Assay for (1→3)-β-D-Glucan in Serum

FUNGITELL® STAT

Instructions For Use

Rx only

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INTENDED USE

The Fungitell® STAT assay is a protease zymogen-based colorimetric assay for the qualitative detection of (1→3)-β-D-glucan in the serum of patients with symptoms of, or medical conditions predisposing the patient to, invasive fungal infection. The serum concentration of (1→3)-β-D-glucan, a major cell-wall component of various medically important fungi[1], can be used as an aid in the diagnosis of deep-seated mycoses and fungemias[2]. A positive result does not indicate which genus of fungi may be causing infection.

(1→3)-β-D-glucan index values should be used in conjunction with other diagnostic procedures, such as microbiological culture, histological examination of biopsy samples and radiological examination.

SUMMARY AND EXPLANATION

There is an increasing incidence of fungal infections by opportunistic pathogens, especially in immuno-compromised patients[3,4,5]. Invasive fungal diseases, as opportunistic infections, are common among hematological malignancy and AIDS patients and account for a growing number of nosocomial infections, particularly among organ transplant recipients and other patients receiving immunosuppressive treatments[4,7]. Many fungal diseases are acquired by inhaling fungal spores originating from the soil, plant detritus, air-handling systems and/or exposed surfaces. Some opportunistic fungi are present in/on human skin, the intestinal tract, and mucous membranes[4,8]. Diagnosis of invasive mycoses and fungemias is usually based on non-specific diagnostic or radiological techniques. Recently, biological markers of fungal infection have been added to the available diagnostic methods[2].

Opportunistic fungal pathogens include *Candida spp., Aspergillus spp., Fusarium spp., Trichosporon spp., Saccharomyces cerevisiae, Acremonium spp., Coccidioides immitis, Histoplasma capsulatum, Sporothrix schenckii, Exserohilum rostratum,* and *Pneumocystis jirovecii.* The (1→3)-β-D-glucan produced by these organisms, and others, can be detected by the Fungitell® STAT assay[3,10,11].

PRINCIPLE OF THE PROCEDURE

The Fungitell® STAT assay is a design modification to the Fungitell® assay format. The Fungitell® STAT assay was developed to answer the need for a single use test format and smaller kit size relative to the 96-well plate format of the Fungitell® assay.

The Fungitell® STAT assay provides a qualitative measurement of (1→3)-β-D-glucan. The assay is based upon a modification of the Limulus Amebocyte Lysate (LAL) pathway[12,13,14,15], **Figure 1**. The Fungitell® STAT Reagent is modified to eliminate bacterial endotoxin reactivity and, thus, to only react to (1→3)-β-D-glucan, through the Factor G-mediated side of the pathway. (1→3)-β-D-glucan activates Factor G, a serine protease zymogen. The activated Factor G converts the inactive pro-clotting enzyme to the active clotting enzyme, which in turn cleaves para-nitroanilide Boc-Leu-Gly-Arg-pNA, creating a chromophore, para-nitroaniline (pNA), that absorbs at 405 nm. The Fungitell® STAT kinetic assay, described below, is based upon the determination of the rate of optical density increase produced by a sample. This rate is compared to the rate of optical density increase of the Fungitell® STAT Standard to produce an index. This patient sample index value is qualitatively interpreted as a Negative, Indeterminate, or Positive result according to the index value ranges provided in **Table 1** below.

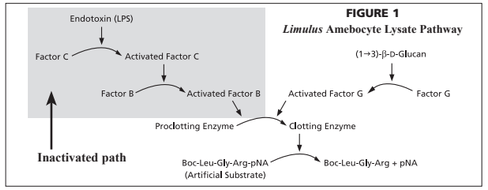


FIGURE 1
***Limulus* Amebocyte Lysate Pathway**

TABLE 1 FUNGITELL® STAT INDEX RANGES

Result	Index Value
Negative	≤ 0.74
Indeterminate	0.75 – 1.1
Positive	≥ 1.2

MATERIALS SUPPLIED WITH THE FUNGITELL® STAT PRODUCT

The Fungitell® STAT product is for *in vitro* diagnostic use. The following materials supplied with each product are sufficient for a total of 10 reactions (based on the 10 vials of Fungitell® STAT Reagent). Each product also contains 5 Fungitell® STAT Standard vials and thus can support up to five runs when 1 Fungitell® STAT Standard vial and 1 Patient Sample are tested per run. Alternatively, a single Fungitell® STAT Standard vial may be run with up to 9 patient samples.

1. Fungitell® STAT Reagent, a lyophilized (1→3)-β-D-glucan specific LAL (10 vials)
   Fungitell® STAT Reagent is free of interfering levels of (1→3)-β-D-glucan.
2. Fungitell® STAT Standard (5 vials), with the lot# specific reconstitution volume on the labeling
3. Instructions for Use
4. Quick Visual Guide

MATERIALS REQUIRED BUT NOT SUPPLIED

All materials must be free of interfering glucan. Glassware must be dry-heat depyrogenated for at least 7 hours at a minimum of 235°C (or a validated equivalent) to be considered suitable for use.

1. LAL Reagent Water* (5.5 mL vial, catalog # W0051-10)
2. Alkaline Pretreatment Solution 0.125 M KOH and 0.6 M KCl * (2.5 mL vial, catalog #APS51-5)
3. Pipettes capable of delivering 20-200 µL and 100-1000 µL volumes
4. Pipette tips* (250 µL catalog # PPT25 and 1000 µL catalog # PPT10)
5. Long Pipette tips* (20-200 µL, catalog # TPT50)
6. Test tubes* for patient sample preparation and combining serum pretreatment solution. (12 x 75 mm, catalog # TB240-5)
7. Tube reader and kinetic assay software
   a) An automated Lab Kinetics Incubating 8-well Tube Reader (PKF08 instrument) and Beta Glucan Analytics (BG Analytics™) software supplied by Associates of Cape Cod, Inc. (ACC) catalog # PKF08-PKG** **or**
   b) Incubating (37°C) tube reader capable of reading at 405 nm and 495 nm with a range of at least 0 – 1.0 Absorbance Units and to accommodate vials of 12 mm diameter; coupled with appropriate computer-based kinetic assay software capable of observing and analyzing reaction kinetics as well as supporting the review of the criteria listed in the Quality Control section of the IFU.
8. Sterile, glucan-free, screw-cap storage tubes for aliquotting samples (most tubes that are certified to be RNAse, DNAse, and pyrogen-free are free of interfering levels of (1→3)-β-D-glucan).
9. Parafilm®

**These products, supplied by Associates of Cape Cod, Inc. (ACC), are certified free of interfering glucans.*

***Hard copies of both the BG Analytics™ Software and PKF08 Instrument User Manuals can be downloaded from the ACC website: www.acciusa.com.*

Caution - glass pipettes with cotton plugs and micro-pipette tips with cellulosic filters are potential sources of glucan contamination.

WARNINGS AND PRECAUTIONS

This product is for IN VITRO DIAGNOSTIC USE.

The Fungitell® STAT assay requires rigorous attention to technique and the testing environment. Thorough training of the technician in the assay method and in the avoidance of contamination is critical for the effectiveness of the assay.

1. Certain fungal species produce very low levels of (1→3)-β-D-glucan and are not usually detected by the Fungitell® STAT assay. These include the genus Cryptococcus[16,17] well as Mucorales such as Absidia, Mucor and Rhizopus[1,17]. In addition, Blastomyces dermatitidis, in its yeast form, produces low levels of (1→3)-β-D-glucan and is therefore not usually detected by the Fungitell® STAT Reagent[18].
2. Do not pipette any material by mouth. Do not smoke, eat or drink in areas where specimens or kit reagents are handled. Follow company and local safety regulations.
3. Establish a clean environment in which to perform the assay. Use materials and reagents that are certified to be free of detectable background levels of (1→3)-β-D-glucan. Note that glucan as well as fungal contamination from the human body, clothes, containers, water and airborne dust may cause interference with the Fungitell® STAT assay. Cellulosic materials such as gauze, paper wipes, and cardboard may contribute (1→3)-β-D-glucan to the environment where the assay is performed.
4. Do not use materials beyond their expiry date.
5. Off-color or turbid samples such as those that are grossly hemolyzed, lipemic, or contain excessive bilirubin may cause optical interference with the assay. If such samples are tested, test results should be examined for evidence of optical interference and/or unusual kinetic patterns.
6. Use suitable protective clothing and powder free gloves when handling patient specimens.
7. The serum of hemodialysis patients may contain high levels of (1→3)-β-D-glucan when certain cellulose dialysis membranes are used[19,20,38]. Hemodialysis with cellulose triacetate, polysulfone membrane or polymethyl methacrylate membranes does not appear to interfere with the assay.
8. Surgical gauzes and sponges can leach high levels of (1→3)-β-D-glucan that may contribute to a contamination-based transient positive result for the Fungitell® assay as has been observed in post-surgical patients[21,22].
9. Blood fractionation products such as intravenous immunoglobulin and albumin may also have burdens of (1→3)-β-D-glucan which, if injected or infused, will elevate serum (1→3)-β-D-glucan titers for a number of days[23].
10. Products with damaged contents should not be used.
11. Materials exposed to potentially contaminated (pathogen-containing) fluids must be disposed of in a manner consistent with local regulation.

REAGENT STORAGE

Store all reagents, as supplied, at 2-8°C in the dark. Fungitell® STAT Reagent and Fungitell® STAT Standard should be used within 1 hour following reconstitution.

SPECIMEN HANDLING

1. Specimen Collection: Blood samples may be collected in sterile serum preparation tubes or serum separator tubes (SST) for the preparation of serum.
2. Specimen Storage: Serum samples can be stored temporarily at 2-8°C before assay, or frozen at -20°C or colder for longer term storage.
3. Specimen Labeling: Specimens should be clearly labeled according to the approved practices of the institution.

PROCEDURE

A Quick Visual Guide with a summary of the automated PKF08 instrument and BG Analytics™ software Procedure is also included in the Fungitell® STAT product package.

A number of the steps that are described in the Procedure below are automated when using the PKF08 instrument and BG Analytics™ software and including: Instrument setting, Quality Control and Interpretation of results. Please refer to the BG Analytics™ Software User Manual or contact the manufacturer for additional information.

Note:

- *Use good laboratory practices according to your local regulations. This assay is sensitive to contamination and pipetting inaccuracy.*
- *It is recommended to perform Steps 3-5 and 7 within a biological safety cabinet to increase the operator safety while working with patient samples and to reduce the potential for contamination by environmental (1→3)-β-D-glucan during the procedure.*
- *To reduce unnecessary glass vial movements in and out of the biological safety cabinet, it is recommended to bring the vortex device within the biological safety cabinet (as long as the critical airflow is maintained).*
- *It is recommended to use long pipette tips to help prevent cross-contamination between vials.*
- *A Fungitell® STAT Standard (red cap and red line label) should always be processed under the same conditions and at the same time as the patient sample(s) within a run. This is critical since the outcome of the assay is an Index (sample/standard) of the kinetic reaction rates (or slopes, OD/sec) from the Patient sample and the Fungitell® STAT Standard.*
- *It is recommended to use 2 tube racks during the procedure, one for the sample preparation vials (Steps 4-6) and one for the reagent vials (Steps 7-8). This will help prevent the potential for vial mix-up and cross contamination during the procedure.*
- *It is recommended to place the Fungitell® STAT Standard at a defined and consistent position within the tube rack, incubator and reader. When using the PKF08 instrument and BG Analytics™ software, use the first well on the left which is labeled "Standard".*
- *At the end of each mixing step, visually confirm that the solution is homogeneously mixed.*
- *Do not over mix the Fungitell® STAT Reagent. A maximum setting of 2000 RPM is recommended for any vortex device. Do not vortex for more than 5 seconds.*

1. Instrument setting

Settings may vary with different instruments and software. In general, the following conditions should be met: The instrument should be able to achieve and hold a temperature of 37°C±1°C. The instrument and software must be able to read optical density over time (kinetic) at two wavelengths. Specifically, these wavelengths should be set to 405 nm and 495 nm. Set the kinetic mode to a read period of 40 minutes (2400 seconds). Set the kinetic read interval to the minimum allowed by the software/instrument over the 40-minute period of the test and to initiate read upon sample insertion. Check the software manual to determine how to calculate a rate (slope) measurement from the data set. For the purposes of this test, this is generally achieved by executing a linear regression on the kinetic data over the time frame suggested. Set the linear regression calculation to execute over the range between 1900 and 2400 seconds using the "slice" function of the software. Reading should commence without any lag time.

2. Confirm Fungitell® STAT Standard Lot# specific information

a. The Lot# specific reconstitution and pretreatment solution volumes are found on the Fungitell® STAT Standard package label, on the Fungitell® STAT product Certificate of Analysis, and available on the ACC website. This information will be required to complete the Step 5 below.
b. It is recommended to note the specific Lot# information within the Quick Visual Guide provided with the Fungitell® STAT product before starting the procedure.

Note: *Each product (Fungitell® STAT Standard and Fungitell® STAT Reagent pair) is tested and released independently. Thus, it is important to note and use the Lot# specific information for each pair.*

3. Label tubes

a. Label one empty tube for each patient sample to be tested.
b. Label one Fungitell® STAT Reagent tube for each patient sample to be tested.
c. Label one Fungitell® STAT Reagent tube for the Fungitell® STAT Standard.

4. Prepare patient sample tubes

a. Vortex patient samples for at least 20 seconds to ensure homogeneity.
   Note: *The freezing process can produce sample heterogeneity due to water abstraction to the growing ice crystal, thus excluding solutes.*
b. To the appropriate labeled empty tube, add the patient sample and Alkaline Pretreatment Solution in a ratio of 1:4. The recommended volumes are 50 µl of patient sample and 200 µl of Alkaline Pretreatment Solution.
   Note: *The Alkaline Pretreatment Solution converts triple-helix glucans into single-stranded glucans[14,15] which are more reactive in the assay. Additionally, the alkaline pH serves to inactivate serum proteases and inhibitors that can interfere with the assay[24].*
c. Vortex for 15 seconds and cover.

5. Prepare Fungitell® STAT Standard tube

a. Reconstitute one vial of the Fungitell® STAT Standard with the Lot# specific volume of LAL Reagent Water and vortex for 15 seconds.
b. Add the Lot# specific volume of Alkaline Pretreatment Solution.
   Note: *The Lot# specific reconstitution and pretreatment solution volumes are stated on the Fungitell® STAT Standard package label, on the Fungitell® STAT product Certificate of Analysis, and are available on the ACC website.*
c. Vortex for 15 seconds and cover.

6. Pretreatment Incubation in tube reader

Incubate the patient sample tubes (from Step 4) and the Fungitell® STAT Standard vial (from Step 5) for 10 minutes at 37°C.

7. Prepare Fungitell® STAT Reagent tubes

a. Reconstitute each of the Fungitell® STAT Reagent vials (labeled in Step 3 above) with 300 µl of LAL Reagent Water.
b. Vortex gently for no more than 5 seconds.
   Note: *The Fungitell® STAT Reagent contains a number of active proteins required for the assay and it is recommended to gently handle the solution. A maximum setting of 2000 RPM is recommended for any vortex device. Do not over mix.*
c. At the end of the pre-incubation treatment:
   - Transfer 75 µl of each patient sample solution into its corresponding Fungitell® STAT Reagent tube.
   - Transfer 75 µl of Fungitell® STAT Standard into its corresponding Fungitell® STAT Reagent tube.
   - Vortex all tubes for no more than 5 seconds and cover.

8. Start the run

a. Insert the tubes into tube reader while confirming that each one is in the intended well.
b. Start the kinetic reading for a period of 40minutes, at 37°C.

9. Review the Quality Control criteria

See Quality Control section below and ***Figure 2****.*

10. Interpretation of results

See Interpretation of Results section below and ***Figure 3****.*

END-OF-PROCEDURE VIAL DISPOSAL

- It is recommended to discard the open Alkaline Pretreatment Solution and LAL Reagent Water vials in accordance with your laboratory procedures. Do not use these materials for more than one run to avoid potential contamination.
- As part of the product manufacturing, the Fungitell® STAT Reagent and Fungitell® STAT Standard are released as a paired Lot and because of this, Fungitell® STAT Reagent and Fungitell® STAT Standard components from different product Lots should not be used. Therefore, when all the Fungitell® STAT Reagent vials within a package have been used, it is recommended to discard the remaining vials, if any, of the Fungitell® STAT Standard.

QUALITY CONTROL

- **For all well numbers,** confirm Fungitell® STAT Standard or Sample # assignment
- **For the Fungitell® STAT Standard result,**
  1. the correlation coefficient (r) must be ≥ 0.980 and
  2. the slope must be within the expected slope range of 0.00010 – 0.00024 OD/second.

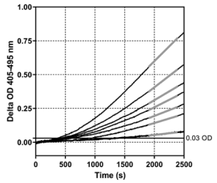
  If the Fungitell® STAT Standard result does not meet criteria #1 and #2, the run is invalid and all samples have to be run again.
- **For all patient sample results:**
  - **A. Determine if the result is out of the assay index range**
    - The result is likely out-of-range on the positive side if:
      - The Y intercept is positive and
      - The kinetic curve passes 0.4 OD before 1000 seconds.
    - The result is likely out-of-range on the negative side if:
      - The kinetic curve is positive after 500 seconds and
      - Has an OD >0.03 and <0.07 at the end of the test.

  If the Sample result meets both criteria for either the positive or negative out-of-range, the general QC criteria below do not need to be completed, and the index value should ***not*** *be calculated. All out of range results on the positive side should be reported as "Positive" and all out of range results on the negative side should be reported as "Negative".*

  - **B. If the result does not meet the out-of-range criteria, verify the general QC:**
    1. the kinetic curve must be positive after 500 seconds,
    2. the kinetic curve must have an OD ≥ 0.03 at the end of the test,
    3. the slope must be numerically positive,
    4. the correlation coefficient (r) must be ≥ 0.980 and
    5. the kinetic curve must have an upward increasing curve shape consistent with examples presented in **Figure 2**.

If the Sample result does not meet all general QC criteria #1-5, the sample result is invalid and the sample has to be tested again. Alternatively, a different method should be used.

FIGURE 2
EXAMPLES OF APPROPRIATE KINETIC CURVE SHAPES



Control samples (negative, near the assay cutoffs, or at highly positive levels) may be run to verify that the reagents and the assays are performing properly. Each user of the test should establish a quality control program to assure proficiency in the performance of the test in accordance with the regulations applicable to their location.

INTERPRETATION OF RESULTS

The Fungitell® STAT test results should be used as an aid in the diagnosis of invasive fungal infection. The patient sample and Fungitell® STAT Standard rates are derived from calculating the slope (rate) between the 1900 and 2400 from the delta OD 405 - 495 nm results. The Fungitell® STAT index results are derived by dividing the rate (slope) of the patient sample by the rate (slope) of the Fungitell® STAT Standard (***see Figure 3***). The index results range from approximately 0.4 to 3.5, covering the full Standard curve (31 – 500 pg/mL) of the Fungitell® predicate. Fungitell® STAT index values should be interpreted as described below:

NEGATIVE RESULT

Index values ≤ 0. 74 are interpreted as negative results.

INDETERMINATE RESULT

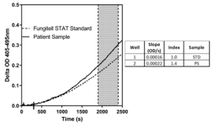
Index values from 0.75 to 1.1 suggest a possible fungal infection. Additional sampling and testing of sera is recommended. Frequent sampling and testing improve the utility for diagnosis.

POSITIVE RESULT

Index values ≥ 1.2 are interpreted as a positive result. A positive result means that (1→3)-β-D-glucan was detected. A positive result does not define the presence of disease and should be used in conjunction with other clinical findings to establish a diagnosis.

The laboratory performing the test should inform the ordering physician that not all fungal infections result in elevated levels of serum (1→3)-β-D-glucan. Some fungi, such as the genus Cryptococcus[16,17] produce very low levels of (1→3)-β-D-glucan. *Mucorales*, such as *Absidia, Mucor* and *Rhizopus*[1,17] are not known to produce (1→3)-β-D-glucan. Similarly, *Blastomyces dermatitidis,* in its yeast phase, produces little (1→3)-β-D-glucan, and blastomycosis patients usually have undetectable levels of (1→3)-β-D-glucan in the Fungitell® STAT assay[18].

FIGURE 3
EXAMPLE OF FUNGITELL® STAT KINETIC CURVES AND DATA ANALYSIS



The region highlighted in grey is the area of the slope determination (1900 to 2400 seconds (s)), the solid line is an example Patient sample (PS) and the dashed line is the Fungitell® STAT Standard (STD). The slope of the sample (i.e. 0.00022 OD/s) divided by the slope of the 80 pg/mL Fungitell® STAT Standard (i.e. 0.00016 OD/s) leads to an Index of 1.4 for the sample. Slope and rate are synonymous in this application.

LIMITATIONS OF THE TEST

1. The tissue locations of fungal infection[10], encapsulation, and the amount of (1→3)-β-D-glucan produced by certain fungi may affect the serum concentration of this analyte. Reduced ability to contribute (1→3)-β-D-glucan to the bloodstream can reduce the ability to detect certain fungal infections. *Cryptococcus spp.* produce low levels of (1→3)-β-Dglucan[16,17]. *Mucorales*, including *Absidia spp., Mucor spp.* and *Rhizopus spp.* are not known to produce (1→3)-β-D-glucan[1,17] *Blastomyces dermatitidis*, in its yeast phase, produces little (1→3)-β-D-glucan, and test results are usually negative[18]. This information should be provided to the requesting physician.

2. Some individuals have (1→3)-β-D-glucan index values that fall into the indeterminate zone. In such cases, additional surveillance testing is recommended.

3. The frequency of patient testing will depend upon the relative risk of fungal infection. Sampling rates of at least two to three times per week are recommended for at risk patients.

4. Positive results have been found in hemodialysis patients[19,20], subjects treated with certain fractionated blood products such as serum albumin and immunoglobulins and in specimens or subjects exposed to glucan-containing gauze and surgical sponges. Patients require 3 – 4 days for the restoration of baseline levels of serum (1→3)-β-D-glucan, after surgical exposure to (1→3)-β-D-glucan containing sponges and gauze[21,22]. Accordingly, the timing of sampling of surgical patients should take this into account.

5. Samples obtained by heel or finger stick methods are unacceptable as the alcohol-soaked gauze used to prepare the site (and, potentially, the skin surface-pooling of blood) has been shown to contaminate the specimens. In studies to date, no differences have been observed between samples obtained by line draws or venipuncture[25,26].

6. Test levels were established in adult subjects. Infant and pediatric normal and cut-off levels are under investigation[27,28].

INTERFERING SUBSTANCES

The following sample conditions can interfere with an accurate Fungitell® STAT assay result:

- Hemolysis
- Sample turbidity caused by lipemia
- The presence of visually apparent bilirubin
- Turbid serum
- Elevated levels of Immunoglobulin G, such as may exist in the serum due to multiple melanomas, may result in precipitation in the reaction mixture upon the addition of Fungitell® STAT to the pre-treated serum[29].

EXPECTED VALUES

A multi-center, prospective study conducted to determine the performance characteristics of the Fungitell (predicate) assay found that β-glucan values are elevated in a variety of fungal infections. When signs and symptoms are present at the 80 pg/mL level or greater, the predictive value that the subject is positive for a fungal infection ranges from 74.4 to 91.7%. In the absence of signs and symptoms at less than 60 pg/mL, the negative predictive values ranged from 65.1% to 85.1%.

The Fungitell® STAT β-glucan index values ≥ 1.2 are interpreted as a positive result in alignment with the Fungitell® predicate product's 80 pg/mL cutoff while index values ≤ 0. 74 are interpreted as negative results in alignment with the Fungitell® predicate product's 60 pg/mL cutoff.

PERFORMANCE CHARACTERISTICS

METHOD COMPARISON TESTING

De-identified, frozen patient serum samples collected for routine clinical care of the intended population and received at Beacon Diagnostics® Laboratory, Inc for Fungitell® predicate testing were used for the purpose of the method comparison study. Beacon Diagnostics® Laboratory, Inc is a licensed Clinical Laboratory Improvement Amendments (CLIA) laboratory part of ACC. A population of 488 de-identified patient serum samples was included in the study with (1→3)-β-D-Glucan concentrations distributed over the full range of the Fungitell® predicate standard curve. These included 309 samples that fell within the Negative zone of the Fungitell® predicate test results, 143 samples that fell within the Positive zone of the Fungitell® predicate, and 36 samples that fell within the Indeterminate zone of the Fungitell® predicate (**Table 2**). All samples were tested with both the Fungitell® STAT and Fungitell® assays during this study. When samples falling within the Indeterminate zone of the Fungitell® STAT were excluded from analysis, there were 290 samples remaining for the negative percent agreement analysis and 119 samples remaining for positive percent agreement analysis

TABLE 2
FUNGITELL® STAT PERFORMANCE COMPARED TO FUNGITELL®

		Fungitell® Predicate			
		Negative	Indeterminate	Positive	Total
Fungitell® STAT	Negative	283	17	1	301 (61.7%)
	Indeterminate	19	17	24	60 (12.3%)
	Positive	7	2	118	127 (26.0%)
	Total	309 (63.3%)	36 (7.4%)	143 (29.3%)	488 (100%)
		NPA: 97.6%* (283/290) 95% CI: (95.4, 99.9)		**PPA:** 99.2%* (118/119) 95% CI: (95.4, 99.9)	

**Indeterminate (i.e., equivocal) results not included in analysis; if all indeterminate results are considered discordant results (e.g., false positive or false negative), performance is as follows: PPA - 73.8% (118/160), 95% CI: (66.4%, 80.0%); NPA - 91.0% (283/311), 95% CI: (87.3%, 93.7%)*

NEGATIVE PERCENT AGREEMENT

Two hundred eighty-three (283) of the 290 samples that were negative when tested with the Fungitell® predicate device were also negative with the Fungitell® STAT assay. The calculated negative percent agreement (NPA) with the predicate method was 97.6% (95% Confidence Interval: 95.4%, 99.9%) (**Table 2**)

POSITIVE PERCENT AGREEMENT

One-hundred eighteen (118) of the 119 samples that were positive when tested with the Fungitell® predicate device were also positive with the Fungitell® STAT assay. The calculated positive percent agreement (PPA) with the Fungitell® predicate method was 99.2% (95% Confidence interval: 95.4%, 99.9%) (**Table 2**).

REPRODUCIBILITY STUDY

The Fungitell® STAT was evaluated for reproducibility by spiking human serum with Saccharomyces cerevisiae (1→3)-β-D-Glucan to produce a five-member panel consisting of a low negative sample, high negative sample (just below the lower cut-off of 0.74), indeterminate (equivocal) sample, low positive sample (just above the upper cut-off of 1.2) and high positive sample (~2x above the upper cut-off of 1.2). The panel was distributed to three CLIA laboratories for testing with the Fungitell® STAT assay. Each laboratory provided 150 data points (i.e. 5 samples x triplicate per run x two operators performing a run per day x 5 days) for a total of 450 data points. The mean study Index values presented in **Table 3** below are derived from the data provided by the three laboratories. The Percent Positive column represents the percentage of samples for a given panel member that fell within the Positive zone. Among all three laboratories, the Percent Positive results were 1.1% for the Low Negative sample, 0% for the High Negative sample, 3.3% for the Indeterminate sample, 96.7% for the Low Positive sample and 100% for the High Positive samples.

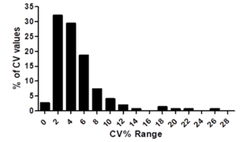
TABLE 3 REPRODUCIBILITY STUDY RESULTS

Panel Member	Mean Index	Standard Deviation	% CV	Percent Positive (Number pos./Number tested)
Low Negative	0.55	0.10	20.4%	1.1% (1/90)
High Negative	0.75	0.08	11.1%	0% (0/90)
Indeterminate	0.94	0.10	11.1%	3.3% (3/90)
Low Positive	1.6	0.30	18.7%	96.7% (87/90)
High Positive	2.6	0.40	15.4%	100% (90/90)

PRECISION

The intra-assay variation (i.e. %CV) ranged from 0.4% to 26.8% and the Inter-assay values ranged from 11 to 20.4%. Regarding the Intra-assay variation range, the distribution of the % CV range is presented below in **Figure 4**. Overall, 94% of CV values were 10% or less and 75% of CV values were 6% or less.

FIGURE 4
DISTRIBUTION OF INTRA-ASSAY % CV VALUES



META-ANALYSES

In addition, numerous peer-reviewed studies have been published on the subject of serum (1→3)-β-D-glucan-based support for invasive fungal disease diagnosis, including meta-analyses of diagnostic performance[30,31,32,33,34,35,36,37].

SYMBOLS LEGEND

	"Use By"		"Temperature Limitation"
	"Contains Sufficient For 'N' Tests"		"Manufacturer"
LOT	"Batch Code"		"Consult Instructions For Use"
IVD	"In Vitro Diagnostic Medical Device"	EC REP	"Authorised Representative"
REF	"Catalogue No."	CE	"CE Mark"

℞only "For prescription use only"

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