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Data Sheet

Validation of a cell line-based Monocyte Activation Test method according to USP <1225> Validation of compendial procedures guideline

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Introduction

The Monocyte Activation Test (MAT) was introduced in the European Pharmacopeia (Chapter 2.6.30) in 2010, as a compendial method that can be used to replace the Rabbit Pyrogen Test (RPT). Also, the new version of USP <151> Pyrogen Test, effective since May 2017, indicates the possibility to use a validated and equivalent *in vitro* pyrogen test in place of the *in vivo* RPT. A new cell line-based MAT was developed to detect the full range of pyrogens.

Using the Mono-Mac-6 (MM6) ready-to-use cell line, monocytes are activated if the sample is contaminated with pyrogens (endotoxins and non-endotoxin pyrogens) and produce cytokines including interleukin-6 (IL-6) which is detected in an immunological assay (ELISA).

This method validation study was organized according to EP 2.6.30 MAT, USP <1225> Validation of compendial procedures, and ICH Q2(R1) November 2005, Validation of analytical procedures: text and methodology guidelines.

The method validation characteristics evaluated in the different guidelines were: accuracy, precision, specificity, detection limit, linearity and range. To cover all the aspects of the method performance, ruggedness and robustness were also considered.

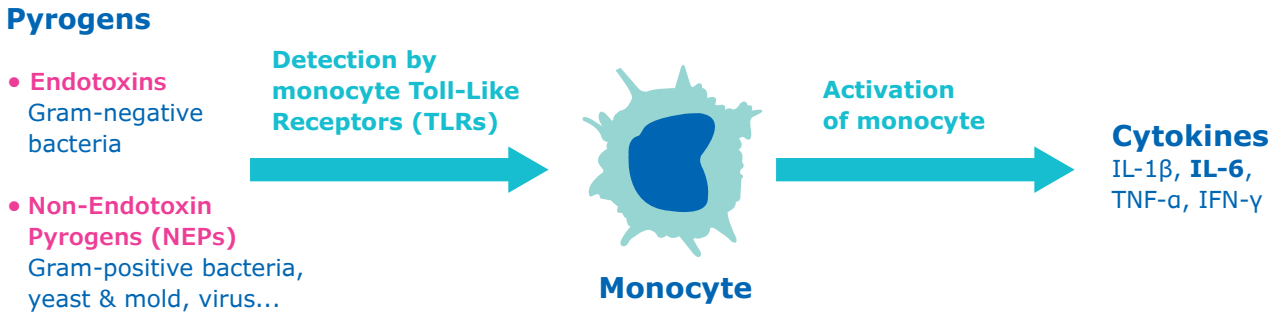


Fig. 1: Monocyte activation test principle Monocyte activation test method detects pyrogenic and proinflammatory contaminants, including endotoxins from Gram-negative bacteria and non-endotoxin contaminants

Methods

Endotoxin standard curves and spiked samples were used for evaluating the monocyte activation test according to the following workflow (see Fig. 2).

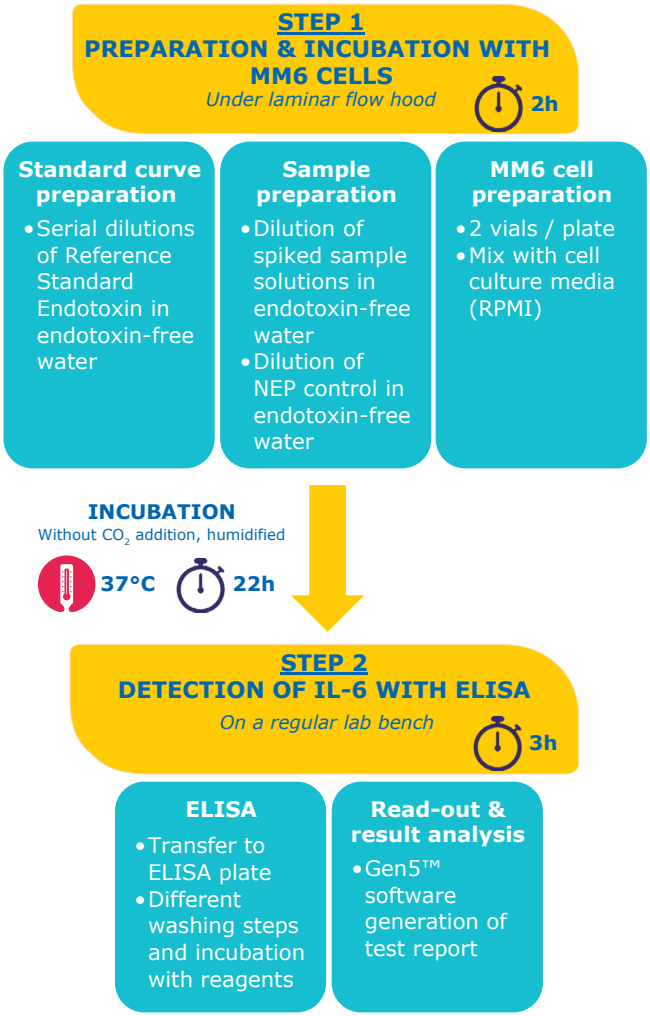


Fig. 2: **Diagram of the test method**
Workflow of the monocyte activation test with the PyroMAT® system

The performance assessment of the monocyte activation test method was challenged through the following parameters:

- Robustness: incubation time
- Ruggedness: cell lots, culture medium lots, ELISA kit lots, operator
- Accuracy, precision, linearity, range, limit of quantification
- Limit of detection: 0.05 EU/mL
- Specificity: panel of non-endotoxin pyrogens (NEP)

Several concentrations of spiked sample (from 0.05 to 0.6 EU/mL) were used for the robustness test (incubation time), for the ruggedness test (testing different cell lot, culture medium lot and ELISA kit lots) and for the accuracy. Data from accuracy were used for precision, linearity, range and limit of quantification analysis. Cell lot ruggedness was carried out by two different operators. A single concentration of spiked sample at 0.05 EU/mL was used for the limit of detection. Three independent preparations were tested for each spiked sample. All the tests were realized with standard and specific ELISA protocols and handled at 2 different sites (except specificity, only one site). To test specificity, NEPs targeting different surface TLRs were challenged.

Results

Incubation time robustness demonstrates a superposition of the standard curves over the three time-points tested (20, 22 and 24 hours) without variation of endotoxin quantification. Therefore, an incubation time of 22 ± 2 hours is recommended.

Accuracy. Endotoxin recoveries from sample spiked with a known pyrogenic concentration meet acceptance criteria (between 50-200%).

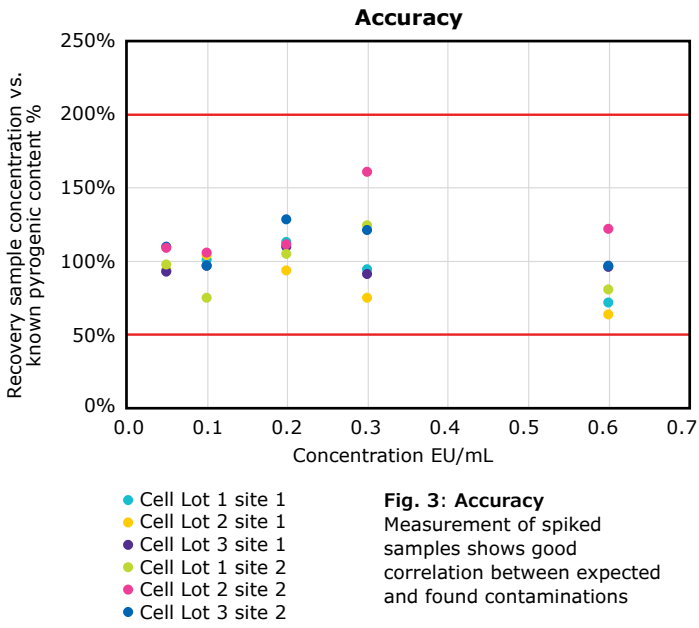


Fig. 3: **Accuracy**
Measurement of spiked samples shows good correlation between expected and found contaminations

Ruggedness test: cell, culture medium and ELISA kit lots and operators. Endotoxin recoveries from sample spiked with a known pyrogenic concentration (not represented here) meet acceptance criteria (between 50-200%). Respective Relative Standard Deviation (RSD) calculation (< 25%) between different variables demonstrates the reproducibility of results for all parameters tested.

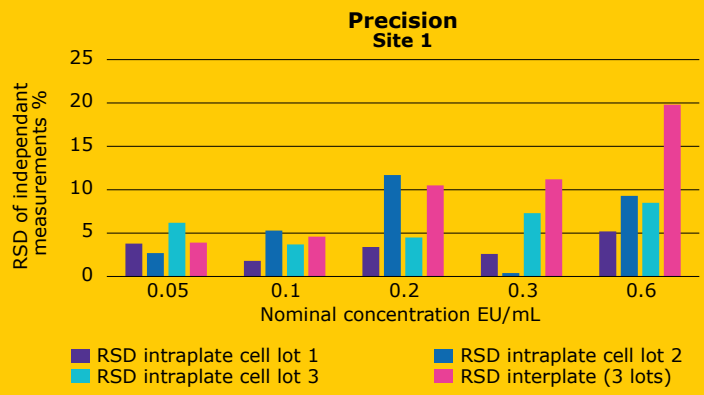
Specificity. Monocytes react to endotoxin and non-endotoxin pyrogens targeting different surface TLRs. Positive signal is demonstrated with lipoteichoic acid (LTA), Heat-Killed *Staphylococcus aureus* (HKSA), peptidoglycan, PAM3CSK4, PAM2CSK4 and flagellin.

RSD (%) for ruggedness of cell lot	Cell lot 1 Operator 1	Cell lot 2 Operator 1	Cell lot 1 Operator 2	Cell lot 2 Operator 2
0.05 EU/mL	3.3	12.3	9.9	6.1
0.1 EU/mL	3.2	1.9	2.4	10.2
0.2 EU/mL	2.2	3.0	11.2	5.8
0.3 EU/mL	8.7	1.2	5.8	7.0

Fig. 4: **Ruggedness of cell lot (site 1)**
Test results with each cell lot and each operator are reproducible (RSD < 25%)

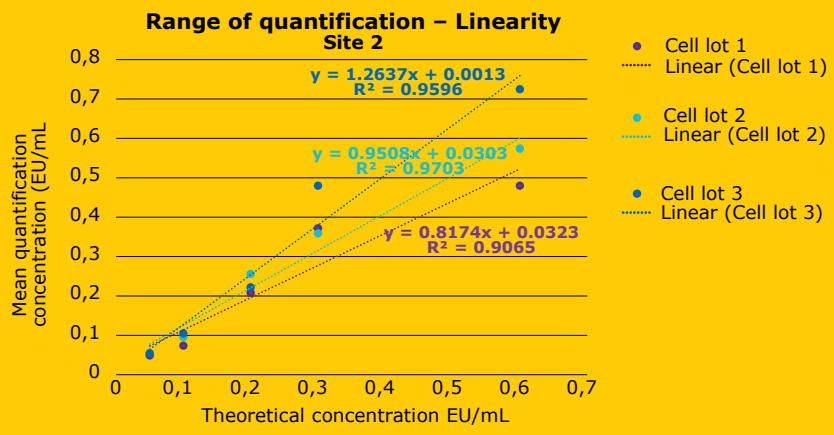
Precision

3 independent measurements of 5 spiked sample concentrations showed a RSD < 25%. The analysis of interplate RSD (< 25%) corresponding to the comparison of the sample measurement from 3 independent monocyte activation tests demonstrates a **reproducibility of test results with precision**.



Range of quantification

The interval between the upper and lower concentrations is derived from accuracy and linearity studies. The specified range of quantification that has been demonstrated with precision, accuracy and linearity is **0.05 to 0.4 EU/mL**.



High sensitivity LOD 0.05 EU/mL

3 independent preparations of Reference Standard Endotoxin at 0.05 EU/mL, with a quantification confirmed by LAL method, were evaluated. At least 23 out of 24 wells for each preparation show a positive signal, confirming the **LOD of 0.05 EU/mL**.

LOD 0.05 EU/mL	Cell lot 1	Cell lot 2	Cell lot 3
Site 1	4	4	4
Site 2	4	4	4

Summary

All the performance assessment was performed in parallel with standard and **specific protocol of ELISA**, standard protocol showing an optimized and reduced time to result (3 hours) and giving reliable and reproducible results with a variety of parameters such as cell lots, operator or site.

The results of this study with the PyroMAT® system are in accordance with the specification given by USP <1225> Validation of compendial procedures and ICH Q2(R1) November 2005 Validation of analytical procedures: text and methodology guidelines, using the monocyte activation test according to EP 2.6.30 MAT.

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