Nomination of a validated *in vitro* test method for assessing pyrogenicity of pharmaceuticals and other products for further evaluation in order to expand its applicability domain to non-endotoxin pyrogens to the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)

Background information on the recognition and status of validation processes regarding national and international regulations - The extent to which the Monocyte Activation Test (MAT) is applicable to regulatory testing needs

Following international validation studies, the MAT has been peer-reviewed and accepted with restrictions to Gram-negative endotoxins by ICCVAM, ECVAM and JaCVAM. Here, the work suggested in the peer-review process shall be carried out to expand the applicability domain and fully replace the rabbit assay.

National pharmacopoeias limit the pyrogen content of pharmaceutical products for parenteral application and require the use of appropriate tests. Several decades after the Rabbit Pyrogen Test (RPT) and the Bacterial Endotoxin Test (BET) the Monocyte Activation Test (MAT) has been established as an *in vitro* method for the detection of pyrogens (1). The method was incorporated in the European Pharmacopoeia (EP 6.7 Chapter 2.6.30) in April 2010 as a replacement for the RPT (2). The respective reception into the Pharmacopoeias of the United States (USP) and Japan (JP) so far has not taken place, though a positive peer-review was carried out by JaCVAM. The test method was recommended by ICCVAM following a comprehensive evaluation of its validation status. FDA endorsed the ICCVAM recommendation that these in vitro pyrogen test methods may be considered on a case by case basis for the detection of Gram negative endotoxin in parenteral drugs, subject to product-specific validation(http://iccvam.niehs.nih.gov/methods/pyrogen/pyrogen.htm)

In the ICCVAM Test Method Evaluation Report "Validation Status of Five *In Vitro* Test Methods Proposed for Assessing Potential Pyrogenicity of Pharmaceutical and Other Products" (3) the MAT was not recommended as a full replacement of the RPT mainly due to a lack of evidence that pyrogens other than Gram-negative endotoxins could be detected and that such pyrogens could be detected in all categories of samples (e.g. pharmaceuticals, biologicals, and medical devices).

The extent of expected use and application of the MAT and its impact on human health

The MAT differs from the currently accepted other methods in the origin of the components responding to a potential pyrogen. Because of the use of human blood, it can be expected that the response *in vitro* will be closely related to reactions in the human body. In fact, experimental evidence has been accumulated, that the MAT in addition to endotoxin, preferentially and specifically detected in the BET and the only pyrogen defined by international standards (4), responds to additional substances known to trigger pyrogenic responses via different Toll-like receptors (TLRs) in humans (5). Although it is expected that such pyrogens are also detected in the RPT, a formal proof of this assumption is lacking and would require individual animal tests for each substance.

The potential for the MAT, compared to current accepted test methods, to refine, reduce, or replace animal use

After a successful validation of the MAT following the ICCVAM recommendations for future studies the RPT can be completely replaced, thus saving the lives of thousands of rabbits.

The potential for the MAT to provide improved prediction of adverse health effects, compared to current accepted test methods

Responses of the innate immune systems of humans and animals are not identical. In fact the decision for rabbit tests emerged after comparing several animal models as a compromise of convenience and economics (6). The MAT offers the chance to analyze the effects of pyrogens on the cells which regulate the inflammatory responses in the human body.

In contrast to the RPT (and also the BET) the MAT allows a differentiation between the effects of interfering factors and pyrogens. An example for a factor enhancing the pyrogen response is peptidoglycan (7), whereas for corticosteroids an inhibitory effect on the production of inflammatory cytokines is observed (8, 9). The MAT allows to detect and measure the activities of all relevant substances in a sample and may help to resolve discrepancies in results obtained with current methods.

A number of drugs influencing temperature regulation or causing immunological reactions cannot be tested in the rabbit. Also the rabbit can not be used for tests of cellular preparations and medical devices. On the other hand, drugs interfering with the clotting system or substances masking LPS cause problems in the LAL test. The MAT offers the possibility to overcome such problems (10).

The extent to which the MAT provides advantages (e.g., reduced cost and time to perform) compared to current methods

In a direct comparison with the RPT the MAT was found to provide at least the same level of security for the products. 58 positive (> 0.5 EU) and 29 negative samples of pharmaceutical albumin were clearly identified in the MAT, whereas in the RPT especially borderline samples required repeated testing (11), resulting not least in additional costs. With respect to sensitivity the MAT thus appears to be superior to the RPT. Similar data were obtained for immunoglobulins. In addition, in contrast to the RPT, the MAT allows a quantitation of pyrogens, facilitating the exact determination of the pyrogen content in a sample.

A further advantage of the MAT with cryo-preserved blood is its constant availability in the laboratory in standardized form, pretested for possible blood-born pathogens. This results in benefits for lab logistics and a reduction of the time from experimental concepts to results.

The MAT is an initial step to replace animal models for complex metabolic or regulatory circuits. In the basic version presented here, the induction of IL-1 beta is used to quantitate pyrogenic effects. It is obvious that modifications of the set-up and readout of the MAT will allow to extend its use to quantitate and differentiate the activity of numerous immunoregulatory substances affecting infection and inflammation. Examples can be found in ref. 12.

The extent to which the MAT could be considered applicable to multiple agencies or programs

Pyrogen testing is a crucial safety requirement for injectable drugs, eye drops, medical devices, cell therapies, baby food etc. It has been considered for work safety (air-born pyrogens) and microbiological water control. While primarily governed by FDA, putative further uses of the method must be considered.

Purpose of the evaluation

The purpose of the proposed evaluation is to overcome the limitations in applicability of the MAT by complying with the ICCVAM recommendations for future studies as detailed in the ICCVAM Test Method Evaluation Report: Section 2.0 (3, page 6).

Proposed Validation Study design

The proposed validation study will involve the analysis of samples spiked with endotoxin and non-endotoxin pyrogens. As an endotoxin standard a Reference Standard Endotoxin (RSE, 4) will be used. Lipoteichoic Acid (LTA) is proposed as the non-endotoxin standard. The substance is available in a highly purified form essentially free of endotoxin activity (13). Crude preparations from Gram-positive bacteria will also be considered. At least one substance with pro-inflammatory properties will be included in the study.
Spiked samples analyzed in the MAT will cover the categories of pharmaceutical and biological products as well as medical devices.

Tests in rabbits and LAL tests will be included but limited to the extent required for reference purposes regarding the activities of the pyrogens. A formal sample size calculation will be made to determine the number of replicates needed to fulfill the requirements for a given significance and power.

This nomination is for coordination of an independent validation study to evaluate the MAT for its abillity to detect non-endotoxin pryogens. In accordance with the validation functions of NICEATM and the International Cooperation on Alternative Test Methods(ICATM), we propose that NICEATM serve as as the lead validation organization for the Validation Management Team, and include partners from the other ICATM validation organizations to participate on the VMT. Ideally three laboratories in different geographic regions should participate. Following completion of the validation study, the results would be presented to ICCVAM for scientific peer review and evaluation, which would also be coordinated with the ICATM validation organizations.

References

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