

# Assay for (1→3)-β-D-Glucan in Serum

**FUNGITELL®**

## Instructions For Use



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### 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 class of fungi may be causing infection.

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

**Important - It is recommended that this information be provided to the requesting physician:**

The Fungitell assay does not detect certain fungal species such as the genus *Cryptococcus* which produces very low levels of (1→3)-β-D-Glucan(3,4). The assay also does not detect Zygomycetes such as *Absidia*, *Mucor* and *Rhizopus*(1,4) which are not known to produce (1→3)-β-D-Glucan. 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 Glucan Assay test results.**

### SUMMARY AND EXPLANATION

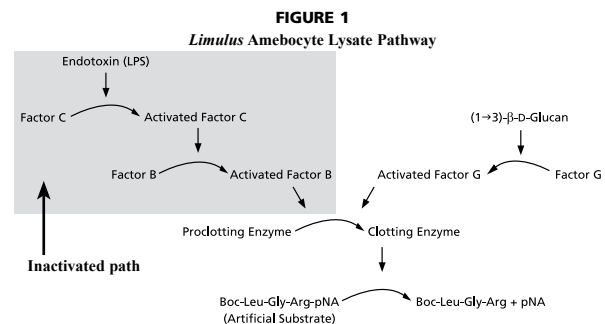
There is an increasing incidence of fungal infections by opportunistic pathogens, especially in immuno-compromised patients(6,7,8). Invasive fungal diseases, as opportunistic infections, are common among hematological malignancy and AIDS patients and account for a growing number of nosocomial infections, 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*, 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).

### PRINCIPLE OF THE PROCEDURE

The Fungitell assay measures levels of (1→3)-β-D-Glucan. The assay is based upon a modification of the *Limulus* Amebocyte Lysate (LAL) pathway(14,15,16,17), Figure 1. The Fungitell reagent is modified to eliminate Factor C 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 proclotting enzyme to the active clotting enzyme, which in turn cleaves pNA from the chromogenic peptide substrate, Boc-Leu-Gly-Arg-pNA, creating a chromophore 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.



### 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):

1. Fungitell® Reagent, a lyophilized (1→3)-β-D-Glucan specific LAL (two vials)
2. Pyrosol® Reconstitution Buffer, Tris HCl 0.2 M pH 7.4 (two vials). Additional vials of Pyrosol Reconstitution Buffer (catalog number BC051) may be purchased separately.
3. Glucan standard, lyophilized pachyman and inert filler with the (1→3)-β-D-Glucan content stated on the label (two vials)
4. Reagent Grade Water (RGW) (two bottles)
5. Pyroplates: Flat-bottom, 96-well, uncoated microplates, with lids, free of interfering glucans (two)
6. KCl 1.2 M (one vial)
7. KOH 0.25 M (one vial)

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

### MATERIALS REQUIRED BUT NOT SUPPLIED

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

1. Pipette tips\* (250 µL - Cat# PPT25, 1000 µL - Cat# PPT10)
2. Pipettors capable of delivering 5-25 µL and 100-1000 µL volumes
3. Stepper pipettor, with syringe tips, capable of delivering 100 µL
4. Test tubes\* for standard series preparation and combining serum treatment reagents. (13 x 100 mm borosilicate glass - Cat# TB013)
5. Incubating (37°C) plate reader capable of dual wavelength monitoring, at 405 and 490 nm, with a dynamic range up to, at least 2.0 Absorbance Units, coupled with appropriate computer-based kinetic assay software.
6. 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).
7. Parafilm®

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

**Caution**-glass pipettes with cotton plugs are a potential source of glucan contamination.

### WARNINGS AND PRECAUTIONS

**This product is for IN VITRO DIAGNOSTIC USE.**

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.

1. Certain fungal species produce very low levels of (1→3)-β-D-Glucan and are not usually detected by the Fungitell assay. These include the genus *Cryptococcus*(3,4) as well as the Zygomycetes such as *Absidia*, *Mucor* and *Rhizopus*(1,4). 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 assay(5).
2. Do not pipette any material by mouth. Do not smoke, eat or drink in areas where specimens or kit reagents are handled.
3. Establish a clean environment in which to perform the assay. Use materials and reagents that are certified to be free of interfering 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 assay.
4. Do not use reagents beyond their expiry date.

5. Off-color or turbid samples such as those that are grossly hemolyzed, lipemic, or contain excessive bilirubin may cause interference. If tested, test results should be examined for evidence of optical interference and/or unusual kinetic trace 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(18,19). Hemodialysis with cellulose triacetate membranes or polymethyl methacrylate membranes does not appear to affect 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(20,21).
9. Kits with damaged contents should not be used.
10. 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. 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.

### Specimen Handling

1. Specimen Collection: Serum samples should be collected in sterile vacuum tubes (red tops), or serum separator tubes (SST), and allowed to clot. The serum is then separated from the clot and decanted to a suitable container that is free of interfering levels of (1→3)-β-D-Glucan.
2. Specimen Storage: Serum samples can be stored at 2-8°C before assay, or frozen at -20° or colder. Testing should be conducted promptly to avoid the possibility of sample degradation.
3. Specimen Labeling: Specimens should be clearly labeled according to the approved practices of the institution.

### PROCEDURE

Note: Settings may vary with different instruments and software. In general, the following will apply: Set the plater reader software to collect data in the V<sub>mean</sub> 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 datapoints gathered. Set the interval between instrument 'reads' to the minimum allowed by the software and instrument over the 40 minute period of the test. The software wavelength settings should be 405 nm minus the background at 490 nm. If dual wavelength reading is not available, read at 405 nm. The incubation temperature is to be set at 37°C. The plate shaking should occur, for 5 – 10 seconds, prior to the commencement of reading. The curve fit setting should be "linear/linear" or equivalent. Reading should commence without any lag time.

1. Preparation of glucan standard provided in the kit.
  - a. Dissolve one vial of the glucan standard with the volume of RGW stated on the vial, to make a 100 pg/mL solution. Vortex at least 30 seconds to resuspend (solution 1). The glucan solution should be stored at 2-8°C and used within three days. Steps b-e below illustrate an example of a standard curve preparation scheme.
  - b. Prepare 50 pg/mL standard by mixing 500 µL RGW 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 by mixing 500 µL RGW 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 by mixing 500 µL RGW 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 by mixing 500 µL RGW and 500 µL of solution 4 in a glucan-free tube (solution 5). Vortex for at least 10 seconds.
2. Preparation of serum pre-treatment reagent. The alkaline serum pre-treatment reagent converts triple-helix glucans into single-stranded glucans(16,17) which are more reactive in the assay. The high pH also inactivates the serine proteases and serine-protease inhibitors in serum that can give a false positive and a false negative result, respectively(22).
  - a. Prepare the serum pre-treatment reagent by combining equal volumes of 0.25 M KOH and 1.2 M KCl, and vortexing well. Recommended volumes are up to 900 µL of each reagent, permitting two preparations. Cover the vials with Parafilm for use with the second plate. Cover the vial with Parafilm using the side of the Parafilm that faced the paper backing.
    - i. Note: When plotting the standard curve, multiply the concentration of the standards by five so that the range is from 500 to 31 pg/mL. Enter the standards into the software settings as 500, 250, 125, 62.5, and 31 pg/mL, respectively.

*The volume of standard in the assay is 25 µL per well or five times the volume of the serum sample. The microtiter plate with the standards (St), negative controls (Neg) and 21 unknowns (Uk) each assayed in duplicate is set up as follows:*

|   | 1 | 2   | 3   | 4 | 5   | 6   | 7   | 8    | 9    | 10   | 11   | 12 |
|---|---|-----|-----|---|-----|-----|-----|------|------|------|------|----|
| A |   |     |     |   |     |     |     |      |      |      |      |    |
| B |   | St1 | St1 |   | Uk1 | Uk4 | Uk7 | Uk10 | Uk13 | Uk16 | Uk19 |    |
| C |   | St2 | St2 |   | Uk1 | Uk4 | Uk7 | Uk10 | Uk13 | Uk16 | Uk19 |    |
| D |   | St3 | St3 |   | Uk2 | Uk5 | Uk8 | Uk11 | Uk14 | Uk17 | Uk20 |    |
| E |   | St4 | St4 |   | Uk2 | Uk5 | Uk8 | Uk11 | Uk14 | Uk17 | Uk20 |    |
| F |   | St5 | St5 |   | Uk3 | Uk6 | Uk9 | Uk12 | Uk15 | Uk18 | Uk21 |    |
| G |   | Neg | Neg |   | Uk3 | Uk6 | Uk9 | Uk12 | Uk15 | Uk18 | Uk21 |    |
| H |   |     |     |   |     |     |     |      |      |      |      |    |

Note 1: 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 2: To avoid accidental contamination, replace the cover on the microplate after adding samples and reagents to the wells. Remove the cover before placing the plate in the reader to avoid optical interference from condensation.

3. Serum and pre-treatment reagent addition.
  - a. Thaw frozen serum samples at room temperature. Vortex all samples well.
  - 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 serum pre-treatment reagent to each well containing serum. *Note:* Steps b and c can be conducted in reverse order according to technician preference.
  - 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.
4. Reconstitution of Fungitell reagent. Note: This may be conveniently performed while the pre-treatment incubation is in progress.
  - a. Reconstitute one vial of Fungitell reagent by adding 2.8 mL of RGW and then adding 2.8 mL of Pyrosol Reconstitution buffer using the 1000 µL pipettor. 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.
5. Addition of negative controls and glucan standards. At the end of serum pre-treatment incubation (step 3.d), remove the plate from the incubating plate reader and add the standards and negative controls to the plate.
  - a. Add 25 µL of RGW to wells G2 and G3.
  - b. Add 25 µL of the 6.25 pg/mL standard solution 5 to wells F2 and F3..
  - c. Add 25 µL of the 12.5 pg/mL standard solution 4 to wells E2 and E3.
  - d. Add 25 µL of the 25 pg/mL standard solution 3 to wells D2 and D3.
  - e. Add 25 µL of the 50 pg/mL standard solution 2 to wells C2 and C3.
  - f. Add 25 µL of the 100 pg/mL standard solution 1 to wells B2 and B3.
6. 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 pipettor.
  - b. Insert the plate into the microplate reader (equilibrated to 37°C) with the lid on and shake for 5 – 10 seconds. Read the plate without the lid at 405 nm minus 490 nm, for 40 minutes at 37°C. If background subtraction (at 490 nm) is unavailable, it is acceptable to read at 405 nm. If a plate shaking function is unavailable with the microplate reader, an external microplate shaker may be used.
  - c. Collect the data and analyze as follows: Examine optical density plots of test samples and check for kinetic trace patterns other than a smooth increase comparable to those of standards. Invalidate plots indicating optical interference. Calculate the mean rate of optical density change (milli-absorbance units per minute) for all points between 0 and 40 minutes.

### INTERPRETATION OF RESULTS

The Fungitell test results should be used as an aid in the diagnosis of invasive fungal infection. 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 in RGW and retested.

The laboratory performing the test should inform the ordering physician that the Fungitell test does not detect certain fungal species such as the genus *Cryptococcus*(3,4) which produces very low levels of (1→3)-β-D-Glucan. The test also does not detect the *Zygomycetes* such as *Absidia*, *Mucor* and *Rhizopus*(1,4) which 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 is usually undetectable(5).

## NEGATIVE RESULT

(1→3)-β-D-Glucan values <60 pg/mL are interpreted as negative results.

## POSITIVE RESULT

Values ≥80 pg/mL are interpreted as positive. 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.

## INDETERMINATE RESULT

Values from 60 to 79 pg/mL suggest a possible fungal infection. Additional sampling and testing of sera is recommended. Frequent sampling and testing improves the utility for diagnosis.

## QUALITY CONTROL

- The correlation coefficient (r) of the standard curve (linear vs. linear) should be >0.980.
- The wells with 25 µL of RGW are the negative controls. Negative controls should have actual optical density rate (V<sub>mean</sub>) values less than 50% of the lowest standard. If not, the assay should be repeated using all new reagents.
- Handling problem samples. If the analyst observes unusual optical density kinetics in a test of 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 in RGW 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.
- Control samples, at cut-off and highly positive levels, may be run to verify that the reagents and the assay are performing properly. Each user of the test should establish a quality control program to assure proficiency in the performance of the test.

## 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. *Cryptococcus spp.* produce low levels of (1→3)-β-D-Glucan (3,4). Zygomycetes, including *Absidia spp.*, *Mucor spp.*, and *Rhizopus spp.* are not known to produce (1→3)-β-D-Glucan(1,4). *Blastomyces dermatitidis*, in its yeast phase, produces little (1→3)-β-D-Glucan, and test results are usually negative(5).
- Some individuals have elevated levels of (1→3)-β-D-Glucan that fall into the indeterminate zone. In such cases, additional 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(18,19), subjects treated with certain fractionated blood products such as serum albumin and immunoglobulins(23) and in specimens or subjects exposed to glucan-containing gauze. Patients require 3 – 4 days for the restoration of baseline level of serum (1→3)-β-D-Glucan, after surgical exposure to (1→3)-β-D-Glucan containing sponges and gauze(20,21). 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.
- Test levels were established in adult subjects. Infant and pediatric normal levels approach those of adults(24). Data for neonates, and infants less than six months, are lacking.
- The reportable range of the assay is 31 pg/mL to 500 pg/mL. Values below 31 pg/mL are to be reported as <31 pg/mL. Values >500 pg/mL are to be reported as >500 pg/mL, unless the sample has been diluted.

## INTERFERING SUBSTANCES

The following sample conditions can interfere with an accurate Fungitell Assay result:

- Hemolysis
- Sample turbidity caused by lipemia
- The presence of visually apparent bilirubin
- Turbid serum

## EXPECTED VALUES

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% (Table 2). In the absence of signs and symptoms at less than 60 pg/mL, the negative predictive values ranged from 65.1% to 85.1%.

## PERFORMANCE CHARACTERISTICS

### Comparison Testing

A multi-center, prospective study to validate the performance characteristics of the Fungitell assay was conducted(25). 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.

The sensitivity for the entire subject population (359) including *Cryptococcus* was 65.0% (60.1 - 70.0% Confidence Interval (C.I.)). The specificity was 81.1% (77.1 - 85.2 % C.I.) (Table 1). The results from the four testing sites had the sensitivity range from 50.0% to 66.7%. The specificity ranged from 70.0% to 93.0% on the 285 samples tested (Table 2).

| <b>Table 1</b> | <b>ACC Test Results at the 60-80 pg/mL Cutoff level by Site</b> |             |                           |                      |             |                           |                     |       |
|----------------|---|-------------|---------------------------|----------------------|-------------|---------------------------|---------------------|-------|
| Site           | Proven/Probable Sensitivity ≥=80pg/mL                           |             |                           | Specificity <60pg/mL |             |                           | Equivalent 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   |

**\*Includes one sample from Site 6.**

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 (Table 2).

| <b>Table 2</b> | <b>Testing Sites Results at the 60-80 pg/mL Cutoff level by Site</b> |             |                           |                      |             |                           |                     |       |
|----------------|--|-------------|---------------------------|----------------------|-------------|---------------------------|---------------------|-------|
| Site           | Proven/Probable Sensitivity ≥=80pg/mL                                |             |                           | Specificity <60pg/mL |             |                           | Equivalent 60<=X<80 | Total |
|                | Pos/Clin. Pos  | Sensitivity | Positive Predictive Value | Neg/Clin. Neg        | Specificity | Negative Predictive Value |                     |       |
| 1              | 32/50  | 64.0        | 74.4                      | 28/40                | 70.0        | 65.1                      | 4                   | 90    |
| 2              | 12/24  | 50.0        | 75.0                      | 15/20                | 75.0        | 65.2                      | 5                   | 44    |
| 3 *            |  |             |                           |                      |             |                           |                     |       |
| 4              | 22/33  | 66.7        | 91.7                      | 40/43                | 93.0        | 85.1                      | 5                   | 76    |
| 5              | 22/36  | 61.1        | 78.6                      | 30/39                | 76.9        | 75.0                      | 7                   | 75    |
| 6 *            |  |             |                           |                      |             |                           |                     |       |
| Total, Sites   | 88/143   | 61.5        | 79.3                      | 113/142              | 79.6        | 73.9                      | 21                  | 285   |
| ACC            | 92/143   | 64.3        | 91.1                      | 123/142              | 86.6        | 74.1                      | 18                  | 285   |

**\* Not a Testing Site**

### 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. 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).

## SPECIFICITY

A total of 170 subjects were negative for fungal infection and were apparently healthy individuals. 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 % C.I.).

## 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 results. The Associates of Cape Cod correlations with the different testing sites ranged from 90.6 to 99.2%.

## PRECISION

In the Precision Studies, ten (10) different samples were each tested by three testing sites, on three different days. The intra-assay variation ranged from 0.9 to 28.9%. The Inter Assay values ranged from 3.9 to 23.8%. The four (4) negative samples were excluded from both anal-yses.

## REFERENCES










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## SYMBOLS LEGEND

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|    | <b>“Use By”</b>                                    |
|    | <b>“Contains Sufficient For ‘N’ Tests”</b>         |
|    | <b>“Batch Code”</b>                                |
|   | <b>“<i>In Vitro</i> Diagnostic Medical Device”</b> |
|  | <b>“Catalogue No.”</b>                             |
|  | <b>“Temperature Limitation”</b>                    |
|  | <b>“Manufacturer”</b>                              |
|  | <b>“Consult Instructions For Use”</b>              |
|  | <b>“Authorised Representative”</b>                 |

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