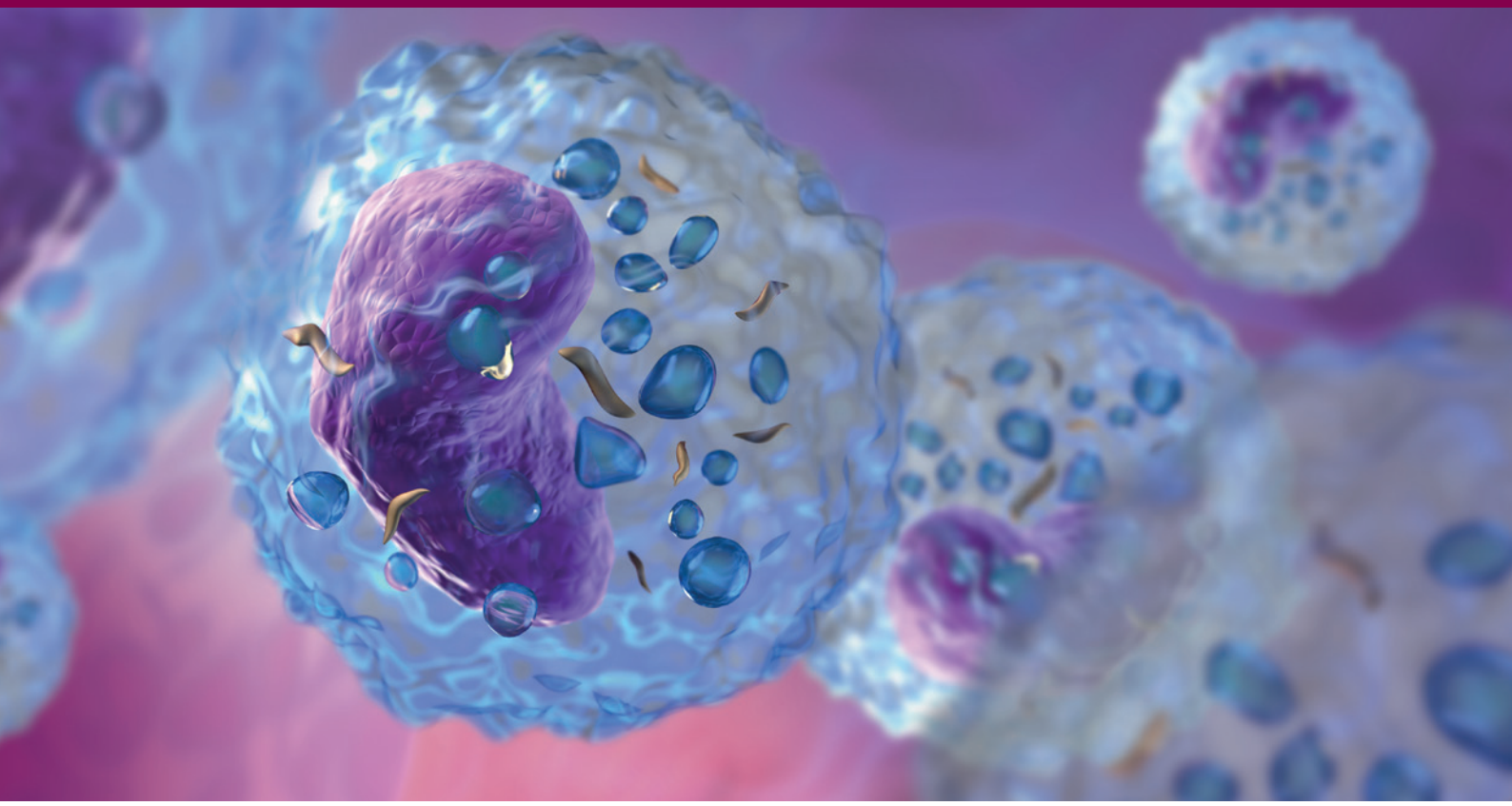
confarma

Monocyte Activation Test

In vitro pyrogen test for QC release with a validated cell line



The measurement of pyrogens is an essential safety measure for pharmaceutical products with parenteral administration and for medical devices. Due to major changes in the Ph. Eur. 5.1.10, and because of the limitations of the BET / LAL test, the MAT acc. to Ph. Eur. 2.6.30 becomes an important in vitro *test to assess pyrogens in your products.*

Amazing where you can go

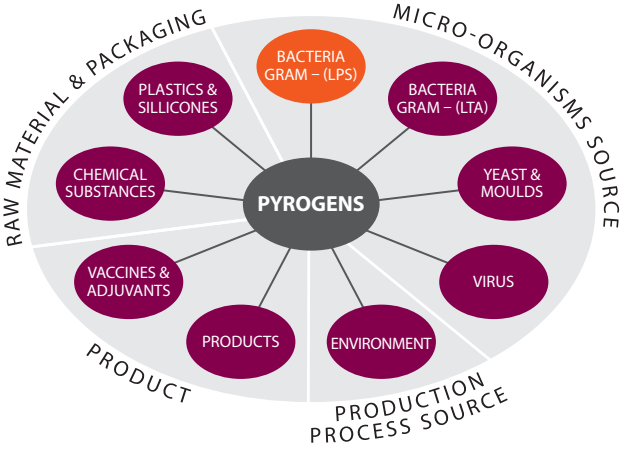
What is a pyrogen?

Pyrogens comprise a chemically heterogeneous group of fever inducing compounds derived from various origins: micro-organisms (bacteria, viruses and fungi), environment, process, product. Contamination by pyrogens is considered a serious health hazard and can result in symptoms ranging from vascular alterations to shock and death.[1]

General principle of fever reaction

PYROGENS + HUMAN WHITE BLOOD CELLS → INTERLEUKINS → FEVER

Pyrogens can be classified in two distinct categories:
Endotoxin pyrogens (LPS) and Non-Endotoxin pyrogens (NEP)



ENDOTOXINS

Endotoxins are part of the outer membrane of the cell wall of gram-negative bacteria. Although the term "*endotoxin*" is occasionally used to refer to any cell-associated bacterial toxin, in bacteriology it is properly reserved to refer to the lipopolysaccharide complex associated with the outer membrane of gram-negative pathogens such as Escherichia coli, Salmonella, Shigella, Pseudomonas, Neisseria, Haemophilus influenzae, Bordetella pertussis and Vibrio cholerae.[2]

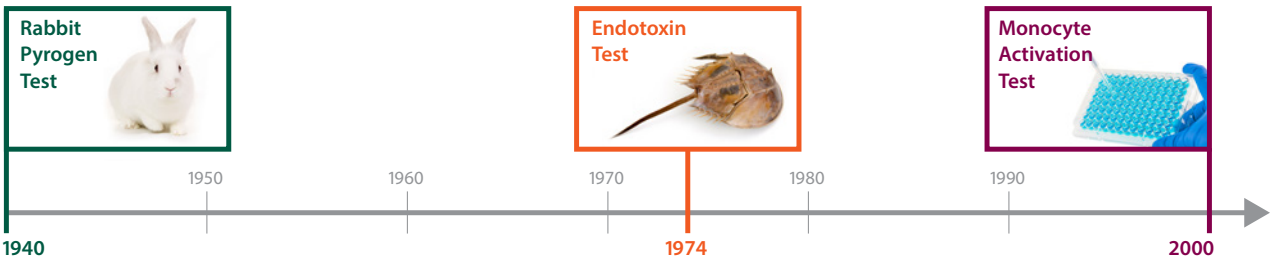
NON-ENDOTOXIN PYROGENS (NEP)

Non-endotoxin pyrogens are all the other substances that can induce fever reaction.

Non-endotoxin pyrogenic substances[1]

Endotoxin associated proteins	Exotoxins
Peptidoglycans (components of bacterial cell wall)	Lipoarabinomannans (from mycobacteria)
DNA (bacterial)	Fungal components for example, mannans, glucans, mannoproteins)
Porins (proteins from the bacterial cell wall)	Parasite components (for example, phosphoinositol)
Bacterial outer surface proteins	Viruses
Muramylpeptides (MDP and other subunits of petidoglycan synergise with endotoxins)	Non-microbiological contaminations (for example, cytokines, media, cells, breakdown products)
Lipoteichoic acids and further gram-positive bacterial cell-wall components	Solid materials (for example, medical devices, plastic)
Superantigens	Drugs (for example steroids, bile salts, dapsone, cytokines)

Evolution of pyrogen detection



RABBIT PYROGEN TEST (RPT)

Limit of detection (IU/mL)	0.3–5
Quantification	No
Human based reaction	No
Spectrum	+ all rabbit pyrogens
Animal testing	*In vivo*

The first method described for the detection of pyrogens is the rabbit pyrogen test (RPT). The RPT is an *in vivo* test that allows detecting all substances pyrogenic for the rabbit.

ENDOTOXIN TEST (LAL / BET)

Limit of detection (IU/mL)	0.03
Quantification	Yes
Human based reaction	No
Spectrum	- (only LPS)
Animal testing	*Ex vivo*

Whenever possible, RPT has been replaced by the BET (Bacterial Endotoxin Test) / LAL (Limulus amoebocytelysate) test, mainly relying on the fact that bacterial endotoxin is the most frequent pyrogen found in pharmaceutical products. The test is an *ex vivo* assay, based on the coagulation of the lysate of amoebocytes (white blood cells) from the horseshoe crab.

MONOCYTE ACTIVATION TEST (MAT)

Limit of detection (IU/mL)	0.03–0.1
Quantification	Yes
Human based reaction	Yes
Spectrum	+ all human pyrogens
Animal testing	*In vitro*

The MAT based on the reaction of human monocytes, now allows predicting the human response to all pyrogens (i.e. endotoxin and non-endotoxin) *in vitro*. The MAT can be used as alternative to animal testing, in accordance with EU legislative framework, as stated in the Ph. Eur. 2.6.30.[3]

Regulation: Major changes for pyrogen detection

- Major change of the Ph. Eur. chapter 5.1.10[4] "*Guidelines for using the test for bacterial endotoxins*": a risk assessment is required when using the bacterial endotoxin test to evaluate the potential presence of non-endotoxin pyrogens. The MAT is recommended as a useful part of this risk assessment. When the BET appears to be not sufficient, the MAT is preferred to the rabbit pyrogen test.
- The Ph. Eur. chapter 2.6.8[5] "*Pyrogens*" mentions the MAT as possible replacement for the RPT.
- The Ph. Eur. chapter 2.6.30[3] includes the MAT as limit test / quantitative test and as test for batch-to-batch comparison, the choice depending on the characteristics of the targeted test item.
- USP <151>: To encourage the use of *in vitro* alternatives to *in vivo* animal tests, the new chapter allows the use of a validated, equivalent *in vitro* pyrogen or bacterial endotoxin test, instead of the *in vivo* rabbit pyrogen test, where appropriate. There is no impact on any of the monographs that refer to this chapter.

ETHICS AND LAWS ACCORDING TO THE ANIMAL WELFARE

- According to the European law (Directive 2010/63/EU[6]), if an *in vivo* test can be replaced by a validated *in vitro* test, it is an obligation to change to an *in vitro* test. According to this law, the MAT has to replace the RPT.
- For the moment, the LAL test is not considered as an *in vivo* test, although the major compound is animal blood. "*The industry says that not that many of the animals die. Between 10 and 30% of the bled animals, according to varying estimates, actually die.*"[7]
- Due to human activity three of four species of the Horseshoe crab have been classified "*Data Deficient*".
- "*UCN SSC Horseshoe Specialist Group is currently revising the IUCN Red List Assessment for the three Asian species of horseshoe crab, (Tachypleus tridentatus, Tachypleus gigas and Carcinoscorpius rotundicauda) which are currently listed as Data Deficient.*"[8]
- Ph. Eur. 2.6.30[3]: "*In accordance with the provisions of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes, tests must be carried out in such a way as to use the minimum number of animals and to cause the least pain, suffering, distress or lasting harm. Wherever possible and after product-specific validation, the pyrogen test is replaced by the monocyte-activation test.*"

Monocyte Activation Test

"*The MAT is used to detect or quantify substances that activate human monocytes or monocytic cells to release endogenous mediators which have a role in the human fever response. The MAT is suitable, after product-specific validation, as a replacement for the RPT.*

The MAT offers significant advantages over animal testing: based on the human fever response, it provides a more relevant prediction of pyrogenic activity than the RPT, it can detect endotoxin and non-endotoxin pyrogens and is applicable to a greater variety of products than the RPT; moreover, it is more accurate as well as more cost- and time-effective than the RPT."[9]

General principle of MAT

ADVANTAGES OF THE MONOCYTES ACTIVATION TEST[10]

- Based on the human fever response, the MAT provides a more relevant prediction of pyrogenic activity than the RPT or BET/LAL.
- It can detect endotoxin and non-endotoxin pyrogens and is applicable to a greater variety of products than the RPT or BET/LAL (e.g., certain drugs and herbal formulations; see Hartung T. 2015. ALTEX. 32(2):79-100).
- It has a lower limit of detection and is more accurate as well as more cost- and time-effective than the RPT.
- Three variants of the MAT have been standardized and validated.
- Protocols using cryopreserved monocytes derived from individual donors are available (Koryakina A. et al2014. J Immunol Methods. 405:181-191).

DIFFERENT MAT FORMATS

Three different formats of the MAT are internationally validated:

- Blood based
- Peripheral Blood Mononuclear Cell (PBMC) based
- Cell line based

	BLOOD BASED	PBMC BASED	CELL LINE BASED
ORIGIN OF THE CELLS	Pooled human blood	Cellular fraction of human blood	Origin of the Cell line: human
RANGE	0.2 to 1.0 EU/mL	0.05–5.0 EU/mL	0.05–5.0 EU/mL
REACTION STANDARDIZED	No	No	Yes
LIMITS	Raw material from blood banks, batch size limited	Raw material from blood banks, batch size limited	Only cell line available: Mono Mac 6
KIT AVAILABILITY	Yes	Under development	Yes

MAT WITH THE MONO MAC 6 CELL LINE

The MAT can be performed based on a cell line. Confarma has implemented the method based on the cell line Mono Mac 6. The focus of Confarma is to offer batch release testing with good sensitivity and very high reproducibility. The reproducibility of a method for batch release analysis is key to guarantee stability in batch-to-batch analysis. The MAT format chosen concurs with Confarmas vocation.

Other formats do not have the same stability and reproducibility (donor-to-donor variability) and are difficult to manage due to the risks associated with the human blood or derived human blood (HIV, viruses…).

The MAT with a cell line is able to detect all human pyrogens and has the advantage of the stability of an assay based on a cell line.

The MAT with the Mono Mac 6 cell line was included in the international MAT evaluation (Hoffmann et al., 2005).

PYROGEN	DETECTION LIMIT WITH MONO MAC 6
Endotoxin	0.05 EU/mL
Flagellin of *B. subtilis*	0.2 µg/mL
Heat-killed *S. aureus*	0.5 x 10^7 cells/mL
Pam3CSK4	50 ng/mL
Peptidoglycan of *S. aureus*	0.1 µg/mL
Lipoteichoic acid	0.25 µg/mL

REFERENCES

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2. Novel Pyrogen Tests Based on the Human Fever Reaction, The Report and Recommendations of ECVAM Workshop 43
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5. Ph. Eur. 2.6.8.: Pyrogens
6. Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes
7. The Blood Harvest The Atlantic, 2014.
8. WCC 2012 Res 030 – Activity Report IUCN, International Union for Conservation of Nature, 2016
9. European Pharmacopoeia Commission adopts revised general chapter on Monocyte-activation test to facilitate reduction in testing on laboratory animals. EDQM, 2016
10. The monocyte activation test for assessing pyrogenicity, PETA, 2010

*scope available on www.cofrac.fr

CONFARMA SERVICES

Confarma is the first lab worldwide to be accredited ISO 17025 (COFRAC*) for MAT for pharmaceutical products and medical devices.

- MAT based on Mono Mac 6 cell line, which is a valuable, stable assay in routine
- Sensitive test system, stable low background
- First optical readout under the microscope → serves as assay control
- True *in vitro* test, completely standardized
- Sample preparation is simple
- Analysis of difficult samples possible
- In routine use at Confarma as investigation tool, and in the context of method development / validation
- Analysis of medical devices possible in direct contact with the cells
- Analysis of cell therapy products possible
- Analysis of non-sterile products possible
- Assessment of finished products as well as raw materials

WHY CONFARMA?

- First to use a robust MAT for QC release with a worldwide validated cell line
- More than 10 years experience with MAT
- MAT with all 3 formats available: cell line, PBMC, blood
- Experience with difficult products such as coagulation factors, cell-based therapeutics, strong antibiotics, products with low pH, gels…
- Strong expertise with endotoxin testing (all methods) as well
- Dedicated project team and flexible capacity planning to ensure the respect of the given timelines

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