eurofins | BioPharma Product Testing



Monocyte Activation Test
In vitro pyrogen test

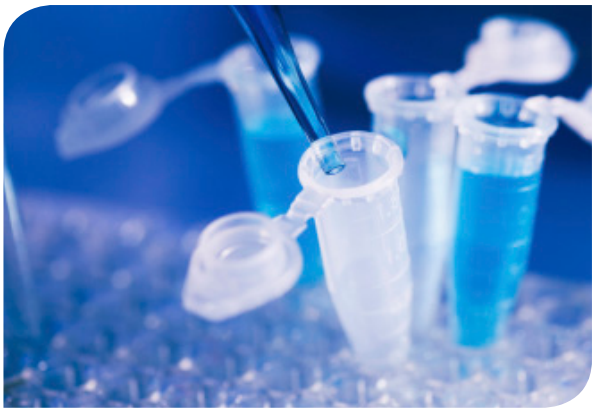
Parenteral administered pharmaceutical products must be free of pyrogenic (fever-inducing) contamination as these substances may induce life-threatening systemic response of the patient's innate immune system. It is imperative to ensure biological products are free of contaminating pyrogenic material prior to administration to patients.

A sterile product does not mean "pyrogen-free" product. The Pyrogen Test is designed to limit the risks of febrile reaction to an acceptable level in the patient from the administration of a parenteral drug. The rabbit pyrogen test (RPT) was originally developed to confirm the absence of pyrogens in large volume parenteral preparations. Despite its intense animal consumption, low sensitivity, and qualitative nature only allowing a pass/fail interpretation this test remained the standard pyrogen detection method for many decades. Also, this test was not applicable to products with intrinsic pyrogenicity like modern vaccine formulations or modified endotoxins.

For many products the RPT was replaced by the Bacterial endotoxin Test (BET) / Limulus Amebocyte Lysate Assay (LAL). The BET is specific for endotoxin (Lipopolysaccharide, LPS), the main constituent of the outer cell wall of gram-negative bacteria. The test is an ex vivo assay, based on the coagulation of the lysate of amoebocytes (white blood cells) from the horseshoe crab. The BET is much more sensitive for endotoxin than the RPT, and is typically performed as a quantitative assay with a wide dynamic range. Though replacing the RPT for many drugs, the BET is a specific assay for endotoxin. Non-Endotoxin Pyrogens (NEPs) are undetectable by the bacterial endotoxin test, and there is therefore a risk of overlooking a NEP contamination.

Given the limitations of the RPT and the BET and due to the manufacturing increase of more more complex products, the general chapter for endotoxin testing in the European Pharmacopoeia (chapter 5.1.10) introduced the necessity for an evaluation of the product, production process and raw materials with respect to the risk for pyrogens that are non-detectable by the bacterial endotoxin test.

In recent years an alternative in vitro pyrogen test, the Monocyte Activation Test (MAT), has been developed to detect and quantify endotoxin and NEP contaminations. The assay is based on the human immune response by measuring cytokine production of human monocytes or monocytic cells.



Since January 2010, the Monocyte Activation Test has been described as a compendial method for Pyrogen Detection in the European Pharmacopeia (chapter 2.6.30) and since the 2016 revision, recommendations have been given to replace tests on rabbits with the Monocyte Activation Test, wherever possible and after product specific validation (EP 2.6.8, Rev. July 2016). Changing from the RPT to the MAT was also in line with the 3Rs principle and EU Directive 2010/63/EU concerning the protection of animals.

The Monocyte Activation Test (MAT) simulates the humane immune response and combines the advantages of the RPT (assessment of pyrogenicity beyond gram-negative endotoxin) with the benefits of an in-vitro method. In contrast to the RPT, the MAT can be applied as a fully quantitative test without the use of animals, making it more appropriate for vaccines which are inherently pyrogenic and is physiologically relevant since it uses human cells.

Pyrogen Tests	BET	RPT	MAT
Non animal, human-based test	-	-	+
Detection of endotoxin	+	+	+
Detection of NEPs - Human Specific	-	-	+
Detection of NEPs - Yeasts and molds	-	+	+

Figure 1. Pyrogen tests comparative table

eurofins | BioPharma Product Testing



Our Services

In order to ensure batch release testing with very high reproducibility and stability in batch-to-batch analysis Eurofins BioPharma Product Testing Italy has implemented the method based on the Mono Mac 6 cell line (MM6 cell line was part of the MAT validation by ECVAM and ICCVAM).

If the sample is contaminated with pyrogens, the monocytes (MM6 cell-line) will be activated and will produce cytokines which will be detected in an immunological assay (ELISA). Moreover this approach has the advantage of greater stability and reproducibility compared to the use of whole blood or peripheral blood mononuclear cells (PBMC) as sources of monocytes.

The target of the ELISA is the interleukin 6 produced by the cell line in presence of pyrogens and the quantitative result is obtained through the application of method A described in EP 2.6.30 and expressed in Endotoxin Equivalent Unit.

Why choose Eurofins BioPharma Product Testing Italy

- MAT for QC release with a validated cell line able to detect all relevant pyrogens
- in vitro test, completely standardized according to 3Rs principle and EU Directive 2010/63/EU
- Sensitive test system
- Wide testing range of drug products and medical devices as well as products that can't undergo in-vivo testing (eg. products containing hyaluronic acid)
- We are able to detect pyrogenic activity in more complex pharmaceuticals, like:
  - Blood-derived products
  - Cell-derived products
  - Biologics and vaccines
- Strong expertise with endotoxin testing
- Laboratory dedicated to MAT and BET

Comprehensive GMP Testing Services

Chemistry/Biochemistry
Cell Banking Services
Facility & Process Validation
Method Development & Validation
Microbiology
Molecular & Cell Biology
Raw Materials Testing
Release Testing
Packaging Testing
Stability Testing & Storage
Viral Clearance & Viral Safety
Professional Scientific Services℠

Our sites in Italy

Eurofins Biolab Srl
Via Bruno Buozzi, 2
20090 Vimodrone - Milan

Eurofins Biolab Srl
Via Reno, 2
53036 Poggibonsi - Siena

The largest network of harmonized bio/pharmaceutical GMP product testing labs worldwide.

Contacts:
Phone: +39 022507151
Email: InfoFarma@eurofins.com
www.eurofins.com/BPT
www.eurofins.it/Pharma