Title: 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

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

TEXT

The Dynamiker® Fungus (1-3)-β-D-Glucan Assay (D-BDG) (Dynamiker Biotechnology (Tianjin) Co., Ltd) has recently become available in the 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 90.8-95.5%, and 78.1-86.3%, 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 comercial 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[1] ,performance must be determined and sensitivity clarified to understand utility.

A performance evaluation was performed using excess clinical material, as an anonymous retrospective case/control study across two centres (Public Health Wales Microbiology Cardiff and the Royal Free Hospital, London) with no impact on patient management Patients were initially tested for PcP (immuno-fluorescent microscopy (IF)/PcP PCR/Associates of Cape Cod BDG (A-BDG)) by the local centre, based on risk factors and symptoms. 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 95pg/mL. All samples (n=73) were tested in duplicate, and when required a third replicate was tested to resolve discordant results. When calculating the final concentration of BDG for each sample the mean value was used. There were 33 samples from PcP cases, five samples from other forms of fungal disease and 35 samples from patients without IFD.

PcP was considered proven (n=2) if a respiratory specimen was positive by IF, probable (n=20) 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 A-BDG positive (15 positive by both, three positive by PCR only and two positive by A-BDG only). Possible PcP (n=7) was classified in symptomatic cases with non-specific, or absence of chest radiology, but with positive PcP PCR (n=2) or strongly positive by A-BDG (>250pg/ml, n=5). No IFD (control) patients (n=29) were classified as symptomatic cases with non-specific, or absence of chest radiology with PcP PCR negativity and/or A-BDG concentrations <250pg/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 22 proven/probable cases of PcP was 260.5pg/mL (range: 19.1->628pg/mL), compared to 198.0pg/mL (range: 26.7->628pg/mL), 292.9pg/mL (range: 38.5->628pg/mL) and 52.8pg/mL (range: <9.4-306.3pg/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 73.9% (51/69 samples, 95% CI: 62.5-82.8. Four samples did not have an ACC-BDG result available), generating a Kappa statistic of 0.461, representing moderate/fair agreement. In relation to cases of PcP (proven/probable/possible) the observed qualitative agreement was 83.9% (26/31 samples, 95% CI: 67.9-95.5. Two samples did not have an ACC-BDG result available). Of the five discordant results two were positive by the D-BDG alone (Mean BDG concentration: 196.0pg/mL) and three were positive by the ACC-BDG alone (Mean BDG concentration: 284.7pg/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 68.6% (24/35, 95% CI: 52.0-81.5). Of the 11 discordant results, five were positive by the D-BDG alone (Mean BDG concentration: 240.7pg/mL, SD±55.0) and six were positive by the ACC-BDG alone (Mean BDG concentration: 108.5pg/mL, SD±24.3). Five 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. There was a trend towards improved sensitivity (36.4%, 95% CI: -0.57 to 68.8, *P*: 0.0913) over the previous

study, but this was not sufficient to enable PcP to be confidently excluded when negative (LR –tive: 0.19). The three false negative results had mean BDG concentrations ranging from 19.1-67.6pg/mL, not close to the positive threshold, although in one sample 1/3 replicates did generate a positive result of 128.2pg/ml, but 2/3 had a concentration of <9.4pg/mL. One false negative D-BDG result was both PcP PCR and ACC-BDG positive (>500pg/mL), one was ACC-BDG positive (255pg/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-HIV-infected population, and lower sensitivity has been associated with this population.[4] The performance of the D-BDG assay was similar when testing cases of possible PcP (Table 1). The performance for the detection of combined IFD (Sensitivity: 85.3% 95% CI: 69.9-93.6; Specificity: 72.4%, 95% CI: 54.3-85.3) was similar to the previous evaluation (Sensitivity: 81.4% 95% CI: 67.4-90.3; Specificity: 78.1%, 95% CI: 66.6-86.5).

Receiver operator characteristic curve analysis generated an area under the curve of XXX, 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 95.5% (95% CI: 78.2-99.2) and samples with a BDG concentration below this threshold would be highly unlikely to be associated with PcP (LR-tive> 0.12). Conversely, a threshold of 300pg/mL is required to achieve a specificity of 96.6% and LR+tive of 14.7 where disease can be confirmed. Given that the BDG concentrations for 3/5 IFD other than PcP were also greater than 300pg/.mL it was not possible to use the D-BDG assay to differentiate between different fungal diseases. While there have been successful attempts to differentiate PcP infection, from colonization and false positivity in controls, BDGcannot differentiate between causes of IFD. [6-8] aA 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] The sensitivity remains slightly 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 300pg/mL, but unlike previous studies this willcompromise sensitivity.[6] Prospective evaluation is required to confirm clinical validity.

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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 (n/N; %, 95% CI)	Fungal disease		
	Proven/Probable PCP vs NEF	Proven/Probable/Possible PCP vs NEF	Other IFD vs NEF
Sensitivity	19/22; 86.4%, 66.7-95.3	25/29; 86.2%, 69.4-94.5	4/5; 80.0%, 37.6-96.4
Specificity	21/29; 72.4%, 54.3-85.3	21/29; 72.4%, 54.3-85.3	21/29; 72.4%, 54.3-85.3
LR +tive	3.13	3.12	2.90
LR -tive	0.19	0.19	0.28
DOR	16.47	16.42	10.36

Key: **PcP:** *Pneumocystis* pneumonia

IFD: Invasive fungal disease

NEF: No evidence of fungal disease

LR +tive: Positive likelihood ration

LR -tive: Negative likelihood ration

DOR: Diagnostic Odds ratio

176 **Table 2.** The effect of varying the positive threshold on the clinical performance of the

177 Dynamiker® Fungus (1-3)-β-D-Glucan Assay for the detection of proven/probable

178 *Pneumocystis* pneumonia versus no evidence of fungal disease

Positivity Threshold (pg/mL)	Performance parameter				
	Sensitivity (%)	Specificity (%)	LR +tive	LR-tive	DOR
20	100	17.2	1.2	<0.006[a]	>200.0[a]
45	95.5	37.9	1.5	0.12	12.5
95 (Current)	86.4	72.4	3.1	0.19	16.5
150	72.7	72.4	2.6	0.38	6.8
200	54.5	79.3	2.6	0.57	4.6
250	54.5	89.7	5.3	0.51	10.4
300	50	96.6	14.7	0.52	28.3
350	45.5	100	>455.0[a]	0.55	>827.3[a]

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