LAL UPDATE®

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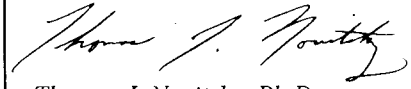
Dear LAL User,

Once again the LAL UPDATE is addressing the issue of CSE potency and certificates of analysis. In September 1993, this topic was discussed from a theoretical point of view. In this issue, practical information is presented on how to cope with the problems that were raised. Until the regulatory agencies can agree on a single standard, we will have to deal with differences in potencies between standards despite efforts to ensure their equivalence.

*Recently the FDA, USP, and WHO have taken the first step toward a universal standard. The standard which will eventually replace EC-5 (USP's lot F) has now been bottled. Since the production of the new standard (EC-6) was a joint effort by the aforementioned agencies, I sincerely hope that this standard will assume international status and be adopted by the regulatory agencies around the world as the reference standard. The ideal would be a single universal standard accessible to all LAL users at a reasonable cost. This would eliminate the need for certificates of analysis. In practical terms, a single, global reference standard is the best we can hope for. Be assured ACC will continue to produce control standard endotoxin and supply certificates of analysis for specific LAL/CSE/RSE combinations in order to make your job easier.**

Sincerely,



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Editor

**Note: For gel-clot and chromogenic LAL/CSE ordered simultaneously, certificates of analysis are automatically provided. When LAL or CSE are ordered separately, our customer service department has no way of knowing the combination required. In this case, please request a certificate of analysis when ordering. Turbidimetric users and anyone using international standards should call our technical services department if a certificate of analysis is required.*

Potency, Certificates of Analysis and International Standards

The September 1993 issue of the LAL UPDATE covered the various endotoxin standards and discussed how the potency of a control standard endotoxin (CSE) can be different when determined with different LAL lots. Because of this, the USP Bacterial Endotoxins Test (BET) chapter requires that, "Calibration of a CSE in terms of the RSE must be with the specific lot of LAL reagent and the test procedure with which it is to be used." Associates of Cape Cod provides certificates of analysis (C of A) that meet this need. The RSE is the USP endotoxin reference standard lot F. Lot F is the same preparation as the US Reference Standard Endotoxin (EC-5) used to determine the label claim sensitivity of LAL reagent produced under license from the FDA.

Associates of Cape Cod's certificates of analysis are detailed, providing the user with as much information as possible. Lot numbers of CSE, reference standard and the LAL reagent are clearly stated. The certificate of analysis shows the raw data from which the CSE potency is calculated, the technician who performed the determination, and the date upon which it was performed. The certificate is then signed by a reviewer.

We strongly caution against re-labelling vials of CSE in EU (or IU). To do so assumes that a single potency applies to all LAL lots which can lead to apparent failure to confirm label claim. Instead, keep documentation of potency for all combinations of LAL and endotoxin used in your laboratory and record potency on all data sheets and reports. Certificates of analysis can be obtained from Associates of Cape Cod for gel-clot lots. C's of A from LAL manufacturers are acceptable to the FDA. The validity of the potency on the certificate is confirmed when you confirm label claim.

Alternatively, potency determination can be performed in your own laboratory. If you use endotoxin from a source other than ACC, you will have to do this for each lot of Pyrotell® using RSE obtained from the USP.

The CSE supplied in Pyrochrome™ chromogenic test kits is the only ACC standard labelled in EU. This is possible because it is a kit component only to be used with the kit. A certificate of analysis is supplied with the kit.

Working with International Standards

Companies releasing product for sale in the USA are required to use an FDA licensed reagent with performance characteristics determined with the RSE in Endotoxin Units (EU). Results must be expressed in EU/ml and standards must be traceable to the RSE. LAL reagent manufactured under FDA license for the gel-clot test is labelled with a sensitivity in EU/ml determined using the RSE. As the LAL (endotoxins) test has replaced the pyrogen test around the world, a number of pharmacopeia have included endotoxins test chapters that specify a reference standard other than the RSE. Consequently, companies increasingly have to deal with different standards and units for products being sold in different countries. Most companies use LAL reagent produced under an FDA license. FDA licensed gel-clot lysates have sensitivities expressed in EU/ml but several pharmacopeia specify standards other than the RSE and require results expressed in units other than EU/ml. This leaves the testing laboratory with the problem of reconciling different standards and units. For example, the European Pharmacopoeia requires the user to confirm the label claim (which will probably be given in EU/ml) using their own reference standard expressed in International Units (IU).

With a view to harmonization, the units of the major international and national standards have been standardized to be equivalent to the US Endotoxin Unit in laboratory studies of relative potency. These standards include the WHO International Standard, the European Biological Reference Preparation (BRP) and the Japanese Pharmacopoeial standard. Standardization studies result in a mean potency that may not hold true for all lots of LAL reagent. Consequently, sometimes it is not possible to confirm the label claim sensitivity of a gel-clot lysate, determined with the US RSE, when using one of the other standards.

For FDA licensed chromogenic and turbidimetric reagents the problem is less acute because, while RSE is used to determine lot characteristics, there is no label claim sensitivity to confirm. The only requirement is to produce a standard curve with a correlation coefficient of at least 0.980 (absolute value).

The question of CSE potency and the difficulty of establishing equivalence between standards was discussed in the September 1993 issue of the LAL UPDATE, but no solutions were offered. Two approaches to this matter are presented here. The gel-clot method is a special case because it is the only method for which a sensitivity, determined using a specific endotoxin standard, must be confirmed before testing can begin. Importantly, both convention and the major pharmacopeia require that the labelled sensitivity be used to calculate results of gel-clot assays. The discussion that follows addresses the issues involved for the gel-clot method. References here are to the BRP, the standard endotoxin referred to in the European Pharmacopoeia, but the principles apply equally to other standards.

Approach 1. Refer to the assigned equivalence of the IU and EU.

Perform all testing using the CSE with a certificate of analysis giving the potency in EU/ml (or the RSE can be used directly) and express results in EU/ml. Then convert results to IU/ml referencing the stated equivalence of the EU and IU.

Advantages:

1. Very straight forward and simple.
2. Based on the equivalence of the IU to the EU.
3. Only one certificate of analysis required (CSE *v* RSE in EU/ng).

Disadvantages:

1. BRP is not used to conduct the test or to produce a C of A, so the requirement of the European Pharmacopoeia is not met.
2. If the potency of the BRP is not equal to 1 IU/EU, but equivalency is assumed, results will differ from those obtained when the actual potency is used. Usually differences are within a factor of two and therefore do not exceed the error of the gel-clot test.

Approach 2. Determine the potency of the BRP relative to the RSE.

Treat the BRP as if it were a CSE and determine its potency with each specific lot of LAL. Use the BRP with the C of A to confirm label claim in EU/ml. Then use the potency expressed in IU/EU in the calculation of results to express final concentrations in IU/ml. If the potency of the BRP is not 1 IU/EU, both the MVD and results of assays in IU will be different from those expressed in EU.

To calculate the MVD using an endotoxin limit expressed in IU and the LAL sensitivity (λ) in EU/ml (as stated on the label), include the BRP potency in the equation:

$$\text{MVD} = \frac{\text{Limit (in IU/unit*) x product concentration (unit*/ml)}}{\lambda\ \text{(EU/ml) x potency of BRP (IU/EU)}}$$

* unit may be mg, ml, unit, etc.

e.g.
If product A has an endotoxin limit of 1 IU/mg, a product concentration of 10 mg/ml, λ = 0.125 EU/ml and the potency of the BRP = 0.5 IU/EU, then:

$$\text{MVD} = \frac{1\ \text{IU/mg} \times 10\ \text{mg/ml}}{0.125\ \text{EU/ml} \times 0.5\ \text{IU/EU}} = 160$$

To express test results in IU/ml, include the potency of the BRP in the calculation:

Endotoxin concentration =
λ (EU/ml) x BRP potency (IU/EU) x end point dilution factor

e.g., for a titer with an endpoint at 1:16

$$0.125\ \text{EU/ml} \times 0.5\ \text{IU/EU} \times 16 = 1\ \text{IU/ml}$$

Advantages:
1. BRP is used as the endotoxin standard and testing can be performed in accordance with the European Pharmacopeia.
2. This approach does not assume that the potency of the BRP is 1 IU/EU with all LAL lots and so avoids the failure to confirm label claim because of an erroneous assumption.

Disadvantages:
1. BRP potency will vary with different lots of LAL.
2. Results in IU/ml will differ from those in EU/ml when potency is not 1 IU/EU.
3. Requires an additional potency determination and C of A (BRP *v* RSE in IU/EU).
4. The MVD will change if the BRP potency changes with different LAL lots.

A third approach that may seem initially attractive can become confusing. This is to determine the potency of CSE relative to both the RSE and the BRP. Then label claim can be confirmed in EU/ml using the RSE potency and it might appear that the BRP potency could be used to express results directly in IU. The problem is that you are required to calculate endotoxin concentrations in samples by multiplying the endpoint dilution factor by the label claim sensitivity. This gives results in EU/ml but the BRP potency is expressed in IU/ng. There is no commonality of units so a direct conversion to IU/ml cannot be made. It is necessary to first express the label claim sensitivity in ng/ml using the CSE potency in EU/ng. Then multiply the endpoint dilution factor by the label claim sensitivity in ng/ml and by the CSE potency in IU/ng to give results in IU/ml. This requires two certificates of analysis, which means twice as much work if the CSE or LAL lot is changed. Also, the calculations are more complex, increasing the danger of confusion. Consequently, it is strongly recommended that Approach 2 be followed.

Chromogenic and Turbidimetric Methods

The situation is rather different in the case of chromogenic and turbidimetric methods because there is no requirement to confirm a labelled sensitivity. The matter is simplified because endotoxin concentrations are determined using a standard curve that can be prepared using any standard endotoxin: RSE, CSE, WHO, BRP, JP or any other. If a CSE is used, its potency can be determined with reference to any other standard endotoxin (RSE, BRP, JP, etc.). Be sure to consult the relevant regulatory documents to determine requirements for a specific standard endotoxin in an official test. For example, the European Pharmacopoeia requires that the BRP be used.

For the great majority of products, the endotoxin limit in IU is the same as that in EU. A problem can arise if a company requires results in both units. If different potencies are obtained for the CSE with the RSE and BRP the results will differ. It is therefore possible that a batch of product might fail when results are expressed in EU, but will pass when they are expressed in IU, or *vice versa*.

One way around this potentially awkward situation is to express results in one unit and then to state that the result is the same in the other unit based on the stated equivalence of the standards and units. (This is equivalent to Approach 1 above.) In Europe, it is likely that the primary unit selected will be the IU while in the US it will be the EU. However, a more conservative course of action is to take the highest result and then express it in the other units.

None of the solutions to this rather thorny problem are ideal, but they do offer ways of dealing with it. These issues will not be properly resolved until a single global primary endotoxin standard is widely available and adopted by all Pharmacopeia.

Reference

Poole, S. and M. V. Mussett. 1989. The international standard for endotoxin: evaluation in an international collaborative study. *J. Biol. Stand.* **17**:161.