Roundtable Session 2 – Table 4 – Moving Away From Animal Testing and Animal Derived Components Including Alternate Endotoxin Test

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Abstract:

Appropriate endotoxin and pyrogen controls throughout the manufacturing process, including in-process and release testing, ensures quality and safety for parenteral biological products. Pyrogens are a chemically heterogeneous group of fever inducing compounds derived from microorganisms and non-microbial substances. Endotoxin pyrogens are a cell wall component of gram negative bacteria, lipopolysaccharide. Per FDA 21 CFR 610.13b test for pyrogenic substances, each lot of final containers of any product intended for use by injection shall be tested for pyrogenic substances by intravenous injection into rabbits. The rabbit pyrogen test is performed per USP<151> for Initial Marketed Authorization. The compendial endotoxin test for in-process and release samples is performed per USP<85>, which relies on the use of a Limulus Amoebocyte Lysate (LAL), a reagent derived from the blood of horseshoe crabs.

The requirements in FDA 21 CFR 610.13(b) may be waived if a method equivalent to the rabbit pyrogen test is demonstrated in accordance with 21 CFR 610.9. In accordance with the 3R principle for animal welfare, the pharmaceutical industry is interested in adopting methods that do not rely on animal testing and animal-derived components. The European Pharmacopoeia is in the process of removing the rabbit pyrogen test from 57 pharmacopeial monographs together with a new general chapter on Pyrogenicity (5.1.13). Ph. Eur. 2.6.30 Monocyte Activation Test (MAT) is a suitable in vitro test for pyrogen testing. Alternative methods to the compendial test method may be used after validation according to USP<1223> Alternative Microbiological Methods, USP<1225> Validation of Compendial Procedures, and Ph. Eur. 5.1.6 Alternative Methods for Control of Microbiological Quality. Ph. Eur. 2.6.32 Test for Bacterial Endotoxins using Recombinant Factor C has been effective since 2021 and is already referenced in some product monographs. USP<86> Bacterial Endotoxins Test Using Recombinant Reagents, which will become official in May 2025, provides additional information to use non-animal derived reagents for endotoxin testing.

Discussion Questions:

1. What are the considerations and challenges in validation of MAT as a replacement for the rabbit pyrogen test?

2. What is the current industry experience in switching from LAL-based to non-animal derived reagents (Recombinant Factor C; rFC, Recombinant Cascade Reagent; rCR) for

endotoxin testing?

3. What are the current regulatory expectations and industry experience in submitting sustainable test methods (i.e. MAT, rFC, and/or rCR)?

4. Are there additional barriers and/or challenges that are delaying industry adoption of MAT and/or non-animal derived endotoxin test methods?

Notes:

1. Roche has developed a general method and validation strategy. FDA has released a guidance for endotoxin and pyrogen testing in 2024, states that a rabbit pyrogen test is required but can be waived if an equivalent method is developed. Rabbit pyrogen test is a qualitative test that looks for fever as a response whereas the monocyte activation test looks at human monocytes for the pyrogen and is quantified using an ELISA. There is guidance also provided in European Pharmacopeia – Chapter 2.6.30. The chapter was recently revised to provide clear guidance on how to perform the testing. This chapter goes into details on equivalence. Generic validation is performed per USP 12.25, 12.23, ICHQ2, the European Pharmacopeia – Chapter 2.6.30. To consider which parameters to validate – Consider LOD, accuracy, specificity, precision, robustness and then range in linearity. After completion of general method validation, product specific validation is performed per European Pharmacopeia – Chapter 2.6.30 and that includes test for interfering factors. Considering for demonstrating equivalency is to leverage literature and vendor provided data on the MAT kit.

2. Industry experience is varied. Some companies have been using recombinant reagents for many years, other companies are in validation or evaluation phase. Once USP <86> becomes official in May 2025, it should reduce the barrier for companies to make the switch. Since the details are outlined in USP <86>.

3. EMA is encouraging the adoption of rFC per Chapter 2.6.32. Health Canada is also open to submission per USP <86>. We assume that FDA should be fine with USP<86> since it was reviewed by them. One consideration for EMA is that rCR is an additional method that would require additional validation activities whereas rFC method.

4. Legacy products data is a barrier.

Additional notes

Sometimes MAT doesn’t work and it’s hard to get that side by side data. Depends on the product, sometimes you get an enhancement with antibodies in the MAT assay (enhancement over 200%) (IL-6 was the readout).

In a CDMO environment, it is hard to implement MAT. Large customers are using the old techniques. What about sustainability goals? CDMO's are still sticking to older techniques since the bigger customers have legacy data using them. Smaller countries also have no bandwidth when it comes to it. EMA is asking for rFC data but recognize they can't force companies to do this.

Bioforums trying to advocate for newer sustainable techniques – BIO is supporting USP <86>. WHO brought out guidelines on phasing out animal based testing for quality control.

ThermoFisher Scientific and other bigger companies need to get on board with switching to MAT. It was highlighted in the press about the horseshoe crab in the last several years.

What conferences are CDMO going to ? Drug, Chemical & Associated Technologies Association (DCAT) – this could be a good place to influence regarding switching to MAT. Bigger issues at hand than innovation. Not every CDMO has the capabilities, CROs are used – BioReliance and Charles River. Charles River makes the handheld endotoxin readers which comes along with cartridges. Fujifilm makes a kit. Lonza and Biomerieux makes kits for rFC. Strategy could be to have a rFC secondary supplier. Since rFC is expressed in a bioreactor, it should be easy to scale up.

Good reviews on this topic ? Latest regulatory chapters. Publications on these topics are available.

https://www.americanpharmaceuticalreview.com/Featured-Articles/583996-Validation-Strategy-for-New-Recombinant-Factor-C-Users/

https://www.europeanpharmaceuticalreview.com/article/225373/endotoxin-testing-the-international-regulatory-landscape/

Is there any way health regulations can come together and agree on a common path for the horseshoe crab issue?