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\(^2\)
- 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

![Diagram describing the steps performed on Day 1](image)

- MAT Serial Dilutions
- Thawing of PBMCs
- Cell Counting
- Co-incubate PBMC with Product dilutions or controls
- Incubation of Plates at 37°C + 5%CO\(_2\) for ~22h

References and Acknowledgments


I would like to thank my colleagues from the Microbiology Unit and my previous department at Analytical and Process Technology (AP&T) Team at Sanofi Pasteur for all the support, knowledge and guidance.