Pyrogenicity

Pyrogenicity is the capacity of a substance to induce a febrile (fever) response when introduced into the bloodstream, lymphatic system, or cerebrospinal fluid of an organism. The test methods below can be used to reliably assess a substance's pyrogenic potential and replace the rabbit pyrogen test (RPT) and the bacterial endotoxin test (BET)/limulus amoebocyte lysate (LAL) test.

	Method	Test Principle	Rationale
In Vitro	Monocyte Activation Test (MAT)	• The MAT can detect both endotoxin and non-endotoxin pyrogens in a diverse set of products including pharmaceuticals, biologics, and medical devices, thus serving as a complete replacement for both the BET/LAL and RPT. • The test system uses human monocytes from either whole blood, cryopreserved blood, peripheral blood mononuclear cells (PBMCs), or monocyte cell lines. • The test sample is incubated with monocytes, and if pyrogens are present, monocytes are activated via toll-like receptors, triggering an immune response in the form of cytokine release. • The MAT quantifies cytokine release from monocytes using ELISA. • Cytokine concentrations are then compared to a standard curve to determine endotoxin equivalent unit concentration (EEU/mL), indicating the pyrogenic potential of the test sample.	• Uses human monocytes for pyrogen detection, providing direct relevance to human immune responses. • The mechanism of monocyte activation and cytokine release mimics the human response. • Delivers quantitative cytokine measurements by ELISA, eliminating the subjectivity of animal temperature readings. • Produces results within hours after incubation instead of requiring days of animal monitoring. • Offered as commercially available kits with built-in positive and negative controls to ensure reliable performance. • Accepted by major global regulatory bodies as a valid method for detecting endotoxin and non-endotoxin pyrogens (replacing the RPT and BET/LAL).
In Chemico	Recombinant Factor C (rFC) Assay	• The rFC is designed to only detect endotoxin pyrogens and serves as a complete replacement of the BET/LAL. • The test system uses a reagent that contains recombinant Factor C and a quenched fluorogenic (or chemogenic) peptide substrate. • The reagent is added to test samples and incubated. If endotoxins are present, they will bind and activate Factor C. • Activated Factor C cleaves the substrate and releases a signal that is proportional to the concentration of endotoxins in the sample. • This signal is read using a microplate reader and compared to a standard curve to determine endotoxin equivalent unit concentration (EEU/mL), indicating the pyrogenic potential of the test sample.	• Provides high-specificity endotoxin detection, avoiding interference from non-endotoxin contaminants. • Uses a defined synthetic system with fully recombinant proteins to ensure batch-to-batch consistency and eliminate lot variability. • Delivers quantitative fluorescence data via microplate reader analysis, replacing subjective clot assessment or animal-temperature measurements. • Produces results with faster setup, less waste, and minimal variability when compared to the BET/LAL. • Offered as validated, commercially available kits with built-in positive and negative controls to ensure reliable performance. • Accepted by major global regulatory bodies as a valid method for detecting endotoxins (replacing the BET/LAL).

Guidance

- European Pharmacopoeia chapter 2.6.30, "Monocyte activation test." **As of July 1, 2025, mandated the transition from RPT to MAT. Must be validated for each product type.**
- European Pharmacopoeia chapter 2.6.32, "Test for bacterial endotoxins using recombinant Factor C." **As of July 1, 2020, rFC is recognized as an official method to detect endotoxins. Must be validated for each product type.**
- US Pharmacopeia chapter <86>, "Bacterial Endotoxins Test Using Recombinant Reagents." **Allows for the use of rFC if validated for each product.**
- US Pharmacopeia chapter <151>, "Pyrogen Test." **Allows the use of MAT if validated for each product.**
- The International Council for Harmonisation (ICH). **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 interchangeably in the ICH regions subject to the [specific] conditions.**
- US FDA's CDRH guidance (2020). **Formally recognizes rFC-based assays as acceptable for endotoxin testing with scientific justification and product-specific validation. Continues to support MAT with product-specific validation.**
- 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.**
- 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.**

Many companies offer MAT kits. For more information, please see ThePSCI.eu/our-work/pyrogenicity

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