

Editor's Comment

This issue completes a two-part series on implementing and documenting validation of a BET application. A comprehensive validation report addresses all issues that impact on test reliability and regulatory compliance. A sound convincing report is concise, complete and focuses on the validation studies and specified validated method.

*The FDA's LAL-Test Guideline has served us well, but the needs of the industry require an update. The harmonized BET has reached a level of final acceptance and appears for comment in the June issue of **PharmEuropa**. A similar document will appear soon in the **Pharmacopeial Forum**.*

Dr. James F. Cooper

DOCUMENTING VALIDATION OF A BET APPLICATION

James F. Cooper

In the May 1999 *LAL Times*, nine steps were proposed for validating a BET (Bacterial Endotoxins Test) application. (1) As discussed in the USP 24, validation of a compendial method, such as the BET, for a parenteral product is not validation of accuracy and reliability of the method, but documentation that the selected test conditions are suitable to accomplish the intended purpose. (2) This discussion identifies critical components of a validation document that would be prepared for an official submission such as a New Drug Application. Less is required for validation reports of established products.

Elements that should be addressed in a validation report are highlighted below. Products that have unique considerations may require additional documentation.

Responsibility: The first section of the report contains the signature, date and title of those responsible for review and approval of the report.

Objective and Scope: The report should open with a clearly stated purpose for the validation study. The study's objective is validation of conditions necessary to test a product for bacterial endotoxin, without interference, by one or more methods and reagents.

REPORT FOR A BET METHOD VALIDATION

- Identify responsibility
- State objective and scope
- Show representative calculations
- Describe materials & methods
- Discuss experimental findings
- Assess robustness
- Specify the validated method
- Present conclusions
- Cite references

The scope defines the product or product lines for which the LAL tests were validated. The product is fully described with name, potency, and product code. If more than one dosage form exists, a table can relate all product types, potencies and fill volumes with appropriate names and codes. All characteristics for a device may be tabulated to include configuration, fluid pathway and dimensions, where applicable.

Calculations and Statistical Procedures:

Published limits in drug compendia should be cited. Limits may vary with compendia and impact on products distributed internationally. The endotoxin limit for a new drug is calculated from the tolerance limit (K) and the maximum dose per hour (M) using the K/M formula.

Representative calculations must be presented clearly for MVD, MVC, and test sensitivity. Gel-clot sensitivities of 0.0625 EU/mL and greater must not be rounded off in the first step to prevent overestimation of these calculations. (3) Some auditors will be interested in the safety factor for the test. Should there be different gel-clot sensitivities or kinetic-LAL standard curves used in a BET laboratory, a table relating lambda, test sensitivity and MVC/MVD can clarify this issue. For example, Table 1 relates test sensitivity with lambda for an antibiotic that has an endotoxin limit of 0.15 EU/mg and a validated test concentration of 2 mg/mL. The calculation applies to finished product and bulk drug. All of the possibilities are valid because test sensitivity is greater (lower value) than the endotoxin limit.

Table 1. Lambda versus Test Sensitivity

λ (EU/mL)	Method	PSS* (EU/mg)
0.125	GC	0.06
0.0625	GC	0.03
0.05	Kinetic	0.025
0.01	Kinetic	0.005

*PSS = lambda/test concentration; the test concentration is 2 mg/mL.

Materials and Methods:

All information regarding the reagents, including names, expiration dates and vendors must be presented. It is important to specify the reagents and vendors that are part of the validation process. Consumables such as pipettes, tubes and microplates should not be vendor-specific unless the vendor specifies a specific tube or other material. Rather, the report should indicate that

consumables meet acceptance criteria for general use in the BET laboratory, such as, “glassware depyrogenated by a validated cycle.”

Validation Experiments:

Experimental results should be tabulated, discussed and evaluated to assure suitability of the validated method. Data sheets may be included in an appendix. Compendial methods of various nations and the FDA’s LAL-test Guideline require a minimum of 3 lots of each product for validation with a vendor-specific LAL reagent. It is prudent and industry practice to show results for at least one compatibility screen. (1) This is done with each product, vendor and method studied.

The goal of each gel-clot validation study is to show that an endpoint assay is the same for endotoxin in water and in the diluted test sample. For kinetic LAL studies, the goal is to demonstrate that endotoxin controls are recovered in the diluted test sample without detectable interference. The results should be tabulated and presented by product and lot number.

The **pH issue** must be clarified because this matter continues to be cited frequently during FDA inspections, even when proper controls are used. As previously described, pH measurements must be made throughout sample-preparation stages of the study to show that mixtures of LAL and test sample are pH neutral. (4) Also, the validated method must be effective over the entire pH range specified for a product. For example, the monograph for Mannitol in Sodium Chloride, USP, has a pH range of 4.5 to 7.0. Data should prove that the validated method will neutralize a product adjusted to a pH of 4.5 and provide full recovery of the positive product control.

Table 2: Summary of Endotoxin Recovery and pH Measurement Studies

Lot	Gel-Clot LAL		pH	Kinetic LAL	
	PWC	PPC		Recovery (%)	pH
A	λ	0.5λ	6.9	94	6.8
B	0.5λ	0.5λ	7.0	112	6.9
C	λ	λ	7.0	89	6.9

Table 2 exemplifies a summary table where three lots of an antibiotic were validated with two LAL methods. The gel-clot studies met acceptance criteria where: 1) valid endpoints of the positive water control (PWC) and positive product control (PPC) were within a two-fold dilution, and 2) reagent-and-sample mixtures yielded a neutral pH. Kinetic LAL studies met acceptance criteria by yielding recovery of $100 \pm 25\%$ and neutral pH of reaction mixtures.

Robustness:

A measure of the reliability of a method during routine use is an indication of robustness. (2) The most important way to enhance robustness in a validated LAL method is to make sure that the test concentration selected for routine testing is clearly defined by a compatibility profile and, ideally, confirmed by two LAL methods. (1)

To explain how to achieve robustness in a LAL protocol, let us consider what typically happens when a therapeutic drug is screened for compatibility. At high concentrations, the drug is very incompatible with LAL reagent. Then, there is a 4-fold concentration range that is border-line; i.e., PPC recovery is variable because of lot-to-lot variation in the drug product and/or LAL reagent. As concentration is reduced by additional dilution, the range of complete compatibility is reached where the test method is resistant to the influence of variables. MVC and MVD values can be extended into the out-of-limit range by using a more sensitive reagent or LAL method.

Table 3 illustrates how to define the compatible range and select a robust test concentration for a validated method. A recombinant drug with a potency of 40 mg/mL was screened for reagent compatibility using both gel-clot and kinetic-turbidimetric methods, by testing 2-fold dilutions from 40 mg/mL. There was no valid recovery above 8 mg/mL, the incompatible range. At 8 and 4 mg/mL, recovery of the 2λ PPC was variable and the KTA recoveries were 40 to 75%; these two levels constituted the borderline region for compatibility where a routine test might underestimate impurity. The compatible concentration range gave consistent kinetic recovery near the 100% level. A routine-test concentration of 0.8 mg/mL was selected because it was clearly in the compatible range, could be conveniently prepared by a 1:50 dilution, and had a safety factor of about 5. The safety factor is determined by dividing the test concentration by the MVC ($0.8 \text{ mg/mL} \div 0.14 \text{ mg/mL} = 5.7$), or by dividing the endotoxin limit by the test sensitivity.

A robust kinetic procedure is one that consistently meets acceptance criteria for linearity and recovery, and yields little or no invalidity. The keys to robustness in kinetic LAL studies are a compatible concentration and the selection of the standard curve range. A 2-to-3 log range curve by linear regression, prepared with reliable standards, will seldom yield invalid recoveries. Also, a lambda selection of 0.01 EU/mL or 0.05 EU/mL will avoid the noise-related artifacts that are associated with the use of microplates.

Table 3. COMPATIBILITY RANGES for a Recombinant Drug

40 mg/mL	CONCENTRATION (mg/mL)			
	8	4	2	<0.8>
Incompatible	Borderline		COMPATIBLE Range	
(Inhibition)	(Unreliable PPC recovery)		0.8 mg/mL selected as test concentration	
Dilution 0	1:5	1:10	1:20	<1:50>
				<0.14 (MVC)
				OUT-OF-LIMIT
				1:285 (MVD)

Additional Experiments:

If a product requires unique procedures for sample preparation or resolution of interference, studies should clearly indicate the need for a special additive or treatment, and prove that it solves the interference problem. Additives such as buffers and surface active agents should be minimized. (1)

Choice of regression analysis impacts heavily on validation of kinetic-LAL methods. Linear regression is well established by regulatory guides and consensus opinion. The correlation coefficient, $|r|$, and PPC recovery combine to assure sufficient linearity to produce reliable results. In contrast, curve-fitting analysis was not decided by consensus opinion. It is less reliable because it minimizes recovery as a check for linearity and increases the probability of hot wells. Use of curve-fitting must be preceded by validation to prove accuracy and reliability. (2)

The Validated Method:

A structured but simplified validated procedure should follow the validation studies. **The validated method defines the optimum test concentration or test dilution, preparation of samples and controls, maintenance of test conditions and acceptance criteria.** The report should focus on the specific, routine-test procedure and avoid discussing an ancillary issue such as glassware depyrogenation that is covered under a separate standard operating procedure (SOP). Auditors are particularly interested to know that the test sample and interference controls are identical except for the added endotoxin. “Hot spike” techniques, as previously described, assure continuity in samples and controls. (1)

Interpretation and reporting of results must be explicit. All issues regarding validity of controls and samples must be clear. Representative calculations can exemplify how routine test results are interpreted to comply with endotoxin limits.

Finally, reasons for **revalidation** may be briefly addressed or referred to a general SOP on this topic. Since formulae for LAL reagent and sample product are the focus of a BET validation, any change in the product formula, reagent or method constitutes a need for revalidation.

Conclusions:

The conclusions of the study address the reagent, method, compatible concentration and dilution range, robustness and pH measurements. For example: “All potencies of Sodium Chloride Injection, USP, produced by Rx Drugs were compatible with gel-clot and kinetic turbidimetric LAL reagents at a concentration of 10mg/mL and less. No routine pH measurements are needed because all sample/LAL mixtures were in the neutral range.”

References: All relevant compendia, guidelines and vendor documents are informative.

References:

1. Cooper JF, Validation of bacterial endotoxins test methods, LAL Times, Vol. 6, No. 2, 1999.
2. “Validation of Compendial Methods, “USP 24/NF 19 (U.S. Pharm. Convention Inc., Rockville, MD), p. 2149.
3. Cooper JF, Rounding BET related calculations, LAL Times, Vol. 5, No.3, 1998.
4. Cooper JF, Using validation to reduce LAL pH measurements, LAL Times, Vol. 4, No. 2, 1997.

The harmonized BET appears for comment in June issue of PharmEuropa

Worldwide acceptance of LAL methods in place of rabbit assays has occurred in the past decade. This transition has fostered regional guides for LAL tests that often lacked continuity with other countries. The (ICH) International Conference on Harmonization selected the JP (Japanese Pharmacopoeia) to lead harmonization of the Bacterial Endotoxins Test. Harmonization of this ICH process has progressed to the final, consensus level; this draft was published in PharmEuropa for information and comment on open points.

The harmonized BET is the most important LAL document since the FDA's LAL-Test Guideline. It is well written and easy to follow. For example, there are tables for gel-clot and kinetic methods that explicitly describe the components of a test. There is true harmony on key issues in the EP draft with the JP version. The preparation of samples, measurement of pH, assignment of endotoxin limits and MVD calculation are in agreement. For efficiency, replicates needed for the positive water control in validation is reduced to two. Whereas earlier accounts of the BET encouraged testing at the MVD, the current draft suggests testing a sample at a dilution less than the MVD. The EP will persist with the IU, but this will not pose any problem since it clearly states that one IU is equal to one EU.

The harmonized draft describes six LAL methods that have been developed, including the endpoint turbidimetric test that has enormous limitations. The greater improvement in harmony is found in the kinetic LAL methods. The spike point and test for inhibition and enhancement are very consistent with the JP version as well as labeling associated with US-licensed reagents. The recovery range is 50-to200% of the interference control. However, a critical issue, the type of regression analysis, is not addressed.

In case of dispute, the final decision is based on the gel-clot result (Method A).

In summary, the EP version of BET harmonization is a great step forward and will benefit the BET community.

CALENDAR

Seminars and workshops presented by Dr. Cooper and local representatives:

- Frankfurt: 29 SEPT-1 OCT 99
- Milano 7 OCT 99
- Warsaw 11-12 OCT 99
- Budapest 14 OCT 99
- Seoul 4 NOV 99

Look for the Charles River Endosafe booth at the 1999 PDA Annual Meeting, 30 NOV-3 DEC 99.

Watch for further announcements concerning the U. S. Western Mid-Winter Kinetic Workshop. Contact your sales representative or Frances Cooper for further details.

CONTACTS

Dr. James F. Cooper, Editor
Frances Cooper, Associate Editor
Phone: 843-795-7316; Fax: 843-795-7221