Development of Monocyte Activation Test: An Assay to Detect Pyrogen Contaminants in Pharmaceuticals

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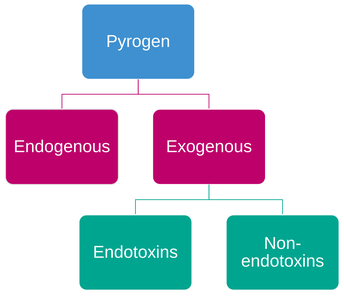
Introduction to Pyrogens

- **Pyrogens** are fever inducing substances[1]
  - **Endogenous pyrogens** are low molecular weight proteins such as cytokines
  - **Exogenous pyrogens** originate from external sources
    - **Endotoxins**
      - Gram negative bacteria
      - Lipopolysaccharide (LPS)
    - **Non-endotoxins**
      - Gram positive bacteria
      - Yeast, mold, virus
- Pyrogens can cause:
  - Fever, rash, swelling at injection site, severe soreness/redness and life-threatening complications (in extreme cases)[1]
- Control of pyrogenic content is crucial to assure the safety and quality of vaccines for patients[3]
- MAT is being developed to replace the Rabbit Pyrogen Test (RPT) at Sanofi Pasteur as a release test for final products[3]



Objective: To develop the Monocyte Activation Test to detect pyrogenic contaminants in vaccines

Monocyte Activation Test

- The MAT is an *in vitro* cell-based assay[2]
- Detects presence of pyrogens that activate human monocytes (eg. Peripheral Blood Mononuclear Cells; PBMCs) to release pro-inflammatory cytokines (eg. IL-6)[2]
  - Test artificial contamination of the Product with pyrogens being used as spike
    - **Lipopolysaccharide (LPS)**
      - Found in outer membrane of Gram-negative bacteria
    - **Pam3CSK4**
      - Mimics Bacterial lipoproteins (found in Gram-negative and Gram-positive bacteria)
    - **Zymosan**
      - Found on the cell wall of yeast

Methodology

Summary of the experimental flow for performing the Monocyte Activation Test

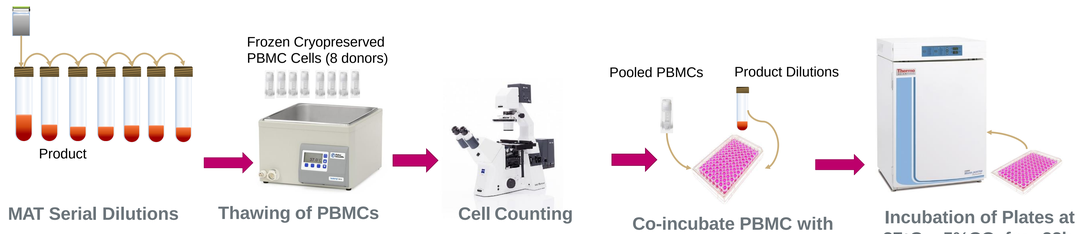


Figure 1. Diagram describing the steps performed on Day 1

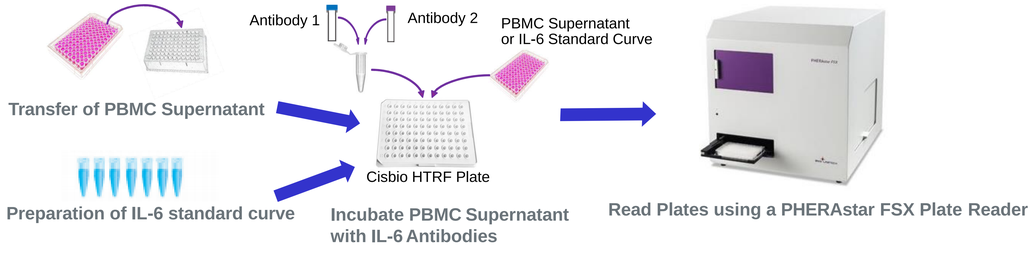


Figure 2. Diagram describing the steps on Day 2, which consists in measuring IL-6 concentration using the Homogenous Time Fluorescence Assay (HTRF Assay)

Results

12 lots were tested successfully in three independent experiments performed on different days

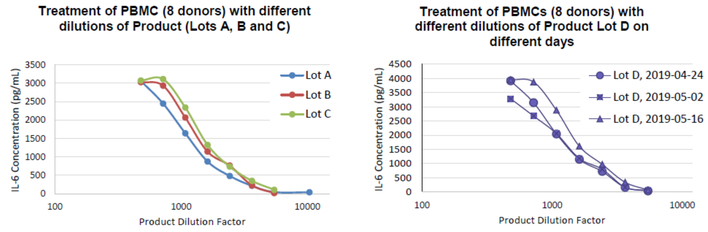


Figure 3. Product stimulates IL-6 in PBMCs in a concentration dependent manner

Artificial contamination of the Product using pyrogens

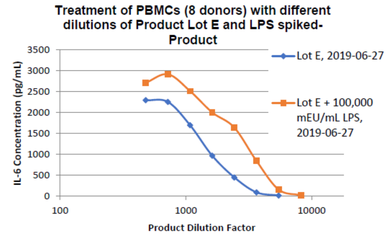


Figure 4. Spiking the Product with LPS further elevated IL-6 response

Detection of a spike in the assay was observed with all three pyrogens (LPS, Pam3CSK4 and Zymosan) amongst different lots and users

Conclusions and Next Steps

- Conclusions:
  - Product dilution range was successfully optimized
  - MAT can detect pyrogen contaminants
- Next Steps:
  - Additional spike investigations:
    - Determine the level of spike that would trigger a fever in the RPT for the specific Product
    - Assess if the MAT can distinguish the spiked from the non-spiked Product with other pyrogens

References and Acknowledgments

[1] Anochie, I. P. Mechanisms of Fever in Humans. *Int. J. Microbiol. Immunol. Res.* **2013**.
[2] Stang, K.; Fennrich, S.; Krajewski, S.; Stoppelkamp, S.; Burgener, I. A.; Wendel, H. P.; Post, M. Highly Sensitive Pyrogen Detection on Medical Devices by the Monocyte Activation Test. *J. Mater. Sci. Mater. Med.* **2014**. https://doi.org/10.1007/s10856-013-5136-6.
[3] 2.6.30. 2.6.30 Monocyte-Activation Test. European Pharmacopoeia (9.2). **2017**, 4299-4304.

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