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Contract Analytical Services

Big Pharma Heads to Wickham Laboratories - Case Study on Bacterial Endotoxin Testing

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The market release of a pharmaceutical product is achieved by ensuring it has met its product specification, which varies dependent on the type and use of the product, says Wickham Laboratories' Louise Rigden.

Introduction

Microbiological testing for most products requires non-sterile specification or Total Aerobic Microbial count (TAMC) and absence of specific pathogens such as *Escherichia coli* and *Salmonella*. Parenteral (injectable) products have more stringent testing and are required to be both sterile and bacterial endotoxin free.

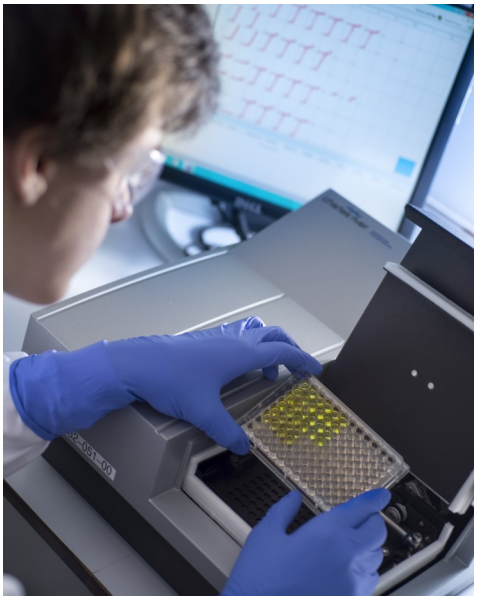
In this case study, we have chosen to focus on endotoxin testing. Endotoxins are constituents of the bacterial cell wall of Gram-negative bacteria and are released during the break down of Gram-negative bacterial cell membranes. Quality control measures for parenteral drugs require testing for endotoxins as they can illicit an immune response resulting in a pyrogenic reaction when they enter the bloodstream. Each drug or medical device has a limit specifying the amount of endotoxin that can be safely present. Endotoxin levels that are higher than that limit are dangerous due to their pyrogenic response and at higher doses, toxic effects, so it is vital that all drug compounds and medical devices undergo this safety screening.

We utilise the internationally established pharmacopeial method Limulus Amebocyte Lysate (LAL) test to assess products for bacterial endotoxins. This test is based on an extract from the blood of the horseshoe crab (*Limulus polyphemus*), which has a primitive immune response clotting mechanism triggered by bacterial endotoxin which

will be present following the breakdown of the Gram-negative bacterial cell wall.

Challenge

We were recently approached by a leading pharmaceutical company regarding endotoxin testing on their product, the finished dose of which was in powdered form. Bacterial endotoxin testing is one of our specialities and we were able to quickly allocate personnel to the project. As this sample was cytotoxic, its preparation required a slightly different handling process and required the use of a safety cabinet to ensure the safety of our technicians. Usually, a



powdered product would be weighed during the preparation stage, but in this instance, we reconstituted the powder within its vial using endotoxin free LAL reagent water and then diluted it down to the 0.5 mg/ml validated concentration. At this time, a method suitability test was conducted to ensure there was no inhibition or enhancement of the test system by the product.

We then proceeded with the gel clot method, which is an endpoint assay giving a positive or negative result at the sensitivity of the lysate used.While this is the most time-consuming method of bacterial endotoxin detection, it is both efficient and extremely sensitive, detecting very low levels of endotoxin with fewer interactions inhibiting the reaction. This method remains the reference method as it produces fewer false-positive and false-negative results.

Results

Upon completion of the testing, we were pleased to report to our client that bacterial endotoxin levels were well within specification. If endotoxin levels higher than the limit had been found, our standard procedure would be to initiate an investigation to determine whether there were any laboratory errors on our part that may have contributed to the out of specification result. This investigation would include a review of the entire process, including environmental monitoring, operator interview, testing procedures and materials.

The most likely cause of a product failure from the presence of bacterial endotoxins is due to a contamination event during the manufacturing process. Focus on the investigation should start with a close examination of any aqueous processes within production where the Gram-negative bacteria may proliferate. While we cannot retest a sample that has failed, the client may choose to send additional samples for testing to determine whether the manufacturing issue is widespread or an isolated incident.

Conclusion

In order to comply with regulatory requirements and maintain an efficient manufacturing environment, it is important to identify any manufacturing issues at an early stage. Process validation is one way in which companies address the needs for a sterile environment, however batch quality control testing such as the type we performed on this occasion may still be required. It is also important to note that there are some chemical pyrogens which cannot be detected by the LAL test and some molecules which are incompatible with the test system, thus alternative methods of testing may still be required depending on the product or device. Any uncertainties regarding process validation or testing requirements should be directed to an organisation or consultant with regulatory expertise to ensure that all products and devices are safe and fit for their intended purposes.

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