THE ADVANTAGES OF THE MONOCYTE ACTIVATION TEST FOR ASSESSING PYROGENICITY

The monocyte activation test (MAT) is a total replacement for the rabbit pyrogen test (RPT) and the bacterial endotoxin test (BET)/limulus amoebocyte lysate (LAL) test. The MAT measures cytokine release from monocytes when human blood is exposed to a test substance. Cytokines released in the activation process are quantified by the enzyme-linked immunosorbent assay (ELISA).

HUMAN-RELEVANT TESTING

- The MAT measures the pro-inflammatory response of human blood to the detection of pyrogens and represents the most human-relevant test.[1,2]
- The MAT outperforms and replaces current animal tests:
  - There is a lower limit of detection,[3] and outcomes are more accurate[4,5] as well as more cost- and time-effective than with the RPT.
  - Human monocytes can detect more diverse types of pyrogens[6] than the BET and LAL tests can.
  - There is greater applicability to a variety of products, such as certain drugs[7-9] and herbal formulations.[1]

FLEXIBLE TESTING FOR ANY FIT-FOR-PURPOSE APPLICATION

- The MAT detects pyrogens in diverse products, including pharmaceuticals, biologics, and medical devices.[10-14] Medical devices can be directly incubated within the MAT system.
- Assays can have multiple endogenous controls to monitor the performance of the test system.[15]
- The MAT is validated[16-18] and commercially available as an assay kit.[19]
  - Protocols can use whole blood, cryopreserved blood, peripheral blood mononuclear cells (PBMCs), or monocyte cell lines.[20]
  - There are five standardised variants of the assay.[21]

GUIDANCE

- The **European Pharmacopoeia** general method 2.6.30 Monocyte activation[22,23] test allows the MAT to serve as a full replacement for the RPT after product-specific validation.
- The International Conference on Harmonisation "recommends that the analytical procedures described in the official pharmacopoeial texts, [*European Pharmacopoeia*] Ph.Eur. 2.6.14. Bacterial Endotoxins, [*Japanese Pharmacopoeia*] JP 4.01 Bacterial Endotoxins Test, and [*United States Pharmacopeia*] USP General Chapter <85> Bacterial Endotoxins Test, can be used as interchangeable in the ICH regions subject to the [specific] conditions". See The **US FDA's CDER and CBER**, Guidance for Industry: Q4B Evaluation and Recommendation of Pharmacopoeial Texts for Use in the ICH Regions, Annex 14, Bacterial Endotoxins Test General.
- US Pharmacopeia general chapter <151>, "Pyrogen Test", allows the use of a "validated, equivalent *in vitro* pyrogen or bacterial endotoxin test" in place of the *in vivo* RPT.
- **The US FDA**'s Guidance for Industry Pyrogen and Endotoxins Testing: Questions and Answers (2012) states that alternatives, specifically the MAT, may be used after product-specific validation for biological products, drugs, and devices, even when US Pharmacopoeia monographs require the RPT. The FDA encourages companies to contact the agency to discuss alternative test methods (e.g. in Guidance for Industry: Formal Meetings Between the FDA and Sponsors or Applicants and FDA's guidance on its Pre-Submission Program and Meetings).
- A proposal to evaluate the MAT as a stand-alone release test for medical devices in place of the RPT and BET/LAL when satisfying biocompatibility testing requirements is underway through the US FDA's Medical Device Development Tools programme.
- **ISO 10993-1:2018** "Biological evaluation of medical devices – Part 1: Evaluation and testing within a risk management process" gives preference to *in vitro* models when they yield equally relevant information.
- The **US FDA's Center for Devices and Radiological Health (CDRH)** guidance (2020) states that the CDRH accepts validated methods equivalent to the RPT.
- In 2006 and 2007, respectively, **ECVAM** and **ICCVAM**[21] endorsed the MAT for identifying gram-negative endotoxins and recognised its capacity to detect a wider range of pyrogens.

Companies offering MAT kits or services include MAT Research, PyroDex, MilliporeSigma, CTL-MAT, Zwisler Laboratorium, Sandquin, Haemochrom Diagnostica, Confarma, and Microcoat Biotechnologie GmbH.

For more information, please see ThePSCI.eu/our-work/pyrogenicity.

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