the Fungitell® Bulletin
volume 9, issue 3

Topic:

FROM SERUM TO RESULT IN ONE HOUR: FUNGITELL STAT® EXPLAINED

THE FUNGITELL STAT® ASSAY: PRINCIPLE, METHOD, AND INTERPRETATION

Fungitell® Bulletins are intended as technical advisory communications and as such are disseminated to the general public in order to highlight the significance of (1→3)-β-D-Glucan on human health. These communications do not promote a specific drug, therapy nor make any representation or suggestion concerning the suitability or effectiveness of a particular drug or therapy in patients harboring (1→3)-β-D-Glucan. Fungitell® is an adjunct diagnostic assay to be utilized in conjunction with clinical signs and symptoms for the diagnosis of invasive fungal infection. Fungitell® is currently 510(k) cleared for the detection and quantification of (1→3)-β-D-Glucan in human serum and should be used and interpreted only in a manner consistent with the current Instructions for Use.

Discussion:

Single or small numbers of (1→3)-β-D-glucan (BDG) tests are required in emergent and acute care as well as in low patient sample number settings. Fungitell STAT® is a simple, fast (1 hour), and small footprint approach to testing 1-7 patient serum samples *[Figure 1]*.

Figure 1: Fungitell STAT® PKF08 Instrument *The instrument is small in size: 174mm x 119mm x 37mm weighing approximately 1 kg. The power supply is EU friendly. The tablet and barcode reader are presented for size reference.*

Based upon the well-known Fungitell microplate-based assay, Fungitell STAT® is also a *Limulus* Amebocyte Lysate kinetic assay that is specific for (1→3)-β-D-glucan (BDG) [*Figure 2*][1,2,3,4,5]. The Fungitell STAT® method employs a standard in place of the standard curve utilized in the Fungitell® method. This Fungitell STAT® standard is a critical element of the test and is designed to represent the rate of a reaction for a sample of glucan at 80 pg/mL. This pg/mL value is based upon the cutoff in, and derived from, the execution of the Fungitell® predicate assay. The Fungitell STAT® standard is run in parallel with a sample (or samples) to which it is compared using the same treatments and materials. A simplified method for execution is outlined below in *Table 1*. With this approach, an emergency department lab or main lab can quickly run one to seven patient tests at a time, saving days over a send-out approach.

Fungitell® (1→3)-β-D-Glucan Assay
Bulletin Volume 9, issue 3
Publish Date: November 2020
CORP_0251

Corporate Headquarters
Associates of Cape Cod, Inc.
124 Bernard E. Saint Jean Drive
East Falmouth, MA 02536 USA
Tel: (508) 540–3444
www.acciusa.com

United Kingdom
Associates of Cape Cod Int'l., Inc.
Deacon Park, Moorgate Road
Knowsley, Liverpool L33 7RX
United Kingdom
Tel: (44) 151–547–7444
www.acciuk.co.uk

Europe
Associates of Cape Cod Europe GmbH
Opelstrasse 14
D-64546 Mörfelden-Walldorf, Germany
Tel: (49) 61 05–96 10 0
www.acciusa.de

FROM SERUM TO RESULT IN ONE HOUR: FUNGITELL STAT® EXPLAINED

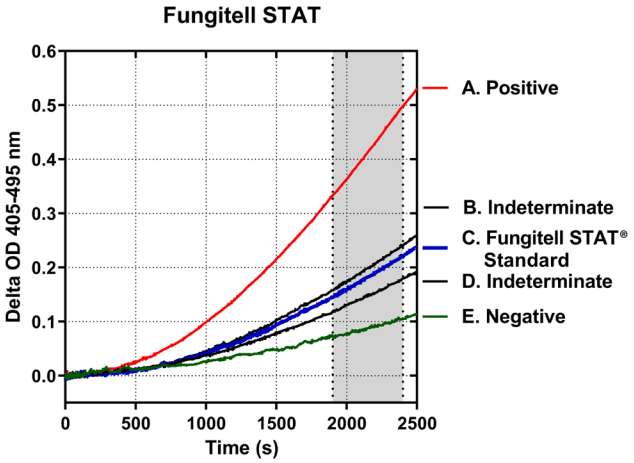

Figure 2: Illustration of underlying kinetic curves derived from the Fungitell STAT® method. Samples on the graph: A. is positive and B. and D. are Indeterminate, C. Fungitell STAT® Standard; E. Negative. All plots are delta OD 405-495 nm. The gray zone between 1900 and 2400 seconds is the area of linear regression from which rates are determined.

Table 1: Fungitell STAT® simplified method outline

Step	Action
1	Add serum sample (50 µL)* to an empty vial /test tube
2	Add alkaline pretreatment to sample (200 µL), mix
3	Reconstitute the Fungitell STAT® Standard (STD) with LRW (100 µL) *, mix
4	Add alkaline pretreatment solution (APS) to STD (400 µL)*, mix
5	Incubate samples and STD at 37°C for 10 min
6	Reconstitute Fungitell STAT® reagent with LRW (300 µL), mix
7	Transfer 75 µL of STD to a Fungitell STAT® reagent reaction vial, mix
8	Transfer 75 µL of sample to another Fungitell STAT® reagent reaction vial, mix
9	Place reaction vials into instrument
10	Collect data

**The ratio, 1:4, with pretreatment for sample and standard is fixed, however, reconstitution volumes of the Fungitell STAT® Standard (STD) will vary depending on lot. For example, the reconstitution volumes for the standard lot used in this table are 100 µL LRW:400 µL APS.*

The output for the Fungitell STAT® method is a comparative index (beta glucan index, BGI, *Figure 3*) computed by dividing the patient sample rate by the Fungitell STAT® standard rate. This patient sample BGI value is qualitatively interpreted as a Negative, Indeterminate, or Positive result according to the ranges provided in *Table 2* below. The relationship of the STAT BGI output back calculated into the pg/mL values of the more familiar Fungitell® microplate kit is described in *Table 2* as well.

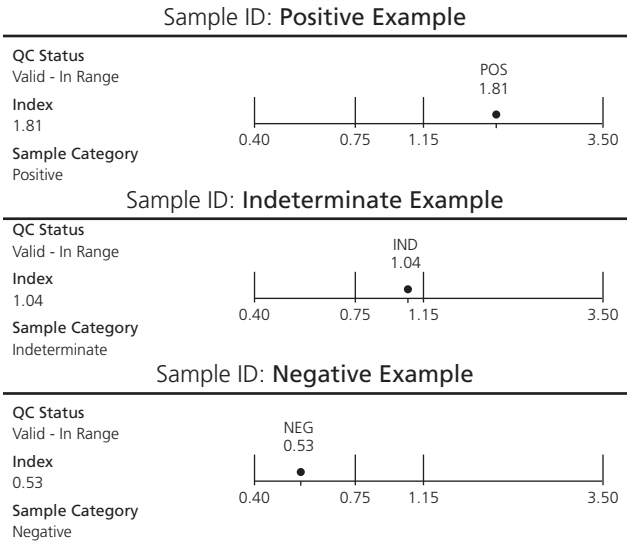

Figure 3: Example output from a Fungitell STAT® assay as reported by the new BG Analytics® (BGA) software.

Table 2: Fungitell STAT® (BGI) and comparison to Fungitell® (pg/mL)

	A	B	C
Cutoff	**Fungitell STAT® IFU (BGI)**	**Fungitell® Predicate (pg/mL)**	**FSTAT to FTELL Back calc. (pg/mL)**
Negative	≤0.74	<60	<60
Indeterminate	0.75 – 1.1	60 – 79	60-88
Positive	≥ 1.2	≥ 80	≥96

Complementing the traditional Fungitell® assay, used with high sample volumes, the Fungitell STAT® permits clinical settings of any size to utilize rapid BDG testing in their patient care. Additional information is available at www.fungitell.com/fungitell_stat.

Discussion References:

1. D'Ordine RL, Garcia KA, Roy J, Zhang Y, Markley B, Finkelman MA. Performance characteristics of Fungitell STAT®, a rapid (1→3)-β-D-Glucan single patient sample in vitro diagnostic assay. Med Mycol. 2020 May 13. pii: myaa028. doi:10.1093/mmy/myaa028.
2. Iwanaga, S., Miyata, T., Tokunaga, F., and Muta, T. 1992. Molecular mechanism of hemolymph clotting system in *Limulus*. Thrombosis Res. 68: 1-32.
3. Tanaka, S., Aketagawa, J., Takahashi, S., Tsumuraya, Y., and Hashimoto, Y. 1991. Activation of a *Limulus* coagulation factor G by (1→3)-β-D-Glucans. Carbohydrate Res. 218:167-174.
4. Saito, H., Yoshioka, Y., Uehara, N., Aketagawa, J., Tanaka, S., and Shibata, Y. 1991. Relationship between conformation and biological response for (1→3)-β-D-Glucans in the activation of coagulation factor G from Limulus amebocyte lysate and host-mediated antitumor activity. Demonstration of single-helix conformation as a stimulant. Carbohydrate Res. 217:181-190.
5. Aketagawa, J., Tanaka, S., Tamura, H., Shibata, Y., and Saito, H. 1993. Activation of *Limulus* coagulation factor G by several (1→3)-β-D-Glucans: Comparison of the potency of glucans with identical degree of polymerization but different conformations. J. Biochem 113:683-686.

Fungitell® (1→3)-β-D-Glucan Assay
Bulletin Volume 9, issue 3
Publish Date: November 2020
CORP_0251

Corporate Headquarters
Associates of Cape Cod, Inc.
124 Bernard E. Saint Jean Drive
East Falmouth, MA 02536 USA
Tel: (508) 540–3444
www.acciusa.com

United Kingdom
Associates of Cape Cod Int'l., Inc.
Deacon Park, Moorgate Road
Knowsley, Liverpool L33 7RX
United Kingdom
Tel: (44) 151–547–7444
www.acciuk.co.uk

Europe
Associates of Cape Cod Europe GmbH
Opelstrasse 14
D-64546 Mörfelden-Walldorf, Germany
Tel: (49) 61 05–96 10 0
www.acciusa.de