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## Mycology

# Comparison of two $\beta$ -D-glucan assays for detecting invasive fungal diseases in immunocompromised patients

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### ABSTRACT

(1-3)- $\beta$ -D-glucan (BDG) is a major biomarker of invasive fungal diseases (IFDs), which are life-threatening for immunodeficient patients. We compared the clinical performance of two BDG-detection assays. The precision, linearity, reference interval, and limit of quantitation of the Wako BDG assay were analyzed and the performance was compared with that of the Goldstream BDG assay using 272 clinical serum samples. The repeatability, within-laboratory imprecision, and limit of quantitation of the Wako BDG assay were 3.8%, 5.9%, and 7.35 pg/mL, respectively (linearity, 23.8–557 pg/mL;  $R^2 = 0.998$ ). The correlation coefficient, slope, and y-intercept for the Wako BDG assay versus Goldstream BDG assay were 0.29, 3.82, and 0.04, respectively. The sensitivity and specificity were 43.8% and 94.9% for the Wako BDG assay and 39.6% and 83.5% for the Goldstream BDG assay, respectively. In clinical settings, the Wako BDG assay is suitable for diagnosing patients with IFDs.

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## 1. Introduction

The polysaccharide (1-3)- $\beta$ -D-glucan (BDG) is a major component of the fungal cell wall. Its backbone, comprising approximately 1500 glucose subunits, is cross-linked by the  $\beta$  (1-3) glycosidic bond (Wright et al., 2011). BDG exists as an insoluble structure in the fungal cell wall. When BDG is released in human body fluids, it transforms into a triple helix or single helix, and is consequently rendered soluble (Inoue et al., 1996; Yoshida et al., 1997). After the discovery of clotting reaction with  $\beta$ -glucan and the blood of *Limulus polyphemus* (North American horseshoe crab), several BDG assays were developed based on the *Limulus* amoebocyte lysate (LAL) pathway (Ohno et al., 1990). Owing to the widespread presence of BDG in clinically relevant fungi, its presence in the serum or plasma of patients has been recognized as an important biomarker for invasive fungal diseases (IFDs) (Obayashi et al., 1995; Horiguchi, 2004).

IFDs, including invasive candidiasis, invasive aspergillosis, and *Pneumocystis jirovecii* pneumonia (PCP), cause high morbidity and mortality among immunocompromised patients (Brown et al., 2012; Schmiedel and Zimmerli, 2016). Although blood culture has a limited sensitivity of approximately 50% for candidemia and a long turnaround time of over 2 days, it remains the primary tool for detecting *Candida* infection (Dichtl et al., 2019). As delayed initiation of therapy

for IFD results in increased mortality (Schmiedel and Zimmerli, 2016), rapid and accurate testing is needed in patients at risk of IFDs, for guiding immediate pre-emptive therapy or for discontinuing unnecessary administration of fungal drugs.

In this context, assaying BDG, which can rapidly identify the presence of various fungi, is of high value (Horiguchi, 2004; Odabasi et al., 2004; Pickering et al., 2005; Senn et al., 2008; Presterl et al., 2009; Alexander et al., 2010; Son et al., 2017; Clancy and Nguyen, 2018). BDG assays typically yield faster results than other methods, including blood culture and polymerase chain reaction (PCR), and the time required from pre-treatment to the final report of the results is up to 120 min (Friedrich et al., 2018). Various BDG assays invariably rely on the detection of the clotting reaction resulting from the LAL pathway as the measurement principle (Mori et al., 1997). However, the type of standard material and detection technique used for the clotting reaction in each assay differs, resulting in differences between the reference values and the possible interfering substances (Friedrich et al., 2018). Two widely used assays, the Goldstream Fungus BDG assay (Gold Mountain River Tech Development, Beijing, China) and the Wako BDG assay (Fujifilm Wako Pure Chemical Industries, Osaka, Japan) differ in several aspects. The Goldstream BDG assay uses pachyman as the BDG standard material, a colorimetric method for detection, and a cut-off value of 60 pg/mL. In contrast, the Wako BDG assay uses lentinan as the BDG standard material, a turbidimetric method for detection, and a cut-off value of 11 pg/mL.

The Wako BDG assay has multiple advantageous features. It accepts single-sample vials, allowing it to efficiently address small volume workloads. Additionally, the assay is relatively free of

Abbreviations: AMR, analytical measuring range; BDG, (1-3)- $\beta$ -D-glucan; IFD, invasive fungal disease; LAL, *Limulus* amoebocyte lysate; LOQ, limit of quantification; PCP, *Pneumocystis jirovecii* pneumonia

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interference from hemolysis, lipemia, and icterus in samples, as it is based on turbidimetry. Considering this, we evaluated the technical performance of the Wako BDG assay, and compared the results of 272 clinical samples using the Wako BDG assay and the Goldstream BDG assay to assess the clinical applicability of the Wako BDG assay. Moreover, we evaluated the optimal cut-off value in order to improve the diagnostic accuracy of the Wako BDG assay.

## 2. Materials and methods

### 2.1. Analytical performances

#### 2.1.1. Precision

A LAL control (Fujifilm Wako Pure Chemical Industries), the quality control material for the BDG assay, was used to evaluate precision using a Toxinometer MT-6500 (Fujifilm Wako Pure Chemical Industries). The test was repeated five times per day for five consecutive days, in accordance with the Clinical and Laboratory Standards Institute (CLSI) EP15-A3 guidelines, to assess its repeatability and within-laboratory imprecision. The mean concentration from 25 measurements was 23.18 pg/mL.

#### 2.1.2. Linearity

The analytical measuring range (AMR) suggested by the manufacturer (Fujifilm Wako Pure Chemical Industries) is 6–600 pg/mL. As per the CLSI EP6-A guidelines, two patient samples, with respective BDG concentrations of 119.0 pg/mL and 557.0 pg/mL, were diluted at ratios of 5:0, 4:1, 3:2, 2:3, and 1:4. Each of the diluted samples was measured in triplicate. We then performed polynomial regression to determine the best-fitting model, and validated linearity when deviation from the first order model was below 5%.

#### 2.1.3. Reference interval

The reference interval suggested by the manufacturer (Fujifilm Wako Pure Chemical Industries) was validated according to the CLSI EP28-A3C guidelines. We measured 21 samples referred from the health check-up center, to determine whether no more than 10% of the results fell outside the originally reported cut-off limit.

#### 2.1.3. Limit of quantitation (LOQ)

According to the CLSI EP17-A2 guidelines, a standard material with a low concentration was diluted at ratios of 4:0, 4:9, 2:9, and 1:10 to obtain expected concentrations of 23.9, 7.35, 4.35, and 2.39 pg/mL, respectively. Each diluted sample was measured in triplicate over 4 days. The lowest concentration at which the diluted sample showed bias between the expected and measured value below 20% was validated as the LOQ.

#### 2.1.4. Comparison of the methods

The methods were compared according to the CLSI EP9-A2-IR guidelines. Along with correlation between quantitative results using the Wako BDG assay and Goldstream BDG assay, we also assessed the classification concordance of the interpreted results based on the cut-offs recommended by the respective manufacturer.

### 2.2. Specimen collection and BDG measurement

From December 2019 to January 2020, 272 serum samples from different patients referred to the laboratory for routine BDG measurement were tested. The Goldstream BDG assay and the Wako BDG assay were performed simultaneously; the Platelia *Aspergillus* galactomannan Ag assay (Bio-Rad, Marnes la Coquette, France) and Cand-Tec *Candida* Ag assay (Ramco Laboratories, Texas, US) were also tested on the same day. The tests were performed following the respective manufacturers' instructions. The Wako BDG and Goldstream BDG assays both exploit the action of a clotting enzyme

triggered by factor G. While the Wako BDG assay measures the gelation time required for achieving the predefined specific turbidity by sample clotting, the Goldstream BDG assay measures the absorbance of the chromophore p-nitroaniline, which is generated by the activated clotting enzyme. This study protocol was reviewed and approved by the Institutional Review Board of Severance Hospital in Seoul, Korea (IRB No. 1-2020-0021) and informed consent was waived.

### 2.3. Clinical data

The patients' age, sex, types of underlying diseases related to immunosuppression, results of culture tests using blood and various body fluids, results of PCR for *P. jirovecii*, and history of other laboratory tests and treatments were comprehensively reviewed from the electronic medical records. In addition, episodes of hemolyzed, lipemic, and icteric blood samples were reviewed to address potential interferences in the BDG test results.

Diagnosis of proven, probable, and possible IFDs were based on the revised definitions of IFD from the European Organisation for Research and Treatment of Cancer/Fungal Infections and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) consensus (De Pauw et al., 2008; Donnelly et al., 2019). Only proven and probable IFDs were regarded as true-positive IFDs. With respect to the mycological criteria for probable diagnosis, blood culture and PCR results from clinical samples drawn at up to  $\pm 3$  days from the BDG test were included, as described previously (Friedrich et al., 2018).

All of the episodes were classified into four groups. Positive IFD episodes with a positive BDG result were classified as true positives. Positive IFD episodes with a negative BDG result were classified as false negatives. Negative IFD episodes with a positive BDG result were classified as false positives. Negative IFD episodes with a negative BDG result were classified as true negatives.

### 2.4. Statistical analysis

We performed statistical analysis and presented the results as graphs using Analyse-it (v2.30, Analyse-it Software Ltd, Leeds, UK). Receiver operating characteristic (ROC) analysis was performed to evaluate the diagnostic accuracy of the Wako BDG assay. The highest Youden index indicated the optimal cut-off. Relationships among BDG assays were evaluated by Spearman's correlation analysis. The slope and y-intercept for the results obtained using each instrument were determined by performing the Passing-Bablok regression analysis. The agreement for categorical items, namely Cohen's kappa, was calculated to analyze the classification concordance. Kappa values of 0.01–0.20, 0.21–0.40, 0.41–0.60, 0.61–0.80, and 0.81–1.0 represented slight, fair, moderate, substantial, and perfect agreement, respectively (Landis and Koch, 1977). *P*-values <0.05 were considered statistically significant.

## 3. Results

The repeatability and within-laboratory imprecision of the Wako BDG assay, presented as the coefficient of variation, were 3.8% and 5.9%, respectively. The BDG concentrations using the Wako BDG assay showed linearity in 23.8–557 pg/mL ( $Y = -0.6333 + 1.027X$ ;  $R^2$ , 0.996). The reference interval of 0–11 pg/mL was validated, and no single result fell outside the suggested limit. The measured concentrations of 21 samples from healthy individuals were distributed in 2.54–6.09 pg/mL. The %bias calculated between the expected value and actual measured value for the diluted standard materials with concentrations of 23.9, 7.35, 4.35, and 2.39 pg/mL was -1.4%, 7.8%, 22.2%, and 51.0%, respectively. Consequently, the determined LOQ was 7.35 pg/mL (Table 1).

**Table 1**  
Technical parameters of BDG assays.

| Parameters                         | Wako BDG assay         |                        | Goldstream BDG assay   |
|------------------------------------|------------------------|------------------------|------------------------|
|                                    | Evaluated              | Indicated <sup>a</sup> | Indicated <sup>a</sup> |
| Imprecision                        |                        |                        |                        |
| Within-run (%)                     | 3.8                    | 6                      | n/a <sup>b</sup>       |
| Within-laboratory (%)              | 5.9                    | 7                      | n/a <sup>b</sup>       |
| Analytical measuring range (pg/mL) | 23.8–557               | 6–600                  | 31.25–500              |
| Reference interval (pg/mL)         | validated <sup>c</sup> | 0–11                   | 0–60                   |
| Limit of quantitation (pg/mL)      | 7.35 <sup>d</sup>      | 6.00                   | 31.25                  |

BDG = (1-3)- $\beta$ -D-glucan

<sup>a</sup> Each set of parameters is indicated by the respective manufacturer.

<sup>b</sup> Imprecision of the Goldstream assay was not provided by the manufacturer.

<sup>c</sup> The reference interval of 0–11, indicated by Fujifilm Wako, was validated in this study.

<sup>d</sup> Since diluted samples of 7.35 pg/mL showed a bias of 7.8% and those of 4.35 pg/mL showed a bias of 22.2%, a concentration of 7.35 pg/mL was determined as the limit of quantitation.

In total, 272 patients were identified during the study period. Among them, 96 were in the positive IFD category and 176 were classified as negative (Fig. 1). The clinical characteristics of the patients are shown in Table 2. The *Candida* species isolated from blood culture were *C. albicans* (n = 5) and *C. krusei* (n = 1). The serum *Aspergillus* and *Candida* antigen assays were positive for 38 and 59 patients, respectively. The sputum PCR for *P. jirovecii* was positive in eight patients. There were 13 patients for which two or more test results were simultaneously positive. Most patients had one or more underlying diseases that result in immunodeficiency. There were no significant episodes of hemolyzed, lipemic, and icteric blood samples, which could interfere with the BDG test results.

BDG results obtained using the Wako BDG assay and Goldstream BDG assay and the diagnostic performances of these two assays for positive and negative IFD patients are summarized in Table 3. The median BDG levels were analyzed for BDG results surpassing the cut-offs suggested by the respective manufacturer (Goldstream BDG assay,  $\geq 60$  pg/mL, and Wako BDG assay,  $\geq 11$  pg/mL). Using the Wako BDG assay, the median BDG level was 35.1 pg/mL in positive IFD patients and 31.1 pg/mL in negative IFD patients. Using the Goldstream BDG assay, the median BDG level was 132.8 pg/mL in positive IFD patients and 109.3 pg/mL in negative IFD patients. Using the cut-off value recommended by the manufacturer, the sensitivity and specificity were 39.6% and 83.5% for the Goldstream BDG assay and 43.8% and 94.9% for the Wako BDG assay, respectively (Table 3). While the sensitivities of two assays showed no significant difference,

the specificity of the Wako BDG assay was significantly higher than that of the Goldstream BDG assay.

When compared quantitatively, the Wako BDG assay showed a negative bias when compared with the Goldstream BDG assay, with a slope of 0.04, y-intercept of 3.82, and R of 0.29 (Fig. 2), indicating substantial discrepancy between the assay results. We also performed an ROC analysis to determine the optimal cut-off value, besides the recommended cut-off of 11 pg/mL, to improve the diagnostic accuracy of the Wako BDG assay, which was found to have the highest Youden index at an optimal cut-off value of  $\geq 8.31$  pg/mL (Fig. 3). The area under the ROC curve (AUC) was 0.751 (95% confidence interval [CI], 0.689 to 0.813).

We also assessed the effects of this systemic bias on the clinical interpretation of the BDG concentration. As the two assays differ with regard to the BDG standard materials and detection methods used for the clotting reaction, their reference ranges and cut-off values vary greatly. The BDG test result classifications using these two assays and Cohen's kappa between them are described in Table 4. Upon reducing the cut-off value for the Wako BDG assay from 11 pg/mL to 8.31 pg/mL, there were a total of nine samples whose Wako BDG results changed from negative to positive due to the change in the cut-off values; of these nine samples, two were negative with the Goldstream assay, while seven were positive. Accordingly, the Cohen's kappa value increased from 0.44 to 0.52.

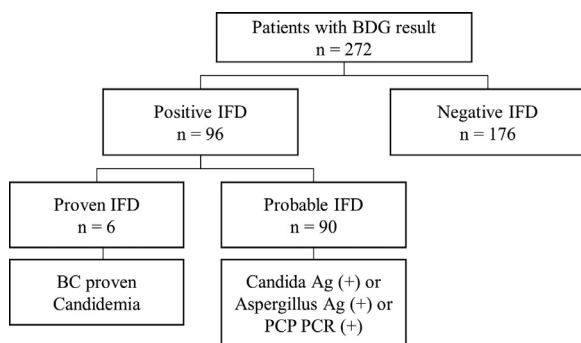
#### 4. Discussion

Since being recognized as a biomarker discovered in virtually all clinically relevant fungal species, the serum BDG level is becoming increasingly important to indicate cases of IFD (Ostrosky-Zeichner et al., 2005; Pazos et al., 2005, 2006; Held et al., 2011; Karageorgopoulos et al., 2011, 2013; Tissot et al., 2013; Li et al., 2015, 2019). However, from the standpoint of individual measurement methods selected in clinical laboratories worldwide, there has been a substantial amount of discrepancy according to the locations of the laboratories due to their specific regulations and economic limitations. Therefore, we measured the actual analytical performance of the Wako BDG assay and compared its clinical performance to that of the Goldstream BDG assay.

In the present study, we observed excellent concordance between the precision, linearity, reference range, and limit of quantitation of the Wako BDG assay suggested by the manufacturer and those acquired by actual measurements. Further, the Wako BDG assay allows single-sample testing, does not need sample collection for cost-effective measurement, and consequently shows excellent economic performance, particularly in smaller laboratories.

Thus far, few studies have compared the analytical performances of different BDG assays (Hossain et al., 1997; Moro et al., 2003; Yoshida et al., 2011; Friedrich et al., 2018; Mercier et al., 2019). Friedrich et al. (2018) compared the Wako BDG assay with the Fungitell assay (Associates of Cape Cod, East Falmouth, MA, USA) and reported that after lowering the cut-off value from 11 pg/mL to 3.8 pg/mL, the Wako BDG assay demonstrated satisfactory sensitivity, higher specificity, and better technical flexibility compared to the Fungitell assay. Mercier et al. (2019) also compared the analytical performance of the Wako BDG assay and Fungitell assay and reported that the Wako BDG assay was more accurate compared with the Fungitell assay after lowering the cut-off value of Wako BDG assay from 11 pg/mL to 6.2 pg/mL.

Together with these previously published results, our study is meaningful as it establishes the utility of the Wako BDG assay in clinical settings for the rapid diagnosis of patients at risk of IFD and evaluates the equivalence of different BDG assays. In line with the adjusted cut-off values suggested in previous studies, we identified that at a reduced cut-off of 8.31 pg/mL, the Wako BDG assay showed improved overall diagnostic performance. We also observed



**Fig. 1.** Flow chart representing study sample collection. Positive invasive fungal disease (IFD) comprised proven IFD and probable IFD. Proven IFD was defined as patients with blood culture-positive candidiasis. Probable IFD was defined as patients with positive results from *Candida* antigen, *Aspergillus* galactomannan antigen, and *Pneumocystis jirovecii* pneumonia (PCP) PCR tests.

**Table 2**  
Clinical characteristics of the subjects.

| Characteristics                                       | Analyzed episodes with:         |                                    |                        |
|---|---------------------------------|------------------------------------|------------------------|
|   | Proven IFD <sup>a</sup> (n = 6) | Probable IFD <sup>b</sup> (n = 90) | Negative IFD (n = 176) |
| Mean age, years (range)                               | 57 (2–80)                       | 56 (1–85)                          | 48 (1–92)              |
| Sex (%)   |                                 |                                    |                        |
| Male  | 6 (100)                         | 64 (71.1)                          | 106 (60.2)             |
| Female  | 0 (0.0)                         | 26 (28.9)                          | 70 (39.8)              |
| Underlying disease, no. of episodes (%)               |                                 |                                    |                        |
| Hematologic malignancy                                | 0 (0.0)                         | 18 (20.0)                          | 55 (31.3)              |
| Solid tumor   | 1 (16.7)                        | 25 (27.8)                          | 25 (14.2)              |
| Allogeneic stem cell transplant                       | 0 (0.0)                         | 2 (2.2)                            | 8 (4.5)                |
| Solid organ transplant                                | 3 (50.0)                        | 18 (20.0)                          | 41 (23.3)              |
| Prematurity   | 0 (0.0)                         | 1 (1.1)                            | 4 (2.3)                |
| Miscellaneous   | 2 (33.3)                        | 26 (28.9)                          | 43 (24.4)              |
| BC proven <i>Candida</i> species, no. of isolates (%) |                                 |                                    |                        |
| <i>Candida albicans</i>                               | 5 (83.3)                        | -                                  | -                      |
| <i>Candida krusei</i>                                 | 1 (16.7)                        | -                                  | -                      |
| Antigen assay, no. of episodes (%)                    |                                 |                                    |                        |
| <i>Aspergillus</i> galactomannan                      | -                               | 27 (30.0)                          | -                      |
| <i>Candida</i>  | -                               | 47 (52.2)                          | -                      |
| Sputum PCR, no. of episodes (%)                       |                                 |                                    |                        |
| <i>Pneumocystis jirovecii</i>                         | -                               | 3 (3.3)                            | -                      |
| Positive for 2 ≥microbiological evidence criteria (%) |                                 |                                    |                        |
| Both antigen assays                                   | -                               | 8 (8.9)                            | -                      |
| <i>P. jirovecii</i> PCR and one antigen assay         | -                               | 3 (3.3)                            | -                      |
| <i>P. jirovecii</i> PCR and both antigen assays       | -                               | 2 (2.2)                            | -                      |

BC = Blood culture; IFD = Invasive fungal disease; PCR = Polymerase chain reaction

<sup>a</sup> Positive blood culture or histological examination result is needed for a diagnosis of proven IFD.

<sup>b</sup> At least one host factor, microbiological, or clinical criterion need to be fulfilled for a diagnosis of probable IFD.

**Table 3**  
Performances of the two evaluated assays.

| Parameters                                  | Goldstream BDG assay | Wako BDG assay   | P-value <sup>a</sup> | Wako BDG assay (modified cut-off) | P-value <sup>b</sup> |
|---|----------------------|------------------|----------------------|-----------------------------------|----------------------|
| Applied cut-off value (pg/mL)               | ≥ 60                 | ≥ 11             |                      | ≥ 8.31                            |                      |
| Median BDG for results above cut-off values |                      |                  |                      |                                   |                      |
| Median BDG (pg/mL) of positive IFD (IQR)    | 135.6 (88.6–248.7)   | 36.8 (21.5–63.6) |                      | 34.4 (18.3–62.5)                  |                      |
| Median BDG (pg/mL) of negative IFD (IQR)    | 150.3 (89.3–221.3)   | 22.1 (17.0–35.1) |                      | 17.0 (10.9–25.0)                  |                      |
| Diagnostic performance                      |                      |                  |                      |                                   |                      |
| Overall positive IFD                        |                      |                  |                      |                                   |                      |
| True positives <sup>c</sup>                 | 38                   | 42               |                      | 46                                |                      |
| False negatives <sup>d</sup>                | 58                   | 54               |                      | 50                                |                      |
| True negatives <sup>e</sup>                 | 147                  | 167              |                      | 163                               |                      |
| False positives <sup>f</sup>                | 29                   | 9                |                      | 13                                |                      |
| Sensitivity, % (95% CI)                     | 39.6 (30.4–49.6)     | 43.8 (34.3–53.7) | 0.4142               | 47.9 (38.2–57.8)                  | 0.0736               |
| Specificity, % (95% CI)                     | 83.5 (77.3–88.3)     | 94.9 (90.6–97.3) | 0.0002               | 92.6 (87.8–95.6)                  | 0.0017               |
| Positive predictive value, % (95% CI)       | 56.7 (44.8–67.9)     | 82.4 (69.7–90.4) |                      | 78.0 (65.9–86.7)                  |                      |
| Negative predictive value, % (95% CI)       | 71.7 (65.2–77.4)     | 75.6 (69.5–80.8) |                      | 76.5 (70.4–81.7)                  |                      |
| Candidiasis                                 |                      |                  |                      |                                   |                      |
| Sensitivity, % (95% CI)                     | 38.7 (27.6–51.2)     | 46.8 (34.9–59.0) | 0.1967               | 50.0 (37.9–62.1)                  | 0.0522               |
| Specificity, % (95% CI)                     | 79.5 (73.6–84.4)     | 89.5 (84.6–93.0) | 0.0006               | 86.7 (81.4–90.6)                  | 0.0090               |
| Positive predictive value, % (95% CI)       | 35.8 (27.0–45.7)     | 56.9 (45.0–68.0) |                      | 52.5 (42.0–62.9)                  |                      |
| Negative predictive value, % (95% CI)       | 81.5 (78.1–84.4)     | 85.1 (81.8–87.8) |                      | 85.4 (82.0–88.3)                  |                      |
| Aspergillosis                               |                      |                  |                      |                                   |                      |
| Sensitivity, % (95% CI)                     | 47.5 (32.9–62.5)     | 47.5 (32.9–62.5) | 1.0000               | 57.5 (42.2–71.5)                  | 0.2059               |
| Specificity, % (95% CI)                     | 79.3 (73.6–84.0)     | 86.2 (81.2–90.1) | 0.0094               | 84.5 (79.3–88.6)                  | 0.0455               |
| Positive predictive value, % (95% CI)       | 28.4 (20.8–37.4)     | 37.3 (27.3–48.4) |                      | 39.0 (30.0–48.8)                  |                      |
| Negative predictive value, % (95% CI)       | 89.8 (86.6–92.2)     | 90.5 (87.6–92.8) |                      | 92.0 (88.9–94.3)                  |                      |
| PCP   |                      |                  |                      |                                   |                      |
| Sensitivity, % (95% CI)                     | 12.5 (2.2–47.1)      | 12.5 (2.2–47.1)  | 1.0000               | 25.0 (7.1–59.1)                   | 0.3173               |
| Specificity, % (95% CI)                     | 75 (69.4–79.8)       | 81.1 (75.9–85.3) | 0.0237               | 78.4 (73.1–82.9)                  | 0.1797               |
| Positive predictive value, % (95% CI)       | 1.5 (0.2–8.8)        | 2.0 (0.3–11.3)   |                      | 3.4 (1.0–10.6)                    |                      |
| Negative predictive value, % (95% CI)       | 96.6 (95.6–97.4)     | 96.8 (95.9–97.6) |                      | 97.2 (95.8–98.1)                  |                      |

CI = confidence interval; IQR = interquartile range; IFD = invasive fungal disease; BDG = (1-3)- $\beta$ -D-glucan; PCP = *Pneumocystis jirovecii* pneumonia.

<sup>a</sup> Goldstream vs Wako (cut-off 11 pg/mL).

<sup>b</sup> Goldstream vs Wako (cut-off 8.31 pg/mL).

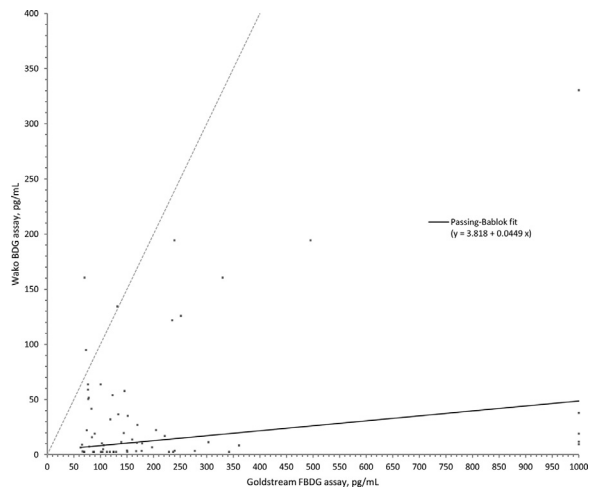
<sup>c</sup> Positive IFD episodes with positive BDG result.

<sup>d</sup> Positive IFD episodes with negative BDG result.

<sup>e</sup> Negative IFD episodes with negative BDG result.

<sup>f</sup> Negative IFD episodes with positive BDG result.

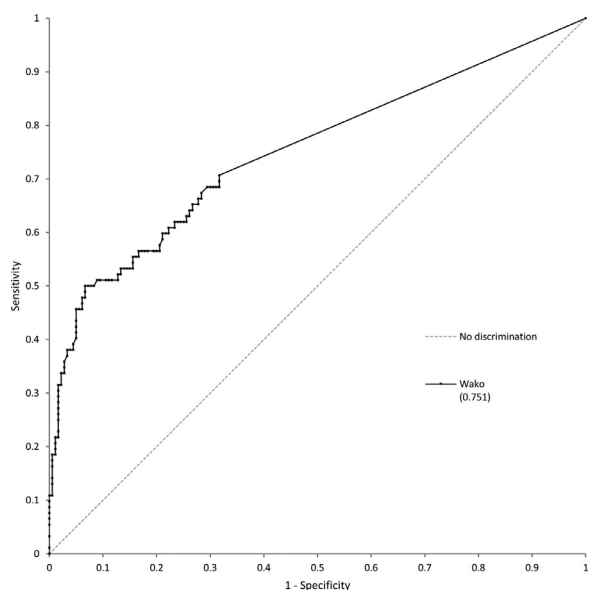




**Fig. 2.** Passing-Bablok regression for all data points. Results obtained using the Wako (1-3)- $\beta$ -D-glucan (BDG) assay compared with those obtained using Goldstream BDG assay were plotted with the Passing-Bablok regression and identity line.

discrepancy in the measured BDG levels between the two assays. This was similar to the results of previous reports (Friedrich et al., 2018; Mercier et al., 2019) comparing the Wako BDG assay and Fungitell assay. This discrepancy could be attributed to methodical differences including the BDG standard materials and clot detection methods used in the assays.

Whereas the Fungitell assay was the only available commercial BDG assay in Europe and the United States until recently, the Goldstream BDG assay is one of the most commonly used BDG assays in clinical laboratories in East Asia (Son et al., 2017; Chang et al., 2020). Son et al. (2017) measured the serum BDG of 50 patients with PCP, 15 with candidemia, six with chronic disseminated candidiasis, 15 with invasive aspergillosis, 10 with mucormycosis, and 40 controls. They showed that the sensitivity and specificity of the Goldstream BDG assay were 86% and 68%, respectively, when the cut-off value of BDG was set as 60 pg/mL. Chang et al. (2020) reported that the sensitivity and specificity of the Goldstream BDG assay were 50.0% and 84.2%, respectively, based on a cut-off value of 80 pg/mL. One



**Fig. 3.** Receiver operating characteristic analysis of (1-3)- $\beta$ -D-glucan (BDG) for discrimination between positive and negative invasive fungal disease. BDG levels measured by the Wako BDG assay are depicted. The cut-off with the highest Youden index was  $\geq 8.31$  pg/mL.

**Table 4**

Agreement of interpretive BDG test result classifications.

|                                     |          | Goldstream BDG assay ( $\geq 60$ pg/mL) |          | $\kappa$ |
|-------------------------------------|----------|---|----------|----------|
|                                     |          | Negative                                | Positive |          |
| Wako BDG assay ( $\geq 11$ pg/mL)   | Negative | 187                                     | 34       | 0.44     |
|                                     | Positive | 18                                      | 33       |          |
| Wako BDG assay ( $\geq 8.31$ pg/mL) | Negative | 186                                     | 27       | 0.52     |
|                                     | Positive | 19                                      | 40       |          |

BDG = (1-3)- $\beta$ -D-glucan.

hundred and fifty-five patients with hematologic malignancies were included in the study population and eight of them were proven or probable positive IFD cases.

To our knowledge, no comparative study between the Wako BDG assay and Goldstream BDG assay has been conducted so far. In the present study, we observed that the sensitivity, specificity, positive predictive value, and negative predictive value of the Goldstream BDG assay was 39.6%, 83.5%, 56.7%, and 71.7%, respectively. The sensitivity of the Goldstream BDG assay in our study was lower than that reported in other studies (Son et al., 2017; Chang et al., 2020) assessing the analytical performance of the Goldstream BDG assay, whereas its specificity was substantially higher (Table 5). The difference in the BDG cut-off applied might have attributed to this discrepancy. The BDG cut-off of 31.25 used by Son et al. (2017) could at least partially explain the substantially higher sensitivity they reported. Moreover, since positive cases included in the study by Chang et al. (2020) are fewer than others, the 95% confidence interval (CI) of sensitivity was determined to be quite wide. Although Chang et al. (2020) reported a somewhat higher sensitivity than us, the CI included 39.6%, the sensitivity reported in the present study. We hope future studies will draw more solid analytical performance values from more data.

Using the manufacturer-suggested cut-off values, the Wako BDG assay showed superior sensitivity and specificity compared with the Goldstream BDG assay. The analytical performances of the Wako BDG assay demonstrated in the present study are similar to those described previously (Friedrich et al., 2018); however, our study is a prospective study that conducted simultaneous measurements using the Wako BDG assay and the routinely used Goldstream BDG assay, with fresh serum samples from patients.

In concordance with previous studies, we obtained the highest Youden index and sensitivity of 47.9% when we applied a decreased cut-off of 8.31 pg/mL in our study. However, this is markedly lower than that described in other studies that assessed sensitivity by applying an optimal cut-off after performing an ROC analysis (Friedrich et al., 2018; Dichtl et al., 2019; Mercier et al., 2019). Mercier et al. (2019) reported a sensitivity of 84% (95% CI 77%–91%) when a cut-off of 6.222 pg/mL was applied to distinguish PCP cases and Friedrich et al. (2018) reported a sensitivity of 72.5% (95% CI 63.6%–80.3%) when a cut-off of 3.8 pg/mL was applied to distinguish candidemia cases. One possible reason for this discrepancy is the difference in the inclusion criteria for the IFD patient group. Unlike previous research, our study included probable IFD cases without blood culture-proven fungemia, along with all culture-proven or microscopically proven IFD cases. Therefore, the present study may better reflect the assay sensitivity in actual clinical settings, where cases with equivocal, indeterminate amounts of fungal biomass are common.

When reducing the cut-off to 8.31 pg/mL, the Youden index for the Wako BDG assay as well as the concordance of interpretive results using this assay with those using the Goldstream BDG assay were increased. The agreement, represented as Cohen's kappa, increased from 0.44 to 0.52. The cut-off value could be set by each laboratory based on the characteristics of patients referred to the hospital, the major purpose of routine BDG orders (surveillance vs diagnostic), and the availability of other mycological methods.

**Table 5**  
Comparison of studies evaluating the performance of the Goldstream BDG assay.

| Studies           | IFD cases (n) / Study population (n) | Target disease(s)                                   | BDG cut-off (pg/mL) | Sensitivity (%)  | Specificity (%)  |
|-------------------|--------------------------------------|---|---------------------|------------------|------------------|
| Son et al.        | 50 / 186                             | PCP   | BDG > 31.25         | 92 (81–98)       | 55 (39–71)       |
| Chang et al.      | 8 / 141                              | Invasive aspergillosis, candidiasis, trichosporosis | BDG > 80            | 50.0 (15.7–84.3) | 84.2 (76.9–90.0) |
| The present study | 96 / 272                             | Invasive aspergillosis, candidiasis, PCP            | BDG > 60            | 39.6 (30.4–49.6) | 83.5 (77.3–88.3) |

IFD = invasive fungal disease; BDG = (1–3)- $\beta$ -D-glucan; PCP = *Pneumocystis jirovecii* pneumonia.

Additionally, in each laboratory, validation for the appropriateness of the cut-off value, indicated by the reagent instructions, is recommended.

Our study has two main strengths compared with other related studies in the field. First, this is the only report describing a head-to-head comparison between the Wako BDG assay and Goldstream BDG assay. Secondly, this is the first prospective study conducted with fresh serum among studies addressing the analytical performance of the Wako BDG assay.

The limitations of this study are as follows. First, our study included only blood culture or PCP PCR-positive results in  $\pm$  3 days from the BDG test. As previous studies included results obtained up to 6 days from the BDG test, differences in study design must be considered when comparing with previous studies. Secondly, the prevalence of IFD in our study is up to 33.0% higher than that in previous studies (Yoshida et al., 2011; Morjaria et al., 2019). When comparing the predictive value of the assay with that reported in other studies, the differences in prevalence need to be considered. Thirdly, the proportion of cases with positive blood culture was lower than that in other studies (Friedrich et al., 2018; Li et al., 2019). However, fungal infection can become fatal even without a sizable amount of fungal expansion in blood, especially in immunocompromised patients. Thus, indirect mycological tests, including the BDG assay, are of particularly high value in cases that are not readily detectable by blood cultures (Tissot et al., 2013).

Future clinical studies conducted in a more calculated and deliberate manner are thus required to yield more accurate and precise data regarding BDG assays. Applying a set of combined antigen assays that cover a wide variety of clinically relevant fungi such as blood cultures, PCR, and BDG assay to every subject included in the study will further assist in assessing the exact diagnostic parameters of the BDG assay.

## 5. Conclusion

Overall, our study establishes that the Wako BDG assay can be applied in clinical settings for the rapid diagnosis of patients at risk of IFDs, compared to the Goldstream BDG assay; however, the choice of the assay to be used should be based on the specific settings of each laboratory. Additionally, determination or validation of the optimal cut-off value for each laboratory is recommended. The BDG assay can provide valuable mycological evidence for diagnosing IFDs, similar to the specific antigen assays for *Aspergillus* galactomannan and *Candida* sp. Furthermore, the BDG assay can be used for the rapid detection of infections caused by a wide variety of fungi. However, the results of BDG assays should be considered in combination with other diagnostic methods to obtain their full benefit, because of their limited sensitivity.

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## Author contributions

Conception and design of study: Younhee Park; Data acquisition: Jungyong Park; Data analysis and interpretation: Jungyong Park,

Younhee Park; Drafting of manuscript: Junhyup Song; Review and final approval of manuscript: Sinyoung Kim, Younhee Park, Hyon-suk Kim

## Declaration of conflict of interests

The authors report no conflicts of interest relevant to this article.

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