

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY**

I. Background Information:

A. 510(k) Number

K191167

B. Applicant

Associates of Cape Cod, Inc

C. Proprietary and Established Names

Fungitell STAT

D. Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
NQZ	Class II	21 CFR 866.3050 - Beta-Glucan Serological Assays	MI - Microbiology

II. Submission/Device Overview:

A. Purpose for Submission: To obtain a substantial equivalence determination for the Fungitell STAT

B. Measurand: Beta-glucan [(1→3)-β-D-glucan]

C. Type of Test: Qualitative protease zymogen-based colorimetric assay

III. Intended Use/Indications for Use:

A. Intended Use(s):

See Indications for Use below.

B. Indication(s) for 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, can be used as an aid in the diagnosis of deep-seated mycoses and fungemias. 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.

C. Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

D. Special Instrument Requirements:

Spectrophotometric tube reader, capable of kinetic reading at 405 nm and background subtraction at 495 nm, while maintaining a temperature of 37°C ± 1°C.

IV. Device/System Characteristics:

A. Device Description:

The Fungitell STAT assay provides a qualitative measurement of (1→3)-β-D glucan. The Fungitell STAT assay is a design modification to the Fungitell (predicate) assay format. The Fungitell STAT assay is designed as a single use test format and has smaller kit size relative to the 96-well plate format of the Fungitell assay.

The Fungitell STAT assay and the Fungitell assay are based upon a modification of the *Limulus* Amebocyte Lysate (LAL) pathway.

The Reagent for both assays 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 substrate, Boc-Leu-Gly-Arg-pNA, creating a chromophore, para-nitroaniline (pNA), that absorbs at 405 nm. The Fungitell STAT kinetic assay is based upon the determination of the rate of optical density increase produced by a sample. This rate is interpreted against the rate of optical density increase of the Fungitell STAT Standard to calculate an index value. This sample index value is qualitatively interpreted as a Negative, Indeterminate or Positive result.

B. Principle of Operation:

The Fungitell STAT assay is based upon a modification of the *Limulus* Amebocyte Lysate (LAL) pathway. The Reagent for the Fungitell STAT 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 substrate, Boc-Leu-Gly-Arg-pNA, creating a chromophore, para-nitroaniline (pNA), that absorbs at 405 nm. The Fungitell STAT kinetic assay is based upon the determination of the rate of optical density increase produced by a sample. This rate is interpreted against the rate of optical density increase of the Fungitell STAT Standard to produce an index.

C. Instrument Description Information:

Modes of Operation	Yes	No
Does the applicant's device contain the ability to transmit data to a computer, webserver, or mobile device?	☐	☒
Does the applicant's device transmit data to a computer, webserver, or mobile device using wireless transmission?	☐	☒
Software		
FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types.	☒	☐

V. Substantial Equivalence Information:

A. Predicate Device Name(s):

Fungitell

B. Predicate 510(k) Number(s):

DEN040003

C. Comparison with Predicate(s):

Similarities		
Device & Predicate Device(s):	Fungitell STAT K191167 (Device)	Fungitell DEN040003 (Predicate)
Intended Use/ Indications for 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, can be used as an aid in the diagnosis of deep-seated	Same

	mycoses and fungemias. 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.	
Reagent	Lyophilized (1→3)-β-D-glucan specific <i>Limulus</i> Amebocyte Lysate (LAL)	Same
Reagent composition (major constituents)	LAL lysate, Boc-Leu-Gly-Arg-pNA colorimetric substrate and Tris buffer	Same

Differences		
Device & Predicate Device(s):	Fungitell STAT K191167 (Device)	Fungitell DEN040003 (Predicate)
Glucan Standard Source	<i>Saccharomyces cerevisiae</i> (1→3)-β-D-glucan for threshold standard (80 pg/mL)	<i>Pachyman</i> (1→3)-β-D-glucan for standard curve equivalent to 31-500 pg/mL
Reagent Reconstitution Buffer	Pyrosol is included in the lyophilized Reagent	Pyrosol Reconstitution Buffer provided in separate vials
Spectrophotometric Reader	Incubating (37°C) plate reader capable of reading at 405 nm and background reading at 495 nm with a range of at least 0-1.0 absorbance units, coupled with appropriate computer-based kinetic assay software capable of observing and reaction kinetics as well as supporting the review of the Quality Control.	Incubating (37°C) plate reader capable of reading at 405 nm and background reading at 490 nm with a dynamic range up to at least 2.0 absorbance units, coupled with appropriate computer-based kinetic assay software.
Output	Index value derived from the ratio of the patient sample kinetic rate (slope) over the Fungitell STAT Standard kinetic rate. Index value analytical measuring range of 0.4 to 3.5, which is equivalent to the analytical measuring range of the Fungitell assay (30-500 pg/mL). Results are interpreted qualitatively as Negative, Indeterminate, or Positive depending on the derived index value.	Estimated (1→3)-β-D-glucan concentration (in pg/mL) based on Fungitell analytical measuring range of 30-500 pg/mL. Results are interpreted qualitatively as Negative, Indeterminate, or Positive depending on the derived glucan concentration.
Assay format	Tubes (for a single test)	96 well Microplates (for multiple tests)

VI. Standards/Guidance Documents Referenced:

- Class II Special Controls Guidance Document: Serological Assays for the Detection of Beta-Glucan - Guidance for Industry and FDA Staff. Document issued on: September 23, 2004.
- Format for Traditional and Abbreviated 510(k)s - Guidance for Industry and FDA Staff. Document issued on: August 12, 2005.
- CLSI document EP05-A3, Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline - Third Edition.
- Guidance for Industry and FDA Staff; Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Tests. March 13, 2007.
- Guidance on Informed Consent for *In Vitro* Diagnostic Device Studies Using Leftover Human Specimens that are Not Individually Identifiable. Guidance for Sponsor, Institutional Review Boards, and Food and Drug Administration Staff. Document issued on April 25, 2006.
- CLSI EP24-A2. Assessment of Diagnostic Accuracy of Laboratory Tests Using Receiver Operating Characteristics Curves; Approved Guideline- Second Edition.

VII. Performance Characteristics (if/when applicable):

A. Analytical Performance:

1. Precision/Reproducibility:

Reproducibility Study

The Fungitell STAT was evaluated for reproducibility by spiking human serum with *Saccharomyces cerevisiae* (1→3)- β -D-Glucan to produce a 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). This panel was tested twice per day, in triplicate, at three sites by multiple operators over a five-day period (1 panel member x twice per day x 3 replicates x 3 sites x 5 days = 90 measurements per panel member) to determine the intra-lab and inter-lab reproducibility of the assay

Intra-lab reproducibility was calculated for each panel member at each testing site. The percent coefficient of variation (CV) for the positive samples ranged from 10.23% to 25.44% at the individual sites. The percent CV of the negative samples ranged from 6.13% to 30.30% at the individual sites. The percent CV of the indeterminate sample ranged from 8.44% to 12.47% at the individual sites. These results are similar to those observed for the Fungitell, and support that the Fungitell STAT is substantially equivalent to the Fungitell assay.

Inter-lab reproducibility was calculated by averaging Fungitell STAT results across testing sites. The percent CV for the positive sample results averaged over all test sites ranged from 15.44% to 18.69%. The percent CV for the negative sample results averaged over all sites ranged from 11.07% to 20.44%. Results are shown in **Table 1**. Results are acceptable.

Table 1. Inter-lab Reproducibility Results

Panel Member	Combined Data (3 Sites)			
	Mean Index Value	Std. Dev	% CV	% Positive (Number positive/Number tested)
Low Negative	0.56	0.11	20.44	1.1% (1/90)
High Negative	0.75	0.08	11.07	0.0% (0/90)
Indeterminate	0.94	0.10	11.14	3.3% (3/90)
Low Positive	1.61	0.30	18.69	96.7% (87/90)
High Positive	2.57	0.40	15.44	100% (90/90)

Precision Study

Precision (intra-run repeatability) was evaluated using the same 5-member panel tested during the Reproducibility Study. The panel was tested twice per day, in triplicate, at three sites by multiple operators over a five-day period.

For each panel member, precision was determined by averaging results during a single run. In total, 30 runs were conducted for each panel member. Intra-run percent CV for all panel members ranged from 0.41% to 26.76%. Overall, 94% of CV values were < 10% and 75% of CV values were < 6%. These results are within the range of precision results observed for Fungitell, and support that the Fungitell STAT is substantially equivalent to Fungitell.

Lot-to-Lot Variability Study

To demonstrate that assay performance is not affected by Fungitell STAT Reagent or Fungitell STAT Standard lot, a Lot-to-Lot Variability Study was conducted at a single internal site (Beacon Diagnostic Laboratory, Inc.) using three lots of Fungitell STAT Reagent and three lots of Fungitell STAT Standard. For this study, a panel consisting of 30 low negative, 20 high negative, 30 indeterminate, 18 low positive, and 12 high positive archived, clinical specimens were tested. Clinical specimens were chosen for inclusion in this study based on Fungitell re-testing results. A greater number of negative samples were tested because the Sample Stability Study (see “*Sample Stability*” below) suggested that negative samples were the most affected by sample instability. Overall, all % CV values were 27% or less except for two negative samples that had % CV values of 34% and 48% and one indeterminate sample that had a % CV of 30%. This is acceptable. Linear correlations derived by plotting Fungitell STAT index values obtained for lots 1-3 ranged from 0.97 to 0.98, indicating that lot is not a major source of variability.

2. Linearity:

Not applicable.

3. Analytical Specificity/Interference:

Not applicable.

4. Assay Reportable Range:

The Fungitell STAT index results range from approximately 0.4 to 3.5, covering the full standard curve (31 – 500 pg/mL) of the predicate Fungitell assay.

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

Quality Control

Fungitell STAT Standard

The standard curve generated with the Fungitell STAT Standard serves as the positive control for the assay. For the Fungitell STAT Standard to pass QC, the following criteria must be met: (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 (i.e., Fungitell STAT Standard and patient samples) must be run again.

Patient Samples

For all patient samples, the following general QC criteria must be met:

1. The kinetic curve must be positive after 500 seconds,
2. The kinetic curve must have an OD ≥ 0.03 at the end of the test,
3. The slope must be numerically positive,
4. The correlation coefficient (r) must be ≥ 0.980 and
5. The kinetic curve must have an upward increasing curve shape

Samples can be outside of the index range of the Fungitell STAT assay and will not meet general QC criterion (4) above: “The correlation coefficient (r) must be ≥ 0.980 above.” For such samples, the following QC steps should be performed to verify that the result is acceptable, but out of the Fungitell STAT index range:

- The result is likely out of range on the positive side if:
 - The Y intercept is positive and
 - The kinetic curve passes 0.4 OD before 1000 seconds
- The result is likely out of range on the negative side if:
 - The kinetic curve is positive after 500 seconds and
 - Has an OD >0.03 and <0.07 at the end of the test

If the Sample result meets both QC criteria for either the positive or negative out of range, 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”.

Sample Stability

To demonstrate that freezing samples does not affect sample stability, 611 archived serum samples stored at -80°C with Fungitell results were re-tested with the Fungitell assay. Samples were considered stable if there was no categorical change from a Positive to a

Negative or a Negative to a Positive result based on the clinical reference values found in the Instructions for Use of the Fungitell assay. Overall, 98.9% (604/611) samples had no categorical change. The seven (7) samples that did have categorical changes originally tested negative by Fungitell and re-tested as positive by Fungitell. These results indicate that freezing samples at -80°C does not affect sample stability.

6. Detection Limit:

Not applicable, as the analytical measuring range of the Fungitell STAT and Fungitell (predicate) assays are the same.

7. Assay Cut-Off:

The assay cut-off was determined through Receiver Operator Curve (ROC) analysis using the Fungitell (predicate) assay to classify a specimen as a true positive, indeterminate, or true negative. ROC analysis was performed on a dataset of 93 archived, de-identified patient serum samples that were selected randomly from the Beacon Diagnostic Laboratory (BDL) sample bank based on concentrations on file from previous testing with Fungitell. All samples were re-tested with Fungitell and re-test results were used to generate the sample distribution illustrated in **Table 2**.

Table 2. Sample Distribution Used to Determine Fungitell STAT Assay Cut-Offs

	Fungitell Testing Results (pg/mL)								
	N=34			N=15	N=44				
Index Value Range	<43	44-51	52-59	60-79	80-91	92-99	100-107	108-500	>500
# Samples	14	13	7	15	15	13	1	13	2
Interpretation	Negative			Indeterminate	Positive				

The negative cut-off was determined using 49 data points, which included 34 negatives and 15 indeterminates. A negative cut-off value of ≤ 0.751 was chosen based on the highest Youden index calculated from the negative vs. indeterminate ROC analysis. At a negative cut-off value of ≤ 0.751 , negative percent agreement was 94.1% compared to Fungitell.

The positive cut-off was determined using 59 data point points, which included 44 positives and 15 indeterminates. A positive cut-off value of > 1.076 was assigned based on the highest Youden index calculated from the positive vs. indeterminate ROC analysis. At a positive cut-off value of > 1.076 , positive percent agreement was 75.0% compared to Fungitell.

Based on these results, the index cut-off values for Fungitell STAT were set at <0.74 , $0.75 - 1.1$, and > 1.2 corresponding to the Negative, Indeterminate and Positive zones, respectively (see **Table 3**).

Table 3. Fungitell STAT Index Ranges

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

*Index values are rounded to the nearest two decimal figures

These cut-offs were confirmed during the Method Comparison Study, which compared results from the Fungitell STAT to the Fungitell assay.

B. Comparison Studies:

1. Method Comparison with Predicate Device

Method comparison testing was conducted to compare performance of the Fungitell STAT to that of the predicate assay, Fungitell. Testing was performed on archived, frozen serum specimens collected from hospital in-patients with signs and symptoms consistent with invasive fungemia and mycoses (the intended use population) at a single internal site (Beacon Diagnostic Laboratory, Inc.). In total, 488 specimens with (1→3)-β-D-Glucan concentrations distributed over the full range of the Fungitell predicate standard curve were evaluated, including 309 samples that fell within the negative zone of the predicate, 36 samples that fell within the indeterminate (equivocal) zone of the predicate, and 109 samples that fell within the positive zone of the predicate assay (**Table 4**).

Table 4. Sample Population Distribution According to Fungitell (Predicate) Re-test Results

	Fungitell Testing Results (pg/mL)							
	N=308			N=37	N=143			
Index Value Range	<31	31-47	48-59	60-79	80-120	121-300	301-500	>500
# Samples	203	72	33	37	34	58	38	13
Interpretation	Negative			Indeterminate	Positive			

The positive percent agreement (PPA) and negative percent agreement (NPA) were 99.2% and 97.6%, respectively (**Table 5**). The linear correlation between the Fungitell concentration and Fungitell STAT index results was 0.92 with a two-sided 95% CI of 0.899 and 0.936.

Table 5. Fungitell STAT Performance Compared to Fungitell

		Fungitell			Total
		Positive	Indeterminate	Negative	
Fungitell STAT	Positive	118	2	7	127
	Indeterminate	24	17	19	60
	Negative	1	17	283	301
	Total	143	36	309	488
		PPA: 99.2%* (118/119) 95% CI: (95.4, 99.9)		NPA: 97.6%* (283/290) 95% CI: (95.1, 98.8)	

*Indeterminate (i.e., equivocal) results excluded; 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)

2. Matrix Comparison:
Not applicable.

C. Clinical Studies:

1. Clinical Sensitivity:
Not applicable.
2. Clinical Specificity:
Not applicable.
3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

D. Clinical Cut-Off:

Not applicable.

E. Expected Values/Reference Range:

Not applicable.

VIII. Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.