LAL UPDATE®

Volume 9, No. 2 **June 1991**

Dear LAL User:

This UPDATE addresses the influence of glass on the LAL test. A previous LAL UPDATE (Vol. 6(3)) addressed the problems with plastics while treating glass as a control. Two papers, "Factors affecting the recovery of endotoxin adsorbed to container surfaces" and the recently published "Plastics, endotoxins, and the Limulus amebocyte lysate test" also compared plastic to glass (see "References" for the complete citations). Much of the material for this UPDATE was compiled by Dr. Michael Dawson, Assistant Director, who has lectured on the subject and by Dr. Priscilla Roslansky, Senior Research Scientist.

Glass is used as the primary container for LAL as well as endotoxin. Glass test tubes are also recommended for the gel-clot test. Historically, glass was chosen because plastics were not in common use in the early '60's when the original research on LAL was performed. Also, glass is easy to depyrogenate. Like plastics, however, glass comes in different chemistries and configurations. The choice of glass, not only as storage containers, but also as reaction vessels, can be critical to the successful outcome of an LAL test.

This UPDATE concludes with the "GLUCAN CORNER", "USP SAYS", and our "CALENDAR."

Sincerely,

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Editor

Glitches with Glass

There are two classes of problems that can be caused by glass (or plastics):
1. The material (chemical and/or physical properties) can have a direct influence on the LAL enzymes/substrate, and
2. the material may remove endotoxin (through adsorption or aggregation).
The distinction between these classes, however, is not often clear.

Interference With LAL

The type of glass used as the reaction vessel for the LAL test can influence the sensitivity of the reaction. The two most common types of glass encountered in laboratory test tubes are flint (soda lime, Type III), and borosilicate (Type I). Depending on the lot of LAL used and its sensitivity, and lot (or manufacturer) of glass used, endpoints ranging from no difference to up to 8-fold have been obtained when flint and borosilicate tubes are compared. This difference is not due to leachable ions or pH, but to the surface chemistry of the glass. Flint glass often contains more crystals of Na_2CO_3 and NaO on its surface than does borosilicate. These crystals possibly serve as "nucleation" sites for the LAL gel. Not only would these sites serve to "anchor" the gel to the tube, they could also provide an "initiation" point for gel formation. If initiation is facilitated, the gel forms faster, resulting in increased sensitivity. Our research has also shown that for the turbidimetric test, where turbidity rather than a gel clot is preferred, borosilicate glass performs better than flint.

One of the problems with flint glass from a chemist's point of view is that it "weathers," i.e. additional Na_2CO_3 and NaO crystals form on the surface. This can lead to a localized corrosive attack of the glass and would manifest itself in the LAL test by either an **increase** in sensitivity, or in the extreme case, where weathering causes a pH change due to leaching, a **decrease** in sensitivity. Some tubing manufacturers treat the glass to prevent or reduce the weathering. One such treatment includes flooding the surface with freon. These treatments, unlike the type of glass, are usually not apparent to the end user. It is relatively easier, however, to distinguish different tube types and chemistries by their color or ultraviolet fluorescence. Some manufacturers include small amounts of metal salts in their glass which cause the glass to fluoresce. If you experience a change

in LAL sensitivity and suspect a change in the tube chemistry/type, compare the color of the tube rim in daylight and under UV. Although it is impossible to determine the exact chemistry by this method, it is usually a reliable indicator that the tubes are different. Using this method, we have found tubes of obviously different chemistries in the same shelf pack of one manufacturer.

ACC has recognized the effect of glass on LAL sensitivity since the commercialization of LAL in 1974. We have therefore recommended certain suppliers of flint glass. For several years, a consistent and reliable supplier has been Fisher Scientific. Recently, however, we have experienced unexplained differences in glass from Fisher that has affected the reactivity (i.e. sensitivity) of LAL. Because of this problem, ACC has decided to switch to a supplier that is able to provide more control of glass chemistry and manufacture (see last page). This new supplier will maintain complete accountability as to chemistry, date and place of manufacturer, and storage of the glass reaction tubes.

Interference through Endotoxin Adsorption or Aggregation

Although we have not compared flint glass to borosilicate glass with respect to the adsorption of endotoxin, adsorption is relatively slow (on the order of hours at room temperature) and is concentration dependent, i.e. the lower the concentration, the greater the percentage loss. Endotoxin storage vessels (including reaction tubes containing dilutions prior to testing which are left for 30 minutes or longer), on the other hand, could differ dramatically in their adsorptivity. Endotoxin adsorption to borosilicate glass is reviewed in the papers "Factors affecting recovery of endotoxin adsorbed to container surfaces" and "Plastics, endotoxins, and the Limulus amebocyte lysate test."

Table 1 shows the loss of endotoxin in glass when endotoxin in solution is frozen. While additional work is needed, these data suggest that the amount of endotoxin recovered is significantly affected by storage temperature. There is no evidence to suggest, however, that storing endotoxin dilutions in the cold for the short time required to set up an LAL test results in any loss of endotoxin. While adsorption is probably the mechanism by which recoverable endotoxin is reduced in glass stored in the refrigerator, another mechanism may be responsible for losses in frozen samples. Since almost the original amount of endotoxin can be recovered from frozen samples to which triethylamine (TEA) is added, it can be argued that freezing changes the conformation or aggregation state of endotoxin, making it less reactive with LAL.

Conclusion

The caution about glassware presented here clearly demonstrates that glass chemistry is a variable that must be controlled. It is recommended that each lot of glass be tested and released for use in the LAL test. Similarly, it should not be assumed that standards or samples can be stored indefinitely in the refrigerator or freezer. Storage procedures should be tested, and yes, validated if necessary. If these precautions are taken with glassware and plasticware too, the problems discussed here can be avoided.

TABLE 1. Recovery of Endotoxin after seven days storage in glass

Temp.	% endotoxin remaining	
	Day 0	Day 7
5°C	100*	82
	100	79
-20°C	100	61
	100	64

*100% = 0.5 EU/ml

References

Roslansky, P.F., M.E. Dawson, and T.J. Novitsky. 1991. Plastics, endotoxins, and the Limulus amebocyte lysate test. J. Parenter. Sci. Technol. *45*:83-87.

Novitsky, T.J., J.Schmidt-Gengenbach, and J.F.Remillard. 1986. Factors affecting recovery of endotoxin adsorbed to container surfaces. J. Parenter. Sci. Technol. *40*:284-286.

USP Says

The following proposed revisions concerning the *Bacterial Endotoxins Test* <85>and the *Pyrogen Test* <151> are included in the Pharmacopeial Forum, May-June,1991, vol. 17, no.3.

1. Sterile Cilastatin Sodium (p.1793). "**Pyrogen** — It meets the requirements of the *Pyrogen Test* <151>, the test dose being 1.0 mL per kg of a solution in pyrogen-free saline TS containing the equivalent of 50 mg of cilastatin per mL."
2. Cyclophosphamide for Injection (p. 1798). "**Bacterial endotoxins** — When tested as directed under *Bacterial Endotoxins Test* <85>, it contains not more than 0.20 USP Endotoxin Unit per mg of cyclophosphamide."
3. Sterile Gentamicin Sulfate (p.1814). "**Bacterial endotoxins** — When tested as directed under *Bacterial Endotoxins Test* <85>, it contains no more than 1.17 USP Endotoxin Units per mg of gentamicin."
4. Chorionic Gonadotropin for Injection (p. 1814). "**Bacterial endotoxins** — When tested as directed under *Bacterial Endotoxins Test* <85>, it contains not more than 0.03 USP Endotoxin Unit per USP Chorionic Gonadotropin Unit."
5. Sterile Imipenem (p. 1822). "**Pyrogen** — It meets the requirements of the *Pyrogen Test*<151>, the test dose being 10.0 mL per kg of a solution in pyrogen-free saline TS containing the equivalent of 5.0 mg of imipenem per mL."
6. Imipenem and Cilastatin Sodium for Injection (p. 1824). "**Pyrogen** — It meets the requirements of the *Pyrogen Test* <151>, the test dose being 10.0 mL per kg of a solution in pyrogen-free saline TS containing the equivalent of 5.0 mg of imipenem per mL."
7. Sterile Imipenem and Cilastatin Sodium (p. 1827). "**Pyrogen** — It meets the requirements of the *Pyrogen Test* <151>, the test dose being 10.0 mL per kg of a solution in pyrogen-free saline TS containing the equivalent of 5.0 mg of imipenem per mL."
8. Potassium Chloride in Lactated Ringer's and Dextrose Injection (p. 1863). "**Bacterial endotoxins** — When tested as directed under *Bacterial Endotoxins Test* <85>, it contains not ~~less~~ more than 0.5 USP Endotoxin Unit per mL."
9. Ringer's and Dextrose Injection (p. 1872). "**Bacterial endotoxins** —

When tested as directed under *Bacterial Endotoxins Test* <85>, it contains not ~~less~~ more than 0.5 USP Endotoxin Unit per mL."

10. Lactated Ringer's and Dextrose Injection (p. 1875). "**Bacterial endotoxins** — When tested as directed under *Bacterial Endotoxins Test* <85>, it contains not ~~less~~ more than 0.5 USP Endotoxin Unit per mL."
11. Half-strength Lactated Ringer's and Dextrose Injection (p. 1877). "**Bacterial endotoxins** — When tested as directed under *Bacterial Endotoxins Test* <85>, it contains not more than 0.5 USP Endotoxin Unit per mL."
12. Modified Lactated Ringer's and Dextrose Injection (p. 1879). "**Bacterial endotoxins** — When tested as directed under *Bacterial Endotoxins Test* <85>, it contains not ~~less~~ more than 0.5 USP Endotoxin Unit per mL."
13. Sterile Tobramycin Sulfate (p. 1898). "**Bacterial endotoxins** — When tested as directed under *Bacterial Endotoxins Test* <85>, it contains not more than 2.00 USP Endotoxin Units per mg of tobramycin."

Fourth Supplement to the USP XXII.

1. Sterile Cefmetazole Sodium (p. 2454). "**Bacterial endotoxins** — When tested as directed under *Bacterial Endotoxins Test* <85>, it contains not more than 0.2 USP Endotoxin Unit per mg of cefmetazole."
2. Sterile Cefotetan Disodium (p. 2455). "**Bacterial endotoxins** — When tested as directed under *Bacterial Endotoxins Test* <85>, it contains not more than 1.17 USP Endotoxin Unit per mg of cefotetan."
3. Sterile Indomethacin Sodium (p. 2473). "**Pyrogen** — It meets the requirements of the *Pyrogen Test* <151>, the test dose being 1.0 mL per rabbit of a solution in Sterile Water for Injection containing 1.0 mg of indomethacin per mL."
4. Oxacillin Sodium Injection (p. 2483). "**Pyrogen** — It meets the requirements of the *Pyrogen Test* <151>, the test dose being a volume of undiluted Injection providing the equivalent of 20 mg of oxacillin per kg. "
5. Ranitidine in Sodium Chloride Injection (p. 2493). "**Bacterial endotoxins** — When tested as directed under *Bacterial Endotoxins Test* <85>, it contains not more than 7.0 USP Endotoxin Units per mg of ranitidine."
6. Ticarcillin Disodium and Clavulanate Potassium Injection (p. 2499). "**Pyrogen** — It meets the requirements of the *Pyrogen Test* <151>, the test dose being a volume of undiluted Injection providing the equivalent of 100 mg of ticarcillin per kg."

Glucan Corner

In this issue of the Update are references to the biological activity of LAL-RM, the cellulosic factor from cuprophane filters, and two papers on the cholesterol-lowering effect of β(1,3) glucans. Also, there are papers on the reaction of LAL to dextrans and simple polysaccharides, two papers on conformational changes of glucans, and several papers on the biological modification of infections by β(1,3) glucans.

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