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LAL test for biomedical devices

The history of the evolution of biomedical devices and their role in improving human life shows lessons learnt and tasks ahead. New materials for biomedical devices are a challenge to materials scientists and engineers. For toxicologists, device-testing systems that will be more and more sensitive, more economical to adopt, highly reproducible, quicker and less labour-intensive are important goals. The Limulus amoebocyte lysate (LAL) test, a quality-control test conducted on a finished product meant for clinical application, screens products for bacterial endotoxins, which are pyrogens, and may produce fever and shock, and even death of users of 'a' contaminated product. The range of products amenable to the LAL test is wide, from drugs to medical devices[1-6].

The addition of LAL to endotoxin from gram-negative bacteria results in granulation and increased viscosity or gelation of the incubation mixture. The rate at which this occurs and the degree of gelation seems to be proportional to the amount of endotoxin present. This feature is now being used effectively for detection of pyrogenicity in various application areas. The present United States Pharmacopoeia (USP) rabbit test for pyrogen[7] is satisfactory, but there are limitations imposed by the test itself. Some of them are: restricted variety of samples that can be tested between- and within-animal variations that may arise in spite of proper preconditioning. requirement of extra space, longer test time, and expense of maintaining a rabbit facility.

In the Indian context, the medical-devices industry, which has to cater to a market demand of 3500 million rupees, needs many companies with manufacturing facilities to shoulder the burden. Many product-volume needs are below medium- and large-scale production. In fact the demand can be met almost entirely from small-scale industrial ventures. These small-scale industrial units can organize the necessary mandatory tests to test their production batches in-house, or use the services of a central testing agency. Implementing in-house mandatory testing will become attractive for such industries if reliable and qualified *in vitro* testing procedures and the know-how for setting up such a facility as an integral part of their production set-up are made available. Especially when the Medical Devices Act is being drafted and will be statutorily enforced, it is important that latest-technology methods for quality-testing of end-products are validated and made available as implementable know-how packages.

Validation of the LAL test calls for a three-tier approach:

(i) Data generation to demonstrate sensitivity and reproducibility with respect to the reagent used

(ii) Check of each particular device or product line, utilizing different materials or methods of manufacture, for inhibition or enhancement of the LAL test

(iii) Routine testing of production samples using prescribed test protocols and controls.

The US Food and Drug Administration (FDA) has approved three types of LAL test: (i) gel-clot technique. (ii) chromogenic and endpoint-turbidimetric technique, and (iii) kinetic-turbidimetric technique.

We used the gel-clot protocol as recommended by FDA, using the readily available kit E-Toxate[8] from Sigma Chemical Company, USA, to test ten numbers of Indian Pharmacopoeia (IP)-grade injectable fluid and 17 numbers of infusion sets, all picked from stock kept for use. Preparation of endotoxin-free equipment, and reading and interpretation of the LAL assay were conducted according to the Sigma protocol[8]. The results (see Table) show that, out of eight samples of sodium chloride injectable packs, only one contained endotoxin. However, both the samples of Dextrose packs had no endotoxin contamination. All but four samples of infusion sets passed the test, and four samples of the 'passed' infusion sets were found to have inhibitors, which made the test invalid for these samples. The validity of this test system depends on the absence of inhibitors. Hence the three-tier procedure as suggested by FDA is meaningful and essential.

From our experience with the LAL test we are optimistic that it is an economically viable and practically feasible *in vitro* procedure that can be adopted for detection of endotoxins in quality-

Sample	Test for endotoxin	Test for E-Toxate inhibitor	Negative control	Positive control
Sodium chloride inj. IP (8 nos)	7 −	+	−	+
	1 +	+	−	
Dextrose inj. IP (2 nos)	2 −	+	−	+
Infusion sets (17 nos)	13 −	4 −	−	+
	4 +	+		

'−' Denotes absence of endotoxin in columns 2 and 4 and presence of inhibitor in column 3; '+' denotes presence of endotoxin in columns 2 and 5 and absence of inhibitor in column 3

control protocols in biomedical-device development and production.

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Surface sugars of cancer cells: The sweeter the deadlier?

In tumorigenesis and metastasis of tumours, although factors such as protease production and organ-specific microenvironment have been implicated in target selection, recent evidences assign a decisive role to oligosaccharide structures on cell-surface glycoconjugates, interaction of which with sugar-specific molecules on other cells or on extracellular matrix governs cell sociology. For example, in reactivity towards the carbohydrate-binding proteins (lectins) from peanut (peanut agglutinin, PNA), soybean (soybean agglutinin, SBA) and *Ulex europeus* (*Ulex europeus* agglutinin-1, UEA-1), primary subcutaneous tumours induced in rats using Lewis lung carcinoma cells had the phenotype PNA^-, SBA^-, UEA-1^-, while its liver and lung metastases were PNA^+, SBA^+, UEA-1^-, and PNA^+, SBA^+, UEA-1^+ respectively[1]. Penno *et al.*[2] observed in *in vitro* studies that galactosylation in general, and that of a 110-kDa cell-surface glycoprotein in particular, correlates with invasiveness of a murine adrenal carcinoma cell line. In more detailed investigations by Dennis and colleagues[3], using the highly metastatic lymphoreticular tumour cell line MDAY-D2 and its non-metastatic glycosylation mutants, two events have been shown to enhance metastatic potential: (i) sialylation of oligosaccharides and (ii) addition of $\beta1\rightarrow6$ branched *N*-acetylglucosamine (GlCNAc) to which galactose is also attached as part of a tri- or tetra-antennary structure in N-linked ogligosaccharides. The above conclusion is supported by earlier *in vitro* tests that showed that natural-killer (NK)-cell attachment to cells is inversely proportional to their sialylation and to masking of mannose groups by substitution such as by $\beta1\rightarrow6$ GlcNAc branching[4]. Also, swainsonine, an alkaloid that inhibits oligosaccharide processing, has been found to reduce growth and increase susceptibility to NK cells of tumour cells *in vitro*[5]. MDAY-D2, unlike its undersialylated mutants or its desialylated derivative, adhered very weakly to plastic surfaces coated with fibronectin, laminin or collagen type IV, thus providing an explanation for its mobility and metastasis[6].

Recent results suggest that endogenous lectins, as complementary molecules or receptors to oligosaccharide groups, may be most effective, though glycosidases and anticarbohydrate antibodies may be operative. Many animal tissues contain a β-galactoside-binding lectin (galaptin)[7]. Galaptins are easily solubilized from tissue by soluble galactosides, indicating their association *in vivo* with complementary glycoconjugates.

Lotan and Raz[8] also demonstrated two β-galactoside-binding lectins in human and murine tumour cells. Interestingly, both the β-galactoside-containing glycopeptides from asialofetuin and monoclonal antibodies to the lectins could inhibit tumour-cell aggregation as well as attachment to the substratum. Further evidence for an active role of glycoconjugate–lectin interaction in growth regulation was provided by the recent results of Sanford and Harris–Hooker[9], who found that rat-lung galaptin was mitogenic towards pulmonary arterial cells and smooth-muscle cells in culture (as measured by incorporation of radiolabelled thymidine) in a sugar-dependent manner.

Changes in oligosaccharide structures *vis-à-vis* their corresponding lectins is a popular topic in cancer research today. Inasmuch as a tumour is regarded as a retrogression to the embryonic stage, it will be interesting to examine if reexpression of differentiation antigens, like the stage-specific embryonic antigen [Gal $\beta1\rightarrow4$ (Fuc$\alpha1\rightarrow3$) GlcNAc-R] or oversialylated form of neural cell adhesion molecule, and their recognition by lectins facilitate tumour mobility and anchorage.

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