Pyrogen testing: MAT and the good practices

Sravan K. Goparaju

Pyrogen testing and good MAT practices

1. Pyrogens
2. Detection of Pyrogens
3. MAT and Good practices

Pyrogens

Pyrogens are substances that trigger the body's **innate immune system**. Pyrogens can cause:

- **Fever**
- **Chills**
- **Hypotension**
- **Septic shock–like symptoms**
- **Multi-organ failure** (in severe cases)

Pyrogens

Drug products - especially injectables—bypass natural barriers, **any contaminating pyrogen is delivered directly into the bloodstream or tissues**, making reactions potentially life-threatening.

Pyrogens

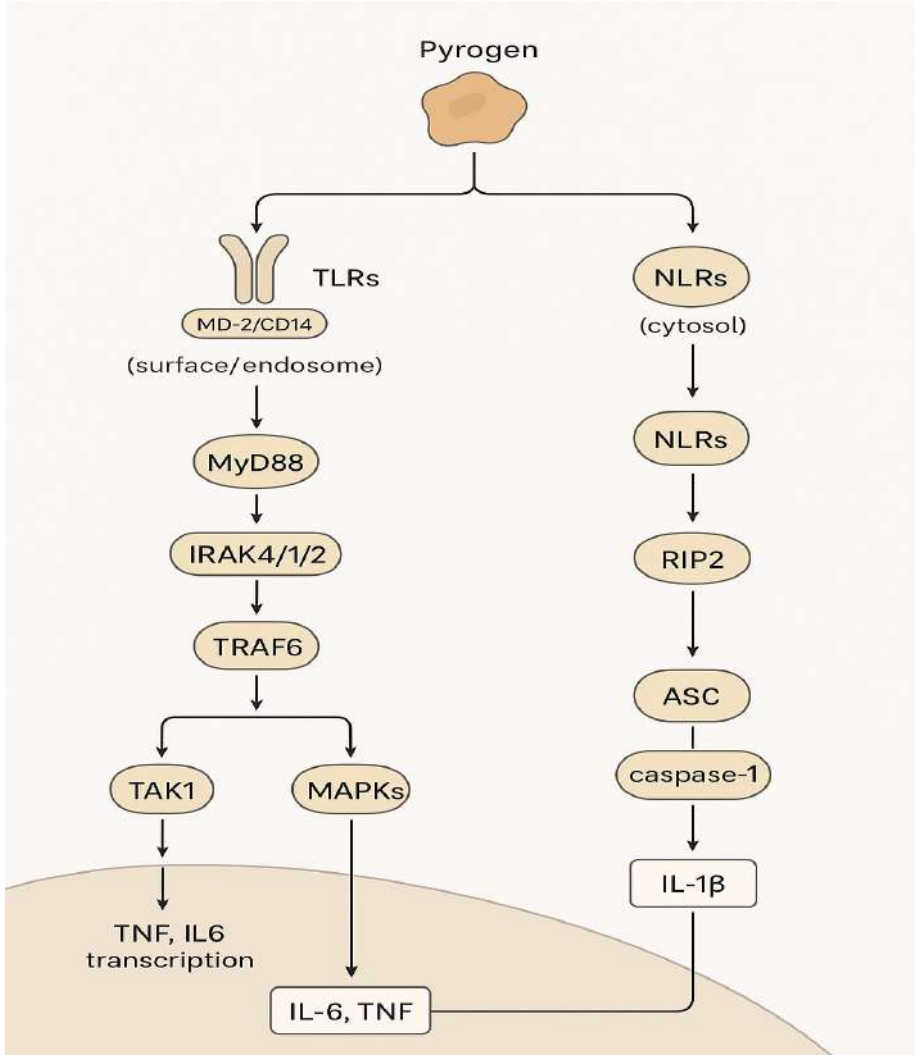
- **Two types**:

- **Endotoxin pyrogens**: Lipopolysaccharides (LPS from Gram-negative bacteria)

- **Non-endotoxin pyrogens** (**NEP**): lipoteichoic acid, flagellin, peptidoglycan, lipopeptides, viral RNA, residual cell debris, impurities from biologics, liposomes, nanoparticles

Pyrogen signaling in Monocytes

Toll-like receptors



NOD-like receptors

Pyrogen detection assays: LAL

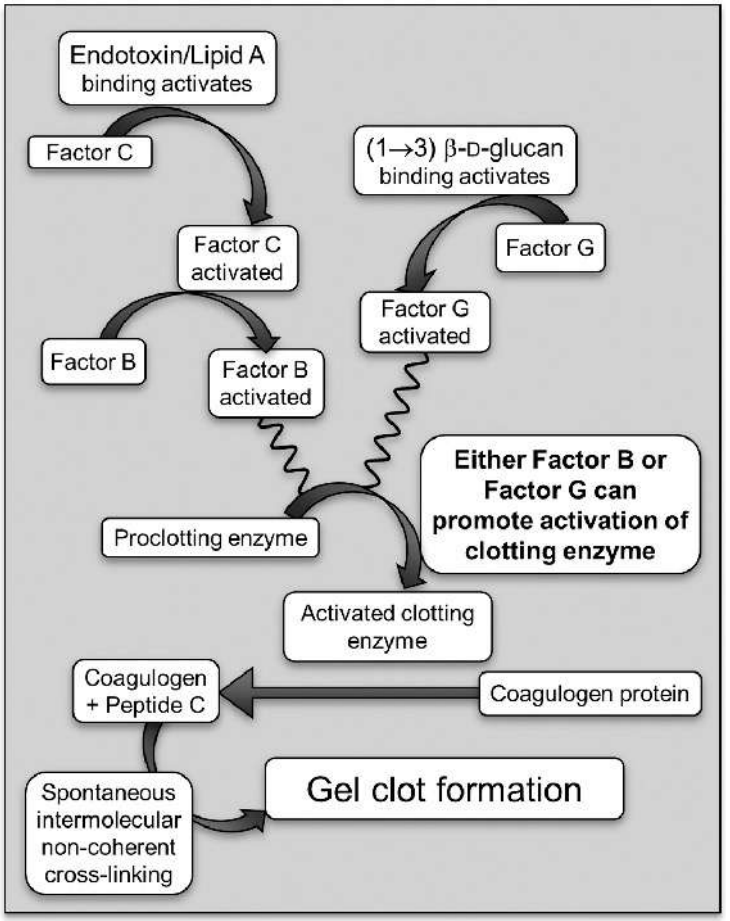
Limulus Amoebocyte Lysate (LAL) assay

•Detects **lipid A component of LPS** from Gram-negative bacteria

•Mechanism: activation of Factor C in horseshoe crab lysate → cascade → gel/clot or color change

•Purely **endotoxin-specific**, does *not* detect:

- •Gram-positive pyrogens
- •Viral pyrogens
- •Host cell impurities
- •Process contaminants (e.g., leachables)



Pyrogen detection assays: RPT

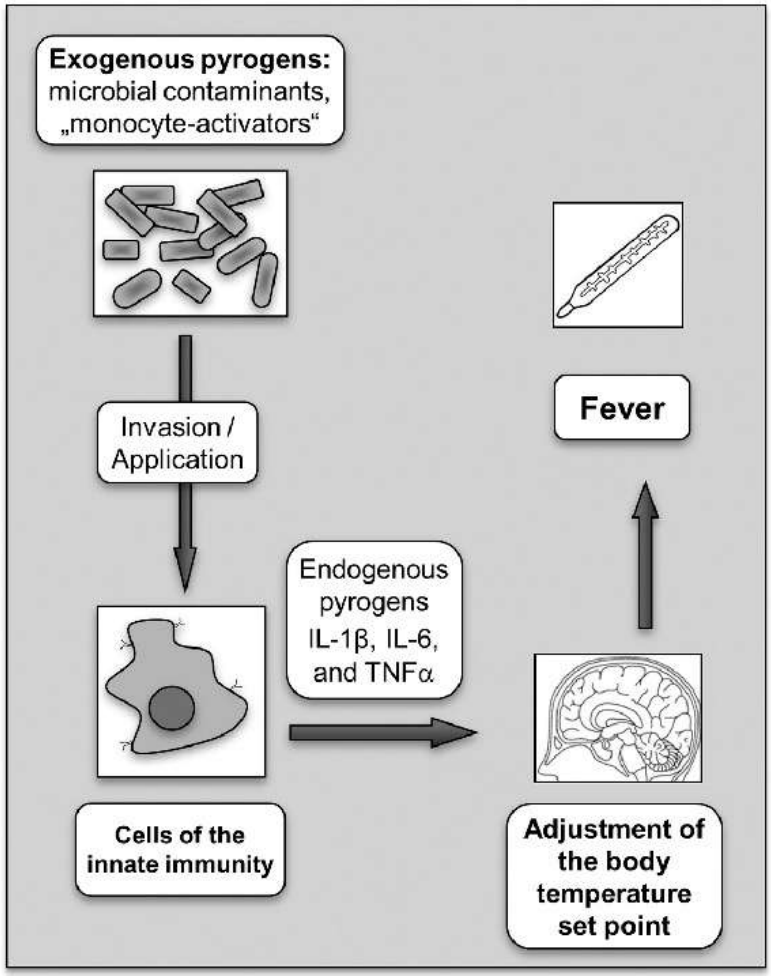
Rabbit Pyrogen Test (RPT)

•Detects substances that cause **fever in rabbits**

•Very broad sensitivity to pyrogens

•But physiologically **not directly reflective of human responses**

•Large ethical, logistical, and variability problems

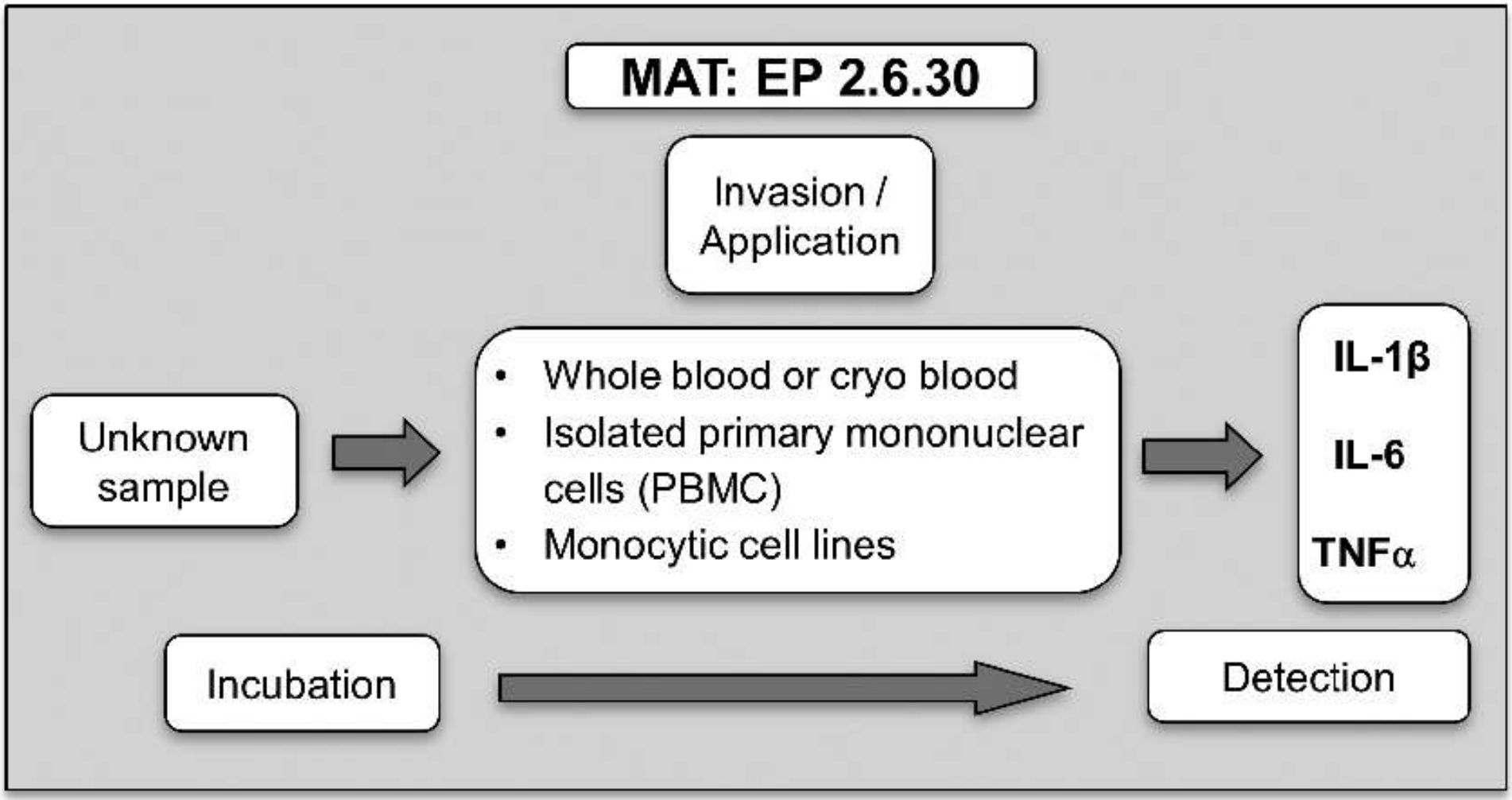


Pyrogen detection assays: MAT

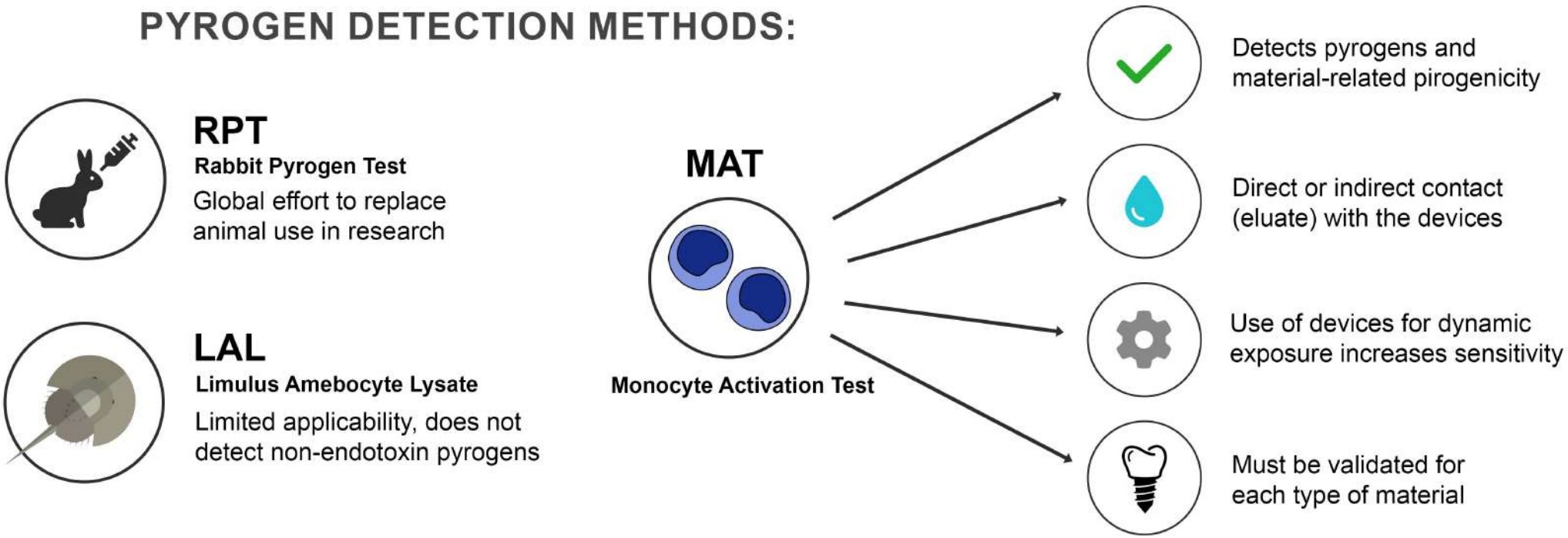
Monocyte Activation Test (MAT)

- Detects **pyrogens that activate human innate immune receptors** (TLR2, TLR4, NLRs etc.)
- Measures **human cytokine release** → more physiologically relevant
- Detects both:
  - **Endotoxin (LPS)**
  - **Non-endotoxin pyrogens** (NEP): lipoteichoic acid, flagellin, peptidoglycan,
  - lipopeptides, viral RNA, residual cell debris, impurities from biologics,
  - liposomes, nanoparticles

MAT procedure



Pyrogen detection assays: schematic summary



Good MAT practices

The MAT is a compendial, human-cell–based pyrogen test in the European Pharmacopoeia (Ph. Eur. 2.6.30) and is recommended as a replacement for the rabbit pyrogen test after product-specific verification.

Good MAT practices: Pre-analytical & assay setup

- **Choose an appropriate cell system**

Use human peripheral blood mononuclear cells (PBMC), whole blood, or validated monocytic cell lines (e.g., THP-1) depending on intended use and sensitivity requirements. Justify choice in method SOP/validation.

- **Donor selection / pooling**

Screen healthy donors for baseline variability. Donors should be free of any bacterial and viral infections at least 1 week before blood donation. Donors should not be on medications (NSAID/SAID) or medications that influence cytokine levels.

Pooling donors is commonly used to reduce inter-donor variability, but pooling strategy must be justified and validated (pool size, lot-to-lot consistency).

Document donor consent and biosafety approvals.

Good MAT practices: Pre-analytical & assay setup

• When cell lines are used, they need to be mycoplasma free, robustly proliferating, should be of relatively low passage and should respond to endotoxin-stimulii consistently

Validation of pooled cells
Freshly pooled cells or pooled cells cryopreserved and thawed shoud be validated both with endotoxin standards and NEPs.

•Use endotoxin-free materials & aseptic technique
All plastics, reagents, and buffers must be certified endotoxin-free handled under clean conditions to avoid false positives.

•Appropriate controls every run
Negative control, positive control (standard LPS or a validated reference), and a sample-spiked control (to assess interference / recovery). Include blanks and assay QC samples.

•Select the read-out and time points
IL-6 is a commonly used primary readout. Optimize incubation time (typical MAT plate incubation ~20–24 h) and ELISA timing in development/robustness experiments.

Good MAT practices: Validation / method performance

•**Define and demonstrate the validation parameters**

Sensitivity / limit of detection (LOD), **linearity**, **accuracy / recovery (spike-recovery)**, **precision** (intra- and inter-assay), **specificity** (distinguish endotoxin vs non-endotoxin pyrogens), **robustness** (critical times/temps/volumes), and **stability** of reagents and samples.

•**Assess matrix effects and interference**
Test product formulations, excipients, and buffers for inhibition or enhancement (spike/parallelism experiments). If inhibition occurs, adapt sample preparation (dilution, buffer exchange) and re-validate.

Good MAT practices:Practical run-day recommendations

- **Run plate layout thoughtfully**
  Randomize donors/conditions across plates to avoid plate position bias; include replicate wells.
  Use plate maps in batch records.

- **Optimize ELISA conditions**
  Validate antibody incubations, substrate times and stop times. Small timing shifts can affect OD readings — include these in robustness testing (e.g., ±10% windows).

- **Recordkeeping & traceability**
  Full SOPs, reagent batch numbers, donor lot information, raw data, curve fits, and deviation records are mandatory for GMP use.

- **Reagent and reference standards**
  Use traceable LPS/reference materials and document storage/expiry. Verify the performance of new reagent lots before use in release testing.

Good MAT practices:Regulatory / quality considerations

Product-specific validation for compendial compliance
Even when MAT is compendial (Ph. Eur.), perform product-specific verification/validation to demonstrate suitability for your product and intended acceptance criteria.

GMP lab implementation
For release testing, run MAT under GMP with validated instrumentation, trained analysts, defined QC release criteria, and periodic requalification.

Keep up with guidance
Follow Ph. Eur. chapter 2.6.30 updates, applicable USP/WHO guidance, and national regulator guidance (FDA Q&A on pyrogens, when applicable). Regulatory positions evolve — note recent reviews and papers summarizing best practices.


Thank You