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Original Article

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An evaluation of the performance of the Dynamiker® Fungus (1-3)-β-D-Glucan Assay to assist in the diagnosis of invasive aspergillosis, invasive candidiasis and *Pneumocystis* pneumonia

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Abstract

Invasive fungal disease (IFD) can be caused by a range of pathogens. Conventional diagnosis has the capacity to detect most causes of IFD, but poor performance limits impact. The introduction of non-culture diagnostics, including the detection of (1-3)-β-D-Glucan (BDG), has shown promising performance for the detection of IFD in variety of clinical settings. Recently, the Dynamiker® Fungus (1-3)-β-D-Glucan assay (D-BDG) was released as an IFD diagnostic test. This article describes an evaluation of the D-BDG assay for the diagnosis of invasive aspergillosis (IA), invasive candidiasis (IC) and *Pneumocystis* pneumonia (PCP) across several high-risk patient cohorts and provides comparative data with the Associates of Cape Cod Fungitell® and BioRad Platelia™ *Aspergillus* Ag (GM) assays. There were 163 serum samples from 121 patients tested, from 21 probable IA cases, 28 proven IC cases, six probable PCP cases, one probable IFD case, 14 possible IFD cases and 64 control patients. For proven/probable IFD the mean BDG concentration was 209pg/ml, significantly greater than the control population (73pg/ml; *P*: <.0001). The sensitivity, specificity, and diagnostic odds ratio for proven/probable IFD was 81.4%, 78.1%, and 15.5, respectively. Significant BDG false positivity (9/13) was associated post abdominal surgery. D-BDG showed fair and good agreement with the Fungitell®, and GM assays, respectively. In conclusion, the D-BDG provides a useful adjunct test to aid the diagnosis of IFD, with technical flexibility that will assist laboratories processing low sample numbers. Further, large scale, prospective evaluation is required to confirm the clinical validity and determine clinical utility.

Key words: β-D-Glucan, invasive aspergillosis, *Pneumocystis* pneumonia, invasive candidiasis.

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Introduction

Invasive fungal disease (IFD) presents in a wide range of clinical manifestations and can be caused by increasing array of fungal pathogens. While conventional diagnosis has the capacity to detect most causes of IFD, poor performance and delays in result reporting limit the influence on clinical management. The introduction of nonculture diagnostics is slowly changing clinical confidence in mycological tests with several recent articles highlighting the benefit of screening strategies, incorporating both molecular and serological assays to assist in the management of haematological patients at high risk of invasive aspergillosis (IA).[1–3] In all these studies, IA could be confidently excluded if both assays were consistently negative. Consequently, the use of empirical antifungal therapy was reduced.

While, in the developed world, IA is the commonest IFD in patients with hematological malignancy and undergoing stem cell transplantation (SCT), elsewhere, and in different patient cohorts, other IFD are more prevalent. Evidence of the benefits of nonculture testing in these areas is less apparent. Combining multiple assays targeting individual genera/species can provide broad range detection but will increase both costs and processing time. While pan-fungal PCR could meet this requirement but use has been associated with poor specificity and positive predictive value.[4] The detection of (1-3)-β-D-Glucan (BDG), a cell wall component of most fungi, excluding the Mucorales family and the genus *Cryptococcus*, has shown promising performance when testing serum/plasma for the detection of IFD in variety of clinical settings.[5–8] The overall sensitivity and specificity for proven/probable IFD as determined by meta-analysis was 77% and 85%, respectively, and it was concluded that performance was similar for the diagnosis of IA and invasive candidiasis (IC).[5] For the specific detection of *Pneumocystis* pneumonia (PCP) meta-analysis generated sensitivity and specificity of 95% and 86%, respectively.[6]

One issue with the pooled performance data generated by meta-analysis is the use of multiple commercial BDG kits with different manufacturing processes, methodology and positivity thresholds. Comparisons of the performance of different commercial BDG assays showed significant differences in both sensitivity and specificity between kits.[9] Since the introduction of BDG testing five commercial assays (Fungitell® (Associates of Cape Cod, East Falmouth, MA, USA); Fungitec G-Test MK (Seikagaku Corporation, Tokyo, Japan) B-G Star (Maruha Corporation, Osaka, Japan); B-Glucan Test Wako (Wako Pure Chemicals, Wako, Japan); Dynamiker® Fungus (1-3)-β-D-Glucan Assay (Dynamiker Biotechnology (Tianjin) Co., Ltd, China) have been released. However, in the Western Hemisphere, until recently, only the Fungitell® assay has been commercially available. This changed with the release of the Dynamiker® Fungus (1-3)-β-D-Glucan assay (D-BDG).

It is essential that any new assay is validated before routine clinical application to determine both overall clinical performance and qualitative and quantitative agreement with other similar assays. To our knowledge, this article describes one of the first independent evaluations of the D-BDG assay to aid in the diagnosis of IA, IC, and PCP across several high-risk patient cohorts and provides comparative data with the Associates of Cape Cod BDG assay (ACC-BDG) and Bio-Rad Platelia™ *Aspergillus* Ag (GM) assays.

Methods

Study design

The study was designed to evaluate the performance of the D-BDG assay when testing serum samples from patients in intensive care or post major abdominal surgery at risk of IC, hematology patients, including SCT recipients on fluconazole prophylaxis who were at risk of IA and PCP, patients with renal impairment including transplant recipients at risk of IC and PCP, and those with immunosuppressive conditions, such as human immunodeficiency virus (HIV), at risk of PCP. In addition to generating overall sensitivity/specificity data for IFD as a whole, it was designed to investigate performance for the individual fungal diseases, but also determine if any particular patient population influenced assay performance.

Clinical serum samples from patients with IA and IC, previously defined using the revised EORTC/MSG definitions, were selected as cases.[10] In addition, serum from patients with probable PCP (clinical suspicion supported with radiological evidence in keeping with PCP, plus PCP PCR positivity or ACC-BDG positive >300 pg/ml) or suspected PCP ((clinical suspicion supported with radiological evidence in keeping with PCP) were also included as cases. Serum samples from patients with no evidence of fungal disease not attaining an EORTC/MSG diagnosis were included as controls. All samples had been sent for routine BDG testing for investigation in to suspected IFD or for fungal screening as part of the routine neutropenic care pathway.[2] After testing, all samples were stored at -80° C for quality control and performance assessment purposes and thawed once for this study. The study was a retrospective performance assessment of D-BDG assay and was an anonymous, case/control design, with no impact on patient management not requiring ethical approval.

Platelia™ *Aspergillus* Ag (GM)

GM (Bio-Rad, UK) was performed following the manufacturer's instruction using an index of 0.5 to determine sample positivity.

Associates of Cape Cod (1-3)-β-D-Glucan assay (ACC-BDG)

ACC-BDG was performed using 5 μl of serum according to manufacturer's instructions and a positivity threshold of 80 pg/ml. Samples with a BDG concentration of between 60 and 79 pg/ml were considered indeterminate, and samples below 60 pg/ml negative.

Dynamiker® Fungus (1-3)-β-D-Glucan assay (D-BDG)

ACC-BDG was performed using 20 μl of serum according to manufacturer's instructions and a positivity threshold of 95 pg/ml. Samples with a BDG concentration of between 70 and 94 pg/ml were considered indeterminate, and samples below 70 pg/ml negative. All samples were tested in duplicate. When calculating the final concentration of BDG for each sample the mean value was used.

Statistical evaluation

To determine the clinical accuracy of the D-BDG assay the positivity rate in samples originating from cases was compared to the false positivity rate in control samples. To determine the clinical performance (sensitivity, specificity, positive and negative likelihood ratios, and diagnostic odds ratio) of the D-BDG assay 2 × 2 tables were constructed, using both proven/probable IFD and proven/probable/possible (suspected) IFD as true cases and NEF patients as the control population. Given the case control study design, and artificially high prevalence of IFD (47%), predictive values were not calculated. For each proportionate value ninety-five percent confidence intervals and, when required, P values (Fisher exact test; $P \leq .05$ considered significant) were generated to determine the significance of the difference between rates. When comparing the qualitative (positive/negative) agreement of the D-BDG and ACC-BDG a KAPPA statistic and observed agreement were calculated. The strength of agreement as determined by KAPPA statistic was defined as values >0.75 representing excellent agreement, values between 0.4 and 0.75 representing fair to good agreement, and values below 0.4 representing moderate to poor agreement. When comparing the quantitative agreement between BDG concentrations generated by the D-BDG and ACC-BDG assays, and D-BDG concentrations and GM index values (GMI), linear regression was performed to determine any correlation between values. To determine an optimal threshold for D-BDG assay, receiver operator characteristic (ROC) curve analysis was performed and the area under the curve (AUC) calculated using Graphpad Prism 5 (Graphpad Software, La Jolla, CA, USA). When comparing mean concentrations between different IFD diagnoses an unpaired two-tailed t-test or one-way analysis of variance (ANOVA) with Bonferroni's adjustment were used depending on the number of populations being compared, with a P value $\leq .05$ considered significant. When comparing mean concentrations between assays a paired two-tailed t-test was performed with a P value $\leq .05$ considered significant.

Results

A total of 163 serum samples from 121 patients were tested by the D-BDG assay. There were 33 samples from 21 cases of probable IA, 28 samples from 15 cases of proven IC, six samples from six cases of probable PCP, two samples from one case of probable IFD (radiological evidence plus ACC-BDG), 11 samples from 10 cases of possible IFD (all invasive mould disease, with radiological evidence but no mycological criterion), four samples from four cases of suspected PCP (equating to 84 samples from 57 cases) and 79 samples from 64 control patients with no evidence of IFD. Patient demographics are shown in Table 1.

Sample positivity rates

Seventy-four of the 163 samples (45.4%, 95% confidence interval [CI]: 38.0–53.1) tested by D-BDG generated a positive result. For all cases the true positivity rate (TPR) was 66.7% (56/84; 95% CI: 56.1-75.8), whereas for proven/probable cases only the TPR was 76.8% (53/69; 95% CI: 65.6-85.2). Both of which were significantly greater than the false positivity rate (FPR) of 22.8% (18/79; 95% CI: 14.9–33.2) in controls ($P < .0001$). The TPR for samples originating from the probable cases of IA was 75.8% (25/33; 95% CI: 59.0–87.2), compared to 82.1% (23/28; 95% CI: 64.4–92.1) for samples originating from cases of IC. For the cases of probable PCP, the TPR was 50.0% (3/6; 95% CI: 18.8–81.2), whereas both samples from the case of probable IFD were positive. The combined TPR for the possible/suspected cases was 20.0% (3/15, 95% CI: 7.1–45.2).

Table 1. Patient demographics.

	Mycological diagnoses					
	IC[b] (N = 15)	IA (N = 21)	PCP[c] (N = 10)	IFD[d] (N = 11)	Controls (N = 64)	Combined (N = 121)
Median age	61	48	50	56	54	54.5
Male/female ratio	1.14/1	1.63/1	1.5/1	1.75/1	1.06/1	1.24/1
Underlying condition						
Hematological Malignancy	0	21	2	7	43	73
Renal including transplant recipients	1	0	4	1	5	11
Abdominal surgery	13	0	0	1	13	27
Other[a]	1	0	4	2	3	10

Note: IA, invasive aspergillosis; IC, invasive candidiasis; IFD, invasive fungal disease; PCP, *Pneumocystis* pneumonia.
[a]Includes patients on intensive care and infectious disease units.
[b]Includes candidaemias (n = 7), peritoneal catheter associated *Candida* infections (n = 3) and intra-abdominal candidiasis (n = 5).
[c]Includes both probable (n = 6) and suspected PCP (n = 4).
[d]Includes both probable (n = 1) and possible IFD (n = 10).

Comparison of β-D-Glucan concentrations as generated by the D-BDG assay according to IFD status

The mean BDG concentration for cases of IA, IC, probable PCP, probable IFD, possible IFD/suspected PCP and the control population with no evidence of IFD were 214 pg/ml (95% CI: 155–273), 187 pg/ml (95% CI: 149–224), 225 pg/ml (95% CI: 41–491), 386 pg/ml (95% CI: 323–450), 63 pg/ml (95% CI: 3.4–122), and 73 pg/ml (95% CI: 48–98), respectively (Fig. 1a). For a combined proven/probable cohort the mean BDG concentration was 209 pg/ml (95% CI: 173–245) and was significantly greater to the mean for both the possible/suspected cases and the control population ($P < .0001$) (Fig. 1b). The mean BDG concentration for the possible/suspected cases and the control population were not different ($P = .7374$).

Clinical performance of the Dynamiker® Fungus (1-3)-β-D-Glucan assay

The clinical performance for the D-BDG assay for the diagnosis of IA, IC, PCP, and combined aetiologies is shown in Table 2. The highest sensitivity (93.3%) was for the detection of IC, whereas the sensitivity for PCP was only 50%, although cases were limited. The sensitivity for detecting combined proven/probable IFD was 81.4%, falling to 66.7% if possible IFD cases were incorporated. The overall specificity was 78.1%, but false positivity was associated with particular patient populations. Of the 14 falsely positive patients, nine were tested post abdominal surgery and the subsequent specificity for this cohort was 30.8% (4/13, 95% CI: 12.7–57.7). The remaining five false positive patients were treated for hematological malignancy, albeit the specificity in this cohort was 88.4% (38/43, 95% CI: 75.5–94.9). The best overall clinical performance was

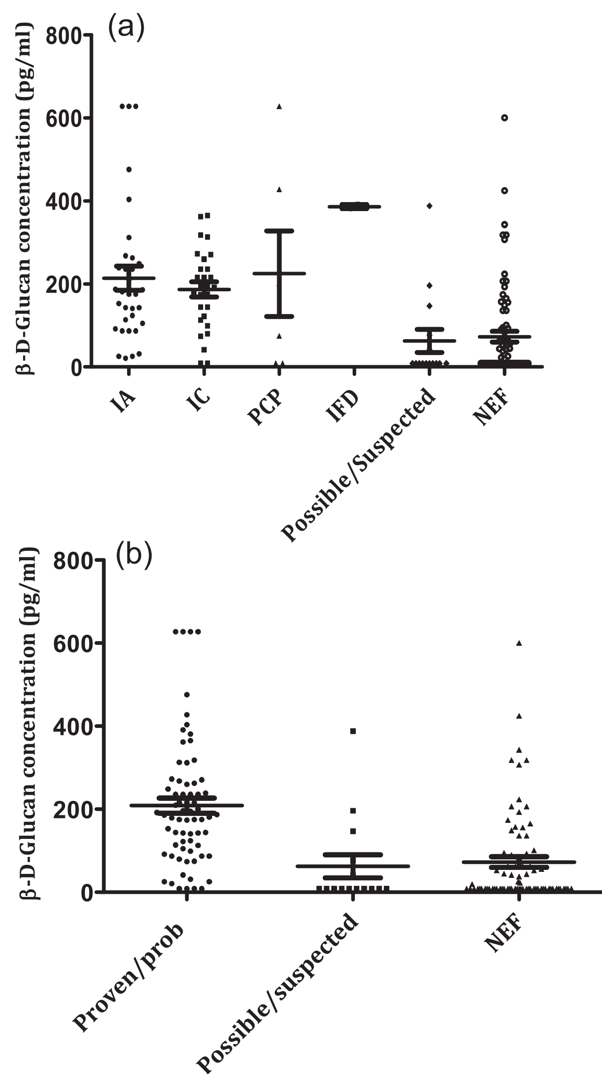

Figure 1. β-D-Glucan concentrations (pg/ml) as determined by the Dynamiker® Fungus (1-3)-β-D-Glucan Assay for patient populations defined according to (a) Individual fungal disease, (b) level of IFD diagnosis.

Table 2. Clinical performance of the Dynamiker® Fungus (1-3)-β-D-Glucan Assay.

Parameter (n/N; %, 95% CI)	Fungal disease IA vs NEF	IC vs NEF	PCP vs NEF	Combined Proven/ Probable IFD vs NEF	Combined Proven/ Probable/Possible IFD vs NEF
Sensitivity	17/21; 81.0, 60.0–92.3	14/15; 93.3, 70.2–98.8	3/6: 50.0, 18.8–81.2	35/43; 81.4, 67.4–90.3	38/57; 66.7, 53.7–77.5
Specificity	50/64; 78.1, 66.6–86.5	50/64; 78.1, 66.6–86.5	50/64; 78.1, 66.6–86.5	50/64; 78.1, 66.6–86.5	50/64; 78.1, 66.6–86.5
LR+tive	3.70	4.26	2.28	3.72	3.05
LR-tive	0.24	0.09	0.64	0.24	0.43
DOR	15.42	47.33	3.56	15.50	7.09

Note: **LR+tive**: Positive likelihood ration; **LR-tive**: Negative likelihood ration; **DOR**: Diagnostic odds ratio.
IA: Probable invasive aspergillosis (n = 21).
IC: Proven Invasive candidiasis (n = 15); includes candidaemias (n = 7), peritoneal catheter associated *Candida* infections (n = 3) and intra-abdominal candidiasis (n = 5).
PCP: Probable Pneumocystis pneumonia (n = 6).
Combined proven/probable IFD: Proven/probable invasive fungal disease (n = 43); comprising IA, IC, PCP and probable IFD (n = 1).
Combined proven/probable/possible IFD: Proven/probable/possible IFD (n = 57); comprising proven/probable IFD (n = 43) plus possible IFD (n = 10) and suspected PCP (n = 4).
NEF: No evidence of fungal disease (n = 64).

for the detection of IC, where the diagnostic odds ratio was 47.3, compared to 15.5 for combined proven/probable IFD (Table 2).

ROC analysis for proven/probable IFD versus the control population generated an area under the curve (AUC) of 0.8192 (95% CI: 0.7496–0.8889), incorporating the possible IFD into the case reduced the AUC to 0.7572 (95% CI: 0.6762–0.8285) (Fig. 2). The overall optimal threshold for the diagnosis of proven/probable IFD determined by ROC analysis was 69 pg/ml, at this cut-off the sensitivity, specificity, positive likelihood, negative likelihood, and diagnostic odds ratios were 90.7% (39/43, 95% CI: 78.4–96.3), 73.4% (47/64, 95% CI: 61.5–82.7), 3.41, 0.13 and 26.23, respectively. Using a 69 pg/ml positivity threshold improved sensitivity for the detection of all individual IFD (IA: 19/21; 90.5%, 95% CI: 71.1–97.4; IC: 15/15, 100%, 95% CI: 79.6–100 and PCP: 4/6, 66.7%, 95% CI: 30.0–90.3) and the sensitivity for proven/probable/possible IFD was 75.4% (43/57; 95% CI: 62.9–84.7).

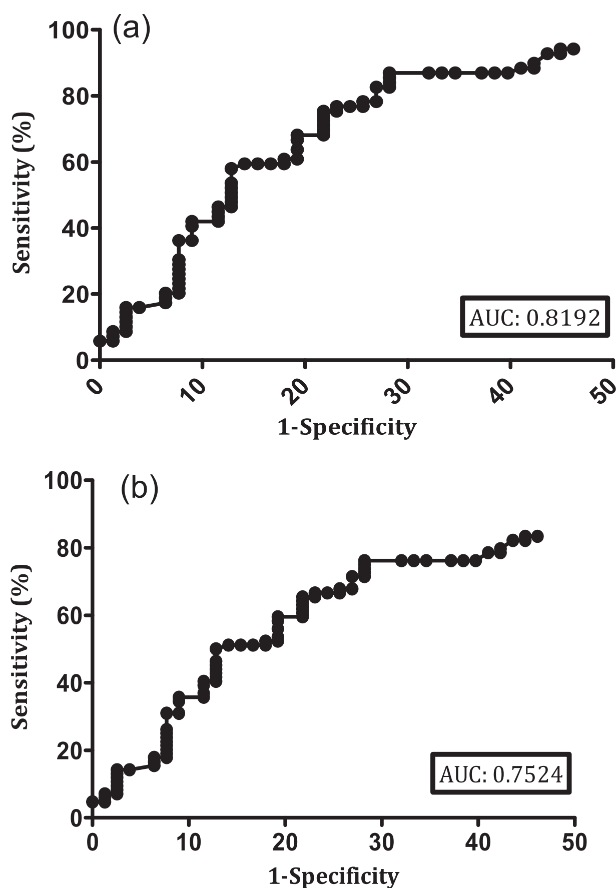

Figure 2. Receiver operator characteristic curve for the Dynamiker® Fungus (1-3)-β-D-Glucan Assay when testing (a) proven/probable IFD cases and controls with no evidence of fungal disease and (b) proven/probable/possible IFD cases and controls with no evidence of fungal disease.

Comparison with the Associates of Cape Cod (1-3)-β-D-Glucan assay

The mean and median ACC-BDG concentrations were 129.9 pg/ml (95% CI: 98.0–161.7) and 46.5 pg/ml (interquartile range [IQR] : 30–183.1), respectively. The mean and median D-BDG concentrations were 130.3 pg/ml (95% CI: 103–157.6) and 78.7 pg/ml (IQR: 9.4–206.6), respectively. There were no significant differences between the mean ($P = .9811$) or median concentrations ($P = .8990$). There was a weak but significant correlation ($P < .0001$)

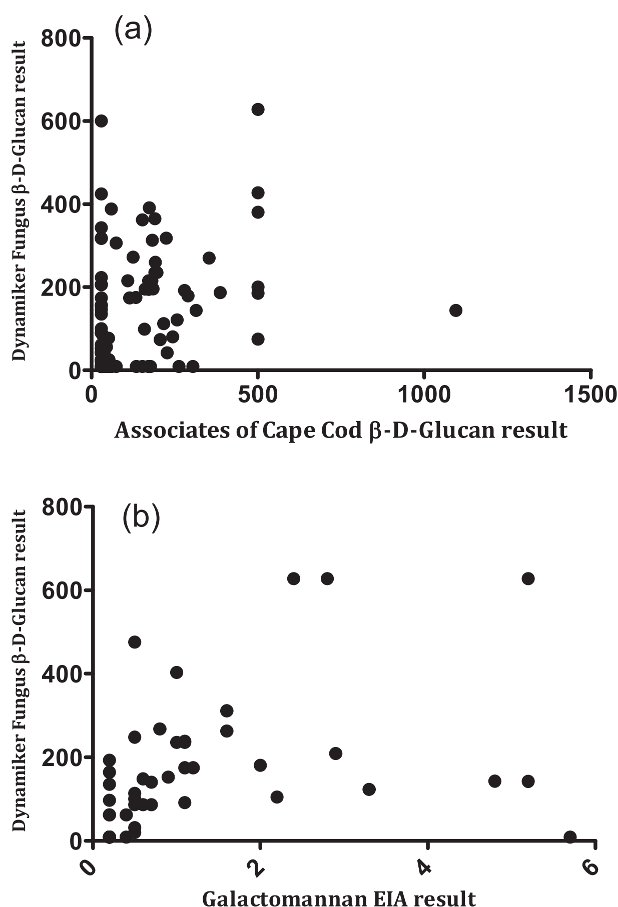

Figure 3. Correlation between (a) β-D-Glucan concentrations (pg/ml) as determined by the Associates of Cape Cod and Dynamiker® Fungus (1-3)-β-D-Glucan Assays, and (b) β-D-Glucan concentration (pg/ml) as determined by the Dynamiker® Fungus (1-3)-β-D-Glucan Assay and Galactomannan index as determined by the BioRad Galactomannan EIA.

between BDG concentrations generated by the ACC-BDG and D-BDG assays, with a Spearman coefficient of 0.3874 (95% CI: 0.2031–0.5453) (Fig. 3a). When considering indeterminate BDG results as negative the observed agreement between the two BDG assays was 75.5% (95% CI: 66.3–82.8), and the Kappa statistic was 0.5, representing fair agreement.

Comparison with the BioRad *Aspergillus* Ag assay

There was a strong correlation ($P < .0001$) between BDG concentration generated by the D-BDG assay and GMI, with a Spearman coefficient of 0.729874 (95% CI: 0.5820–0.8309) (Fig. 3b). When considering indeterminate BDG results as negative the observed agreement between the GM EIA and D-BDG assay was 83.9% (95% CI: 72.8–91.0) and Kappa statistic was 0.678, representing good agreement.

Discussion

This study describes an evaluation of the D-BDG assay to aid in the diagnosis of IFD across a range of patient cohorts. The overall performance of the assay was acceptable generating sensitivity and specificity of 81.4% and 78.1%, respectively, with the AUC determined by ROC analysis being 0.8192. While the sensitivity and overall performance, as indicated by AUC, compared favourably with the sensitivity (70.2–76.8%) and AUC (0.80–0.89) generated by meta-analytical review the specificity was slightly reduced (Specificity for a single positive threshold as determined by meta-analyses: 85.3–91.2%).[5,7] Comparison of the current study with the other previously published evaluation of the D-BDG testing 72 serum samples from patients at risk of IFD generated a similar discrepancy (sensitivity and specificity of the D-BDG assay was 82.9% and 94.6%, respectively).[11] Interestingly, in this study a positivity threshold of 70 pg/ml was used, whereas the defined D-BDG positivity threshold is >95 pg/ml.[11] In this current study, ROC analysis showed a threshold of 69 pg/ml to be optimal, at this cut-off sensitivity and specificity were 90.7% and 73.4%, respectively. Nevertheless, specificity in this current study remains substantially lower, and to get a specificity value >90% or >95% required the positivity threshold to be increased to >200 pg/ml and >330 pg/ml, respectively, compromising sensitivity (<50%).

False positive BDG results have been associated with *Candida* colonization, systemic bacterial infections, antibiotics, cellulose membranes, blood products and surgical gauze.[9,12] Other than the patients underlying clinical condition, there was insufficient information to determine specific factors associated with false positivity. However, the reduction in specificity could be a result of including patients post abdominal surgery, where translocation of gut flora into the bloodstream is likely. Specificity individual to this cohort was only 30.8% (4/13 NEF patients) compared to 90.2% (46/51 NEF patients) for other cohorts (hematology, renal and infectious disease patients), ($P < .0001$). Consequently, abdominal surgery patients are likely to be prone to false positive BDG results. Other than abdominal surgery patients the remaining false positivity was associated with hematology patients (false positivity rate: 5/43), and this has been noted previously.[13] In a study using the ACC-BDG to screen for IFD in haematology patients, Racil et al. generated specificity values of 19.8–55.8%, depending on threshold. Despite intensive efforts they could not identify a single significant source of BDG false positivity but hypothesized that a combination of clinical factors, including antibiotics and bacterial infection, could be responsible.[13] In this current study the specificity in hematology patients was not significantly compromised at 88.4%. This

is in line with meta-analysis of BDG testing in hematology where specificity calculated, with positivity defined using a single positive sample greater than the positivity threshold, was 91%.[7] In the study of Sulahian the specificity when testing haematology patients was 82%, with false positivity potentially associated with bacteremias limiting the utility of the BDG assay for predicting IFD.[14]

While the overall qualitative agreement between the ACC-BDG and D-BDG assays was fair (Kappa: 0.5) the quantitative correlation between the concentration values was weak (Spearman's coefficient: 0.3874). This was lower than the previous study comparing the two BDG assays, where overall observed agreement and Kappa were 89% and 0.77, respectively, representing very good/excellent agreement.[11] This could represent a limitation of the current study where the ACC-BDG testing was performed as part of the routine diagnostic work-up, and the D-BDG testing was performed retrospectively. Another limitation is that secondary samples were only available for 19% (23/121) patients. Consequently, it was not possible to accurately determine if specificity could be improved by requiring two consecutive positive results, as has been seen in the haematology cohort.[7] Both qualitative agreement and quantitative correlation between GM index and D-BDG concentration were good (Fig. 3b), indicating the D-BDG assay was moderately accurate test for the diagnosis of IA with a DOR of 15.42. The performance for the diagnosis of PCP the D-BDG did not provide satisfactory results. In this current study, sensitivity for PCP was only 50%, significantly compromised when compared to a meta-analysis of BDG for PCP that generated sensitivity of 95%.[6] However, cases were limited and compounded by the absence of mycologically proven cases determined by positive immunofluorescence of respiratory samples, although 3/6 of the probable cases were *Pneumocystis* PCR positive, one of which was D-BDG positive, one was indeterminate and one negative. The D-BDG assay provided optimal sensitivity for the diagnosis of IC (93.3%), and while the DOR reflected this (47.3) the result must be considered in the context of the "at-risk" patient population. Currently, this DOR is calculated using the combined control population containing patients at risk of from a range of IFD dependent on clinical condition and subsequent management. If the control population is limited to those mainly at risk of IC (i.e., post abdominal surgery) then the specificity is 30.8% and the DOR is reduced to 6.1. It is therefore important to remember that while the BDG assay provides a broad range of fungal detection all results should be interpreted in relation to the specific patient population. Nevertheless, in the post abdominal surgery cohort a negative D-BDG result is strongly associated with the absence of IFD.

One technical benefit of the D-BDG is it can be performed using eight well strips, comparable to the GM, rather than using 96 well plates as is the case for the ACC-BDG assay. The manufacturer also provides four vials of main-reagent furthering testing flexibility and cost-effectiveness. A local evaluation showed that after re-suspension the main reagent could be frozen and thawed, at least once, for up to 5 days without affecting the reaction kinetics (results not shown). Consequently, post preparation the main-reagent could be dispensed in to single use aliquots to be spread across the working week for daily testing of low sample numbers.

In conclusion, the D-BDG provides a useful adjunct test to aid the diagnosis of IFD, with technical flexibility that place it at an advantage over the ACC-BDG assay, particularly in laboratories processing low sample numbers. As with all BDG assays a single positivity threshold is utilised for all IFD, and although the mean D-BDG concentrations were similar for cases of IA, IC, and PCP, it is still no clear as to whether a single threshold is equally applicable. Furthermore, ROC analysis showed that the positivity threshold could be lowered to 69 pg/ml. Further, large scale, prospective evaluation is required to confirm the clinical validity and determine clinical utility.

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Declaration of interest

PLW: received project funding from Myconostica, Luminex, and Renishaw diagnostics, was sponsored by Myconostica, MSD and Gilead Sciences to attend international meetings, on a speaker's bureau for Gilead Sciences, and provided consultancy for Renishaw Diagnostics Limited.

RAB: received an educational grant and scientific fellowship award from Gilead Sciences and Pfizer, is a member of the advisory board and speaker bureau for Gilead Sciences, MSD, Astellas, and Pfizer, and was sponsored by Gilead Sciences and Pfizer to attend international meetings.

JSP and **RBP** report no conflicts of interest.

The authors alone are responsible for the content and the writing of the paper.

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