Application of the 1,3-β-D-Glucan (Fungitell) Assay in the Diagnosis of Invasive Fungal Infections

Tuan Tran, MD; Stacy G. Beal, MD

- With the high mortality rate associated with invasive fungal infections, methods for timely detection and diagnosis are necessary for appropriate and effective treatment. Testing for 1,3-β-D-glucan, a cell wall component of many medically important fungi, can be a useful adjunct in diagnosing such infections. The Fungitell assay (Associates of Cape Cod, East Falmouth, Massachusetts) is a US Food and Drug Administration–approved laboratory test that quantitatively measures 1,3-β-D-glucan levels and is widely available for clinical use as a relatively noninvasive method to aid in detecting the presence of invasive fungal infections. Numerous studies have evaluated its performance in clinical settings, and results have, overall, been favorable. It is not without its drawbacks, however, and the test must be interpreted and applied with care. Ordering practices are also widely variable among clinicians, and official guidelines have not been readily available. We present the details of this test and aim to propose evidence-based guidance for its use.


With new advances in organ transplantation,1 therapy for hematologic malignancies,2 and immunomodulation therapy,3 the number of patients who are immunocompromised and at increased risk for life-threatening fungal infections is worrisome.4 Early and appropriate treatment is essential,5 which generally requires early detection and identification of the causative organism. Some fungal organisms, however, can often be difficult to or require a long time to culture in the laboratory, making early identification difficult through traditional methods. With some fungal infections occurring nosocomially,6 diligent surveillance of at-risk patients for development of infections is also critical. Fortunately, rapid laboratory tests have been developed that can be invaluable in timely detection and treatment of some fungal infections. The Fungitell assay (Associates of Cape Cod, East Falmouth, Massachusetts) detects 1,3-β-D-glucan (BG), a component of many medically important fungi, such as Candida spp, Aspergillus spp, Fusarium spp, Pneumocystis jiroveci, Coccioidiodes immitis, Histoplasma capsulatum, and Blastomyces dermatitidis.7 We will discuss the strengths and utility, as well as drawbacks, of the Fungitell assay in assessing the presence of invasive fungal infections.

BACKGROUND

Fungitell (formerly called Glucatell) is a US Food and Drug Administration–approved quantitative assay used to aid in the detection of invasive fungal infections. The fungal cell wall, necessary for the survival of many fungal organisms, is a dynamic structure that continually undergoes remodeling during the life of the organism.8 This exoskeletal structure is composed primarily of chitin, glucan, mannan, and glycoproteins,9 of which glucan is an important component used in the Fungitell assay. The BG component is composed mainly of glucose polymers linked via β-1,3-glycosidic bonds, forming the BG backbone of the fungal cell wall. As the fungus grows and divides, this cell wall is continuously remodeled and some BG is released as soluble forms, most of which are multiple strands intermingled as triple helices and the rest as single strands or random coils.9 The basis of the Fungitell assay relies on BG’s ability to activate the limulus amebocyte lysate clotting cascade present in the blood of Limulus polyphemus, the North American horseshoe crab.10 In the presence of BG, a serine protease zymogen called factor G is activated, leading to activation of a proclotting enzyme in the cascade. Activation of factor G occurs via binding of single-stranded BG to the factor G α subunit.11 Because single-stranded BG is required for activation, serum specimens are pretreated with an alkali reagent that encourages conversion of the multimer forms to single strands.11 The resultant clotting can be measured via spectrophotometry, yielding an indirect BG concentration in picograms per millimeter (pg/mL) (Figure). A BG value of less than 60 pg/mL is considered a negative result, 60 to 79 pg/mL is an indeterminate result, and 80 pg/mL or more is a positive result.12 Because the limulus amebocyte lysate clotting cascade can also be activated via another zymogen (factor C) by bacterial endotoxin,10 factor C is manufactured out of the final product so the cascade is specific to BG.12 Several pathogenic fungi are inherently undetectable via Fungitell because of the very low levels of BG they produce, such as Cryptococcus species and the yeast phase of Blastomyces dermatitidis,13 as well as the Zygomycetes (Lichtheimia

Accepted for publication November 19, 2014.

From the Department of Pathology, Baylor University Medical Center, Dallas, Texas (Drs Tran and Beal); and the Infectious Diseases Laboratory, med fusion, Lewisville, Texas (Drs Tran and Beal).

The authors have no relevant financial interest in the products or companies described in this article.

Reprints: Tuan Tran, MD, Department of Pathology, Baylor University Medical Center, 3500 Gaston Ave, Dallas, TX 75246 (e-mail: Tuan.Tran@BaylorHealth.edu).

Arch Pathol Lab Med—Vol 140, February 2016

1,3-β-D-Glucan in Invasive Fungal Infections—Tran & Beal 181
**SENSITIVITY AND SPECIFICITY**

Multiple studies have shown Fungitell to be a beneficial adjunct in the detection of some fungal infections. A study of 456 autopsy cases, to include examination of premortem test results, found that blood culture testing detected fungal infections with a sensitivity of 8.3%, whereas Fungitell had a sensitivity of 78.0% and a specificity of 98.4% using the 80 pg/mL positivity cutoff, with an 86.7% positive predictive value and a 97.1% negative predictive value. Another study compared BG results among 36 healthy blood donors versus 15 candidemic and 25 bacteremic patients, finding a 93.3% sensitivity, 77.2% specificity, 51.9% positive predictive value, and 97.8% negative predictive value. The lower specificity and positive predictive value in this study were attributed to the high instances of false-positives in this group of bacteremic patients; some of which were determined to have false-positivity that could be linked to known causes other than bacteremia (discussed below). A multicenter prospective study comparing 170 patients without signs of invasive fungal infection and 163 patients with proven (142 patients) or probable (21 patients) invasive fungal infection (based on criteria from the European Organisation for Research and Treatment of Cancer and the Mycoses Study Group [EORTC-MSG]) found a 64.4% sensitivity, 92.4% specificity, 89% positive predictive value, and 73% negative predictive value. The researchers identified that sensitivity in the probable group was significantly less than that in the proven group, which they indicated may partly be due to a lack of actual invasive fungal infections in at least some of the probable patients. Furthermore, a number of patients with proven Cryptococcus sp, Mucor sp, and Rhizopus sp infections were included in the study, which are generally not detectable by Fungitell.

**CLINICAL UTILITY**

An important value of BG testing is its potential in prediction of fungal infections, with positivity appearing sometimes days before the clinical diagnosis is made. A prospective study examined serial BG testing in 283 patients with acute myeloid leukemia or myelodysplastic syndrome on antifungal prophylaxis. Researchers looked at serial BG testing from these patients and found that BG became positive a median of 10 days before the clinical diagnosis of proven or probable invasive fungal infection was determined, and the usual trend was that BG levels continued to rise after the initial positive result. Another study looked specifically at blood culture–negative, intra-abdominal candidiasis in patients in surgical intensive care units; BG testing was superior to Candida score and colonization index, and BG became positive a median of 5 days prior to culture-based diagnosis of intra-abdominal candidiasis. Furthermore, severe sepsis (91% versus 28%) and mortality (36% versus 6%) were significantly higher in patients with BG result of 400 pg/mL or more compared to those with a BG result of less than 400 pg/mL. Not surprisingly, it was also found that BG levels decreased in patients responding to therapy but continued to rise or remain elevated in patients who did not respond, a general characteristic that has been identified in other studies as well. A study of 100 patients with hematologic malignancies receiving chemotherapy who developed anti-biotic-unresponsive neutropenic fever found that measuring...
Table 1. Potential Causes of False Fungitell Resultsa

<table>
<thead>
<tr>
<th>Organisms known to contain little or no 1,3-β-D-glucan</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cryptococcus</em></td>
</tr>
<tr>
<td>Zygomycetes (Mucor spp, Rhizopus spp, Lichtheimia corymbifera)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Substances thought to cause false-positive Fungitell results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellulose membranes used in hemodialysis</td>
</tr>
<tr>
<td>Exposure to glucan-containing gauze</td>
</tr>
<tr>
<td>Products (such as blood or albumin, among others) filtered through glucan-containing filters</td>
</tr>
<tr>
<td>High triglycerides, hemoglobin</td>
</tr>
<tr>
<td>Some antibiotics, such as intravenous amoxicillin-clavulanic acid</td>
</tr>
</tbody>
</table>

a Although most fungi produce 1,3-β-D-glucan as part of cell wall synthesis, some organisms, such as *Cryptococcus* and the Zygomycetes species, produce little or none of this substance and, therefore, Fungitell results would likely be negative in these patients. Likewise, some substances are known to cause false-positive results.

BG at the beginning of neutropenia, rather than at onset of antibiotic-unresponsive neutropenic fever, would have identified invasive fungal infections 5 days sooner in 50% of the patients. The researchers also compared daily sampling versus every-other-day sampling, finding that daily sampling was not superior to every-other-day in this study.

As with many laboratory tests, the possibility of false-positivity and false-negativity must be considered when interpreting test results (Table 1). Numerous potential causes of false BG positivity have been reported and include hemodialysis with cellulose membranes; serosal exposure to gauze or other materials that contain glucans, such as during surgery; and administration of blood products, albumin, immunoglobulin, coagulation factors, or plasma protein fraction filtered through BG-containing filters. Additionally, high triglycerides, hemoglobin (hemolyzed samples), and bilirubin have been shown to interfere with the Fungitell assay. There are also reports that some antibiotics may cause false-positive results. One study in the Netherlands reported BG positivity in a group of patients treated with intravenous amoxicillin-clavulanic acid (not available in the United States), where BG turned positive when resampled 20 minutes after administration and became negative once discontinued. It was further noted that galactofuranose antigens were also elevated in the patients treated with this antibiotic, leading the researchers to conclude a likely fungal origin in the cross-reactive components found in the antibiotic. A blinded study examining 44 different intravenous antibiotics (not including intravenous amoxicillin-clavulanic acid) from specific manufacturers found that 7 (colistin, etrapenem, ceftazolin, trimethoprim-sulfamethoxazole, cefotaxime, cefepime, and ampicillin-sulbactam) were BG+ at reconstituted-vial concentrations but were not when diluted to usual maximum plasma concentrations. Interestingly, although all lyophilized ceftazolin vials were BG+, the premixed ceftazolin bags from a different manufacturer did not test BG+, further suggesting that cross-reactivity likely comes from contaminants during the manufacturing process rather than from the drug itself. The authors cautioned that the results should not be generalized to intravenous antibiotics from manufacturers outside of those used in the study and that, although the diluted antibiotics did not test positive in vitro, further research was needed to examine them in relation to in vivo kinetics. Separate studies looking specifically at administered intravenous ampicillin-sulbactam and piperacillin-tazobactam found no notable association to BG positivity in the patients who received these antibiotics. In another study, cefepime was again found to be strongly BG+ at the vial concentration but not when diluted to serum concentration.

There are also reports of BG positivity in patients with bacteremia, with indications that BG levels appear higher with gram-negative than gram-positive bacteremia. Some species of *Streptococcus*, *Escherichia coli*, and *Pseudomonas* have been shown to produce glucan or glucanlike polymers that could potentially interfere with the BG assay. However, a separate study of 83 bacteremia episodes in 71 patients found that 13 of the 14 patients (93%) with positive BG results had concurrent possible, probable, or proven invasive fungal infections based on EORTC-MSG criteria; additionally, the 14th patient with bacteremia had no indication of invasive fungal infections but had a BG value of 126 pg/mL that dropped to 29 pg/mL at the next measurement, indicating false positivity likely unrelated to the bacteremia. The authors of this study concluded that false-positivity associated with bacteremia does occur but is rare and cautioned that the culture-supernatant method used to determine the presence of glucan in these bacteria does not necessarily translate to BG positivity in vivo. Additionally, their reexamination of previous studies noted that the BG+ bacteremia found in those studies could often be linked to other known causes of false-positivity, such as hemodialysis, recent surgery, or hemolyzed serum. In a separate study, it was found that *Candida* spp colonization alone did not appear to cause BG positivity; however, compared with patients without mucositis, those with mucositis (such as from chemoradiation) were seen to have higher BG levels.

Some studies have also looked at variability and reproducibility of BG test results. For 15 patient samples, between-run coefficients of variance were found to range from 3.2% to 16.8% for BG concentrations ranging from 1643 pg/mL to 85 pg/mL, with the average being 9.1%. Within-run coefficients of variance were also examined for 4 samples, finding a range of 1.3% to 4.8% for BG concentrations of 2034 pg/mL to 181 pg/mL. The same study also looked at interlaboratory reproducibility, which found a correlation coefficient of 0.9892 between two laboratories. On a larger scale, interlaboratory reproducibility was found to have an r2 correlation value of 0.93 by another study. The BG cross-contamination was also examined in 3 negative specimens by measuring BG levels following each of 4 successive transfers to new transport tubes, finding that BG levels continually increased after each transfer. Another study tested 40 plastic blood-collection tubes using reagent-grade, glucan-negative water and found that 3 tubes had BG levels considered positive. The same researchers examined potential contamination by fungal colonization in central venous catheters in 37 patients by comparing BG levels in paired blood samples taken from central venous catheters versus peripheral venipuncture, finding that 3 central venous catheter samples were BG+; of these, 2 were also positive in the venipuncture samples of the same patients.

Results of multiple studies have indicated that BG levels are useful for diagnosing invasive fungal infections and can be beneficial in monitoring patient response to therapy. Persistently high levels have been associated with worse outcome than were levels that trended down with
treatment. The assay, however, is not without its drawbacks. Different causes of false-positivity have been reported and should be considered when evaluating test results. Despite the numerous causes of potential cross-contamination and false-positivity, this assay can be a valuable tool in guiding clinicians if interpreted and applied with caution. To minimize effects of cross-contamination, patient specimens should be run in, at least, duplicates and with known controls and concentrations for the calibration curve, and plate wells should be intentionally left empty in a strategic pattern to decrease risk of cross-contamination. Even with such stringent precautions taken during testing, however, the risk of contamination cannot be eliminated. On the other hand, although potential causes of false-positivity may be identified in a patient, that does not necessarily exclude a potential, concurrent, invasive fungal infection. The easy contamination and prevalent causes of false-positivity have made accurate examination of sensitivities and specificities difficult, but the overall assessment of the Fungitell assay has been favorable.

PROPOSED RECOMMENDATIONS FOR FUNGITELL USE

The frequency at which to repeat D-glucan tests has not been clearly established, and there have not been formal guidelines on ordering practices. Some choose not to repeat the test once it is known that a fungal infection exists, whereas others may order repeat testing as often as daily to monitor BG levels. Clinicians are often not privy to the day-to-day operations of a laboratory, and how often a test is actually performed in the laboratory can have an effect on the timing of clinicians receiving test results. Because of logistics, some tests are run in batches, rather than individually as the specimens are received. This is particularly true of tests that require multiple steps and incubation periods in between, where it would be time and labor prohibitive to perform testing on individual specimens at different times. Although a laboratory may be open 24 hours a day, certain tests may be performed only once every few days because specimens are accumulated and stored appropriately while awaiting testing. A clinician may order BG testing separately on consecutive days for the same patient, and the specimens may be batched together as they accumulate and the results reported together. Although BG results would not be available daily in some laboratories, a positive, unintended outcome of batching is a potential decrease in the variability within that group of BG levels from testing under the same conditions, reagent lot, medical technologist, and so on. Furthermore, although data are limited, at least one study has shown that daily testing does not appear to be superior to every-other-day testing. Although formal guidelines for the use of BG testing are lacking, some proposed recommendations are suggested (Table 2).

**Table 2. Proposed Recommendations for Fungitell Use**

<table>
<thead>
<tr>
<th>Recommendation</th>
<th>Source, y</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Testing should be used in conjunction with other methods of diagnosis</td>
<td>ACC, 12 2011</td>
</tr>
<tr>
<td>2. Confirm positive result with a second specimen</td>
<td>Hanson et al, 19 2012</td>
</tr>
<tr>
<td>3. Twice-weekly testing can be used for surveillance in at-risk patients</td>
<td>Hanson et al, 19 2012</td>
</tr>
<tr>
<td>4. Begin surveillance at the onset of neutropenia rather than when symptoms arise</td>
<td>Ellis et al, 22 2008</td>
</tr>
<tr>
<td>5. Avoid lipemic, hemolyzed, or icteric specimens</td>
<td>Pickering et al, 15 2005</td>
</tr>
<tr>
<td>6. Minimize manipulation of specimen to decrease risk of contamination</td>
<td>Pickering et al, 15 2005</td>
</tr>
</tbody>
</table>

**SUMMARY**

It has been repeatedly shown that BG can be a valuable adjunct to early diagnosis of fungal infections. Although BG levels can also be useful in monitoring patient response to therapy, a delicate balance is required to minimize overuse while, at the same time, maintaining optimal patient care. Repeat testing should be performed when it is beneficial for patient outcome and the inherent limitations of Fungitell testing taken into account. The test is not organism-specific and does not detect several types of fungal infections, so it should be used in conjunction with other forms of fungal testing. The prevalence of the causes of false-positivity need to be considered when evaluating BG results, although the culprit can sometimes be difficult to identify. Levels that do not come down or continue to trend upward despite antifungal therapy should be particularly concerning when causes of potential contamination are ruled out. 1,3-β-D-glucan testing can be a powerful tool when used, interpreted, and applied with care.

**References**


