LAL Update®

ASSOCIATES OF CAPE COD, INCORPORATED • NOVEMBER 2001 • VOLUME 19, NO.3

Letter From the President

The term LAL Reactive Material (LAL-RM) was coined in 1981 to describe a non-endotoxin material associated with hemodialysis membranes that caused LAL to gel. Although the exact chemical nature of the original LAL-RM (from the Travenol CF dialyzer) has never been clearly elucidated, it is now well known that LAL contains an alternate pathway specifically activated by beta 1,3 glucans. What has become clear in recent years is that beta 1,3 glucans, originating from fungal contamination, frequently show up in certain pharmaceutical products and medical devices. These fungal glucans, while capable of elucidating a brand-dependent response in LAL similar to LAL-RM, can now be detected and quantified with certainty using a new product from Associates of Cape Cod, Inc., Glucatell. With the possible exception of the original Travenol CF dialyzer, LAL reactive and Glucatell reactive glucans are fungal in origin and must therefore be clearly considered as contaminants---not an intrinsic component of the drug or device in which they are found. Variable reactivity of existing LAL brands and the concept of an "endotoxin-specific LAL" and the treatment of glucan as a substance that causes a "false positive", only serve to hide a contamination problem. Because fungal glucans are clearly contaminants, their presence in pharmaceutical products is unwarranted. Furthermore glucans are not inert. Research has shown that fungal glucans are biologically active and can potentiate the activity of endotoxin. This UPDATE gives an overview of glucan contamination and its detection. As with endotoxin contamination, knowledge of glucan presence and concentration is the first step in elimination and control.

Sincerely,

Thomas J. Novitsky, Ph.D.

Glucatell™- A New Kit for Quantitative Determination of (1→3)-β-D-Glucan

by Malcolm Finkelman, Ph.D.

Introduction

Fungal glucan, like bacterial endotoxin, is a ubiquitous environmental contaminant. It is a constitutent of the cell envelope and is continuously shed into the environment through the life cycle. Although the backbone is typically a homopolymer of glucose, fungal glucan has a variable structure. In addition to linear sequences of (1→3)-β-D-linked glucose, fungal glucan contains variable amounts of branches, and cross-linked side chains, to other polysaccharides and proteins (Griffin). As a result of its complexity, it also presents with a highly variable molecular weight and solubility (Manners, p.411-417). Accordingly, measuring (1→3)-β-D-glucan presents a challenge to researchers who wish to monitor its presence as a contaminant and evaluate its properties as a biological response modifier. In order to address this need, Associates of Cape Cod, Inc. has developed, in conjunction with researchers at Seikagaku Corporation, a Limulus Amebocyte Lysate-based test kit for (1→3)-β-D-glucan measurement. The test kit is presented in the familiar chromogenic, microtiter plate format, and provides picogram level sensitivity to (1→3)-β-D-glucan. The kits are available in both kinetic and end-point diazo formats. In addition, ACC is offering a special serum analysis kit with a specific serum protocol, for those researchers interested in monitoring blood levels of fungal glucan.

Note: The serum kit is for research use only and is not for use in diagnostic procedures.

Appearance of Glucan Contamination

A variety of circumstances may contribute to fungal glucan burdens in pharmaceuticals, biologicals, immunological preparations, medical devices, patient serum, etc. These include production methods based upon yeast or fungal fermentation; use of cellulose-based devices including absorbent pads, clarifying filters, and gauze; and isolation of active ingredients from plant material (Kimura, p.790-794). In some cases, a "moldy" environment or mold growth on stored raw materials may be a source of contaminating fungal glucan. Another area of fungal glucan appearance is in the air of residential and work environments that are "moldy," and may be characterized with "sick building syndrome" (Rylander, p.263-267).

Biology of Fungal Glucan

(1→3)-β-D-glucan forms an important component of the cell wall of most genera of fungi, where it contributes to mechanical and structural integrity. It typically has a complex presentation, including linear, branched, and cross-linked forms. (1→3)-β-D-glucan content is highly variable in cell walls but may form up to half of the dry weight of fungal mycelium or yeast cell walls. Through the fungal life cycle, there is a continual synthesis and degradation of cell wall material, leading to a sloughing of cell wall material, including (1→3)-β-D-glucan. The molecular weight ranges from low molecular weight (10^3 kD) soluble material to large molecular weight, insoluble particulates including mycelial fragments or whole cell sacculi.

(1→3)-β-D-glucan is a biological response modifier and has been extensively studied as an immunomodulator (Muller, p.3418-3425). A variety of mammalian cell types, including macrophages, neutrophils, and fibroblasts have been demonstrated to bind and respond to (1→3)-β-D-glucans. These responses include internalization of the bound glucan, stimulation, or inhibition, of nuclear factor Kappa B, elicitation of pro-inflammatory cytokine cascades, and natural killer cell priming. A variety of firms have conducted tests with (1→3)-β-D-glucan as an immuno-protective agent against sepsis, as a stimulant to wound healing, and for anti-tumor activity.

The complexity of working with (1→3)-β-D-glucan is compounded by its presentation in different structural forms. Major forms include the triple helix, single helix, and random coil configurations (Aketagawa, p.683-686). These forms have been shown to behave differently with respect to receptor binding and their ability to elicit cellular responses. Another confounding factor in working with (1→3)-β-D-glucan has been the difficulty of quantitation, especially at low levels, and between different sources.

Perhaps the most dramatic biological response elicited by (1→3)-β-D-glucan is that observed with the amebocytes of the Horseshoe Crab. Workers familiar with the LAL cascade are well acquainted with the fact that the LAL cascade is triggered by both LPS and (1→3)-β-D-glucan (Morita, p. 318-321). The latter specifically binds to and activates, Factor G, a glycoprotein serine protease zymogen. Once activated, Factor G specifically cleaves pro-clotting enzyme which can then proceed to cleave its native substrate, coagulogen, leading to clot formation. Addition of a chromogenic peptide substrate allows this process to be followed spectrophotometrically.

Specific Quantitation of (1→3)-β-D-Glucan

Specific quantitation of (1→3)-β-D-glucan, in impure solutions, has been very problematic. The availability of Glucatell offers a major advance in this area. ACC's Glucatell kit provides the user with a highly sensitive chromogenic reagent, in a familiar 96 well microtiter plate format. The reagent has picogram level sensitivity in either the kinetic or endpoint diazo modes. The methods are very similar to those employed with the chromogenic LAL tests (Pyrochrome or Chromo-LAL).

Typical standard curves are low range, 3-50 pg/mL and high range, 30 - 500 pg/mL. The standard is Pachyman, a (1→3)-β-D-glucan, with a small amount of (1→6)-β-branching. This highly purified standard is derived from the mushroom, *Poria cocos*.

Verification of (1→3)-β-D-Glucan Content

Users who wish to add an additional verification step to their assessment of (1→3)-β-D-glucan levels, as measured by Glucatell, may wish to utilize a specific Zymolyase digestion protocol, available, from ACC. Utilizing the specific (1→3)-β-D-glucan-degrading properties of Zymolyase, the investigator is able to establish the level of signal measured by the Glucatell reagent, before and after Zymolyase digestion.

To Order

To place an order, please call us at:
1-800-LAL TEST (1-800-525-8378).

For Additional Information

To request a copy of the Glucatell Serum Protocol, a Glucatell insert sheet, or to receive general information on Glucatell or any of our other products, please call 888-395-2221 and ask for our Technical Service Department. One of our scientists will be happy to assist you. You can also visit our website at www.acciusa.com.

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Glucatell™ Applications

LAL Endotoxin Out of Specification Results: Occasionally, LAL false positives results may be encountered. The resulting OOS investigation is made very much simpler if a root cause determination can be made. Glucatell can assist in verifying an alternative explanation for OOS LAL results, truly establishing the basis of a false positive.

Cellulose Filter Preparation: Experience has shown that cellulose filters may leach large quantities of fungal glucan. Monitoring both filter rinse and final product for glucan contamination is easily accomplished with Glucatell

rDNA Yeast Protein Production: A popular host for recombinant protein production, yeast easily contaminate product with cell wall-derived glucan. Glucatell may be used to monitor glucan levels through the purification process.

Cell Culture Fluid: Fungal (1→3)-β-D-glucan is a pro-inflammatory biological response modifier. Avoidance of extraneous, artifact-producing contaminants is a goal for both manufacturers and consumers.

Airborne Contamination: "Sick building syndrome", bad workplace air, and "moldy" environments may all have high levels of airborne glucan in common. This may be monitored using air sampling onto filters and Glucatell.

Mycosis Research: Serum burdens of fungal (1→3)-β-D-glucan can be accurately and reproducibly monitored using the serum version of the Glucatell kit. Additional pre-test sample processing is conveniently accomplished within the microplate wells, using simple, easy to use chemistry, supplied with the kit.

CALENDAR OF EVENTS

Glucatell™ Kinetic Assay Kits

GT002 Glucatell Kit without diazo reagents for kinetic assays (research use only)
110 Tests

GT004 Glucatell Kit without diazo reagents for kinetic assays (research use only)
55 Tests

Glucatell™ End-point Assay Kits

GT001 Glucatell Kit with diazo reagents for end-point assays (research use only)
110 Tests

GT003 Glucatell Kit with diazo reagents for end-point assays (research use only)
55 Tests

Glucatell™ Serum

GT005 Glucatell Serum Kit with diazo reagents for kinetic assays (research use only)
110 Tests

NOVEMBER 2001

November 11-14
Society for Neuroscience
San Diego Convention Center
San Diego, CA
Booth #1324

November 14-16
Glycobiology 2001
Cathedral Hill Hotel
San Francisco, CA

DECEMBER 2001

December 3-4
PDA Annual Meeting
Marriott Wardman Park Hotel
Washington, DC
Booth# 603 & 605

December 8-12
American Society for Cell Biology
Washington Convention Center
Washington, DC
Booth# 755

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