Pyrogen Detection – Results from MAT Ring Trial

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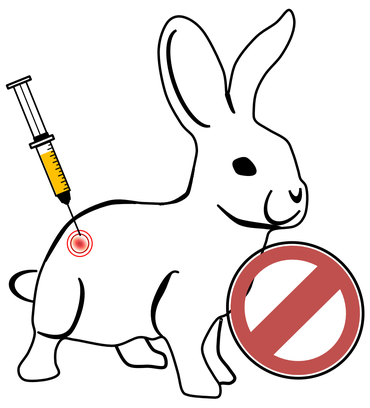
Background



Rabbit Pyrogen Test

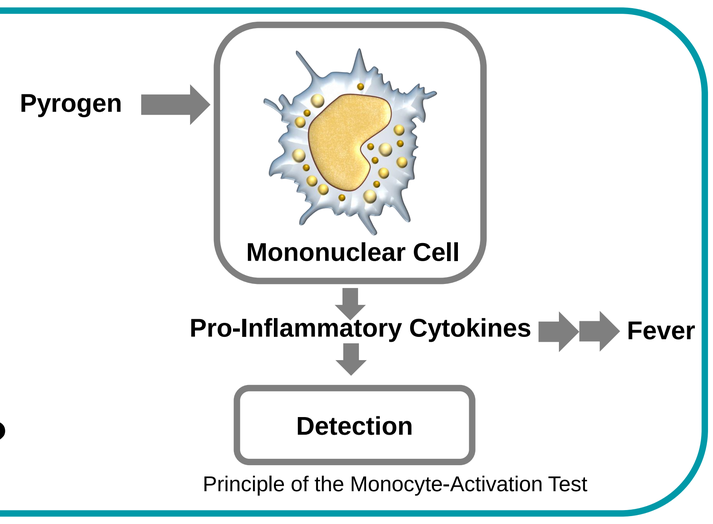
The Rabbit Pyrogen Test (RPT) is designed to limit the risk of febrile reaction in the patient, upon administration by injection of the concerned product, to an acceptable limit. [1,2]. Due to the European Directive 2010/63/EU on the protection of animals used for scientific purpose the routine testing for pyrogens with the RPT is limited. According to European Pharmacopoeia (2.6.30) the Monocyte Activation Test (MAT) is primarily intended to be used as a replacement for the RPT. By this method the use of live animals is avoided [2].

→ The Rabbit Pyrogen Test has to be substituted by the MAT

The Alternative

The MAT, an in vitro test based on monocytes and monocyte derived cells, is used for the detection of pyrogens from different origins. The fundamental principle of the MAT, is an immunological reaction of monocytic cells upon contact with pyrogenic substances (e.g., Lipoteichoic acid, peptidoglycan, endotoxin etc.), by releasing pro-inflammatory cytokines (e.g., IL-1ß, IL-6, TNFα). The expressed and released cytokines can later be detected using various analytical systems (e.g., ELISA, HTRF, Multiplexing etc.).

→ Is the MAT a reliable test method to replace the RPT?



Principle of the Monocyte-Activation Test

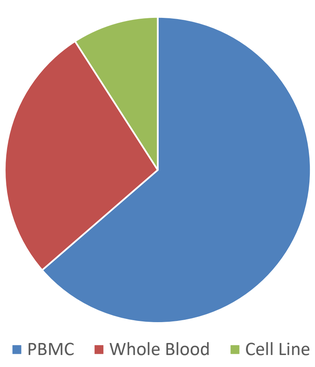
Program

The ring trial (MAT Proficiency Test Program) generally verifies working procedures, method and report validation as well as identifies trends and potential demands for training. Moreover, the resulting data reveals, if the MAT is an appropriate test method. Participating analysts received a blind, contaminated sample. Methodology, source, and readout were chosen freely by respective laboratories. Upon completion of the study, a final report of all results for individual analysis were provided. In this poster the individual results are compiled and displayed by qualitative and quantitative interpretation, respectively [3].

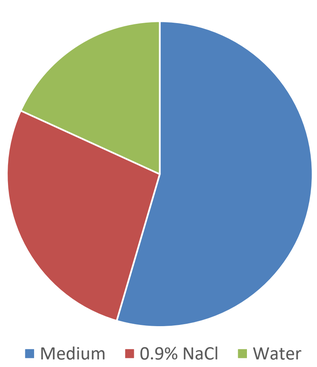
Results

- A defined and blinded sample was distributed to participants.
- The sample was contaminated by >1 EEU*/mL.
- The sample was analyzed in 21 assays in 11 labs.

Used cell sources:

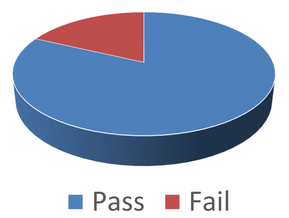


Used sample diluents:



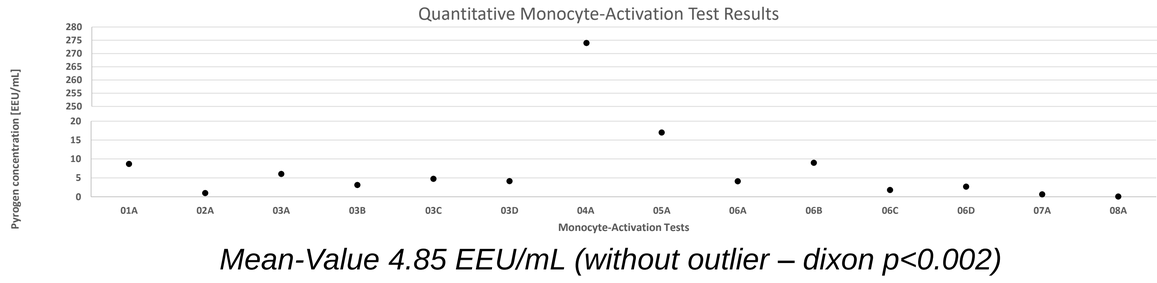
Following qualitative test interpretation:

➢Result > 1 EEU/mL: 90.5 %
➢Result < 1 EEU/mL: 9.5 %



Following quantitative interpretation

- 8 labs provided quantitative results, 3 labs only qualitative (provision of 14 individual results by 8 labs)



Mean-Value 4.85 EEU/mL (without outlier – dixon p<0.002)

➢Results within 50 to 200 %: 64.3 %

➢Result out of 50 to 200 %: 35.7 %

Conclusion

- Results were generated by 11 different laboratories around the world.
- Majority of participants generated correct results (90.5 %).
- Qualitative interpretation of data preferred, because synergistic effects may interfere with quantitative interpretation.
- **MAT is an adequate method to substitute the Rabbit Pyrogen Test, but proper method validation recommended.**

References

[1] United States Pharmacopeial Convention. The United States Pharmacopeia 2011: USP 35 ; The national formulary : NF 30. Rockville, MD: United States Pharmacopeial Convention; 2011.
[2] European Pharmacopoeia, 8th edition 2013, English Subscription to Main volume + Supplement 1 + Supplement 2. Stuttgart: Deutscher Apotheker Verlag; 2013.
[3] https://www.microcoat.de/laboratory-services/endotoxin-and-pyrogen-test-service-2/#Monocyte-activation-test-Proficiency-Test-Program
** Endotoxin Equivalent Unit*