An Interview with

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Could you explain what pyrogens are and why testing for them is crucial in pharmaceutical products?

Pyrogens are contaminant substances in injectable drugs or implants that can provoke harmful reactions in patients, ranging from mild fever to severe, potentially life-endangering symptoms. Among the various pyrogens, endotoxins—lipopolysaccharides of gram-negative bacteria—are the most commonly found in pharmaceutical products. All other pyrogens are classed as non-endotoxin pyrogens (NEPs). This heterogeneous group includes compounds such as peptidoglycans, lipoteichoic acids and lipoproteins from gram-positive bacteria, polysaccharides from fungi, components of myxoviruses but also microscopic rubber or plastic particles and metal compounds in elastomers. Some NEPs remain unidentified.

What exactly was the Rabbit Pyrogen Test (RPT) and why did the European Pharmacopoeia Commission decide to eliminate it from its monographs by July 2025?

The RPT has been used for decades to detect pyrogens in pharmaceutical products by measuring changes in rabbits' body temperature following injection. Its removal from the EP was largely motivated by the 3Rs principle—aimed at replacing, reducing, or refining animal use in pharmaceutical testing wherever feasible. Beyond ethical concerns, the RPT has notable limitations, including low sensitivity, significant result variability, and sometimes non-human specificity, which can result in false positives or negatives.

What are the key alternative methods to the Rabbit Pyrogen Test, and how do they differ in their ability to detect various types of pyrogens?

The EP now recommends the monocyte activation test (MAT) as the replacement pyrogen test for the RPT. Introduced in chapter 2.6.30, the MAT was first included in the EP in 2010 as an alternative method and is now the compendial test method in the EU for the full range of pyrogens. Other in vitro assays, such as the bacterial endotoxin test (BET), also known as the limulus amebocyte lysate (LAL) test, the recombinant factor C (rFC) test and the recombinant cascade reagent (r-CR) test recently introduced in USP <86> have also been adopted by pharmacopoeias; however, these only detect endotoxins. According to EP chapter 5.1.13, an endotoxin-specific test may be used for pyrogen testing only if a thorough risk assessment rules out the presence of NEPs.

In addition to avoiding the ethical concerns liked to animal-based tests, the MAT addresses many of the shortcomings associated with the RPT and the BET. Since it is based on the immune response of humans, its results more accurately reflect human fever reactions. The test is also highly sensitive, reproducible and compatible with a broader range of product types for testing than the RPT, BET and rFC.

How will the pharmaceutical industry need to adapt to comply with the new European Pharmacopoeia requirements after July 2025?

Any company that sells or manufactures parenteral drugs, biologics or medical devices in the European Union now has to switch to the

MAT for full-spectrum pyrogen testing. The MAT is a quantitative in vitro method that mimics the human immune response to both endotoxins and NEPs. It measures the release of inflammatory cytokines—such as TNF-α, IL-1β, or IL-6—produced by activated human monocytes. These cytokines are subsequently identified and quantified through an immunological assay (ELISA), which uses specific antibodies and an enzyme-driven colorimetric reaction. Only monocytes from validated sources may be used for the MAT, including whole human blood (fresh or cryopreserved), peripheral blood mononuclear cells (PBMCs), or established monocytic cell lines like Mono-Mac-6 (MM6).

What challenges might companies face in transitioning from the Rabbit Pyrogen Test to in vitro alternatives like the Monocyte Activation Test?

The MAT is compendial in the EU, so manufacturers do not need to perform full validation. The supplier should be able to provide comprehensive validation data. Our own PyroMAT® system, which utilizes cryopreserved MM6 monocytic cells, has been fully validated in accordance with EP 2.6.30, and following the USP <1225> guidelines. Therefore, customers only need to perform the considerably less substantial product-specific validation (qualification). Since the PyroMAT® system's launch in 2018, we have managed to accumulate extensive data, insights and practical experience with it. Our expertise enables us to offer a range of professional services—including feasibility assessments, trainings, and validation/qualification support—to help ease the path to implementation.

How might other global regulatory bodies respond to the European Pharmacopoeia's decision to ban the Rabbit Pyrogen Test?

The Ph. Eur. Commission moved first to effectively abolish the RPT, and there seems to be a general willingness among the larger nations to follow suit. Whether this will materialize in the coming years remains uncertain at this time. In the US, the MAT has been accepted as an alternative method for pyrogen detection since the release of an FDA industry guidance in 2012, with similar recommendations made in USP <151> in 2017. The MAT is also accepted as a compendial method in the Pharmacopoeia of Russia and Eurasia, as an alternative method in the Pharmacopoeia of India, China, Brazil, and as a supplementary method in Japan.

Learn more about our MAT solution: SigmaAldrich.com/pyromat