



Ensuring the Safety of Parenteral Pharmaceuticals:

How Manufacturers Can Benefit from New Pyrogenicity Testing Methods

by Nancy Beck, Ph.D.

Food and drug recalls due to contamination have been big news lately, with the heparin recall being the most recent pharmaceutical contamination case to result in severe adverse health effects and garner intense media coverage. As a result of such recalls, companies may suffer loss of revenue as well as loss of public confidence. Recalls also may result in intense scrutiny of a company's quality control and manufacturing practices, supply chain and business practices from the Food and Drug Administration (FDA) and even Congress. Another undesirable consequence of a recall is the potential for shortages in critical product that could result in medical crisis.

Parenteral products — those administered via injection, infusion or implantation — represent a vast and growing share of the pharmaceutical market including: biologics (antitoxins, antivenoms, blood, blood derivatives, immune serums, immunologic diagnostic aids, toxoids and vaccines); numerous other injectables; infusion solutions (intravenous, dialysis and nutrition solutions); and devices. Growth of the parenteral pharmaceutical sector can be attributed to both the development of novel parenteral drugs, particularly biologics which

are commonly delivered via this route, as well as the development of parenteral systems to provide precise, targeted of existing drugs.¹ Increasing focus has been aimed at parenteral delivery systems as companies attempt to capitalize on well-characterized products — a profitable enterprise when compared with the time and money required to develop new drugs. Growth of drug delivery systems is expected to increase 10 percent annually, reaching \$132 billion by 2012, with development of parenterally delivered products expected to experience the greatest growth.²

The Dangers of Contamination

Contamination is a particular issue for drugs like heparin and other parenteral pharmaceuticals because these products are delivered directly by injection, infusion or implantation, bypassing many of the body's natural defenses. For that reason, contamination with microbes — bacteria, fungi, viruses and parasites — is a prominent concern. Although good manufacturing practice requires sterilization of parenteral products, certain microbial remnants can persist despite sterilization. Specific testing (pyrogenicity testing) is performed to ensure products are free of these remnants (py-

rogens), which can cause serious adverse health effects including fever (pyrogenic response) and in some cases death.

This article provides an overview of parenteral pharmaceuticals and pyrogenicity testing in the United States and European Union (EU), including the shortcomings of standard testing methods and the potentially costly gamble manufacturers are taking by depending on those methods. Additionally, the benefits of using more modern, predictive and cost-effective methods will be discussed.

Beyond fever, inflammation, chills and low blood pressure, pyrogens can lead to shock and even death. For example, the deadly symptoms of diseases such as bacterial meningitis or toxic shock syndrome are the result of pyrogenic materials in the blood. If a batch of pyrogen-contaminated product goes to market the results could be disastrous: adverse health events, product recall, loss of revenue and damage to the company's reputation. One well-documented exam-



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ple of the damaging effects of pyrogenic contamination was the global outbreak of aseptic peritonitis in 2002. In that case, icodextrin-containing peritoneal dialysis solutions contaminated with pyrogenic material were released onto the market, resulting in a worldwide recall of several hundred batches of the product. This product was considered safe under the current testing called for by United States Pharmacopeia (USP) and European Pharmacopeia (Ph. Eur.) standards, but nevertheless led to serious adverse health effects.³ This case highlights the need to re-examine current standards for pyrogenicity testing of parenteral products.

Manufacturers of parenterals are required by FDA and European Medicines Agency (EMA) to test for pyrogenicity along with safety, purity and potency on each batch of their product. Most U.S. manufacturers follow FDA and USP guidance to satisfy quality control requirements, and pyrogenicity testing is no different. Unfortunately the methods endorsed by USP guidelines have not changed significantly since 1987, and do not reflect the current state of the science. There are more accurate, efficient and cheaper methods available from which manufacturers could benefit.⁴ Beyond financial benefit, adoption of the new methods would reduce the financial and legal liability implicit in the less accurate methods currently used.

Limitations of Standard Test Methods

The two standard methods specified in USP and Ph. Eur. guidance on pyrogen testing are the Rabbit Pyrogen Test (RPT)⁵ and the Limulus Ameobocyte Lysate assay (LAL).⁶ The RPT method entails monitoring a rabbit's body temperature following injection of the product into the rabbit, with a significant rise in body temperature indicating the presence of a pyrogen. Rather than fever,

detection of pyrogen using the LAL depends on agglutination, or clumping, of horseshoe crab blood. Historically, the RPT was the primary method of pyrogenicity testing, but over the last 30 years it has been largely supplanted by the LAL.⁷

Although the RPT and LAL are considered by some to be the gold-standards for pyrogenicity testing, they have significant shortcomings, not the least of which is the simple fact that neither rabbits nor horseshoe crabs share the same physiology or fever response as humans. Measuring fever response in rabbits is not a good predictor of human fever response because humans are more sensitive than rabbits — lower levels of pyrogen contamination can cause a fever in humans, while having no effect on the rabbit.⁸ Additionally, there is considerable individual variation in human fever response, making some members of the population more sensitive than others. Therefore, the most sensitive methods available should be used to protect the most vulnerable members of the population. Another limitation of both the RPT and LAL relates to their incompatibility with some parenteral pharmaceuticals: the RPT is unsuitable for use with certain cellular products, radiopharmaceuticals, certain biologicals and medical devices while the LAL is unsuitable for drugs that prevent blood clotting, some biologicals, and medical devices.⁹ A third major drawback of the LAL is its limited range of detection; although the LAL is capable of detecting the most common type of pyrogenic contamination, that caused by a specific category of bacteria, other less prevalent although equally serious types of (bacterial, viral, fungal and parasitic) pyrogens are not detected.¹⁰

Benefits of Newer Methods-IVPTs

To overcome these limitations, several new methods have been developed.

These methods, referred to as *in vitro* pyrogen tests (IVPTs), measure the fever response in human cells. The IVPTs are based on monitoring a pathway well-accepted as a fundamental mechanism of fever response in humans.¹¹ Five IVPT methods were subject to an intense validation process in the EU involving stakeholders from all industry sectors. These efforts involved the use of IVPTs in over 200 laboratories world-wide,¹² which demonstrated satisfactory reproducibility and performance of these methods with regard to sensitivity and specificity, as compared to the RPT.¹³ It should be noted that several optimized IVPT methods are available commercially as simple test kits, making them readily accessible to manufacturers eager to save time, money and boost sensitivity.

It is thought that the IVPTs are more predictive of pyrogenic potential because they rely on the response of human cells, rather than a rabbit. Another benefit of the IVPTs is their quantitative nature, which allows for more accurate assessment of pyrogen content than the RPT. The RPT only indicates the presence or absence of pyrogen, but gives no indication as to the amount of pyrogen present. Since the rabbit is less sensitive to pyrogens than humans,¹⁴ the RPT could fail to detect low levels of pyrogen contamination that could be dangerous to humans. Increased use of the IVPTs could provide a valuable safeguard for companies by preventing release of contaminated products onto the market. The sensitive detection and quantitative capacity of IVPTs also hold promise for ensuring products are safe for even the most sensitive humans, as certain individuals are more susceptible to very low levels of pyrogen than others. The human-based IVPT methods confer several other advantages: IVPTs are capable of detecting a wide variety of

pyrogens unlike the LAL,¹⁵ in contrast to the RPT or LAL, IVPTs have wide utility for biologicals, blood products, other injectable and infusable pharmaceuticals and medical devices,¹⁶ and IVPTs provide savings in terms of cost, time and efficiency.¹⁷

Using IVPTs for Regulatory Purposes

The 5 IVPT methods, validated in the EU as replacements for the RPT, are being considered for inclusion in the Ph. Eur.¹⁸ Designation of the IVPTs as a reference method by the *Pharmacopeias* will enhance product safety and remove burdens to industry resulting from divergent regulatory policies. Although they have not yet been included in the USP, the use of IVPTs can be considered for regulatory purposes in the United States based on FDA guidance. As the ultimate regulatory authority on pharmaceutical test requirements in the United States, FDA allows flexibility concerning specific methods as long as the required data is generated. FDA does not require a full method validation for a new technique, rather the agency will accept data generated by any method as long as the scientific validity of the method has been demonstrated in the specific product being tested. Numerous scientific publications in peer-reviewed journals as well as the official method validation conducted by the EU support the scientific validity and utility of IVPTs.¹⁹

Further FDA guidance, on biologicals, from the *Code of Federal Regulations* states that a test method may be modified if the manufacturer presents data “demonstrating that the modification will provide assurances of the safety, purity, potency and effectiveness of the biological product equal to or greater than the assurances provided by the method or process specified in the

general standards.”²⁰ FDA’s Center for Biologics Evaluation and Research’s (CBER’s) “Vaccine Product Approval Process” document states that “if the sponsor describes an alternative procedure which provides continued assurance of safety, purity and potency, CBER may determine that routine submission of lot release protocols (showing results of applicable tests) and samples is not necessary.”²¹

To meet the above criteria, a manufacturer could conduct both the current method used and an IVPT in parallel, submitting data for both to demonstrate the equivalence, or superiority, of the IVPT for pyrogen detection. Once equivalency is demonstrated, the IVPT could be used exclusively. Such an approach would be analogous to that currently applied by FDA’s Center for Veterinary Medicine (CVM) for replacement of the RPT with the LAL. CVM guidance calls for submission of data from the RPT in conjunction with data from the LAL for the first three batches of a product in order to support exclusive use of the LAL for future batches.²²

IVPTs are superior testing methods which offer solutions to many of the problems associated with the current pyrogen detection methods including wider applicability and enhanced detection capability. By submitting parallel data from an IVPT and a standard method demonstrating validity of the IVPT, manufacturers could conceivably replace existing standards with minimal cost or effort. Adoption of the new methods means savings for industry as well as production of safer products. **Δ**

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