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Brief Report

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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 *Pneumocystis* pneumonia

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Abstract

The Dynamiker® Fungus (1-3)-β-D-Glucan Assay (D-BDG) has recently become available in the Western Hemisphere for the diagnosis of invasive fungal disease (IFD). Evaluations of its performance for *Pneumocystis* pneumonia (PcP) are limited. A retrospective evaluation of D-BDG diagnosis of PcP was performed (23 PcP cases and 23 controls). Sensitivity and specificity were 87% and 70%, respectively, reducing the positivity threshold to 45 pg/ml increased sensitivity (96%), whereas a threshold of 300 pg/ml increased specificity (96%). The performance of D-BDG for the detection of PcP is comparable to other IFD, but sensitivity is below that required to confidently exclude PcP.

Key words: β-D-glucan, *Pneumocystis* pneumonia, Dynamiker Fungus Assay.

The Dynamiker® Fungus (1-3)-β-D-Glucan Assay (D-BDG) (Dynamiker Biotechnology (Tianjin) Co., Ltd., Singapore) has recently become available in Europe and provides technical flexibility permitting frequent, cost-effective testing. A recent publication describing the performance of D-BDG for the diagnosis of invasive fungal disease (IFD) reported sensitivity and specificity for IFD of 81.4% and 78.1%, respectively.[1] However, sensitivity for the detection of *Pneumocystis* pneumonia (PcP) was lower than expected (50%), albeit cases were limited.[1] The detection of BDG to assist in the diagnosis of PcP has been widely demonstrated. Meta-analyses have confirmed performance, generating sensitivity and specificity ranging from 90.8 to 95.5%, and 78.1 to 86.3%, respectively.[2–4] The high sensitivity and ability to exclude a diagnosis of PcP when negative, has resulted in the inclusion of BDG testing in algorithms for management of PcP.[5]

Data from meta-analyses is encouraging, but variations in performance between different commercial BDG kits needs to be determined. In one study, there was no significant difference in accuracy when performance was stratified according to brand.[3] Accuracy is a combination of both

sensitivity and specificity. Under-performance in one parameter can be masked by over performance in the other, resulting in similar overall accuracy. This can be misleading if the performance of one parameter is more important. This is the case for BDG testing for PcP, where optimal sensitivity is required to confidently exclude disease. Given the poor, previously reported, sensitivity of the D-BDG when detecting PcP, performance must be determined and the sensitivity clarified to understand utility.[1] It was decided to evaluate the performance of D-BDG when testing serum samples from a larger cohort of patients at risk of PcP.

A performance evaluation was performed using excess clinical material, as an anonymous retrospective case/control study across two centers (Public Health Wales Microbiology Cardiff and the Health Services laboratory, London, UK) with no impact on patient management. Patients were initially tested for PcP (Grocott's stain/PcP PCR/Associates of Cape Cod BDG (ACC-BDG)) by the local center, based on risk factors and symptoms and as requested by the consulting physician. D-BDG testing was performed at Public Health Wales Microbiology Cardiff blinded to the diagnosis. D-BDG was performed according to manufacturer's instructions using a positivity threshold of 95 pg/ml. All samples ($n = 73$) were tested in duplicate, and when required a third replicate it was tested to resolve discordant results. When calculating the final concentration of BDG for each sample the mean value was used. There were 41 samples from PcP cases, five samples from other forms of fungal disease, and 27 samples from patients without IFD.

PcP was considered proven ($n = 2$) if a respiratory specimen was positive by Grocott's, probable ($n = 21$) if the patient was immuno-suppressed, had clinical signs consistent with PcP (e.g., bilateral ground glass opacification, reduced O_2 saturation) and was PcP PCR and/or ACC-BDG positive (15 positive by both, three positive by PCR only, and three positive by ACC-BDG only). Possible PcP ($n = 11$) was classified in symptomatic cases with nonspecific, or absence of chest radiology, but with positive PcP PCR ($n = 2$), or a ACC-BDG positive with a concentration >100 pg/ml ($n = 9$) in the absence of evidence of alternative IFD. The threshold for ACC-BDG positivity when clinical evidence was limited was based on a previous publication evaluating the performance of the ACC-BDG assay to assist in the diagnosis of PcP, where all cases had a BDG concentration 100 pg/ml, while all control/colonized patients had a BDG concentration below 90 pg/ml. [6] No IFD (control) patients ($n = 23$) were classified as symptomatic cases with nonspecific, or absence of chest radiology with PcP PCR negativity and/or ACC-BDG concentrations <100 pg/ml. A further five control patients with evidence of other IFD (three invasive aspergillosis and two invasive candidal disease) were also included.

When using the D-BDG the median BDG concentration for the 23 proven/probable cases of PcP was 262.4 pg/ml (range: 19.1 $\geq$ 628 pg/ml), compared to 87.1 pg/ml (range: <9.4 $\geq$ 628 pg/ml), 442.7 pg/ml (range: 38.5 $\geq$ 628 pg/ml), and 48.7 pg/ml (range: <9.4–306.3 pg/ml) for possible PcP, other IFD, and No IFD, respectively. Qualitative agreement between the D-BDG and ACC-BDG result, irrespective of PcP status was 74.7% (53/71 samples, 95% confidence interval [CI]: 63.5–83.3; two samples did not have an ACC-BDG result available), generating a Kappa statistic of 0.461, representing moderate/fair agreement. In relation to all cases of PcP (proven/probable/possible) the observed qualitative agreement was 76.9% (30/39 samples, 95% CI: 61.7–87.4; two samples from patients with probable PcP did not have an ACC-BDG result available). Qualitative agreement when testing samples from proven/probable cases was 87.5% (21/24 samples, 95% CI: 69.0–95.7). Of the three discordant results from proven/probable cases two were positive by the ACC-BDG alone (BDG concentration: 255 and >500 pg/ml) and one was only positive by the D-BDG (BDG concentration: 178 pg/ml). One sample from a probable PcP case was negative by both BDG assays but was PCR positive. Observed agreement when testing samples from the control population was 66.7% (18/27, 95% CI: 47.8–81.4). Of the nine discordant results, six were positive by the D-BDG alone (Mean BDG concentration: 236.3 pg/ml, SD $\pm$ 50.3) and three were positive by the ACC-BDG alone (Mean BDG concentration: 88.0 pg/ml, SD $\pm$ 7.2). Two samples from control patients were positive by both BDG assays. For the five cases of IFD other than PcP there was 100% agreement between the BDG assays.

The clinical performance when testing various populations is shown in Table 1. Compared to the previous study there was a significant improved in sensitivity (37.0%, 95% CI: 0.35–69.3) when testing proven/probable cases, but the assay was not sufficiently sensitive to confidently exclude PcP when negative (LR –tive: 0.19).[1] The three false negative results had mean BDG concentrations of 19.1, 48.9, and 67.6 pg/ml, not close to the positive threshold. One false negative D-BDG result was both PcP PCR and ACC-BDG positive (>500 pg/ml), one was ACC-BDG positive (255 pg/ml) but inhibitory to PCR and one was PcP PCR positive but ACC-BDG negative. All false negative D-BDG results were in the non–human immunodeficiency virus (HIV)-infected population, and lower sensitivity has been associated with this population.[4] Consequently, D-BDG sensitivity for cases of PCP associated with the HIV-infected population was 100% (5/5, 95% CI: 56.6–100) and the mean D-BDG concentration was 296.2 pg/ml

Table 1. Performance parameters of the Dynamiker® Fungus (1-3)-β-D-Glucan Assay for the diagnosis of *Pneumocystis* pneumonia and other invasive fungal diseases.

Parameter	Fungal disease		
(n/N; %, 95% CI)	Proven/Probable PcP vs NEF	Proven/Probable/Possible PcP vs NEF	Other IFD vs NEF
Sensitivity	20/23; 87.0%, 67.9–95.5	27/34; 79.4%, 63.2–89.7	4/5; 80.0%, 37.6–96.4
Specificity	16/23; 69.6%, 49.1–95.5	16/23; 69.6%, 49.1–95.5	16/23; 69.6%, 49.1–95.5
LR +tive	2.86	2.61	2.63
LR −tive	0.19	0.30	0.29
DOR	15.05	8.70	9.07

CI, Confidence interval; DOR, Diagnostic odds ratio; IFD, Invasive fungal disease, includes invasive aspergillosis ($n = 3$) and invasive candidiasis ($n = 2$); LR −tive, Negative likelihood ratio; LR +tive, Positive likelihood ratio; NEF, No evidence of fungal disease; PcP, *Pneumocystis* pneumonia.

Table 2. The effect of varying the positive threshold on the clinical performance of the Dynamiker® Fungus (1-3)-β-D-Glucan Assay for the detection of proven/probable *Pneumocystis* pneumonia versus no evidence of fungal disease.

Positivity threshold	Performance parameter				
(pg/ml)	Sensitivity (%)	Specificity (%)	LR +tive	LR −tive	DOR
20	100	13	1.15	<0.008[a]	>149.28[a]
45	95.6	34.8	1.47	0.13	11.60
70	87	69.6	2.86	0.19	15.32
95 (Current)	87	69.6	2.86	0.19	15.32
150	65.2	69.6	2.15	0.50	4.29
200	56.5	78.2	2.59	0.56	4.66
250	56.5	91.3	6.49	0.48	13.63
300	47.8	95.6	10.86	0.55	19.90
350	47.8	100	>478[a]	0.52	>914.79[a]

[a]To overcome infinity likelihood ratios have been calculated using sensitivity/specificity of 99.9%, instead of 100%. DOR, Diagnostic odds ratio; LR −tive, Negative likelihood ratio; LR +tive, Positive likelihood ratio.

(range: 120.8–520.4 pg/ml). Furthermore, two cases of possible PcP in the HIV-infected population were also D-BDG positive (BDG concentration: 406.6 and >628 pg/ml). It was not possible to calculate a specificity value specific to the HIV-infected population as all control patients were HIV negative. The performance of the D-BDG assay was not significantly different when including cases of possible PcP (Table 1). The performance for the detection of combined proven/probable IFD (Sensitivity: 85.7% 95% CI: 68.5–94.3; Specificity: 69.6%, 95% CI: 63.2–89.7) was similar to the previous evaluation (Sensitivity: 81.4% 95% CI: 67.4–90.3; Specificity: 78.1%, 95% CI: 66.6–86.5).[1]

Receiver operator characteristic curve analysis generated an area under the curve of 0.854, and representative performance according to positivity threshold is shown in Table 2. To achieve a sensitivity >90% the threshold would need to be reduced to 45pg/ml, generating a sensitivity of 95.6% (95% CI: 79.0–99.2), and samples with a BDG concentration below this threshold would be highly unlikely to be associated with PcP (LR−tive > 0.13). Conversely, a threshold of 300 pg/mL is required to achieve a specificity of 95.6% and LR+tive of 10.86 where disease can be confirmed. Given that the BDG concentrations for 3/5 IFD other than PcP were also greater than 300 pg/ml it was not possible to use the D-BDG assay to differentiate between different IFD. While there have been successful attempts to differentiate PcP infection, from colonization and false positivity, BDG cannot be confidently used to differentiate between causes of IFD.[6–8] A single threshold cannot be applied across brands, due to differences in reaction kinetics and it has been noted that BDG concentrations can vary between assays.[8]

In summary, the reported performance the D-BDG assays for the detection of PcP was improved compared to the initial evaluation and is comparable to performance for other IFD.[1] Using the current threshold sensitivity remains below that required for it to be used to confidently exclude PcP, although this may reflect the retrospective nature of the study. Specificity can be enhanced by using a positivity threshold of 300 pg/ml, but unlike previous studies this will compromise sensitivity.[7] Prospective evaluation is required to confirm clinical validity.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

References

1. White PL, Price JS, Posso RB, Barnes RA. 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. *Med Mycol*. 2017; Epub Ahead of print.
2. Karageorgopoulos DE, Qu JM, Korbila IP, Zhu YG, Vasileiou VA, Falagas ME. Accuracy of b-D-glucan for the diagnosis of *Pneumocystis jirovecii* pneumonia: a meta-analysis. *Clin Microbiol Infect*. 2013; 19: 39–49.
3. Onishi A, Sugiyama D, Kogata Y et al. Diagnostic Accuracy of Serum 1,3-β-D-glucan for *Pneumocystis jiroveci* pneumonia, invasve candidiasis, and invasive aspergillosis: Systematic review and meta-analysis. *J Clin Microbiol*. 2012; 50: 7–15.
4. Li WJ, Guo YL, Liu TJ, Wang K, Kong JL. Diagnosis of *Pneumocystis* pneumonia using serum (1-3)-β-D-Glucan: a bivariate meta-analysis and systematic review. *Thorac Dis*. 2015; 7: 2214–2225.
5. Alanio A, Hauser PM, Lagrou K et al. ECIL guidelines for the diagnosis of *Pneumocystis jirovecii* pneumonia in patients with haematological malignancies and stem cell transplant recipients. *J Antimicrob Chemother*. 2016; 71: 2386–2396.
6. Damiani C, Le Gal S, Da Costa C, Virmaux M, Nevez G, Totet A. Combined quantification of pulmonary *Pneumocystis jirovecii* DNA and serum (1-3)-β-D-glucan for differential diagnosis of *Pneumocystis* pneumonia and *Pneumocystis* colonisation. *J Clin Microbiol*. 2013; 51: 3380–3388.
7. Salerno D, Mushatt D, Myers L et al. Serum and BAL beta-D-glucan for the diagnosis of *Pneumocystis* pneumonia in HIV positive patients. *Resp Med*. 2014; 108: 1688–1695.
8. Tasaka S, Kobayashi S, Yagi K et al. Serum (1 $\rightarrow$ 3) β-D-glucan assay for discrimination between *Pneumocystis jirovecii* pneumonia and colonisation. *J Infect Chemother*. 2014; 20: 678–681.