the Fungitell® Bulletin
volume 9, issue 1

Topic:

INFLUENCE OF (1→3)-β-GLUCAN STRUCTURE UPON REACTIVITY

(1→3)-β-GLUCAN STRUCTURAL ELEMENTS THAT INFLUENCE ACTIVITY IN FUNGITELL®, A *LIMULUS POLYPHEMUS* AMEBOCYTE LYSATE-BASED REAGENT

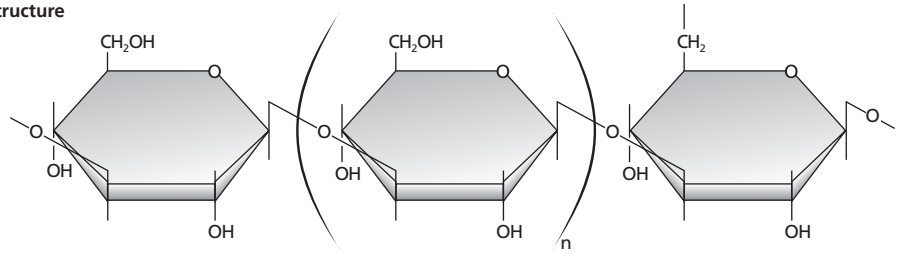
Fungitell® Bulletins are intended as technical advisory communications and as such are disseminated to the general public in order to highlight the significance of (1→3)-β-D-Glucan on human health. These communications do not promote a specific drug, therapy nor make any representation or suggestion 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:

A. (1→3)-β-D-Glucan (BDG) Structure

BDG in its simplest form exists as a linear backbone sequence of β-D-glucose molecules linked through beta-(1→3)-glycosidic linkages (Fig. 1).[1] In addition to the linear backbone, BDG is often substituted with other molecules, including glucans, mannans, proteins, lipids, etc., producing a large range of possible structures.[2] The molecular weight of naturally occurring BDG is extremely heterogeneous, ranging from low thousands of Daltons to large particulate structures, such as fungal hyphae or yeast sacculi, or their fragments.[3] For the purposes of this article, much of the discussion will focus on the linear (1→3)-β-D-glucan backbone. This backbone normally exists in a triple helical conformation.[3] However, heat, alkali, and solvents can convert the triple helical form to single helices that can, slowly, re-anneal to re-form the triple helix.[3,4] BDG is generally insoluble in water, requiring solvents or alkaline solutions, to be rendered soluble. Its presence in fluids is thought to occur as poorly soluble polysaccharide to suspended microparticulates.[3] An additional route to increasing its solubility is branching and substitution.[3,5] Analysis of BDG is complex, with processing conditions having a profound impact upon biological activity results.[6,7] Collectively, the heterogeneity of BDG, the effects of processing, and the diverse bio-analytical approaches, make its study challenging. Interestingly, this has much in common with the study of the structure-function relationships of bacterial endotoxins.[8,9]

Fig. 1 (1→3)-β-D-glucan structure



Fungitell® (1→3)-β-D-Glucan Assay
Bulletin Volume 9, issue 1
Publish Date: April 2020
CORP_0228

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

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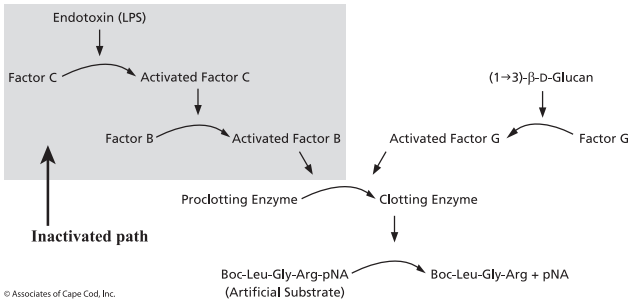
B. LAL-based reagent measurement of (1→3)-β-D-Glucan in biological fluids

The measurement of BDG content of a biological fluid is routinely performed using Fungitell®, a Horseshoe Crab (*Limulus Polyphemus*) blood cell extract-based reagent (Fig. 2).[10] In this assay, the activation of a highly BDG-specific zymogen protease, Factor G, begins the process by which signal is generated. Once activated, Factor G cleaves and activates a second zymogen protease, pro-clotting enzyme, to yield an active protease that is used to hydrolyze paranitroaniline away from a triplet chromogenic peptide, boc-Leu-Gly-Arg-pNA. The elements of the LAL Cascade encompassed by the shaded rectangle in Fig. 2 are inactive in Fungitell, yielding BDG specificity.[11] The free pNA light absorbtion at 405 nanometers is used to monitor the reaction, kinetically. Interpolation of the results against a water-based Pachyman (highly linear, single helical BDG) standard curve yields the estimate of the sample's BDG titer. It is important to note, that the activity and subsequent BDG titer estimate are dependent upon multiple factors including single helix content, molecular weight, degree of branching, degree of backbone substitution, size of adducts, solubility, etc.[12] Thus, in the clinical diagnostic laboratory setting, the estimate of BDG titer represents an integration of the characteristics of the BDG present in the sample. This differs from the circumstance of the measurement of perfectly soluble, monodisperse, moieties with highly conserved composition and structure. However, the cutoff values utilized in the diagnosis of invasive fungal disease have been validated clinically and those values have held up.[13]

The principles and characteristics of biological assays of complex biomolecules need to be understood by users. It is important to be aware of their benefits and limitations. Fungitell® is an exquisitely sensitive reagent for the detection and measurement of (1→3)-β-D-glucans and, used appropriately, can assist in multiple diagnostic, research, and quality control applications.

Figure 2. ***Limulus*** **Amebocyte Lysate (LAL) Cascade**



Discussion References:

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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