Assay for (1→3)-β-D-Glucan in Serum

FUNGITELL® ASSAY

Instructions For Use

Rx only

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Visit www.acciusa.com for instructions for use in your language.
This product is for In Vitro Diagnostic and Professional Use only.

1. Intended Use

The **Fungitell®** 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 titers should be used in conjunction with other diagnostic procedures, such as microbiological culture, histological examination of biopsy samples and radiological examination.

Important

Provide this information to the requesting physician: *Certain fungi, such as the genus Cryptococcus which produces very low levels of (1→3)-β-D-glucan, may not result in serum (1→3)-β-D-glucan sufficiently elevated so as to be detected by the assay[3,4]. Infections with fungi of the Order Mucorales such as Absidia, Mucor and Rhizopus[1,4] which are not known to produce (1→3)-β-D-glucan, are also observed to yield low serum (1→3)-β-D-glucan titers. In addition, the yeast phase of Blastomyces dermatitidis produces little (1→3)-β-D-glucan and may not be detected by the assay[5].*

Include this statement when reporting the Fungitell® assay test results.

2. Summary and Explanation

There is an increasing incidence of fungal infections by opportunistic pathogens, especially in immuno-compromised patients[6,7,8]. 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[9,10]. 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[11,12]. 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® assay[1,8,13,14].

3. Principle of the Procedure

The Fungitell® assay measures (1→3)-β-D-glucan. The assay is based upon a modification of the *Limulus* Amebocyte Lysate (LAL) pathway[15,16,17,18], Figure 1. The Fungitell® 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 (pNA) from the chromogenic peptide substrate, Boc-Leu-Gly-Arg-pNA, creating a chromophore, para-nitroaniline, that absorbs at 405 nm. The Fungitell® kinetic assay, described below, is based upon the determination of the rate of optical density increase produced by a sample. This rate is interpreted against a standard curve to produce estimates of (1→3)-β-D-glucan concentration in the sample.

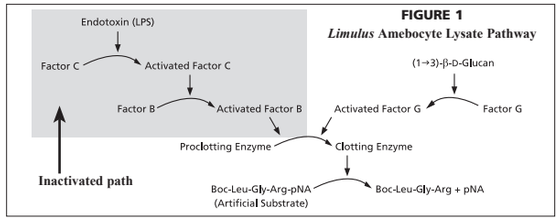

FIGURE 1
***Limulus* Amebocyte Lysate Pathway**

4. Materials Supplied with the Fungitell® Kit

The Fungitell® kit is for in vitro diagnostic use. The following materials supplied with each kit are sufficient to assay 110 wells on two microtiter plates (55 wells on each):

- Fungitell® Reagent, a lyophilized (1→3)-β-D-glucan specific LAL (two vials). *The Fungitell® Reagent is composed of Limulus (i.e., horseshoe crab) Amebocyte Lysate and Boc-Leu-Gly-Arg-pNA colorimetric substrate. It does not contain human or mammalian proteins.*
- Pyrosol® Reconstitution Buffer (two vials). Additional vials of Pyrosol Reconstitution Buffer (catalog number BC051) may be purchased separately. *It is composed of 0.2M Tris buffer.*
- Glucan standard, lyophilized (1→3)-β-D-glucan from Pachyman (two vials). *The volume of reagent water to be added is indicated on the vial label. It is calibrated against an internal reference standard.*
- LAL Reagent Water (LRW) (two bottles)
  Note: 20mL Reagent Grade Water (RGW) and LRW in glass vials are equivalent.
- Alkaline Pretreatment Solution (two vials) which contains *0.125 M KOH and 0.6 M KCl*

All of the above, with the exception of the standard, are free of interfering levels of (1→3)-β-D-glucan.

5. Materials Required but not Supplied

All materials must be free of interfering glucan.

- Pipette tips* (250 µL - Cat# PPT25, 1000 µL - Cat# PPT10)
- Pipettes capable of delivering 5-25 µL and 100-1000 µL volumes
- Repeating pipette with syringe tips capable of delivering 100 µL
- Test tubes* for standard series (calibration curve) preparation and combining serum treatment reagents. (12 x 75 mm - Cat# TB240 or 13 x 100 mm - Cat# TB013)
- Incubating (37°C) plate reader capable of reading at 405 nm (preferably capable of dual wavelength monitoring at both 405 and 490 nm) with a dynamic range up to, at least 2.0 Absorbance Units, coupled with appropriate computer-based kinetic assay software.
- Sterile, glucan-free, tubes for aliquotting samples. Tubes that are certified to be RNAse, DNAse, and pyrogen-free can be used.
- Parafilm®
- 96 well Microplates* **Note:** The Fungitell® assay has been validated with plates that have the following characteristics: Polystyrene, sterile, uncoated, flat-bottomed, no interfering beta glucan to ACC specifications and individually wrapped.

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

6. Reagent Storage

- Store all reagents, as supplied, at 2-8°C in the dark.
- Reconstituted Fungitell® Reagent should be stored at 2-8°C and used within 2 hours. Alternatively, reconstituted Fungitell® Reagent can be frozen at -20°C for up to 20 days, thawed once and used.

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.
- **Note:** Do not use kits with damaged contents.
- 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 all Fungitell® kit components can be downloaded from ACC website: www.acciusa.com.

7.1 Procedural Precautions

The Fungitell® 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.

- Use good laboratory practices according to your local regulations. This assay is sensitive to contamination and pipetting inaccuracy.
- 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® test.
- Possible sources of contamination: 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 release high amounts of (1→3)-β-D-glucan[21,22]. For other patient-related sources of contamination, see the Limitations section of the test.
- 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 institution.

8. Procedure

8.1 Instrument setting and test programming

Settings may vary with different instruments and software. In general, the following will apply: Set the plate reader software to collect data in the Vmean mode. Check the software manual for the proper settings to ensure that the value calculated is the mean rate of optical density change for all of the data points gathered. Set the detector read interval to the minimum allowed by the software/instrument over the 40 minute period of the test. The software wavelength settings should be 405 nm minus the background at 490 nm. It is recommended to use both wavelengths but if dual wavelength reading is not available, read the test at 405 nm and examine each patient sample kinetic curve for signs of interference (see Section 9.0 for more details). The incubation temperature is to be set at 37°C. Set mixing/plate shaking to occur for 5 – 10 seconds prior to the commencement of reading. Select the curve fit setting to "linear/linear" or equivalent. Reading should commence without any lag time.

8.2 Preparation of glucan standard provided in the kit.

a. Dissolve one vial of the glucan standard with the volume of LRW stated on the vial, to make a 100 pg/mL solution. Vortex at least 30 seconds at medium to medium-high speed to reconstitute the standard (solution 1). The glucan solution should be stored at 2-8°C and used within three days. Steps b - e listed below illustrate an example of a standard curve preparation scheme.
b. Prepare 50 pg/mL standard (solution 2) by mixing 500 µL LRW and 500 µL of solution 1 in a glucan-free tube (solution 2). Vortex for at least 10 seconds.
c. Prepare 25 pg/mL standard (solution 3) by mixing 500 µL LRW and 500 µL of solution 2 in a glucan-free tube (solution 3). Vortex for at least 10 seconds.
d. Prepare 12.5 pg/mL standard (solution 4) by mixing 500 µl LRW and 500 µl of solution 3 in a glucan-free tube (solution 4). Vortex for at least 10 seconds.
e. Prepare 6.25 pg/mL standard (solution 5) by mixing 500 µl LRW and 500 µL of solution 4 in a glucan-free tube (solution 5). Vortex for at least 10 seconds.

8.3 Open the Alkaline Pretreatment Solution.

The Alkaline Pretreatment Solution converts triple-helix glucans into single-stranded glucans[17,18] 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]

Discard the vial (in accordance with laboratory procedures) unless it is going to be used in a subsequent test, in which case, cover it with Parafilm, using the side of the Parafilm that faced the paper backing.

8.4 Microtiter plate set up

Set up the microtiter plate layout in the software, with the standards (Std), negative controls (Neg) and 21 samples (Spl). The following layout is recommended:

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		STD1 500	STD1 500		SPL1	SPL4	SPL7	SPL10	SPL13	SPL16	SPL19	
C		STD2 250	STD2 250		SPL1	SPL4	SPL7	SPL10	SPL13	SPL16	SPL19	
D		STD3 125	STD3 125		SPL2	SPL5	SPL8	SPL11	SPL14	SPL17	SPL20	
E		STD4 62,5	STD4 62,5		SPL2	SPL5	SPL8	SPL11	SPL14	SPL17	SPL20	
F		STD5 31,25	STD5 31,25		SPL3	SPL6	SPL9	SPL12	SPL15	SPL18	SPL21	
G		Neg	Neg		SPL3	SPL6	SPL9	SPL12	SPL15	SPL18	SPL21	
H												

Enter the standard concentrations into the software settings as 500, 250, 125, 62.5, and 31 pg/mL, respectively.

Note that the standard concentrations entered are five times greater than those prepared in Section 8.2 above. This is because the volume of standard used in the assay is 25 µL per well, which is five times the volume of the serum sample used (see Section 8.5 b. below). Thus, the serum sample is effectively diluted five-fold relative to the standard. Multiplication of the standard concentrations by five compensates for this dilution.

Note: The outside wells may be used, if it has been demonstrated that the performance of the outside wells is comparable to that of the internal wells.

Note: The negative controls are not utilized in the standard curve.

8.5 Serum and Alkaline Pretreatment Solution addition.

a. Thaw frozen serum samples at room temperature. Vortex all samples well – for at least 30 seconds on medium to medium-high speed setting.
b. Transfer 5 µl of the serum sample to each of its designated wells (Uk) in at least duplicate. Repeat for each serum sample.
c. Add 20 µl of the Alkaline Pretreatment Solution to each well containing serum. Ensure that the serum and the pretreatment droplets come in contact with one another.
   Note: Steps b and c can be conducted in reverse order according to technician preference.
   Note: To avoid accidental contamination, replace the cover on the microplate after adding samples and reagents to the wells.
d. Agitate the plate for 5 – 10 seconds to mix the well contents (the reader's plate agitation function may be used) then incubate for 10 minutes at 37°C in the incubating plate reader.

8.6 Reconstitution of Fungitell® reagent.

Note: This may be conveniently performed while the pretreatment incubation is in progress. Consistency of reconstitution timing will enhance reproducibility as the Fungitell® reaction begins upon reconstitution, albeit at a low level.

Reconstitute one vial of Fungitell® reagent by adding 2.8 mL of LRW and then adding 2.8 mL of Pyrosol Reconstitution buffer using the 1000 µL pipette. Cover the vial with Parafilm using the side of Parafilm that faced the paper backing. Swirl the vial gently to dissolve completely – do not vortex.

8.7 Addition of negative controls and glucan standards.

At the end of serum pretreatment incubation (Section 8.5 d.), remove the plate from the incubating plate reader and add the standards and negative controls to the plate. Recommended standard concentration pattern:
a. Add 25 µL of LRW to wells G2 and G3.
b. Add 25 µL of the 6.25 pg/mL standard solution 5 to wells F2 and F3, labeled as 31.25 pg/mL.
c. Add 25 µL of the 12.5 pg/mL standard solution 4 to wells E2 and E3, labeled as 62.5 pg/mL.
d. Add 25 µL of the 25 pg/mL standard solution 3 to wells D2 and D3, labeled as 125 pg/mL.
e. Add 25 µL of the 50 pg/mL standard solution 2 to wells C2 and C3, labeled as 250 pg/mL.
f. Add 25 µL of the 100 pg/mL standard solution 1 to wells B2 and B3, labeled as 500 pg/mL.

8.8 Fungitell® reagent addition and plate incubation procedure.

a. Add 100 µL of Fungitell® reagent to each well (containing negative controls, standards, and samples) using the stepper (repeater) pipette.
b. Insert the plate into the microplate reader (equilibrated to 37°C), remove the lid and shake for 5 – 10 seconds.

Read the plate **without the lid** at 405 nm minus 490 nm, for 40 minutes at 37°C. Note: If the instrument does not allow time to take the lid off between shaking and reading, shake with lid off to ensure reading without the lid on.

9 Calculate the results.

Collect the data and analyze as follows: Examine kinetic plots of test samples and check for patterns other than a smooth increase comparable to those of standards. Invalidate plots indicating optical interference (e.g. their kinetic patterns do not follow those of the standards). Calculate the mean rate of optical density change (milli-absorbance units per minute) for all points between 0 and 40 minutes (performed by the software). Interpolate the sample (1→3)-β-D-glucan concentrations from the standard curve (performed by the software).

10 Quality Control

- The correlation coefficient (r) of the standard curve (linear vs. linear) should be ≥ 0.980.
- The wells with 25 µL of LRW are the negative controls. Negative controls should have rate results (e.g., milli-absorbance units per minute) values less than 50% of the lowest standard rates. If not, the assay should be repeated using all new reagents.
- Handling complex samples. If the analyst observes unusual kinetics in a test of a sample, e.g., a sample that is cloudy, off-color, or turbid (such as those that are grossly hemolyzed, lipemic or contain excessive bilirubin), the sample must be diluted with LRW and retested. The dilution must be accounted for in the reporting of results by multiplying the result by the dilution factor. **Typically, the dilution factor is entered in the software setup for the sample and the correction is automatically applied.**

Note:

- 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.
- 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® kit.

11 Interpretation of Results

NEGATIVE RESULT

(1→3)-β-D-glucan values <60 pg/mL 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[3,4,] produce very low levels of (1→3)-β-D-glucan. Mucorales, such as *Absidia, Mucor* and *Rhizopus*[1,4] 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® assay[5].

INDETERMINATE RESULT

Values from 60 to 79 pg/mL are considered inconclusive. Additional sampling and testing of sera is recommended. Frequent sampling and testing improve the utility for diagnosis.

POSITIVE RESULT

(1→3)-β-D-glucan values are ≥ 80 pg/mL are interpreted as a positive result. 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[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.
- Some individuals have elevated levels of (1→3)-β-D-glucan 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[19,20,38], subjects treated with certain fractionated blood products such as serum albumin and immunoglobulins[23,25] 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.
- 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[26,27].
- For a comprehensive review of factors contributing to 1→3)-β-D-glucan false positives see Finkelman, M.A., Journal of Fungi (2021)[39].
- Test levels were established in adult subjects. Infant and pediatric normal and cut-off levels are under investigation[28,29].

13 Performance Characteristics

13.1 Cut-off and Expected Values

A multi-center, prospective study[31] conducted to determine the diagnostic sensitivity and diagnostic specificity of the Fungitell® assay (see comparison testing below) has shown that beta 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%.

13.2 Clinical Performance

A multi-center, prospective study to validate the performance characteristics of the Fungitell® assay was conducted[21]. The test was compared to other standard methods of detection, (i.e., blood culture, histopathological examination of biopsy specimen and radiological signs) for mycoses and fungemias.

Three hundred and fifty-nine (359) subjects were tested by the assay. A single sample was obtained from each subject. The low risk subjects included apparently healthy individuals and those at the clinical sites who were admitted to hospitals for reasons other than fungal infections. Subject accrual was conducted at six clinical sites in the United States. Four of the clinical sites performed the assay and tested a total of 285 samples. ACC tested all 359 samples twice but only used the second set of results to determine the assay performance. The results of the second set of analyses were not statistically different from the first set.

- **Diagnostic Sensitivity**

  The sensitivity for the entire subject population (359) including cryptococcosis patients was 65.0% ([60.1 - 70.0% 95% Confidence Interval (CI)](Table 1).
- **Diagnostic Specificity**

  The specificity was 81.1% (77.1 - 85.2% CI). When the 170 subjects negative for fungal infection and apparently healthy individuals were analyzed, the specificity was 86.5% with the assay (82.8% - 90.1% C.I.). When the additional 26 subjects who were negative for fungal infection but with other disorders were included, an 81.1% specificity was observed (77.1 - 85.2 % CI).

Table 1 ACC Test Results at the 60-80 pg/mL Cutoff level by Site

Site	Proven/Probable **Sensitivity** >=80pg/mL			**Specificity** <60pg/mL			Equivocal 60<=X<80	Total
	Pos/ Clin. Pos	**Sensitivity**	Positive Predictive Value	Neg/ Clin. Neg	**Specificity**	Negative Predictive Value		
1	32/50	64.0	97.0	39/40	97.5	69.6	1	90
2	14/24	58.3	93.3	17/20	85.0	70.8	5	44
3	14/19	73.7	46.7	36/54	66.7	90.0	3	73
4	25/33	75.8	92.6	37/43	86.0	86.0	6	76
5	21/36	58.3	80.8	30/39	76.9	69.8	6	75
6	0/1	0.0	N/A	0/0	N/A	0.0	0	1
Total	106/163	65.0	80.9	159/196	81.1	76.8	21	359

When the results obtained by ACC (359 samples) and the by the clinical sites (285 sample) are compared to clinical diagnosis, the sensitivity is 64.3% (58.8% - 69.9% CI) for ACC and 61.5% (55.9% - 67.2% CI) for the sites. The specificity is 86.6% (82.7% - 90.6% CI) for ACC versus 79.6% (74.9% - 84.3% CI) for the sites

Candidiasis

There were 107 subjects who were positively diagnosed with candidiasis in the prospective study. 83 of the 107 were positive by the Fungitell® assay.

One hundred seventy-five candidiasis library samples were furnished to Associates of Cape Cod, Inc. 145 of the 175 were positive by the assay.

Aspergillosis

A total of 10 subjects were positive for aspergillosis. 8 of the 10 were positive by the assay.

Fusariosis

Three subjects were positive for fusariosis. 2 of the 3 were positive by the assay.

Anti-Fungal Drug Therapy

The presence or absence of antifungal drug therapy had no statistically significant effect upon assay sensitivity. 118 subjects were proven positive for invasive fungal infection and on anti-fungal therapy. 82 were positive by the assay (sensitivity, 69.5%; 61.2% - 77.8% CI). In addition, twenty-four (24) subjects were proven positive, but not on any anti-fungal therapy. 18 were positive by the assay (sensitivity, 75%; 57.7% - 92.3% CI).

13.3 Test Correlations

Four of the clinical sites assayed a total of 285 samples. The site test results correlated quantitatively at 96.4% with the Associates of Cape Cod, Inc. results. The Associates of Cape Cod, Inc. correlations with the different testing sites ranged from 90.6 to 99.2%.

13.4 Precision

The Fungitell® assay was evaluated for precision (i.e., repeatability and reproducibility) using ten (10) different samples that were each tested by three testing sites, on three different days. The intra-assay variation ranged from 0.9 to 28.9% and served as a measure of repeatability. The Inter Assay values ranged from 3.9 to 23.8% and served as a measure of reproducibility. The four (4) negative samples were excluded from both analyses.

13.5 Measuring range and linearity

The results are expressed in pg/mL of serum and range from non-detectable (<31 pg/mL) to >500 pg/mL and are printed out by the software or read from the standard curve. Accurate values above 500 pg/mL require that the sample be diluted with LAL Reagent Water and retested. As indicated in the Quality Control section, the correlation coefficient (r) of the standard curve (linear vs. linear) covering the measuring range of the Fungitell® assay should be ≥ 0.980 and the Negative controls should have rate (e.g., milli-absorbance units per minute) values less than 50% of the lowest standard rate. If not, the assay should be repeated using all new reagents.

13.6 Interfering Substances

The following sample conditions can interfere with an accurate Fungitell® 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® to the pre-treated serum[30].
- 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[15]. 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[32,34,35,36,37].

15 Symbols Legend

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

16 Authorized representatives

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 0 to 11: Changed triplicate to duplicate testing. Replaced Reagent Grade Water with LAL Reagent Water. Combined the KCL and KOH components into Alkaline Pretreatment Solution. Removed microplate from Kit and offer as required but not supplied item. Changed EC representative and added Australian sponsor. Minor clarifications, formatting, addition of symbols, additional interfering substances.
Rev 12: Removed EC REP Emergo Europe.

19 References

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