351 and 8.4 pg/ml, respectively. With both assays, the BDG levels in candidemia were significantly higher than those measured in the control group ($P < 0.001$). The sensitivity, specificity, and positive and negative predictive values for the diagnosis of candidemia were 86.7%, 85.0%, 6.0%, and 99.8% for the FA and 42.5%, 98.0%, 19.0%, and 99.4% for the GT, respectively. In PCP patients the median BDG values of the FA and the GT were 963 and 57.7 pg/ml, respectively. The sensitivities for PCP diagnosis were 100% for the FA and 88.9% for the GT. In practical terms, the GT proved to be robust and applicable for testing single samples, whereas for economic reasons the FA required the samples to be tested in batch. The sensitivity of the FA is superior to that of the GT. However, the GT is a valuable alternative to the FA, especially for patients with suspected PCP and in laboratories with low sample throughput.

KEYWORDS BDG, *Candida*, Fungitell, PCP, pneumocystis, Wako, beta-D-glucan, biomarker, candidiasis, candidosis

Invasive fungal diseases (IFD), like invasive candidiasis, invasive aspergillosis, and *Pneumocystis jirovecii* pneumonia (PCP), cause high morbidity and mortality among immunocompromised patients (1). Because a delay in the initiation of adequate therapy is associated with an increased mortality, it is of utmost importance to start antifungal treatment in time (2). However, the diagnosis and therapy of IFD are often delayed, because its clinical presentation is nonspecific and, consequently, the suspicion of treating physicians, especially in intensive care units (ICU), is low (2). Therefore, rapid and highly sensitive diagnostic tests are required to guide preemptive therapy in patients at risk. Assays detecting fungal antigens, like (1→3)-β-D-glucan (BDG), seem to be promising candidates to fulfill this task.

BDG is a major component of the fungal cell wall and is produced by nearly all medically relevant fungi, with the exception of *Mucorales*. During growth, BDG is shed from the fungal cells and is released into the surrounding tissues, body fluids, and the blood. There, it can be detected and used as a biomarker for IFD (3). The diagnostic performance of BDG measurement is depending on the fungal species. The sensitivity is excellent for PCP (sensitivity of approximately 95%) (4, 5) and is good for invasive

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Address correspondence to Jürgen Held, juergen.held@uk-erlangen.de.

candidiasis and invasive aspergillosis (sensitivity of approximately 80% for each) (5, 6). Thus, it is not surprising that BDG measurement is recommended for the diagnosis of IFD by the European Conference on Infections in Leukemia (ECIL) working group (7, 8), the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) (9), the British Society for Medical Mycology (10), and the Infectious Diseases Working Party (AGIHO) of the German Society of Hematology and Medical Oncology (DGHO) (11).

BDG measurement is uniformly based on a modification of the *Limulus* amebocyte lysate (LAL) pathway. The LAL pathway is a bifurcated serine protease cascade that is activated by bacterial lipopolysaccharide and BDG. It leads to cleavage of the so-called coagulogen and the generated peptides form a solid gel clot. The LAL reagent itself is obtained by phlebotomy from horseshoe crabs (3). Until now, all data from the Western Hemisphere on the diagnostic performance of BDG was generated using the Fungitell assay (FA). However, in April 2018 a second commercial BDG assay, the Wako β-glucan test (GT), was launched in Europe. Because the two assays utilize different BDG standards (pachyman versus lentinan) and differ in their detection techniques (colorimetric versus turbidimetric), the BDG concentrations measured by them are not directly comparable. As a consequence, the cutoff values recommended by the manufacturers are different (FA 80 pg/ml and GT 11 pg/ml, respectively). Furthermore, the two assays show significant differences in the workflow. While the FA is performed most economically, with 21 patients per run, the GT is designed for multiple- or single-sample use.

So far, the FA and the GT have been compared only once utilizing plasma samples (12). However, in Europe and the United States, serum is the preferred sample for fungal antigen testing. Therefore, we have conducted a retrospective case-control study in order to evaluate the diagnostic performance and the technical practicability of the two assays from serum samples in patients with candidemia and PCP.

MATERIALS AND METHODS

We conducted a retrospective case-control study at the University Medical Center Freiburg, Germany, a 1,600-bed tertiary care hospital. Archived serum samples (stored at −80°C) from patients with candidemia, bacteremia, negative blood cultures (BC), and PCP were tested for BDG using the FA (Associates of Cape Cod, East Falmouth, MA) and the GT (Wako Pure Chemical Industries, Osaka, Japan). Candidemia and PCP patients receiving intravenous immunoglobulins (IVIG) or albumin in the 7 days prior to serum sampling were excluded, because these substances are known to cause elevated BDG levels (13). The study was approved by the ethics committee of the University of Freiburg, application numbers 105/09 and 293/11. The need for informed consent was waived.

Candidemia patients. All patients presenting between January 2001 and September 2013 with BC-proven candidemia and an archived serum sample from the day of BC sampling (±3 days) were included. BC diagnostic was performed using the BacT/Alert 3D microbial detection system (bioMérieux, Marcy l'Etoile, France).

Candidemia controls. Sera from two control groups were examined. Control group 1 consisted of 100 patients with a negative BC, and control group 2 consisted of 100 patients with bacteremia. In both groups an archived serum from the day of BC sampling (±1 day) was required for inclusion. Bacteremia was caused by the following pathogens: *Bacillus* species ($n = 3$), *Bacteroides* species ($n = 3$), *Citrobacter* species ($n = 2$), *Enterobacter cloacae* ($n = 5$), *Enterococcus faecalis* ($n = 5$), *Enterococcus faecium* ($n = 5$), *Escherichia coli* ($n = 5$), *Haemophilus influenzae* ($n = 2$), *Klebsiella oxytoca* ($n = 3$), *Klebsiella pneumoniae* ($n = 3$), *Listeria monocytogenes* ($n = 2$), *Pseudomonas aeruginosa* ($n = 10$), *Serratia marcescens* ($n = 3$), *Staphylococcus aureus* ($n = 9$), *Staphylococcus epidermidis* ($n = 5$), *Staphylococcus haemolyticus* ($n = 3$), *Staphylococcus hominis* ($n = 5$), *Streptococcus agalactiae* ($n = 3$), *Streptococcus anginosus* group ($n = 3$), *Streptococcus bovis* ($n = 3$), *Streptococcus dysgalactiae* ($n = 3$), *Streptococcus mitis* ($n = 2$), *Streptococcus mutans* ($n = 3$), *Streptococcus oralis* ($n = 2$), *Streptococcus pneumoniae* ($n = 5$), and *Streptococcus pyogenes* ($n = 3$). Patients with bacteremia caused by coagulase-negative staphylococci were included only if at least two different BC sets were positive.

PCP patients. All patients presenting between March 2003 and December 2013 with PCP and an archived serum sample from the day of the diagnostic bronchoscopy (±7 days) were included. PCP was considered proven if the patient showed a clinical picture compatible with PCP and if *P. jirovecii* was detected by specific immunofluorescence staining and/or PCR. Clinical presentation was considered to be typical if pulmonary infiltrates compatible with PCP were present and if at least four of the following criteria were met: existing immunosuppression, fever, dyspnea, cough, elevated lactate dehydrogenase (LDH) level, and hypoxia. Exclusion criteria were culture of any fungus from relevant materials, a positive serum or bronchoalveolar lavage (BAL) galactomannan assay (Platelia Aspergillus enzyme immunoassay [EIA]; Bio-Rad Laboratories, Marnes-la-Coquette, France), or detection of a relevant bacterial pulmonary pathogen.

BDG measurement. The FA and the GT were performed according to the manufacturers' instructions. Briefly, for the FA 5 μl of serum was added to 20 μl of pretreatment reagent in a glucan-free 96-well

plate. After 10 min of incubation at 37°C, 100 μl of Fungitell reagent was added to the pretreated sample. The LAL reaction was then monitored at 37°C for 40 min in an ELx808 microplate reader (BioTek Instruments, Winooski, VT). The mean optical density change over time was calculated and compared to a standard curve to determine the BDG concentration. All samples were tested in duplicate.

For the GT, 100 μl of serum was added to 900 μl of β-D-glucan sample pretreatment solution. After 10 min of incubation at 70°C, the sample was cooled on ice. 200 μl of the pretreated sample were then added to the LAL reagent and the LAL gelation was monitored at 37°C for a maximum of 90 min in a MT-5500 toxinometer (Wako Pure Chemical Industries, Osaka, Japan). The BDG concentration of the sample was calculated by comparing its LAL gelation time to a calibration curve that is supplied with each lot by the manufacturer.

Samples with BDG levels above the upper validation limit (FA, 500 pg/ml, or GT, 600 pg/ml) were diluted and retested. BDG levels below the lower validation limit (FA, 31 pg/ml, or GT, 6 pg/ml, respectively) were calculated by extrapolation (FA and GT) or were considered to have a BDG concentration of 0 pg/ml if the result was "no gelation" (GT).

Both tests were performed with the same serum samples. However, most of the FA analyses had been performed between 2009 and 2011, whereas all of the GT analyses were carried out in 2013. In the meantime, the serum samples were stored at −80°C. To rule out that BDG in archived serum samples was degraded over the years at −80°C, we retested 20 serum samples of patients with BC-proven candidemia after 3 years (2015 to 2018) of storage at −20°C using the FA. The mean difference of the BDG measurements in 2015 and 2018 was −13.2 pg/ml, which means that the BDG values in 2018 were on average 13.2 pg/ml higher than in 2015 (see Table S1 in the supplemental material). Seventeen serum samples (85%) showed a coefficient of variation (CV) between the two measurements of less than 20%, which is the acceptable intra-assay variation given by the manufacturer. The remaining three serum samples uniformly showed higher BDG levels in 2018 than in 2015 (mean difference 108 pg/ml), strongly suggesting contamination of the samples rather than degradation. In addition, a Bland-Altman plot of the BDG measurements in 2015 and 2018 was generated (see Fig. S1 in the supplemental material). In the Bland-Altman plot the difference of the two measurements is plotted against the mean of the two measurements. If there is agreement between the two methods, 95% of the data points should lie within ±2 standard deviations (SD) of the mean difference (14). Our comparison showed good agreement and no evidence for a bias caused by BDG degradation.

Indirect immunofluorescence staining of *P. jirovecii*. Monoclonal antibody staining for *P. jirovecii* was performed with the DETECT immunofluorescence (IF) test (Axis Shield Diagnostics Limited, Dundee, UK) according to the manufacturer's instructions.

***P. jirovecii* nested PCR.** DNA from BAL fluids was isolated by proteinase K digestion followed by phenol-chloroform extraction. Touchdown PCR was performed as previously described (15). All amplification products were sequenced and confirmed to be part of the *P. jirovecii* mitochondrial large-subunit rRNA gene.

***P. jirovecii* real-time PCR.** On the basis of a previously published protocol (16), quantitative real-time PCR was performed on DNA preparations from serum and BAL samples. Briefly, 5-μl aliquots were used as template DNA for subsequent PCR testing on a LightCycler (Roche Diagnostics, Mannheim, Germany). Samples positive for the specific amplicons were identified by the PCR instrument at the cycle number where the individual fluorescence value exceeded that measured for the background. The quantitative interpretation of the results was assisted by a set of external standards that were tested in parallel.

Data collection. Patient demographics and clinical characteristics were collected, including age, sex, underlying diseases, body temperature, prior surgery, type of immunosuppression, C-reactive protein (CRP) level, procalcitonin (PCT) level, leukocyte count, CD4 cell count, creatinine level, LDH level, arterial PaO_2, PCP prophylaxis, antibiotic and antimycotic therapy, existence of central venous catheters, radiological and microbiological results. The therapy was reviewed for possible confounding factors for BDG measurement, including IVIG and albumin.

Statistical methods. Statistical analysis was performed using SPSS-V19 (SPSS Inc., USA) and MedCalc Statistical Software-V12 (MedCalc Software bvba, Belgium). Data are given as means ± SD or medians with interquartile ranges (IQR). Receiver operating characteristic (ROC) analysis was used to evaluate the ability of BDG to distinguish between candidemia and control patients. The highest Youden index indicated the optimal cutoff. The chi-square test and the Mann-Whitney U test were used for comparison of variables. Quantitative and qualitative agreement between the FA and GT were determined by Pearson's correlation and by calculation of Cohen's κ. A Bland-Altman plot was used to assess BDG agreement between measurements in 2015 and 2018. Differences were considered significant at a P value of <0.05.

RESULTS

Candidemia. One hundred twenty patients with BC-proven candidemia and 200 control patients with bacteremia ($n = 100$) and negative BC ($n = 100$) were included in the study. Clinical characteristics are shown in Table 1. The *Candida* species isolated were *C. albicans* ($n = 71$ [57.7%]), *C. glabrata* ($n = 25$ [20.3%]), *C. parapsilosis* ($n = 10$ [8.1%]), *C. tropicalis* ($n = 8$ [6.5%]), *C. guilliermondii* ($n = 3$ [2.4%]), *C. krusei* ($n = 2$ [1.6%]), *C. dubliniensis* ($n = 2$ [1.6%]), *C. fabianii* ($n = 1$ [0.8%]), and *C. lusitaniae* ($n = 1$ [0.8%]). In three candidemia episodes (2.5%) two different *Candida* spp. were detected simultaneously. The mean BC incubation time until detection of *Candida* spp. was

TABLE 1 Clinical characteristics of candidemia and control patients

Characteristic	Value for patients with:		
	Candidemia ($n = 120$)	Bacteremia ($n = 100$)	Negative BC ($n = 100$)
Mean age, yrs (range)	57 (1–84)	60 (21–93)	56 (20–88)
Sex, male/female	60/60	64/36	60/40
Candida species, no. of isolates (%)			
Candida albicans	71 (57.7)		
Candida glabrata	25 (20.3)		
Candida parapsilosis	10 (8.1)		
Candida tropicalis	8 (6.5)		
Candida guilliermondii	3 (2.4)		
Candida krusei	2 (1.6)		
Candida dubliniensis	2 (1.6)		
Candida fabianii	1 (0.8)		
Candida lusitaniae	1 (0.8)		
Underlying disease, no. of patients (%)			
Abdominal surgery	29 (24.2)		
Miscellaneous	26 (21.7)		
Solid tumor	25 (20.8)		
Hematologic malignancy/HSCT[a]	12 (10.0)		
Major surgery	8 (6.7)		
Gastrointestinal disease	7 (5.8)		
Liver cirrhosis	7 (5.8)		
Intravenous drug abuse	6 (5.0)		
ICU patient, yes/no (%)	60/60 (50.0)	32/68 (32.0)	7/93 (7.0)
No. of days on ICU before BC sampling (IQR[b])	7 (1–13.5)	0 (0–1)	3 (1–3)
Central venous catheter, yes/no (%)	102/17 (85.0)	48/52 (48.0)	41/59 (41.0)
Nontunneled	84 (82.3)	39 (81.3)	32 (78.0)
Tunneled (Port-A-Cath)	27 (26.5)	11 (22.9)	9 (22.0)
Preceding antibiotic therapy (−7 days), yes/no (%)	108/12 (90.0)	58/42 (58.0)	70/30 (70.0)
Preceding antimycotic therapy (−7 days), yes/no (%)	33/87 (27.5)	25/75 (25.0)	33/67 (33.0)
Candida colonization, yes (%)	67 (55.8)	24 (24.0)	23 (23.0)
One site	39 (32.5)	20 (20.0)	19 (19.0)
Two sites	24 (20.0)	4 (4.0)	4 (4.0)
Three sites	4 (3.3)	0 (0.0)	0 (0.0)
Median PCT, ng/ml (IQR)	2.3 (0.6–4.3)	3.1 (0.4–26.6)	0.3 (0.1–4.5)
Median CRP, mg/liter (IQR)	86 (37–144)	110 (48–222)	49 (16–124)
Median leukocytes; 10^4/ml (IQR)	11.8 (6.2–17.7)	8.0 (0.8–14.1)	5.2 (1.3–8.7)
In-hospital mortality, no. of patients (%)	46 (38.3)	20 (20.0)	
Death within 7 days from BC sampling, no. of patients (%)	19 (15.8)	12 (12.0)	

[a]HSCT, hematopoietic stem cell transplantation.
[b]IQR, interquartile range.

2,185 ± 1,415 min (36.4 ± 23.6 h). Most candidemia episodes occurred after abdominal surgery (24.2%) and in patients with solid tumors (20.8%) or hematologic malignancy (10.0%). Fifty percent of the candidemia patients required treatment in an ICU. The median time from ICU admission to the onset of candidemia (defined as the day of BC sampling) was 7 days, compared to 0 days for bacteremia and 3 days for negative BC. Furthermore, patients with candidemia had more often a central venous catheter (85.0 versus 44.5%; $P < 0.001$), received more often antibiotic therapy during the 7 days prior to BC sampling (90.0% versus 64.0%; $P < 0.001$), and were more often colonized with *Candida* spp. (55.8 versus 24.0%; $P < 0.001$) than control patients. Colonization with *Candida* spp. at two or more sites was predictive of candidemia. Median PCT and CRP levels in candidemia (2.3 ng/ml and 86 mg/liter, respectively) ranged between those of

TABLE 2 BDG levels of the Fungitell assay and the Wako β-glucan test in candidemia and control patients

Parameter	Fungitell assay	Wako β-glucan test
Median BDG (pg/ml), candidemia (IQR)	351 (274–448)	8.4 (6.2–14.3)
Median BDG, controls (IQR)	21 (16–29)	0 (0–0)
Median BDG, bacteremia (IQR)	15 (0–61)	0 (0–0)
Median BDG, negative BC (IQR)	26 (8–47)	0 (0–0)

patients with negative BC (0.3 ng/ml and 49 mg/liter, respectively) or bacteremia (3.1 ng/ml and 110 mg/liter, respectively). While the PCT and CRP levels of patients with negative BC and candidemia were significantly different from each other ($P < 0.001$ and $P = 0.004$, respectively), those of patients with candidemia and bacteremia were not ($P = 0.152$ and 0.078, respectively). In-hospital mortality in patients with candidemia was significantly higher than in patients with bacteremia (38.3 versus 20.0%; $P = 0.003$). However, mortality within 7 days after BC sampling was similar (15.8 versus 12.0%; $P = 0.385$), suggesting that the attributable mortality of candidemia was comparable to that of bacteremia.

BDG results for candidemia and control patients are shown in Table 2 and Fig. 1A, and the diagnostic performance is summarized in Table 3. Median BDG levels in candidemia were 351 and 8.4 pg/ml for the FA and the GT, respectively. *C. tropicalis* caused the highest median BDG levels (FA, 632 pg/ml, and GT, 17.2 pg/ml), followed by *C. glabrata* (FA, 356 pg/ml, and GT, 7.5 pg/ml) and *C. albicans* (FA, 345 pg/ml, and GT, 8.4 pg/ml). The lowest BDG levels were measured in candidemia with *C. fabianii* (FA, 7 pg/ml, and GT, 0 pg/ml), *C. lusitaniae* (FA, 79 pg/ml, and GT, 0 pg/ml), and *C. guilliermondii* (FA, 88 pg/ml; GT, 5.9 pg/ml).

With both assays, the BDG levels measured in candidemia were significantly higher than those in patients with bacteremia ($P < 0.001$) or negative BC ($P < 0.001$) or in the entire control cohort ($P < 0.001$). Using the manufacturers' cutoff values (FA, ≥80 pg/ml, and GT, ≥11 pg/ml), the sensitivity and specificity were 86.7 and 85.0% for the FA and 42.5 and 98.0% for the GT, respectively. To improve the diagnostic accuracy, we performed a ROC analysis, which showed optimal cutoff values of ≥70 pg/ml (FA) and ≥3.8 pg/ml (GT) (Fig. 2). The area under the ROC curve (AUC) was significantly higher for the FA than for the GT (0.917 versus 0.847; $P < 0.001$).

Using the optimized cutoff values, the sensitivity and specificity were 90.8 and 78.5% for the FA and 70.8 and 91.5% for the GT, respectively. Because the positive and negative predictive values are dependent on the prevalence of the disease, they were calculated for three patient populations (patients with suspected bloodstream infection, patients with proven bloodstream infection, and ICU patients with proven bloodstream infection). Altogether, the FA consistently showed a higher negative but a lower positive predictive value than the GT (Table 3).

Using the manufacturers' cutoff values, the numbers of false-positive results were 30 for the FA and 4 for the GT. The clinical characteristics of the respective patients and potential reasons for the false-positive results are shown in Table S2 in the supplemental material. Interestingly, all four patients with false-positive results by the GT were also highly positive by the FA.

PCP. The clinical characteristics of the 63 patients with PCP included in the study are presented in Table 4. The BDG results are shown in Table 5 and Fig. 1B, and the diagnostic test performance is summarized in Table 6.

P. jirovecii was detected in 44 patients by immunostaining and nested PCR, in 12 patients by immunostaining only (PCR not performed), and in 7 patients by nested PCR only (immunostaining negative). To rule out that nested PCR detected only *P. jirovecii* colonization, all available DNA samples were additionally tested by quantitative real-time PCR. The seven patients who tested only positive by the nested PCR showed medium to very high copy numbers (10^4 to $>10^6$ copies/ml). Given the clinical findings for these patients, it seems highly unlikely that PCR detected only colonization.

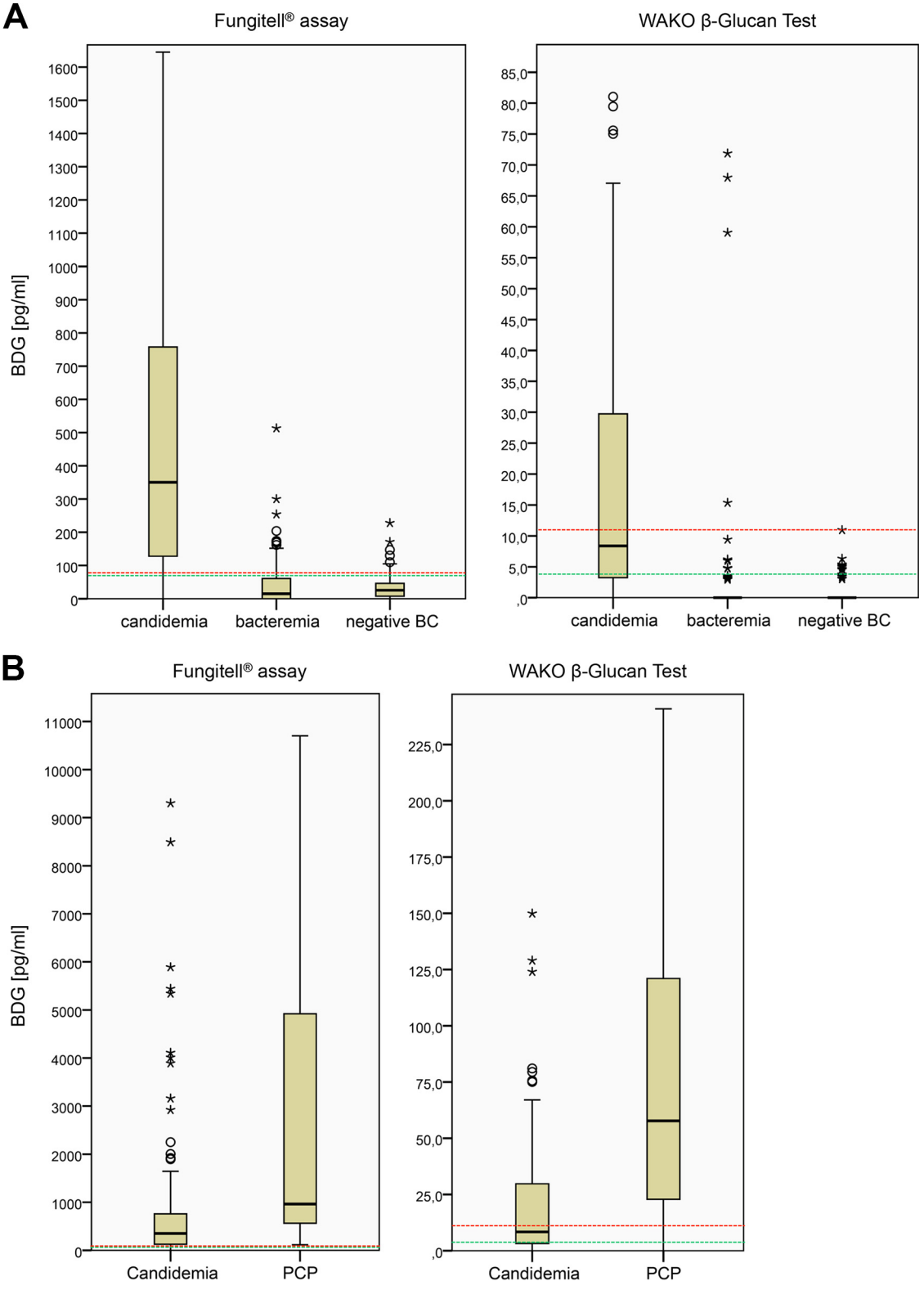


FIG 1 Box plots of the BDG levels measured by the Fungitell assay and the Wako *β*-glucan test. Serum samples from 120 patients with candidemia, 100 patients with bacteremia, and 100 patients with negative blood culture (BC) (A) or from 120 patients with candidemia and 67 patients with PCP (B) were analyzed with the FA or the GT. Due to the different BDG values measured by the FA and the GT, which are not directly comparable to each other, the scaling on the *y* axis (BDG concentration) is different for the two assays. The red dotted line indicates the manufacturer's cutoff value and the green dotted line the optimized cutoff value. The small circles depict outliers and the asterisks extreme outliers. For better display, some of the most extreme outliers are not shown.

TABLE 3 Performance of the Fungitell assay and the Wako β-glucan test in candidemia and control patients at manufacturer and optimized cutoffs

Parameter	Fungitell assay result at:		Wako β-glucan test result at:	
	Manufacturer cutoff of ≥80 pg/ml	Optimized cutoff of ≥70 pg/ml	Manufacturer cutoff of ≥11 pg/ml	Optimized cutoff of ≥3.8 pg/ml
True positives	104	109	51	87
False negatives	16	11	69	33
True negatives (negative BC + bacteremia = total)	91 + 79 = 170	90 + 77 = 167	100 + 96 = 196	91 + 91 = 182
False positives (negative BC + bacteremia = total)	9 + 21 = 30	10 + 23 = 33	0 + 4 = 4	9 + 9 = 18
Sensitivity, % (95% CI)	86.7 (79.3–92.2)	90.8 (84.2–95.3)	42.5 (33.5–51.9)	72.5 (63.6–80.3)
Specificity, % (95% CI)	85.0 (79.3–89.7)	83.5 (77.6–88.4)	98.0 (95.0–99.5)	91.0 (86.2–94.6)
Positive likelihood ratio (95% CI)	5.78 (4.12–8.10)	5.51 (4.01–7.56)	21.25 (7.88–57.32)	8.06 (5.11–12.69)
Negative likelihood ratio (95% CI)	0.16 (0.10–0.25)	0.11 (0.06–0.19)	0.59 (0.50–0.69)	0.30 (0.23–0.41)
Positive predictive value, %				
All patients with blood culture	6.0 (1.3–16.5)	5.7 (1.3–15.5)	19.0 (1.1–62.5)	8.2 (1.4–23.7)
Patients with BSI[a]	36.4 (25.0–48.9)	35.3 (24.5–47.2)	67.8 (42.1–87.4)	44.3 (29.9–59.6)
ICU patients with BSI	39.4 (28.0–51.7)	38.2 (27.3–50.1)	70.5 (45.5–88.8)	47.5 (33.1–62.3)
Negative predictive value, %				
All patients with blood culture	99.8 (98.3–100.0)	99.9 (98.4–100.0)	99.4 (97.7–99.9)	99.7 (98.1–100.0)
Patients with BSI	98.5 (96.1–99.6)	98.9 (96.7–99.8)	94.5 (91.3–96.8)	97.1 (94.3–98.8)
ICU patients with BSI	98.3 (95.8–99.5)	98.8 (96.5–99.8)	93.8 (90.5–96.3)	96.7 (93.8–98.5)

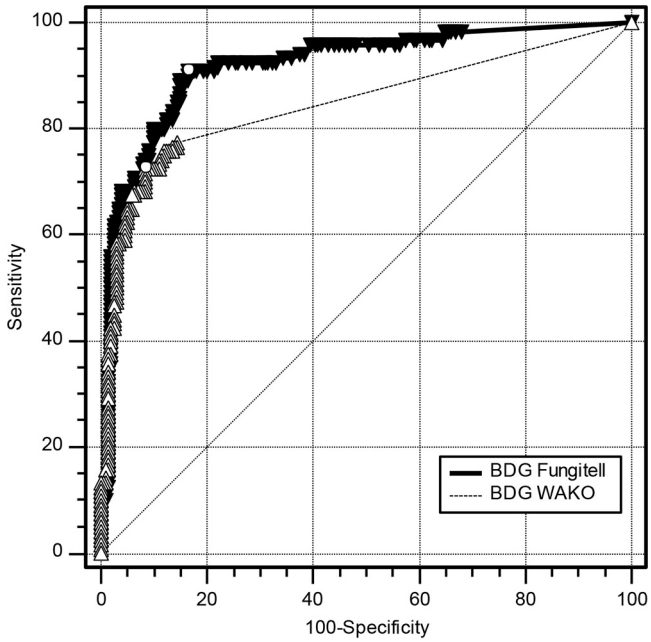
[a]BSI, bloodstream infection.

Most cases of PCP occurred in HIV-positive patients (44.4%), followed by patients with hematologic malignancy (28.6%) and solid tumors (17.5%). Median BDG levels in PCP were 963 pg/ml and 57.7 pg/ml for the FA and the GT, respectively. BDG levels were highest in patients with hematologic malignancy (1780 and 70.4 pg/ml, respectively) and lowest in patients with immunosuppressive therapy (285 and 21.9 pg/ml, respectively). However, the difference in BDG levels between the clinical subgroups was not statistically significant ($P_{FA} = 0.682$ and $P_{GT} = 0.694$, respectively).

By applying the manufacturers' cutoff values, the sensitivities of the FA and the GT for the diagnosis of PCP were 100% and 88.9%, respectively. Because we did not test a control group, a ROC analysis was not possible. However, utilization of the optimized candidemia cutoff values resulted in sensitivities of the FA and the GT of 100% and 95.2%, respectively.

Comparability of the BDG values. To determine if there is quantitative agreement between the two assays, Pearson's correlation was used. It shows that there is a significant correlation between the BDG levels determined by the FA and the GT in candidemia ($r = 0.956$ and $P < 0.001$; $n = 120$) and in PCP ($r = 0.391$ and $P = 0.002$; $n = 63$), respectively. According to Cohen (17) this corresponds to a strong agreement for candidemia and a moderate agreement for PCP. To be able to predict the FA BDG levels from the GT BDG levels, a linear regression was performed (Fig. S3 in the supplemental material). The regression line had a gradient of 21.42, an axis intercept of 212 pg/ml, and a coefficient of determination r^2 of 0.890. To further determine if there was also qualitative agreement between the assays, Cohen's κ was calculated. The agreement matrices are shown in Table S3 in the supplemental material. There was a moderate agreement ($\kappa = 0.556$; 95% confidence interval [CI], 0.482 to 0.630) using the manufacturers' cutoff values and a good agreement ($\kappa = 0.700$; 95% CI, 0.630 to 0.770) using the optimized cutoff values.

Technical practicability of the GT. The mean time to result for a single sample in the GT was 120 min (15 min for preparation of the control, 1 min for pipetting and mixing, 10 min at 70°C, 3 min on ice, 1 min for pipetting and mixing, and 90 min for turbidimetric analysis). For every additional sample, 2 min has to be added, and the analysis of 15 patients in batch (maximum load of the MT-5500 toxinometer) takes 150 min. Altogether, we performed 463 measurements, of which 5 (1.1%) could not be interpreted. For comparison, the FA also takes 120 min; however, for economic reasons,



	AUC (95%-CI)	Optimal cut-off	p
FA	0.917 (0.881-0.945)	≥ 70 pg/ml	< 0.001
GT	0.847 (0.803-0.885)	≥ 3.8 pg/ml	< 0.001

FIG 2 Receiver operating characteristic (ROC) analysis of BDG for discrimination between candidemia and control patients. BDG levels measured by the FA are depicted as black triangles and those measured by the GT as white triangles. The cutoffs with the highest Youden index (circles) were ≥70 pg/ml for the FA (sensitivity of 90.8% and specificity of 78.5%) and ≥3.8 for the GT (sensitivity of 70.8% and specificity of 91.5%). The FA had a significantly higher area under the ROC curve than the GT ($P < 0.001$).

the test should be carried out with the maximal possible sample number of 21 in duplicate. Furthermore, in the FA around 10 to 15% of the samples had to be tested again because the CV of the duplicates was above 20% (data not shown).

DISCUSSION

The measurement of BDG is an important element in the diagnosis of IFD, and multiple studies have confirmed its use in invasive candidiasis, invasive aspergillosis, and PCP (4–6). Currently, there are five commercial BDG assays available worldwide. However, only the FA was distributed in Europe and the United States. Therefore, it is not surprising that all the data on the diagnostic performance of BDG in the Western Hemisphere was obtained using the FA. In April 2018, the GT assay, which has received a Conformité Européenne (CE) marking, was introduced to the European market. So far, the two assays have been compared only once. Yoshida et al. reported that the sensitivity, specificity, and AUC were 83.3%, 92.6%, and 0.904 for the FA and 41.7%, 98.9%, and 0.743 for the GT (12). Although these values are similar to our results obtained with serum samples, it is important to emphasize that the study by Yoshida et al. was a retrospective analysis of a small number of patients with proven ($n = 9$) or probable ($n = 3$) IFD that were compared to 95 patients without IFD.

The data shown in the present study demonstrate that the FA is superior to the GT with respect to sensitivity in patients with candidemia (86.7 versus 42.5%, respectively) and PCP (100% versus 88.9%, respectively). Because of its low sensitivity, the GT is not suitable for the diagnosis of candidemia using the cutoff value of 11 pg/ml recommended by the manufacturer. However, when this cutoff is lowered to 3.8 pg/ml, the sensitivity of the GT strongly increased, from 42.5% to 70.8%. At the same time the

TABLE 4 Clinical and microbiological characteristics of PCP patients

Characteristic	Value for PCP patients (n = 63)
Sex, male/female	47/16
Mean age, yrs (range)	53 (26–78)
Underlying disease, no. of patients (%)	
HIV[a]	28 (44.4)
Hematologic malignancy/HSCT	18 (28.6)
Organ transplantation	11 (17.5)
Solid tumor	3 (4.8)
Rheumatic disease + immunosuppressive therapy	3 (4.8)
Clinical symptoms, yes/no (%)	
Pulmonary infiltrates compatible with PCP	63/0 (100.0)
Dyspnea	63/0 (100.0)
Fever	56/7 (88.9)
Cough	60/3 (95.2)
Hypoxia	61/2 (96.8)
Elevated LDH	56/7 (88.9)
Detection of *Pneumocystis jirovecii*, no. of patients (%)	
Immunostaining and PCR positive	44 (69.8)
Immunostaining positive, PCR not performed	12 (19.0)
Immunostaining negative, PCR positive	7 (11.1)

[a]HIV, human immunodeficiency virus.

excellent specificity of the GT showed only a minor decrease, from 98.0% to 91.0%, which is still higher than the specificity of the FA, at 85.0%. Although lowering the cutoff affected the specificity in candidemia only slightly, it caused a considerable decrease of the positive predictive value of the GT (e.g., from 70.5% to 47.5% in ICU patients with bloodstream infections).

In contrast to candidemia, the sensitivity of the GT in patients with PCP (88.9%) was already good without lowering the cutoff value. This is presumably due to the strongly elevated BDG levels found in PCP, which are twice as high as those found in candidemia (18, 19). However, the sensitivity of the GT in PCP can be further increased to an excellent 95.2% by using the cutoff value optimized for candidemia. Considering the high BDG levels seen in PCP patients, it is fair to assume that the specificity of the GT in PCP would be even higher than in candidemia patients.

Using the manufacturers' cutoff values, the numbers of false-positive results in patients with candidemia were 30 (15%) for the FA and 4 (2%) for the GT. Most of the false-positive patients were in the bacteremia group. None of these patients received IVIG or albumin, substances that are known to cause elevated BDG levels (13). However, three patients had elevated serum levels of mannan antigen, a highly specific biomarker for invasive candidiasis (19), indicating that they might have been true-positive patients and that BC analysis was false negative. One patient had a positive serum galactomannan value, and for another patient *Aspergillus fumigatus* was cultured from respiratory samples. Interestingly, all four patients with false-positive results in the GT

TABLE 5 BDG levels of the Fungitell assay and the Wako β-glucan test in PCP patients

Parameter	Fungitell assay	Wako β-glucan test
Median BDG, pg/ml (IQR)		
Total	963 (555–4,970)	57.7 (22.4–121.0)
HIV	1,408 (649–4,884)	57.7 (30.4–182.1)
Hematologic malignancy/HSCT	1,780 (578–7,098)	70.4 (17.7–286.9)
Organ transplantation	707 (418–4,820)	32.8 (11.5–121.0)
Solid tumor	817 (NA)[a]	43.9 (NA)[a]
Immunologic disorders	285 (163–9,620)	21.9 (0–383.0)

[a]The IQR for solid tumor cannot be calculated because the number of patients (n = 3) is too small. NA, not applicable.

TABLE 6 Performance of the Fungitell assay and the Wako β-glucan test in PCP patients at manufacturer and optimized cutoffs

Parameter	Fungitell assay result at:		Wako β-glucan test result at:	
	Manufacturer cutoff of ≥80 pg/ml	Optimized cutoff of ≥70 pg/ml	Manufacturer cutoff of ≥11 pg/ml	Optimized cutoff of ≥3.8 pg/ml
True positives	63	63	56	60
False negatives	0	0	7	3
Sensitivity, % (95% CI)	100.0 (94.3–100.0)	100.0 (94.3–100.0)	88.9 (78.4–95.4)	95.2 (86.7–99.0)

were also highly positive in the FA. Furthermore, it is striking that a considerable number of patients with false-positive results in the FA and/or GT ($n = 15$) had signs of a gastrointestinal barrier dysfunction (e.g., *Enterococcus* bacteremia, pathological gastrointestinal condition). Thus, the elevated BDG levels might have been caused by occult candidemia or by BDG translocation from the gut to the blood. Altogether, it seems plausible that the specificity of the two assays is underestimated because analysis of BC is a suboptimal gold standard.

Our comparison of the two assays has further shown that the quantitative agreement between the FA and the GT was strong, whereas the qualitative agreement was only moderate. This indicates that both tests quantify the BDG content of a sample correctly but that one of the tests does not categorize the patients properly. In fact, after lowering the GT cutoff value a considerable number of patients could be reassigned to the seropositive group and the qualitative agreement reached a good level. This is another indicator that the manufacturer's cutoff value does not seem to be appropriate for patients with candidemia and PCP.

While the BDG results of the two assays correlated, linear regression analysis showed that the FA-BDG levels are more than 21 times higher than those of the GT. What is the reason for this discrepancy? The FA and the GT have in common that they utilize the LAL pathway for detection and that they obtain the LAL reagent from the same species of horseshoe crab (*Limulus polyphemus*). However, apart from that, the FA and the GT differ in the BDG standards (pachyman and lentinan, respectively) and the exact detection method. In the FA, the coagulogen is replaced by a chromogenic peptide and the cleavage can be monitored at 405 nm photometrically. In contrast, the GT follows the formation of the gel clot turbidimetrically (3). These methodical differences are the reason for the different BDG values measured by the two assays, and we suggest that the lower sensitivity of the GT is mainly due to the reduced sensitivity of turbidimetry compared to that of photometry.

Another explanation for the low sensitivity of the GT in our study could be the use of serum instead of plasma. However, the study by Yoshida et al. described a similar low sensitivity using plasma (12), and serum is listed as an alternative specimen in the GT manual. Therefore, we are convinced that plasma and serum samples will yield comparable results.

Another important aspect for the assessment of both assays is their layout and workflow. The FA is designed to analyze 21 samples in parallel. Testing of a single sample is possible, but each test run requires an extensive standard curve and the glucan-free assay plate cannot be split. As a consequence, samples have to be collected in order to allow for cost-effective measurements, and test frequencies range from daily in large centers to once per week in smaller laboratories. However, this may lead to an unfavorable delay of test results, and the suitability of the FA for rapid diagnosis of candidemia or PCP becomes questionable. At this point it deserves mention that a modification of the FA has been described that uses a blood coagulation analyzer to offer rapid single-sample testing (20). Economic performance of single-sample testing was achieved by using an archived standard curve and by freezing aliquots of the LAL reagent. However, this method is not the one recommended by the manufacturer, and it has to be thoroughly validated in each laboratory.

In contrast, in the GT individual samples can be economically analyzed and up to 15 specimens can be tested in parallel using the standard protocol validated by the

manufacturer. Furthermore, it is possible to sequentially process and load the samples into the analyzer as they arrive in the laboratory, which is a major advantage with respect to the time to result. The standard curve is provided by the manufacturer, and only one positive control is required per day. In terms of practicability, the GT is easy to perform, and the assay is robust, without the need for extensive retesting. In contrast, the FA is more difficult to execute, and the number of samples that required repeated testing was 10 to 15 times higher than with the GT.

Conclusions. The FA showed superior sensitivity compared to the GT, particularly for patients with candidemia. However, by lowering the cutoff value, the sensitivity of the GT became satisfactory in candidemia and excellent in PCP. The strength of the GT, however, is its high specificity as well as the robustness and the technical flexibility which allows individual, parallel, or sequential testing of samples. For these reasons, the GT is a valuable alternative to the FA, especially in laboratories with a small sample throughput. It enables them to offer prompt BDG testing which is of great importance for the timely diagnostic workup for patients with suspected IFD.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/JCM.00464-18.

SUPPLEMENTAL FILE 1, PDF file, 0.4 MB.

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