Assay for (1→3)-β-D-Glucan in Serum

FUNGITELL STAT®

Instructions For Use

Rx only

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IVD

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Visit www.fungitell.com for instructions for use in your language.

1. 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.
This product is for In Vitro Diagnostic Use and Professional Use only.

2. 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[6,7]. 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[8,9]. 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[1,5,10,11].

3. Principle of the Procedure

The Fungitell STAT® (cat# FT007, Associates of Cape Cod, Inc.) assay is a design modification to the Fungitell® (cat# FT001, Associates of Cape Cod, Inc. or ACC) assay format. The Fungitell STAT® assay (2019 CE-marked device) 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® (USA predicate and 2008 CE-marked device) 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 the 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 patient serum sample. This rate is compared to the rate of optical density increase of the Fungitell STAT® Standard to produce an index. The Fungitell STAT® Standard is calibrated at 80 +/- 8 pg/mL which is the Positive cut-off for the Fungitell® assay. This patient serum sample index value is qualitatively interpreted as a Negative, Indeterminate, or Positive result according to the index value ranges provided in **Table 1** below.

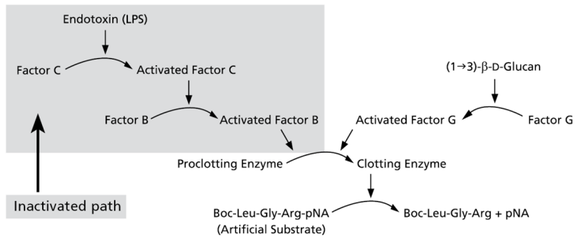


Figure 1. *Limulus* Amebocyte Lysate Pathway

Table 1. Fungitell STAT® Index Ranges

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

4. 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 tubes of Fungitell STAT® Reagent). Each product also contains 5 Fungitell STAT® Standard tubes.

1. Fungitell STAT® Reagent, a lyophilized (1→3)-β-D-glucan specific LAL (10 tubes)
   The Fungitell STAT® Reagent is composed of Limulus (i.e., horseshoe crab) Amebocyte Lysate, Boc-Leu-Gly-Arg-pNA colorimetric substrate and Tris buffer. It does not contain human or mammalian proteins. Fungitell STAT® Reagent is free of interfering levels of (1→3)-β-D-glucan.
2. Fungitell STAT® Glucan Standard (5 tubes) lyophilized (1→3)-β-D-glucan.
   The Fungitell STAT® Glucan Standard is composed of D-lactose and (1→3)-β-D-glucan derived from *Saccharomyces cerevisiae yeast extract.*
   Internal control: The Fungitell STAT® Standard (1→3) β-D-glucan concentration is calibrated to the positive limit value of the Fungitell® product (USA predicate and CE mark 2008) and against an internal reference standard. The Fungitell STAT® Standard, contains a known amount of glucan. The resulting values are described in the Quality Control section and serve as internal control for the Fungitell STAT® assay.
3. Instructions for Use
4. Quick Visual Guide

5. Materials Required but not Supplied

All materials must be free of interfering glucan.

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) PKF08 Incubating 8-Well Tube Reader (PKF08-1, Lab Kinetics, LLC)** with Beta Glucan Analytics (BG Analytics® or BG Analytics® Software), BG Analytics® Software Manual and BG Analytics® System Verification Protocol** (BGA007, Associates of Cape Cod, Inc.).
   The PKF08 device and BG Analytics® software are supplied by Associates of Cape Cod, Inc. (catalog# PKF08-PKG**). The PKF08-PKG has been validated for use with the Fungitell STAT® test. **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, coupled with appropriate computer-based kinetic assay software capable of 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, tubes for aliquoting samples. Tubes that are certified to be RNAse, DNAse, and pyrogen-free can be used.
9. Parafilm®

** These products, supplied by Associates of Cape Cod, Inc. (ACC), are certified free of interfering glucans.*
***User Manuals can be downloaded from ACC website: www.fungitell.com.*

6. Reagent Storage

- Store the kit, as supplied in the original packaging, at 2-8°C away from sunlight
- Fungitell STAT® Reagent and Fungitell STAT® Standard are designed to be used up to 1 hour after reconstitution.

7. ⚠ *Warnings and Precautions*

- Do not pipette any material by mouth. Do not smoke, eat or drink in areas where specimens or kit reagents are handled.
- Follow operational and local safety regulations.
- Wear protective gloves when handling biological samples that may be infectious or dangerous. The gloved hands should be considered contaminated at all times; keep your gloved hands away from your eyes, mouth and nose. Wear an eye guard and surgical mask if there is a possibility of aerosol contamination.
- Products with damaged contents should not be used.
- Disposal: Residues of chemicals and preparations are generally considered to be hazardous wastes. The disposal of this type of waste is regulated by national and regional laws and regulations. Contact your local authorities or waste management companies for advice on the disposal of hazardous waste.
- **The Safety data sheets** for the Fungitell STAT® Reagent, Fungitell STAT® Standard, LAL Reagent Water and Alkaline Pretreatment Solution can be downloaded from ACC website: www.acciusa.com.

7.1 Procedural Precautions

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.

- Establish a clean environment in which to perform the assay.
- Note that glucan as well as fungal particles contamination from the human body, clothes, containers, water and airborne dust may cause interference with the Fungitell STAT® test.
- Possible sources of contamination include cellulose-containing materials such as gauze, paper wipes and cardboard, glass pipettes with cotton plugs and pipette tips with cellulose filters. Surgical gauze bindings and sponges can also secrete high amounts of (1→3)-β-D-glucan[21,22]. For other patient-related sources of contamination, see the Limitations section of the test.
- Use the open vials with alkaline pretreatment solution and LAL reagent water immediately and if potential contamination is a concern, do not re-use these materials.
- The Fungitell STAT® Reagent and the Fungitell STAT® Standard are released as a paired batch. For this reason, no Fungitell STAT® Reagent and Fungitell STAT® Standard components from other product batches should be used. Therefore, it is recommended to dispose of any remaining Fungitell STAT® Standards as soon as all Fungitell STAT® Reagent tubes contained in a package have been used up.
- Do not use materials beyond their expiry date.

7.2 Specimen Handling

- Blood collection and preparation of serum shall be carried out in accordance with applicable local regulations. Specimen Collection: Blood samples may be collected in sterile serum preparation tubes or serum separator tubes (SST) for the preparation of serum.
- Specimen Storage: Serum samples can be stored at 2-8°C for up to 15 days, or frozen at -20°C for up to 27 days or -80°C for up to 4 years.
- Specimen Labeling: Specimens should be clearly labeled according to the approved practices of the medical institution (laboratory).

7.3 Notes on Testing:

- Use good laboratory practices according to your local regulations. This assay is sensitive to contamination and pipetting inaccuracy.
- In order to ensure the safety of the operator while working with serum samples and to reduce the potential for contamination by (1→3)-β-D-glucan from the environment during the process, it is recommended to work in a biological safety cabinet.
- 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 separate tube racks during the procedure, one for the sample preparation tubes and one for the reagent tubes. to avoid confusion and cross contamination.
- It is recommended to place the Fungitell STAT® Standard at a defined and consistent position within the tube rack, incubator and reader. In the PKF08 Reader, 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.

8. Procedure

The Fungitell STAT® product contains a Quick Visual Guide with illustrations and a summary of the features of the PKF08 instrument and BG Analytics® software.

The following procedures are already preset when using the PKF08 device and the BG Analytics® software: Device setting, evaluation of results and quality control. For more information, see the BG Analytics® Software User Manual or contact the manufacturer.

8.1 Instrument setting and test programming

8.1.1 When using PKF08 with BG Analytics® Software:
Turn on the device and follow the instructions of the BG Analytics® software. For detailed information, see the BG Analytics® manual.

8.1.2 When using another instrument and software, the following conditions should be met:
a. The instrument should be able to achieve and hold a temperature of 37°C±1°C.
b. The instrument and software must be able to read optical density over time (kinetic mode) at two wavelengths. Specifically, these wavelengths should be set to 405 nm and 495 nm.
c. Set the kinetic mode to a read length of 40 minutes (2400 seconds). Set the kinetic read interval to the minimum allowed by the software/instrument.
d. The measurement should be initiated immediately upon sample insertion.
e. Refer to 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.

8.2 Label tubes

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

8.3 Prepare patient serum sample

a. Vortex patient serum 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. Add the patient serum sample and Alkaline Pretreatment Solution in a ratio of 1:4 in the corresponding labeled empty tube. 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.

8.4 Prepare Fungitell STAT® Standard

***Note:** Each product (Fungitell STAT® Standard and Fungitell STAT® Reagent pair) is tested and released independently. Thus, it is important to use the Lot# volumes of reconstitution and Alkaline Pretreatment Solution. These can be found on the Fungitell STAT® Standard package label, on the Fungitell STAT® product Certificate of Analysis, and available on the ACC website. Recommendation: Before starting the test, write down this information on the supplied Quick Visual Guide.*

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.
c. Vortex for 15 seconds and cover.

8.5 Pretreatment Incubation in tube reader

Incubate the patient serum sample tubes (from Step 8.3) and the Fungitell STAT® Standard vial (from Step 8.4) for 10 minutes at 37°C.

Note: When using the PKF08 instrument, on inserting a tube into a well, an indicator turns from red to green. Push the tube fully in until the indicator turns green.

⚠ Caution, the tubes are fragile. In case of penetration of shards of glass and liquids into a measuring station of the PKF08, contact Associates of Cape Cod, Inc. Technical Service.

8.6 Prepare Fungitell STAT® Reagent tubes

a. Reconstitute each of the Fungitell STAT® Reagent vials (labeled in Step 8.2 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:
   - Transfer 75 µl of each patient serum 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.

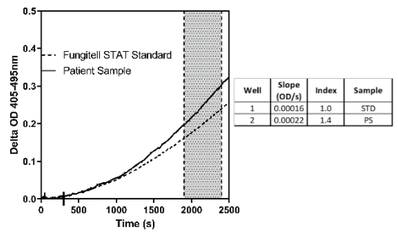
8.7 Start the run

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 40 minutes, at 37°C.

9 Calculate the results

9.1 Measuring Principle

The results of the Fungitell STAT® test should be used as an aid in the diagnosis of an invasive fungal infection. The standard rates of the patient sample and Fungitell STAT® are derived from the calculation of the slope (rate) between 1900 and 2400 from the delta OD 405 - 495 nm results. The results of the Fungitell STAT® index are obtained from the division of the slope of the patient sample by the slope of the Fungitell STAT® Standard (see Figure 2).



Well	Slope (OD/s)	Index	Sample
1	0.00016	1.0	STD
2	0.00022	1.4	PS

Figure 2. 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), 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.

9.2 When using PKF08 with BG Analytics® software:

a. The review of the quality criteria is carried out automatically by the software. The result is displayed in the final report.
b. For valid test runs, the BG Analytics® software determines an index value for each sample, or assigns a clear negative or positive result to the sample.
c. If the software shows any indications of invalid parameters in the results evaluation, follow the instructions in the BG Analytics® Software Manual.

9.3 When using other software:

Verify that all Quality Criteria are met.

10. Quality Control

Listed below are the quality control criteria for the Fungitell STAT® Standard and patient serum sample results, including examples of expected kinetic curve shapes. These quality control criteria were validated during the studies presented in the Performance Features section.

- **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 must be re-prepared and tested.

- **For all patient sample results do the following:**
  A. Determine if the result may be outside the measurement range of the test
  - 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 ≥ 0.00 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 above criteria do not apply, verify the general QC:
  1. the kinetic curve must be positive after 500 seconds,
  2. the kinetic curve must have an OD ≥ 0.00 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 3**.

  If the Sample result does not meet general QC criteria #1, 3-5, the sample result is invalid and the sample has to be tested again. Alternatively, a different method should be used.
  If the Sample result does not meet QC criteria #2, this suggests that the sample signal is low. In that case, the user should carefully review the provided curve in context and determine the validity of the results based on the laboratory internal quality system.

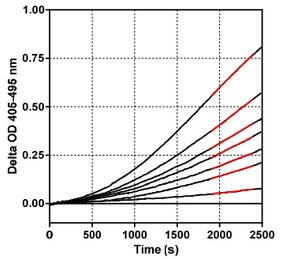


Figure 3. Examples of appropriate kinetic curve shapes

Kinetic curves should have a shape with an upward increasing curve as in the examples above. The sample examples shown here are from across the index range of the Fungitell STAT® assay. Use these examples to review the quality criteria.

Note:

1. 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.
2. It is recommended to test serum control samples (negative, close to the limit value or strongly positive) in the context of further laboratory checks and good laboratory practice. These are not included in the Fungitell STAT® kit.

11. Interpretation of Results

- **Negative Result**
  Index values ≤ 0. 74 are interpreted as negative results.
  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].
- **Indeterminate Result**
  Index values from 0.75 to 1.1 are considered inconclusive (equivocal). Additional sampling and testing of sera is recommended. Frequent sampling and testing improves 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.

12. Limitations of the Test

- The tissue locations of fungal infection[7], 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.
- Some individuals have (1→3)-β-D-glucan index values that fall into the indeterminate zone. In such cases, additional surveillance testing is recommended.
- 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.
- Positive results have been found in hemodialysis patients[19,20,39], subjects treated with certain fractionated blood products such as serum albumin and immunoglobulins[23,24] 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. A comprehensive review of factors contributing to 1→3)-β-D-glucan false positives is found in Finkelman, M.A., Journal of Fungi (2021)[40].
- 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].
- Test levels were established in adult subjects. Infant and pediatric normal and cut-off levels are under investigation[27,28].

13. Performance Characteristics

13.1 Expected Values

- ***Diagnostic sensitivity and diagnostic specificity of the reference method, Fungitell® assay***
  A multi-center, prospective study conducted to determine the diagnostic sensitivity and diagnostic specificity of the Fungitell® assay (USA predicate and 2008 CE-marked) has shown that the (1→3) β-D-glucan values are increased in various 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%[29].

- ***Determination of the Fungitell STAT® cut-off values***
  De-identified, frozen patient serum samples collected for routine clinical care of the intended population and received at Beacon Diagnostics Laboratory, Inc for Fungitell® testing were used for the purpose of this study. Beacon Diagnostics Laboratory, Inc is a licensed Clinical Laboratory Improvement Amendments (CLIA) laboratory part of Associates of Cape Cod (ACC). A population of 93 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® standard curve of 31 – 500 pg/mL. The Fungitell STAT® cut-off assessment followed the ROC curve analysis (Receiver Operating Characteristic Curves)[30]. The results indicated that Fungitell STAT β-glucan index values ≥ 1.2 are to be interpreted as a positive result in alignment with the Fungitell® product's 80 pg/mL cutoff while index values ≤ 0. 74 are to be interpreted as negative results in alignment with the Fungitell® product's 60 pg/mL cutoff. These cut-off values were validated as part of the Method Comparison study and calculation of the Negative Percent Agreement and Positive Percent Agreement presented below.

13.2. Method Comparison

Similarly to the Cut-off value study but using a different set of samples, 488 de-identified, frozen patient serum samples also with (1→3)-β-D-Glucan concentrations distributed over the full range of the Fungitell® standard curve of 31 – 500 pg/mL were used for the purpose of the method comparison study[30]. These included 309 samples that fell within the Negative zone of the Fungitell® test results, 143 samples that fell within the Positive zone of the Fungitell® and 36 samples that fell within the Indeterminate zone of the Fungitell® (**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®			
		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® device were also negative with the Fungitell STAT® assay. The calculated negative percent agreement (NPA) with the Fungitell® 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® device were also positive with the Fungitell STAT® assay. The calculated positive percent agreement (PPA) with the Fungitell® method was 99.2% (95% Confidence interval: 95.4%, 99.9%) (**Table 2**).
- **Measuring Range, Linearity, and Accuracy**
  The index results ranged from approximately 0.4 to 3.5, covering the Standard curve (31 – 500 pg/mL) of the Fungitell®. The linear correlation between the Fungitell® concentration and Fungitell STAT® index results was 0.92 (95% Confidence interval: 89.9% and 93.6%).
  Note: When using PKF08 with BG Analytics® Software, the Fungitell STAT® index result is used to determine the categorization. An estimated pg/mL value is provided for reference only.

13.3 Analytical Inter-laboratory Study

The Fungitell STAT® was evaluated for precision (i.e., repeatability and reproducibility), analytical sensitivity and analytical specificity 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 and including 30 runs (i.e., assays) and 90 datapoints per sample (i.e., panel member). 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. Analytical Inter-laboratory Study

Panel Member	Mean Index	Standard Deviation	% CV	Percent Positive (Number pos./ Number tested)	Analytical Specificity (True Negative) and Analytical Sensitivity (True Positive)
Low Negative	0.55	0.10	20.4%	1.1% (1/90)	89/90 True Negative
High Negative	0.75	0.08	11.1%	0% (0/90)	90/90 True Negative
Indeterminate	0.94	0.10	11.1%	3.3% (3/90)	87/90 Not Positive
Low Positive	1.6	0.30	18.7%	96.7% (87/90)	87/90 True Positive
High Positive	2.6	0.40	15.4%	100% (90/90)	90/90 True Positive

As indicated in the Table 3, the Inter-assay variation (i.e., %CV) ranged from 11 to 20.4% and served as a measure of reproducibility. The intra-assay variation ranged from 0.4% to 26.8% and served as a measure of repeatability. The distribution of the intra-assay % 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.

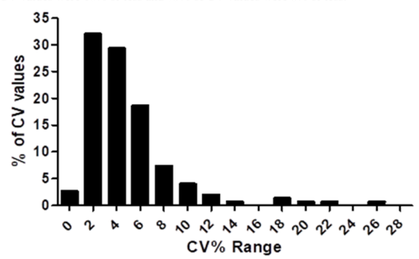


Figure 4. Distribution of intra-assay % CV values

13.4 Trueness

For each lot of the Fungitell STAT® product, the Fungitell STAT® Standard (1→3)-β-D-glucan concentration is calibrated to 80 +/- 8 pg/mL using the Fungitell® reference method and against an internal (1→3)-β-D-glucan reference standard.

13.5 Interfering Substances

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

- 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.
- 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[31].
- As of this writing, no activating Factor G ((1→3)-β-glucan detection element) of Fungitell® reagent have been described other than (1→3)-β-glucan. In some studies, where assertions of cross-reactivity have been made, treatment of the supposed activating material with purified (1→3)-β-glucanase have eliminated the signal, demonstrating that the observed activation had been due to contaminating (1→3)-β-glucan[12]. Serine protease contamination may also result in para-nitroaniline release in Fungitell® reaction mixtures, but these are inactivated as part of the pre-treatment process.

14. *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[32,33,34,35,36,37,38,39].

15. Symbols Legend

Use By		Consult Instructions For Use	
Contains Sufficient For 'N' Tests		EU Authorized Representative	EC REP
Batch Code	LOT	CE Mark	CE
In Vitro Diagnostic Medical Device	IVD	For Prescription Use Only	Rx only
Catalogue No.	REF	Caution	
Temperature Limitation		Keep Away From Sunlight	
Manufacturer		Importer	
Swiss Authorized Representative	CH REP		

16. Authorized Representatives/Importer

EC REP Emergo Europe, Westervoortsedijk 60, 6827 AT Arnhem, The Netherlands

CH REP	**Swiss Authorized Representative** MedEnvoy Switzerland Gotthardstrasse 28, 6302 Zug, Switzerland
	Importer **MedEnvoy Global B.V.** Prinses Margrietplantsoen 33- Suite 123 2595 AM The Hague, The Netherlands

Australian Sponsor:
Emergo Australia, Level 20, Tower II, Darling Park
201 Sussex Street, Sydney, NSW 2000, Australia

Note: serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

17. Contact Information

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18. Revision History

Rev 1-3: Added PKF08-PKG catalog # and related instructions; details about Fungitell STAT® Standard serving as Internal Control, contact information, clarifications and formatting. Clarified general QC criteria #3. Added Specimen stability data and determination of cut-off value, Measuring Range-Linearity-Accuracy and Trueness sections. Rev 4: Changed EC representative, changed 0.03 value to 0.00 in Quality Control section and minor changes for clarification. Rev 5: Removed EC REP Emergo Europe. Rev 6: Updated symbols used. Added MedEnvoy as EU importer and removed ACC Europe GmBh from section 17. Updated symbols used. Added EC-REP, Swiss Importer, and CH-REP name and address. Rev 7: Clarification of reagent storage. Added that an Estimated Fungitell; pg/mL is provided with the PKF08-BGA report, updated logo and reference to the ACC website to www.fungitell.com for labeling access.

19. References

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