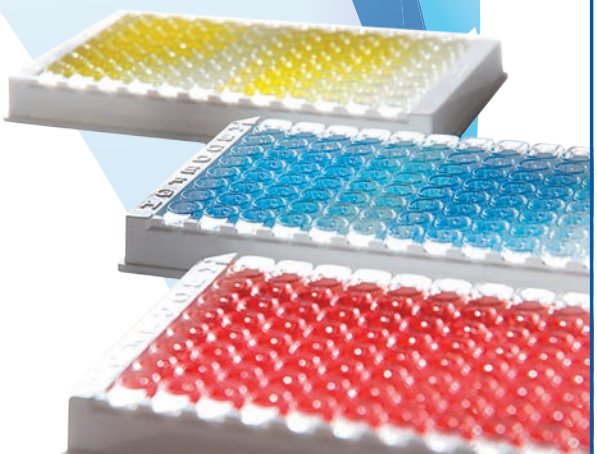
Relief for Rabbits, Greater Safety for Patients

PyroDetect: The Monocyte Activation Test for detection of pyrogens

Benefits:

- **Greater product safety:**
  Detects a wide spectrum
  of pyrogens (including non-endotoxin pyrogens)
- **Highly compatible:**
  Allows testing of a wider range of
  pharmaceutical product types
- **In Vitro test with cryoblood:**
  Involves no animal consumption and mimics
  innate immune reaction of humans
- **Reliable results:**
  Easy-to-perform, robust test system
- **Regulatory recognition:**
  Introduced into European Pharmacopoeia and
  in an FDA Guidance for Industry



Merck Millipore is a business of MERCK

Testing for pyrogens: A core product safety issue

During production or usage of pharmaceuticals, biotherapeutics and medical devices, pyrogen contamination can occur. These pyrogenic substances have the potential to induce life threatening fever in humans. To ensure patient safety, the affected industries must test that their concentration does not exceed certain limits.

The most frequently applied methods to detect pyrogens in the pharmaceutical industry have been the Rabbit Pyrogen Test (RPT) and Bacterial Endotoxin Test (BET), also known as Limulus Amoebocyte Lysate (LAL) test. However, both are limited in the product types they are able to test (see table below) and in the range of pyrogens for which they are suitable. For example, the LAL test can only detect the lipopolysaccharides (LPS) of Gram-negative bacteria, otherwise known as endotoxins. They form the most common but not the only class of pyrogens. Both of these tests are based on immune reactions of animals, rather than humans, and bring with them a high level of animal consumption (rabbits or horseshoe crabs).

To overcome the limitations of the rabbit and LAL test, the Monocyte Activation Test (MAT) was introduced into:

- Chapter 2.6.30 of the European Pharmacopoeia in 2010 as an alternative (wherever possible) for the rabbit test and
- An FDA Guidance for Industry in 2012 (Pyrogen and Endotoxins Testing)

Rabbit Test (RPT)	LAL Test
Cytostatic drugs	Blood products
Sedatives	Lipids
Analgesics	Cell therapeutics
Cytokines	Proteins
Antibiotics	
Chemotherapeutics	
Proteins	

Table: Limitations of the rabbit and LAL test

PyroDetect: The commercially available MAT system

As the only MAT kit on the market, Merck Millipore's PyroDetect system detects and quantifies a uniquely broad range of non-endotoxin pyrogens (NEPs) in addition to endotoxins. Based on a patented procedure, it models the immune response in patients: the innate defense reaction of the human immune system simulates the human fever reaction better than any animal-based pyrogen test. Monocytes of the cryo-preserved human whole blood are activated by any pyrogens contained in the sample. As a consequence, they produce interleukin-1β which is detected in an immunological assay (ELISA) involving specific antibodies and an enzymatic color change reaction. Positive and negative controls are included.

The unique PyroDetect system for a wider spectrum of detectable pyrogens

Pyrogens constitute a heterogeneous group of contaminants comprising microbial and non-microbial substances. Data demonstrating that the MAT is capable of covering a much wider range of pyrogens relevant to humans were not included in the (predominantly LPS focused) validation studies of the past years. However, extensive clinical and scientific research has since proved the MAT reaches further: while LAL tests detect only the LPS of Gram-negative bacteria, PyroDetect also covers pyrogens originating from Gram-positive bacteria, yeasts, molds and viruses.[1] Its spectrum includes:

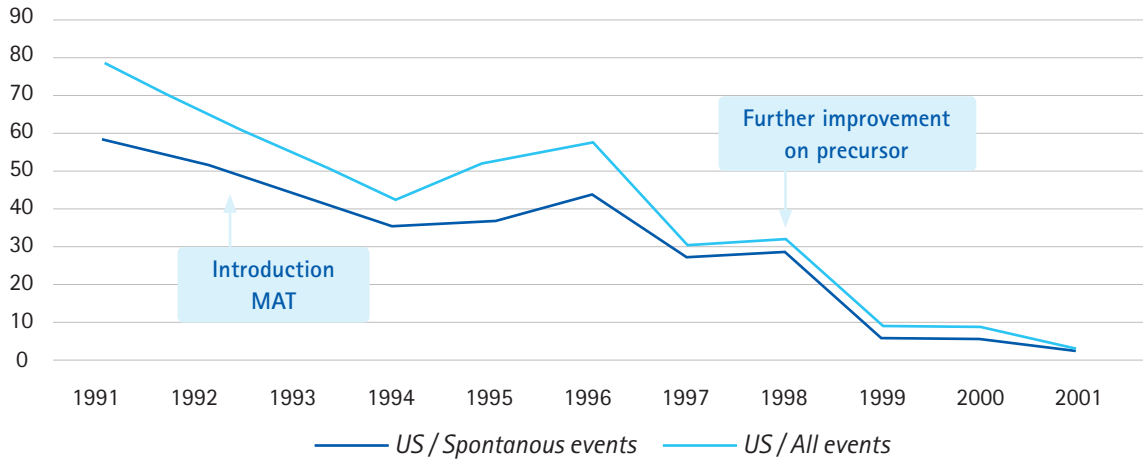
- Endotoxins (lipopolysaccharides, LPS)
- Lipoteichoic acid (LTA)
- Bacterial DNA (CpG-motif)
- Synthetic Toll-like receptor (TLR)-agonists
- Lipoproteins
- Endogenous pyrogens
- Particles from packaging, rubber, plastic, organic dust

1 Hasiwa N, Daneshian M, Bruegger P, Fennrich S, Fleck R, Hochadel A, Hoffmann S, Rivera-Mariani FE, Rockel C, Schindler S, Spreitzer I, Stoppelkamp S, Vysyaraju K and Hartung T. Evidence for the detection of non-endotoxin pyrogens by the whole blood monocyte activation test. ALTEX 2013, 30:169-208.

The MAT detects non-endotoxin pyrogens that the rabbit and LAL tests don't – five cases:

1. Life-Saving Drug:

A major pharmaceutical company received many reports of adverse drug reactions (redness, shivering, fever) for a life-saving drug containing a substance produced by fermentation. The batches responsible had passed LAL and rabbit tests without a detectable response. It became clear that an unknown NEP contamination was impairing patient safety. The MAT was introduced as a testing system for batch release in accordance with FDA. As a consequence, it was possible to improve the production process. As shown in the timeline below, adverse drug reactions had decreased to zero a few years later.[2]



2. Human Serum Albumin:

An experimental study tested pyrogen contaminated batches of human serum albumin (HSA) for clinical use. The influence of NEPs was investigated by blocking LPS with Polymyxin B, which revealed that all three contaminated batches contained significant concentrations of NEPs which could only be detected using the whole blood MAT. The authors concluded that only the whole blood MAT is qualified to ensure consumer safety when it comes to non-endotoxin pyrogenic contaminations.[3]

3. Infusion Solution:

An infusion solution containing gelatin caused fever reactions in hospital patients. The manufacturer withdrew the incriminated batches from the market and re-investigated them using both LAL (the release criterion of this parenteral) and rabbit tests. The manufacturer blinded the batches and sent them to the responsible national control authority for analysis using the whole blood MAT in parallel with blinded, non-incriminated control batches. The fever causing substances were identified as NEPs. The results are summarized below.

Batch	LAL test	Rabbit test	Fever in patients	Whole Blood Pyrogen Test		
				IL-1 (pg/ml)	IL-6 (pg/ml)	TNF-β(pg/ml)
A	negative	negative	no	8.5	28.0	28.2
B	negative	positive	yes	142.6	654.4	67.6
C	negative	negative	yes	421.5	9,444.0	116.7
			cut off:	**32.6**	**127.6**	**43.6**

2 Hasiwa N, Daneshian M, Bruegger P, Fennrich S, Fleck R, Hochadel A, Hoffmann S, Rivera-Mariani FE, Rockel C, Schindler S, Spreitzer I, Stoppelkamp S, Vysyaraju K and Hartung T. Evidence for the detection of non-endotoxin pyrogens by the whole blood monocyte activation test. ALTEX 2013, 30:169-208.

3 Perdomo-Morales, R., Pardo-Ruiz, Z., Spreitzer, I., et al. (2011). 'Monocyte activation test (MAT) reliably detects pyrogens in parenteral formulations of human serum albumin.' ALTEX 28, 227-235.

4. Dialysate:

A global recall was issued for an icodextrin-containing dialysate after patients complained about abdominal pain, nausea, vomiting, diarrhea and fever. The incriminated batches passed the LAL and rabbit tests. An ex vivo pyrogen test was then performed using human peripheral blood mononuclear cells (PBMCs) from healthy donors after exposure to the test substance. The samples led to increased cytokine release, suggesting the presence of NEPs. Further analysis showed peptidoglycan to be the main contaminant. An investigation of the production process revealed that the Gram-positive bacterium Alicyclobacillus acidocaldarius was the source.[4]

5. Dialysis Tubing:

Biofilm contamination within silicone dialysis tubing is a significant patient safety concern. An analysis of tube scrapings showed that the MAT detected greater amounts of pyrogens than the LAL test. The authors concluded that an LAL test is insufficient to quantify potentially hazardous pyrogenic contaminations of dialysis tubing. Many biofilm-creating bacteria are Gram-positive so cannot be detected by LAL tests.[5]

Ordering Information

Product	Pack Size	Ord. No.
PyroDetect System		
Comprises PyroDetect Kit and PyroDetect Cryoblood	1 SET	1.44153.0001
PyroDetect Kit		
Contains all biological and biochemical reagents required for the Monocyte-Activation Test (MAT) which consists of the cryoblood incubation and the Interleukin-1βELISA, 3 – 4 tests following EP, 1 SET, Store at 2 – 8°C	1 SET	1.44154.0001
PyroDetect Cryoblood		
Cryo-preserved and pooled human whole blood preparation, sufficient for the reactions of one entire microculture plate, 2× 2 ml, Store at -80°C or lower	2× 2 ml	1.44155.0001
PyroDetect Endotoxin Standard		
International Reference Standard Endotoxin (RSE) – lyophilized, 1 bottle, 10.000 IU, Store at -20°C (reconstituted at -40°C)	1 PC	1.44161.0001
PyroDetect Interleukin Standard		
Interleukin-1βStandard for control reactions of the Interleukin-1βELISA (optional), up to 10 reactions, Store at 2 – 8°C	1 PC	1.44158.0001
PyroDetect Data Analysis Tool		
Validated excel-based analysis tool to interpretate results according to European Pharmacopoeia	1 PC	1.44299.0001

4 Martis, L., Patel, M., Giertych, J., et al. (2005). 'Aseptic peritonitis due to peptidoglycan contamination of pharmacopoeia standard dialysis solution.' Lancet 365, 588-594.

5 Marion-Ferey, K., Leid, J. G., Bouvier, G., et al. (2005). 'Endotoxin level measurement in hemodialysis biofilm using "the whole blood assay".' Artif Organs 29, 475-481.

For more information:
www.merckmillipore.com/pyrodetect

Find contact information for your country at:
www.merckmillipore.com/offices

For Technical Service, please visit:
www.merckmillipore.com/techservice

Merck KGaA
Frankfurter Straße 250
64293 Darmstadt, Germany
e-mail: mibio@merckgroup.com
www.merckmillipore.com/biomonitoring

Lit. No.: DS6846EN00 09/2015