On June 22, 2011, the U.S. Food and Drug Administration (FDA) withdrew the "Guideline on Validation of the Limulus Amebocyte Lysate Test as an End-product Test for Human and Animal Parenteral Drugs, Biological Products, and Medical Devices," which was issued in 1987. Elements of the Guideline continue to impact current methods because they can be found in other references relied on by the industry. As such, to those of us who helped write it, its retirement is like the passage of a venerated patriarch who lives on through his descendents. This discussion will cover how important aspects of the LAL Guideline influence standards of practice today.

The LAL Test Guideline was a truly remarkable document with a fascinating history. Terry Munson wrote a feature on the Guideline that appeared in this Newsletter last year. He attributed the lasting impact of the Guideline to the collaboration which made it possible and the science-driven nature of its principles. He wrote, "It could be said that the LAL Guideline was the first case product, with the same regulatory status as any other FDA-approved LAL method. The FDA, when approving the licenses for Charles River’s LAL reagent and cartridge determined that the products appropriately conformed with relevant standards, including the Bacterial Endotoxins Test (BET). Some industry claims that the use of the LAL cartridge platform is an alternative method to the BET are incorrect.

The PTS™ system has a huge following and is in use in all geographic regions where LAL testing is conducted. The product has surpassed all of our expectations with nearly 5,000 machines produced, and millions of licensed cartridges produced and sold under the auspices of our Biological License. In all, the LAL cartridge provides the industry with a valuable new tool and the acceptance of rapid photometric methods has been dramatic.
showing what can be accomplished when FDA and industry experts collaborate on guidance documents. The Guideline contained three elements that enabled the LAL test to enter the mainstream of microbiological testing and to replace the problematic rabbit pyrogen test as the required endotoxins test (with blood products as an exception). The first was the concept of an Endotoxin Limit (EL), which addressed the question: How much endotoxin is safe? The second was the acceptance of a Reference Standard Endotoxin (RSE) against which LAL reagents were certified for endotoxin measurement capacity. The third was the appearance of standard methods for conducting the classic gel-clot test and for validating the test conditions that apply to detecting endotoxin in the presence of a parenteral product.

Endotoxin Limit
The creation of the general endotoxin limit of 5 EU/kg/hr enabled the industry to develop dose-related limits for individual, and classes, of parenteral drugs and devices. Numerous rabbit and human studies support the adequacy of this threshold for intravenous injections. However, the EL for intrathecal administration was assigned arbitrarily and was not science-based. The LAL Guideline described ways to develop test parameters by use of the Maximum Valid Dilution and Minimum Valid Concentration to prepare samples for endotoxin testing, independent of the method. A table of drug-specific endotoxin limits was published as an Appendix. This EL role was appropriately assumed by the pharmacopeia where LAL dependent endotoxin limits became a specification in compendial monographs. The first large-scale conversion from rabbit to LAL tests occurred in 1991 when endotoxin limits were adopted for 185 USP articles. This number grew to approximately 650 compendial articles when the harmonized BET appeared in 2001. The determination of an endotoxin limit for new drugs is a critical part of an application for a new product.

Endotoxin Standards
The variability of early endotoxin standards required the creation of a RSE to assess the pyrogenic capacity of a test material. The USP and FDA sponsored the production of an E. coli lipopolysaccharide for this purpose and defined the Endotoxin Unit (EU) as being equivalent to a quantity of the RSE—currently 10 EU per nanogram. As the RSE is exhaustible, LAL reagent suppliers certify secondary standards to serve as a surrogate for RSE. Calibration of Control Standard Endotoxins (CSE) required a determination of the RSE/CSE ratio to inform LAL users of the EU/ng value and to express LAL test results in EU. The calibration of CSE is an example of an LAL Guideline component (originally described in an Appendix) that was not subsequently addressed by the pharmacopeia. Fortunately, the LAL reagent industry seems to perform this function well, without FDA oversight or a compendial method.

Standardized LAL Test Methods
The LAL Guideline was the first source of a standardized way to conduct the gel-clot method and to validate LAL test conditions. The limit test may be used when an endotoxin limit is assigned to a test material. The limit test has four components, as described in the current, harmonized BET. The critical components are a test material (often diluted to an extent that was validated) and a positive control, which must be recovered within a prescribed range. Recovery of the Positive Product Control (PPC) allows the analyst to have confidence in test results. There are several ways to prepare the PPC; fortunately, the BET is not prescriptive on this topic. Rather, LAL users and LAL reagent manufacturers can be innovative with ways to make test methods as robust as possible. For example, the hot-spike method for PPC preparation is recognized for its efficiency and robustness, making it the accepted spiking technique for kinetic LAL methods. The hot-spike approach allows rapid preparation of the PPC for multiple test materials in the same kinetic LAL microplate or gel-clot bath. While not compendial-driven, this hot-spike approach is the most widely used method.

User qualification of LAL sensitivity also differs from the BET. The BET maintained the LAL Guideline procedure for verifying lambda, the labeled sensitivity of gel-clot reagents. The LAL vendor’s certificate of analysis gives the LAL user an external reference to the accuracy of gel-clot results. Even though the USP specifies RSE for this procedure, the industry universally uses CSE, and there appears to be no evidence of regulatory enforcement action. For kinetic BET, there was no reference to the accuracy of the LAL reagent, which opened the possibility of inaccurate preparation of standards and unreliable results (see Dr. Tsuchiya’s article in this issue). Weak standards meant overestimation of endotoxin, which was usually not a problem. However, strong standards led to underestimation of endotoxin and introduced a realistic risk to the patient. Charles River addressed this problem by creating a unique platform where a kinetic chromogenic assay was referenced to RSE by means of an archived standard curve. The only compendial requirement was linearity, which is met by Charles River’s certificate of analysis. The FDA has approved Charles River’s LAL cartridge method for all product classes.
Impact of LAL Guideline Retirement

Without minimizing the role of the LAL Guideline on endotoxin detection, the rapid pace of technological development eventually rendered it obsolete. References to Guideline elements can be found elsewhere. Other issues, such as training and analyst qualification and sampling schemes, are addressed generally in CGMP regulations. Although not applicable to microbiology tests such as endotoxin, OOS (Out of Specification) results must be dealt with in the spirit of the OOS Guidance.

The FDA may address other LAL test issues at a later date. For example, the FDA has indicated a concern for sampling plans in Warning Letters. Good science will lead to a scientifically sound sampling plan. The previous BME (beginning, middle and end) scheme is still applicable to simplistic products that are produced from a single container and free of bioburden during processing. However, we would expect a more arduous plan if there were multiple filling lines, mixing vessels and steam sterilization cycles involved in production. Pooling of BME should be a thing of the past, except for devices.

Medical Devices

Medical devices have always had a unique place in LAL testing. The devices industry was the first to adopt standard procedures and an endotoxin standard.

FDA’s Role

Withdrawal of the LAL Guideline poses no change in the FDA’s role in endotoxin testing. The LAL industry will continue under the stringent regulatory oversight of the Agency, including reagent licensing and facility inspection. FDA inspectors will continue to inspect for CGMP compliance in the BET laboratory, which means they will determine compliance with your company’s procedures and use instructions (i.e., FDA-approved package inserts for LAL reagents).

In summary, elements of the LAL Guideline of 1987 continue to affect BET function because they can be found in compendial documents, CGMP regulations and related documents. The FDA action was prudent, and in the best interest of everyone. Withdrawal of this Guideline presents the opportunity to revise BET-related procedures for relevance and robustness.

References

The LAL cartridge method is one of the kinetic chromogenic methods; bias is caused by the potency of RSE dilutions used for the establishment of the standard curves. Bias in the LAL cartridge method caused by the potency of the RSE dilutions is consistent because of the utilization of the archived standard curves. The degree of bias is expected to be very low, due to the fact that operators at LAL manufacturers are well-trained and product performance is confirmed by additional quality tests for release.

Fact 1–Variability of endotoxin standard potency
If the endotoxin standard shows consistent potency, daily standard curves will give the most accurate results. However, the potency of standard endotoxin dilutions is not very consistent because of the errors in preparation, vial-to-vial variability of the standard endotoxin and potency change of the endotoxin dilutions. As previously published, the data analysis for the potency check of daily RSE dilutions with the kinetic turbidimetric assay (KTA) at the Charles River laboratory revealed the variability of the potency of the RSE dilutions. The total number of measurements was 174 during the period between September 10, 2008 and June 5, 2009. An average standard curve was established by using the average onset times and the endotoxin concentrations between 0.5 and 0.03 EU/mL (5 concentrations). The individual endotoxin concentrations were recalculated by using the average standard curve from the individual onset times. The ratios of the individual endotoxin concentrations against the average endotoxin concentrations were calculated for each assay. The average ratio of each assay was calculated by using the ratios of the five concentrations of endotoxin in each assay, and was used as the average potency ratio. The averages of the potency ratios of each RSE vial were also calculated. The variability of the potency of endotoxin standard curve was between 62% and 147%. This is in the acceptable range of 50% and 200%. However, this range may vary by personal and environmental factors. Higher and lower potency of RSE dilutions gives underestimation and overestimation in the endotoxin assay, respectively. If endotoxin standard dilutions for the standard curve have 250% potency, the calculated endotoxin values will be about 40% of the actual values. Most importantly, the analyst seldom recognizes this error.

Fact 2–Gel-clot method showed higher values
Analysis of the Limulus Proficiency Testing Program (PTP) results in 2008 was reported previously. In this article, the PTP results from 2008 to 2010 were analyzed (Table 1). The gel-clot method showed 14.4% high values (higher than 200% of the control values) in the total reported values. This result supports the theory of the bias in the gel-clot method. The gel-clot method also showed the highest wrong value ratio (19.1%). These results may be caused by operator error. The second highest wrong value ratio was observed in the endpoint chromogenic method, which requires more human operations than other quantitative methods.

### TABLE 1. ANALYSIS OF PTP RESULTS FROM 2008-2010

<table>
<thead>
<tr>
<th>Method</th>
<th>GEL</th>
<th>KTA</th>
<th>KCA</th>
<th>PTS</th>
<th>EPC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>874</td>
<td>100%</td>
<td>1008</td>
<td>100%</td>
<td>1128</td>
</tr>
<tr>
<td>Fail</td>
<td>256</td>
<td>29.3%</td>
<td>151</td>
<td>15%</td>
<td>233</td>
</tr>
<tr>
<td>Wrong Value</td>
<td>167</td>
<td>19.1%</td>
<td>78</td>
<td>7.7%</td>
<td>96</td>
</tr>
<tr>
<td>High Value</td>
<td>126</td>
<td>14.4%</td>
<td>47</td>
<td>4.7%</td>
<td>39</td>
</tr>
<tr>
<td>Low Value</td>
<td>41</td>
<td>4.7%</td>
<td>31</td>
<td>3.1%</td>
<td>57</td>
</tr>
</tbody>
</table>

GEL, Gel-clot method; KTA, Kinetic turbidimetric assay; KCA, Kinetic chromogenic assay; PTS™, LAL cartridge method; EPC, endpoint chromogenic method.

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**FIGURE 1.** Positive sample values calculated by using daily standard curves and archived standard curve with tube reader KTA method.

**FIGURE 2.** Positive sample values calculated by using daily standard curves and archived standard curve with microplate KCA method.
Fact 3–Archived standard curves provided more stable routine endotoxin assays with suitable LAL methods

The comparison between the positive sample assay values with an archived curve and those with daily standard curves was reported in the previous issue of the Endosafe Times.² The results clearly showed the advantage of the application of the archived standard curve in the LAL cartridge method. We also reported the comparison among the KTA, KCA and the LAL cartridge method (Endosafe®-PTS™), and observed that the archived (averaged) standard curve eliminated the error of a standard curve in the KTA with a tube reader (Figure 1). Figure 2 shows the positive sample values calculated by using the daily standard curves and the archived (averaged) standard curve with the microplate KCA method. In that experiment, there were no irregular values in the KCA measurement. The positive sample values calculated with the archived standard curve were not more stable than those with the daily standard curve. This indicates that the microplate KCA method is not suitable for the archived standard curve application.

Discussion

The BET method requires negative control testing, positive controls/standard curve and positive product controls. These controls are useful for finding most of the errors in the BET. This article focused on the errors seldom recognized by analysts, which are high-risk errors in the BET.

The gel-clot method is the so-called "referee test" in the harmonized BET, likely because there are fewer false negatives with this method. This does not mean the gel-clot technique is more accurate than other quantitative methods. The gel-clot method often provides higher values than quantitative photometric methods. It is usually caused by the bias of the gel-clot method.

The errors caused by the potency of endotoxin standard dilutions are seldom recognized by analysts. The results of the daily potency check of the RSE dilutions by well-trained operators demonstrated that the variability of the potency was between 62% and 147%. This indicates that the variability could be larger in the field. The analysis of the PTP supported this expectation. Therefore, LAL users need to observe the trends in the data to find unexpected errors.

Archived standard curves were useful to avoid the errors seldom recognized by analysts with the KTA and the LAL cartridge method. There seemed to be suitable LAL methods for the archived standard curve application. The LAL cartridge method and the tube reader KTA method could be suitable for the archived standard curve application. The microplate KCA method was not suitable for the archived standard curve application—probably because of different starting times for each measurement. The KCA usually includes a standard endotoxin dilution series in a microplate.

An archived standard curve is no different than the regular daily standard curve. According to the BET, the timing of the preparation of the standard curve is not identified. There is not much difference between an archived standard curve and a daily standard curve prepared in the morning. Both types of standard curves are affected by the potency of the endotoxin standard dilutions. Therefore, the establishment of archived standard curves must be controlled to obtain stable results. For example, Charles River’s Endosafe®-PTS™ cartridges are released by several steps to ensure the quality of the archived standard curves. Establishment of the archived standard curves is performed by a well-trained analyst in the Technical Service department. The potency of RSE dilutions are checked daily using a dedicated LAL lot with KTA. The potency test for the PTS™ cartridges is performed with the RSE dilutions by the Quality Control department—a different section from the Technical Service department. All the data are reviewed by the Quality Assurance department as usual. This type of double-checked quality system provides reliable archived standard curves. If the potency of the standard endotoxin dilutions and the quality of the archived standard curves are not controlled, the obtained archived standard curves are not reliable.

In conclusion, utilization of controlled archived standard curves with a suitable LAL method can eliminate the errors seldom recognized by analysts in the BET.

References


Part of this article was presented at the PDA Annual Meeting in 2011.
Evolution of the Endosafe®-PTS™
Foster Jordan, Corporate Senior Vice President, Endotoxin and Microbial Detection, Charles River

Early Development
In the late 1990’s, kinetic Limulus Amebocyte Lysate (LAL) methods began to dominate endotoxin testing. These assays predominately used 96-well incubating microplate readers, validated endotoxin-specific software and FDA-approved kinetic chromogenic or turbidimetric LAL reagents. Although superior to gel-clot methods when it came to throughput, trending, and electronic reporting, none of the technical weaknesses of kinetic assays were resolved. End users still dealt with many technical issues, such as variability of endotoxin standards, contaminated accessories, complexity of test methods, RSE/CSE calibrations and reader-dependent temperature variations. Charles River realized that creating a completely automated and robust LAL test would require a total redesign of existing LAL reagents and photometric readers. The original concept for the PTS™ was not a point-of-use test, but one that could be run without the need of a skilled analyst.

Since Charles River was not an instrument company, we sought collaboration with a partner that could provide a fully automated test platform to support an easy integration of our unique reagents. Our search led us to a company that produced FDA 510K home blood clotting test instruments and reagents: New Jersey-based International Technidyne Corporation (ITC). Although not designed for high sample volume, their system consisted of a disposable, consumable and portable test unit. We recognized the potential for a system that would give us the best of both worlds by (1) delivering a portable “point-of-use” system, which could support the FDA’s evolving real-time testing initiatives, as well as (2) creating a fully automated, high-volume test system.

In 1999, Charles River and ITC entered into a joint development agreement to create the future quantitative BET assay. The project combined Charles River’s FDA-approved chromogenic LAL reagent (Endochrome) with ITC instrumentation. Collaboration continued for several years; however a change in strategic focus at ITC resulted in Charles River buying all rights to the jointly developed intellectual property in 2001. We continued to develop the technology, improving the methods to provide dry and stable reagents in the cartridge format. Several patents were filed and granted. Ultimately, we launched the first unlicensed PTS™ instrument and cartridge in 2003.

The FDA Approval Process
Simultaneously we filed a briefing package with the FDA, specifically the Center for Biologics Evaluation and Research (CBER), outlining the PTS™ product design. During 2003-2004, Charles River and CBER had written communications, teleconferences, and face-to-face meetings negotiating requirements that would be required to gain FDA approval. These discussions included implementation of the archived standard curve, linearity requirements, assay system suitability, negative controls, samples, and positive controls, manufacturing requirements and software design. The reference materials for all these discussions were the existing FDA-approved reagent package inserts, the USP BET (see Figure 1), the 1987 FDA guidelines, the 1991 interim guidance, and of course our existing BLA. In 2004, the FDA and Charles River mutually agreed on the manufacturing and final product specification for the PTS100 instrument and PTS20F cartridges (F stands for FDA-approved). Of all items discussed, most were focused around maintaining compliance to the existing USP and LAL guideline-described methodology; therefore, the discussion focused on three critical points:

1) Archived Standard Curve: Because there are no liquid LAL reagents involved in the use of the PTS™ test method, the FDA understood that archiving a lot-specific, 3-point RSE standard curve and a mid-point PPC was scientifically valid and consistent with the USP BET photometric methods. Therefore, the primary concern was not the actual archived curve validation, but real-time stability testing on the cartridge itself. Our real-time stability data has been reviewed a total of three times: once during the approval, once again during our first FDA inspection post-PTS™ approval, and most recently after the introduction of the high-sensitivity PTS™ cartridge. No issues were found.

2) Negative Controls: We also demonstrated to the FDA that a routine negative control on each cartridge was unnecessary and could actually be detrimental. The FDA’s main concern is the ability of each cartridge to detect the presence of endotoxin, not to avoid false positives. Since there is no need to prepare standard curves or PPC’s when testing a sample, the only use for LAL reagent water is to dilute a sample if necessary. If we had included a negative control on each cartridge, we would have increased the possibility for the user to add the negative control as a sample and report a false negative. We do conduct negative control testing on each lot of cartridges to ensure there are no “hot channels” similar to testing we do on 96-well plates. We state in the insert that any reagent used to dilute a sample should be certified pyrogen-free prior to use.

3) Process Validations: The agency was highly focused on the cartridge manufacturing requirements and QC testing requirements. As part of our filing, they requested we submit all Validation protocols and test data associated with PTS™ cartridge manufacturing and release testing.
Upon completion of final specifications, SOPs, protocols, stability tests, and validation required for the FDA filing, our PTS™ supplement to our CBER license 1197 was filed. Over the next year, there were written communications and teleconferences regarding the filing package and additional requirements requested by the FDA. In July of 2006, the FDA granted approval of the Endosafe-PTS™ LAL cartridge. CBER clarified that the license applied only to the LAL reagent in the cartridge; the photometer would be treated like the current 96-well microplate readers.

The PTS™ system quickly gained a huge following. The product has surpassed all expectations with thousands of users, nearly 5,000 machines produced and millions of cartridges released by the FDA. We recently launched our 5 cartridge packaging for higher volume users, and more innovations are forthcoming. We will soon launch our fully automated high-throughput system. Design specifications have been completed for the next generation PTS™ and LAL cartridge, which promise to improve greatly on the existing system.

Over the last 12 years, Charles River has committed an enormous amount of time and resources to developing revolutionary LAL technology. Our investment has succeeded in meeting the needs of thousands of customers around the world and we are looking forward to continued development of the platform.

FIGURE 1.

<table>
<thead>
<tr>
<th>Method</th>
<th>LAL/TAL</th>
<th>Reagent refers only to a product manufactured in accordance with the regulations of the competent authority</th>
<th>Minimum 3-point curve</th>
<th>Mid-point spike</th>
<th>Negative control</th>
<th>Use of RSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kinetic Turbidimetric</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes/no¹</td>
<td>yes</td>
<td>yes/no²</td>
</tr>
<tr>
<td>Kinetic Chromogenic</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes/no¹</td>
<td>yes</td>
<td>yes/no²</td>
</tr>
<tr>
<td>End-point Chromogenic</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes/no¹</td>
<td>yes</td>
<td>yes/no²</td>
</tr>
<tr>
<td>Recombinant Factor C</td>
<td>no</td>
<td>no</td>
<td>yes</td>
<td>no</td>
<td>yes</td>
<td>yes/no²</td>
</tr>
<tr>
<td>Monocyte Activation Test (MAT)</td>
<td>no</td>
<td>no</td>
<td>yes</td>
<td>no</td>
<td>yes</td>
<td>yes/no²</td>
</tr>
<tr>
<td>Portable Test (PTS™)</td>
<td>yes</td>
<td>yes</td>
<td>yes ³</td>
<td>yes</td>
<td>yes³</td>
<td>yes</td>
</tr>
</tbody>
</table>

¹ Curves such as 5-.005 EU/mL with spike at 0.5 EU/mL would not comply.
² Use of CSE is not defined in USP, but is standard industry practice when valid RSE/CSE calibration is performed.
³ Pre-calibrated by vendor and data supplied on FDA-approved Certificate of Analysis.

WHAT'S NEW

To meet customers' needs in high-volume endotoxin testing labs, Charles River is proud to introduce the Endosafe-PTS™ 5-pack cartridge configuration. These cartridges are identical to the existing single-use cartridges, but are ideal for customers processing high sample volumes. The multi-cartridge packaging will consist of 5 packs of 5 cartridges or 10 packs of 5 cartridges, and must be used within 2 hours of opening each pouch of 5. They can be used with any existing PTS™ or MCS™ with no re-validation required. For more information, please see www.criver.com/endosafe or call 1-877.CRIVER.1.
Participants at the FDA’s March 2nd Public Meeting on PET (Positron Emission Tomography) drug products came away (1) with a better understanding of the agency’s expectations for microbiological control and testing. Dr. Lynne Ensor of OGD (Office of Generic Drugs) and Dr. Frank Perrella of OC (Office of Compliance) presented helpful information. The PET Drug CGMP Guidance of 2009 (2) requires that an NDA (New Drug Application) or ANDA (Abbreviated New Drug Application) must be submitted by December 2011 in order to continue distribution of Fluorine F 18 and Ammonia N 13 radiotracers. This article describes how to address BET issues in the submission.

Dr. Ensor encouraged the use of rapid photometric endotoxin assays, such as the LAL cartridge test (PTS™). The PTS™ was approved for end-product testing of all drugs and devices under the FDA’s regulatory authority. A BET must be completed before patient administration and must meet an Endotoxin Limit (EL) of 175 EU per dose. The EL is calculated by dividing 175 EU by the maximum dose volume per hour (described in product labeling) or total production volume; dose volume is more realistic. Example: A supplier’s labeling specifies delivery of FDG doses in ≤10 mL, resulting in an EL of 17.5 EU/mL. The permissible dilution to avoid test interference is the EL ÷ λ, where lambda is the lowest concentration on the standard curve. With an EL of 17.5 EU/mL and a LAL cartridge with a robust 5-to-0.05 EU/mL range, the permissible dilution is 350. Dilution factors in the range of 50-to-100 achieve valid test conditions and reduce radiation exposure.

The keys to a compliant BET program are a comprehensive SOP and training. All directions for use in PTS™ product labeling and manuals must be embodied in the SOP for compliance. Elements of the SOP include the following, with recommended text:

1. **Purpose and Scope**: This procedure describes a rapid photometric BET for PET drugs using the LAL Cartridge method (Endosafe®-PTS™). The method meets the acceptance criteria for kinetic chromogenic BET, as specified in USP chapter <85>.

2. **References**: The SOP should reference USP <85> BET, the PET Drug CGMP, and all manuals for the PTS™ reader, printer and software.

3. **Equipment and Materials**: All equipment, reagents and consumables, and their approved suppliers, should be specified with part number and other identifiers.

4. **Reagent Qualification**: Each cartridge shipment must be qualified by valid recovery of the positive control, using the Water BET designated for sample preparation. This qualification allows release of the cartridges from quarantine.

5. **Procedures**: All procedures for sample preparation, reader set-up, cartridge inoculation, review of results and printing of results must be described in detail. The directions must conform to Charles River’s FDA-approved package insert and any additional requirements specified in the PET product’s batch record ANDA submission.

6. **Specifications**: The SOP should draw on the endotoxin limit and permissible dilution calculations from the BET section of the ANDA submission. The ANDA must specify actions to be taken in case of BET failure, such as product withdrawal and investigation.

7. **Review and Interpretation of Results**: The review and decision-making process for test suitability and product release must be described. Should the test result exceed the endotoxin limit, the product is Out of Specification (OOS) and must be withdrawn. An investigation must determine whether the results were invalid or due to a true product failure. If the suitability test is FAIL, it means that the test is INVALID, because that result only refers to repeatability (acceptable CV) and recovery of the positive control. An invalid test leads immediately to a new test, not a retest. If the suitability test is PASS, it means that the test conditions were valid and the results can be trusted.

8. **Documentation of Results**: The procedures for documenting the results on the Batch Record and product release must be described.

The PTS™ system is a robust LAL test. However, inadequate dilutions and inaccurate pipetting frequently cause invalid test results. Refer to the **Endosafe®-PTS™ Assay Guide** from Charles River for additional tips. Your Charles River representative can provide an SOP written specifically for PET products.

**References**
