

## Letter From the President



Dear LAL User:

Associates of Cape Cod, Inc. scientists have been actively aware of LAL reactivity to (1,3)- $\beta$ -D-glucans since the first reports appeared in the early 1980's. In the June 1990 issue of the LAL UPDATE (Vol. 8, No. 2), Dr. Roslansky, a former colleague of mine, discussed LAL reactivity to glucans in some detail. Then, as now, it has been our policy to treat glucans as "bioactive" molecules and as "foreign substances" when present in pharmaceutical preparations. We choose to recognize glucans as the contaminants they are through analysis of positive LAL samples with endotoxin and glucan-specific reagents (Endospey<sup>®</sup> and GlucateLL<sup>™</sup> respectively). We believe that the use of glucan-insensitive formulations or masking techniques is inappropriate for product release purposes. While different formulations of ACC LAL vary somewhat in their glucan sensitivity, generally speaking, a negative result with these LALs is a negative result for both endotoxin and (1,3)- $\beta$ -D-glucan. Glucan contamination is relatively rare, given the fact that the great majority of LAL assays on drugs, devices, and biologics conducted in the years since LAL has been in use, have been negative. It is, however, clear from the data reported here that cellulose-based products may contain variable levels of contaminating (1,3)- $\beta$ -D-glucans. This problem extends to any drug or medical device manufacturer whose process employs raw materials, cellulose filters, yeast/molds, etc. that might contribute contaminating levels of (1,3)- $\beta$ -D-glucan. In order to address this issue, we have introduced GlucateLL<sup>™</sup>, a (1,3)- $\beta$ -D-glucan-specific formulation. GlucateLL provides our clients with a tool to assess any potential glucan contamination, design an effective cleaning protocol, or select clean(er) raw material.

ACC has always been cognizant of the ubiquitous nature of glucans in the environment. The opportunity for glucan contamination of transfer pipette tips, tubes, LRW, sample collection containers, and micro plates is similar to that for endotoxin. Our Pyroclear<sup>®</sup> line of LAL accessories have been certified to be free of interfering levels of endotoxin and

(1,3)- $\beta$ -D-glucan. Therefore users of any of our LAL products, including GlucateLL, can potentially eliminate all equipment-related OOS results by exclusively using Pyroclear.

Sincerely,

Thomas J. Novitsky, Ph.D.

## TECHNICAL REPORT

### (1,3)- $\beta$ -D-Glucan Contamination of Cotton-Containing Medical Devices: Sources, Measurement, and Implications

by Malcolm Finkelman, Ph.D.

In the August 2001 LAL Update (Vol. 19 No. 3), we provided a basic description of (1,3)- $\beta$ -D-Glucan, an overview of its biology, and its measurement using the newly introduced GlucateLL<sup>™</sup> kit. In this issue of the Update, we will focus on specific medical devices that have been demonstrated to be contaminated with (1,3)- $\beta$ -D-Glucan and some of the potential implications. This will also be the subject of an ACC poster presentation at the International Endotoxin Society meeting in Washington, DC (July 17-21, 2002).

The two classes of medical device that are ubiquitous in both medicine and every day life are cotton gauze wound dressings/surgical sponges and menstrual tampons. As has been noted earlier, (1,3)- $\beta$ -D-Glucans are pro-inflammatory molecules, capable of immuno-modulatory activity<sup>(1,2,3)</sup>. Researchers in Japan have reported that (1,3)- $\beta$ -D-Glucan leached from medical gauze used during abdominal surgery.



The leaching of the glucan contributed a massive level of LAL signal to the peritoneal fluid, when measured with Toxicolor, a LAL formulation that is sensitive to both endotoxin and glucan<sup>(4,5)</sup>. Subsequent analysis with Endospecy and Gluspecy, formulations that are endotoxin-specific and glucan-specific, respectively, showed that the signal was overwhelmingly from leached (1,3)- $\beta$ -D-Glucan. These reports also showed that some of the (1,3)- $\beta$ -D-Glucan that leached from the surgical gauze, was translocated to the systemic circulation. Analysis of perioperative blood samples showed (1,3)- $\beta$ -D-Glucan levels approximately seven-fold higher than the cut-off used to diagnose fungal infections, using Fungitec-G, a clinical diagnostic for invasive fungal infection (available in Japan).

At ACC, we have begun surveying cotton and cotton gauze-containing medical devices to determine the burdens of leachable, and measurable, (1,3)- $\beta$ -D-Glucan, using the GlucateLL assay. The results have shown that every device tested leached (1,3)- $\beta$ -D-Glucan and that the (1,3)- $\beta$ -D-Glucan burdens are highly variable. Figure 1 illustrates the levels observed in a survey of menstrual tampons, of different absorbencies, from different manufacturers. The results show that the amounts of (1,3)- $\beta$ -D-Glucan leached are highly variable and differ with the levels of absorbency, claimed for the products, within a manufacturer's offerings. The methods used involved measurement of glucan levels after standardized soaks in ACC LAL Reagent Water, which is glucan-free. It remains to be seen what sort of (1,3)- $\beta$ -D-Glucan levels may be observed *in-vivo*.

An analysis of (1,3)- $\beta$ -D-Glucan leaching from wound dressings and surgical sponges is presented in Figure 2. Again the results show that all the examples tested leached (1,3)- $\beta$ -D-Glucan and that there was tremendous variability among the products. From the levels observed, it is clear that there is great potential to contribute (1,3)- $\beta$ -D-Glucan to both wound surfaces, systemic circulation, and intra-cavity spaces, depending upon the source, number, size and type of application of these materials.

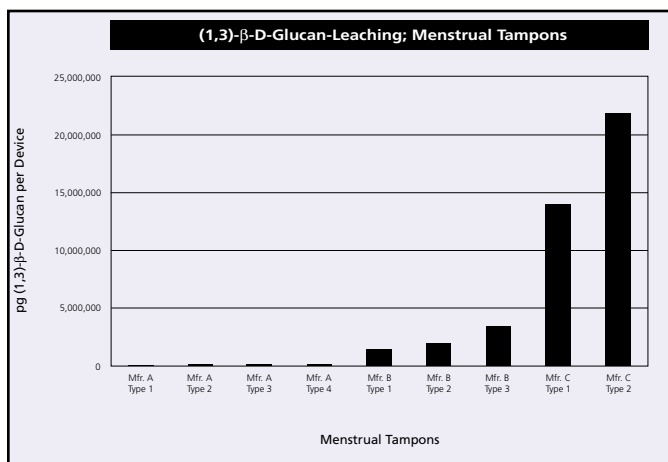


Figure 1 - (1,3)- $\beta$ -D-Glucan Leachate Burdens of Cotton-Containing Tampons

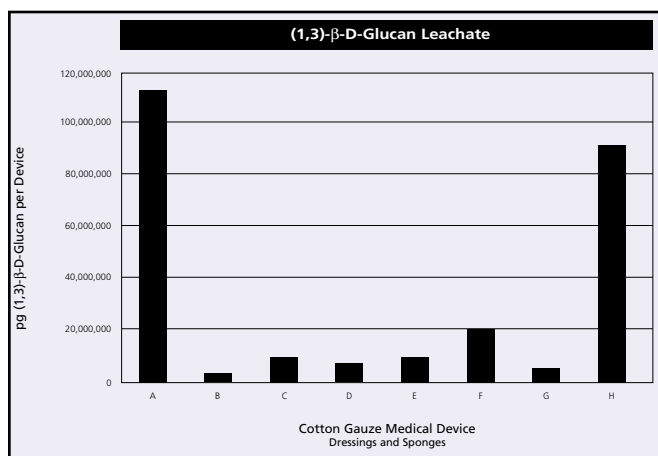


Figure 2 - (1,3)- $\beta$ -D-Glucan Leachate Burdens of Gauze Dressings and Sponges

### Discussion

Although wound dressings and surgical sponges are generally considered to be inert, chemically, they are associated with inflammatory responses *in-vivo*<sup>(6,7,8)</sup>. Given that (1,3)- $\beta$ -D-Glucan is immunologically active, and that it is leached in relatively large amounts from these devices, it is of interest to determine whether such exposure has a physiological impact upon patients. Some reports have noted greater levels of inflammation in wounds covered with gauze dressings than those covered with hydrocolloid dressings<sup>(9)</sup>. Additionally, research has demonstrated that prior systemic exposure to (1,3)- $\beta$ -D-Glucan, primes rodents for a lethal response to normally sub-lethal endotoxin challenge<sup>(10,11)</sup>. Given the clear demonstration of pro-inflammatory activity in a variety of *in-vitro* and *in-vivo* models, and the widespread exposure to patients with highly variable clinical circumstances, the safety of (1,3)- $\beta$ -D-Glucan exposure requires systematic investigation.

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## New Glucan Specific Website Section Under Construction

Since the introduction of the GlucateLL kit for the specific detection of (1,3)- $\beta$ -D-Glucan, in 2001, ACC has received numerous inquiries from a community that includes laboratorians with glucan concerns in end-product release testing and in-process quality testing, as well as process development for new products. In addition, some analysts have expressed concerns that glucan-contaminated reagents and laboratory equipment are introducing artifacts into cell-based assays. In order to assist this community of users with glucan-related questions and concerns, ACC is setting up a special section on its website that will include Frequently Asked Questions and Answers, as well as tips, lore, and bibliographic information associated with both the applied aspects of (1,3)- $\beta$ -D-Glucan measurement and control and basic science. In this issue, we provided a limited bibliography that we have found useful. A more complete list of references and resource links will also be posted on the ACC website. You can find the new GlucateLL section listed under **Product Catalog / Research Products / GlucateLL** and then follow the link for additional GlucateLL information. As always, our Technical Service Department can be reached at (800) 848-3248 and will be happy to answer any questions about LAL testing or GlucateLL that you may have.





## 21 CFR 11 Compliant Software Available This Summer

Associates of Cape Cod, Inc. announces the upcoming release of its 21 CFR part 11 compliant software. This software, which was designed to meet the tough new standard, has been two years in the making and well worth the wait.



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