Goodbye Rabbit, Hello MAT: The Start of a New Era in Pyrogen Testing

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In June 2024, the European Pharmacopoeia Commission adopted 57 revised monographs from which the rabbit pyrogen test (RPT) was deleted, and it added a new general chapter 5.1.13 on pyrogenicity. These changes, published in supplement 11.8 and effective as of July 1st, 2025, mark the end of the RPT in the Ph. Eur. (EP), making it necessary for manufacturers to switch to an *in vitro* technique for pyrogen testing if they manufacture parenteral drugs, biologics or medical devices in the European Union or sell them there. This move was driven by the 3Rs principle to replace, reduce or refine the use of animals in the testing of pharmaceuticals wherever possible. In addition to the animal welfare considerations, the RPT also shows low sensitivity, high result variability and sometimes non-human specificity that leads to false positives or negatives.

Why is pyrogen testing necessary?

Pyrogens are contaminant substances that can cause adverse reactions in patients, ranging from fever to life-threatening shock-like symptoms. The pyrogenic substances most frequently detected in pharmaceuticals are endotoxins, which are lipopolysaccharides from gram-negative bacteria. *In vitro* tests to detect such endotoxins have been introduced into worldwide pharmacopoeias, most notably the bacterial endotoxin test (BET), also known as the limulus amebocyte test (LAL). However, this test is limited to the detection of endotoxins and does not address the diverse group of non-endotoxin pyrogens (NEPs). These include substances such as peptidoglycan, lipoteichoic acids and lipoproteins from gram-positive bacteria, capsular polysaccharides from fungi, virion components of myxoviruses, but also microscopic rubber or plastic particles and metal compounds present in elastomers. The nature of some NEPs is still unknown.

To ensure patient safety, EP 5.1.13 stipulates that an endotoxin test can be used for pyrogen testing only if the presence of non-endotoxin pyrogens can be ruled out in the course of a risk assessment. Since the RPT, for many decades the gold standard of pyrogen testing, is no longer an option, the EP now recommends to perform the monocyte activation test (MAT) as its replacement. Described in chapter 2.6.30, the MAT entered the EP as an alternative test method in 2010 and is now the sole method for assessing the full spectrum of pyrogens. Its compendial status in the EP means that manufacturers do not need to perform full method validation, only product-specific validation (i.e., qualification). In the US, the MAT has been an alternative pyrogen detection method since a 2012 FDA guidance for industry. USP <151> has given similar guidance since 2017. The MAT is recognized as a compendial method in the Pharmacopoeia of Russia and Eurasia, as an alternative in the Pharmacopoeia of India, China, and Brazil, and as a supplementary method in Japan.

What is the monocyte activation test (MAT)?

The MAT is a semi-quantitative *in vitro* test that mimics the human immune response to endotoxins and NEPs by measuring inflammatory cytokines (TNF-α, IL-1β or IL-6) released by activated human monocytes. The cytokines are then detected in an immunological assay (ELISA) involving specific antibodies and an enzymatic color reaction. Only monocytes from approved sources, including whole human blood (or cryopreserved blood), peripheral blood mononuclear cells (PBMCs) and monocytic continuous cell lines such as Mono-Mac-6 (MM6), may be used.

The MAT overcomes many of the limitations of the RPT and the BET. Its results correlate closer with the human fever response because it is based on a human signal transduction pathway. It is sensitive, highly reproducible and is compatible with more product types than RPT and BET. Importantly, the MAT does not raise the ethical questions that the RPT and, to a lesser degree, the BET do – many horseshoe crabs die following the bleeding process to gain their blood cells.

Proven reliability of the MAT over years

The first MAT to become commercially available was a forerunner of the present PyroMAT® system, which is based on cryo-preserved MM6 human monocytic cells. These monocytes express a variety of toll-like receptors (TLRs) that recognize different microbial ligands. Upon binding, these ligands stimulate their corresponding TLRs, which subsequently initiate signaling cascades that trigger specific immunological responses. MM6 cells respond by producing the interleukin-6 cytokine, which is what the PyroMAT® system detects and quantifies. It has been demonstrated that the various TLRs of the MM6 cells recognize a wide spectrum of ligands originating from gram-negative and gram-positive bacteria, fungi, viruses and other microscopic particles.

The PyroMAT® system has been fully validated according to EP 2.6.30 07/2024 including the USP <1225> analytical characteristics. To gain better QC data and to follow the regulatory path established by the EP, many manufacturers switched to using it routinely years before the requirement arose. Having supplied the industry since 2018, we have been able to collect a wealth of information, data and experience with the PyroMAT® system. This has paved the way for our expert services, including feasibility studies, training and validation support, to reduce the work burden to allow implementation.

Learn more about the PyroMAT® system, our monocyte activation test based on the MM6 cell line.
https://www.sigmaaldrich.com/pyromat