charles river

Summary

The Endosafe®-MCS™ and FDA-licensed LAL cartridges are validated for bacterial endotoxin testing and can be used for product validation for both previously validated samples and new products.

MICROBIAL SOLUTIONS

Product Validation Using the Endosafe®-MCS™

Product validation for a Bacterial Endotoxin Test (BET) (USP chapter <85> or equivalent Pharmacopeial chapter) is performed to document that a specific limulus amebocyte lysate (LAL) reagent or test system will detect endotoxin in a specific drug formula or device extract without interference from the sample matrix. This validation is required in accordance with the interference test for photometric methods under the "Preparatory Testing for the Photometric Quantitative Techniques" section of the BET chapter. The Endosafe®-MCS™ system utilizes LAL cartridges that are licensed by the FDA for detection of bacterial endotoxin, and has been validated for bacterial endotoxin testing prior to end-product release. As with any system, we recommend users conduct their own qualification of the MCS™ before using it for product validation.

Validation Approaches

There are two simple approaches for validating products using the Endosafe®-MCS™ and our FDA-licensed cartridges: One for samples already validated with an existing, conventional method and another for new products. Satisfactory recovery of the positive product control (PPC) for three batches of product or extract is the basis for BET validation using the MCS™ method. Successful recovery of the PPC also indicates that buffers in the LAL reagent satisfactorily resolve any potential pH issues. It is useful to record the pH as part of the validation, as USP <85> directs that the pH of lystate and sample mixture be taken. See package insert for instructions on this pH measurement when using a cartridge based system. Additionally, it is important to ensure that the MCS™ system qualification is up to date, and to use FDA-licensed LAL cartridges.

Validating products with an existing test method using the MCS™

1. Select the appropriate standard curve range of the LAL cartridge to allow product testing within the endotoxin limit.
2. Prepare one batch of product sample at the dilution or concentration currently in use as a non-interfering preparation. Screen it using a licensed cartridge to verify the same sample dilution/preparation will work for the cartridge technology. If spike recovery is valid, then proceed; if it is not, then follow the steps listed below for samples not previously validated.
3. Prepare three batches of the product sample at the dilution or concentration currently in use as a non-interfering preparation.
4. Test these sample preparations on the MCS™ system, one batch per cartridge.
5. Confirm test suitability with valid PPC, sample coefficient of variation (CV) and spike CV.
6. Confirm/verify the pH of the lysate and sample mixture are within a range of 6-8.

Once the results are recorded and the acceptance criteria are met, the validation is complete. The acceptance criteria include: a) spike recovery of the positive product control within the BET acceptance range of 50% to 200%, and b) the CVs for reaction times for both the sample and the PPC replicates are less than 25%.

Validating new chemical entities or non-validated products, including an initial screening phase

1. Determine the endotoxin limit (EL).
2. Calculate the maximum valid dilution (MVD) or minimum valid test concentration (MVC) based on the endotoxin limit and test sensitivity.
3. Test sample at several different dilutions or concentrations to find compatible testing level. (Note: Charles River's inhibition/enhancement screening cartridges can be used for the initial step under certain conditions.)
4. Allow for a two- to four-fold safety factor from the endotoxin limit, if possible.
5. Follow screening by using FDA-licensed LAL cartridges for validation. With the results from this screening, a non-interfering sample preparation for the validation can be determined. To complete the validation, follow the steps detailed above with an existing test method.

Product validation using the MCS™ entails the same steps that are followed for other LAL kinetic/photometric methods. Upon completion of the validation, endotoxin testing is streamlined considerably, as analysts no longer have to prepare endotoxin standards and PPCs. The lot-specific calibration code associated with each particular lot of cartridges provides information for the archived standard curve and spike concentration for the positive product control. See Tables 1 and 2 below for an example of a product validation, including an interference screen.

Table 1: Interference Screen for a Pharmaceutical Product

Dilution with LRW	Limit of Detection (EU/mL)	Spike Recovery
1:4	< 0.2	22%
1:8	< 0.4	7%
1:16	< 0.8	16%
1:32	< 1.6	44%
1:64	< 3.2	65%
1:128	< 6.4	68%
1:256	< 12.8	76%
1:512	< 25.6	84%
1:1024	< 51.2	80%

Table 2: Validation of the Pharmaceutical Product

Batch	Dilution	Limit of Detection (EU/mL)	Spike Recovery
A	1:256	< 12.8	83%
B	1:256	< 12.8	88%
C	1:256	< 12.8	87%

Conclusion

The product in the tables above had an endotoxin limit of 100 EU/mL, and there was no experience testing it with LAL. The product was tested with the MCS™ system, which revealed an interfering issue (in this case, pH) that could be resolved by diluting the product with LAL reagent water. A resolution of the LAL test inhibition at a dilution of approximately 1:256 is indicated by valid spike recovery of 76% (Table 1). In this example, the 1:256 dilution was chosen to avoid validating near the interfering level, and also to allow a safety factor from the endotoxin limit. The product was then validated at this dilution using a lot of LAL cartridges with a standard range of 5–0.05 EU/mL. With a test sensitivity of 0.05 EU/mL, the limit of detection was < 12.8 EU/mL, well within the endotoxin limit of 100 EU/mL. Validation was conducted by testing three batches of the new drug and achieving valid recovery of the positive controls (Table 2).