Meeting Report

Using the Monocyte Activation Test as a Stand-Alone Release Test for Medical Devices

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Abstract

Monocyte activation tests (MAT) are widely available but rarely used in place of animal-based pyrogen tests for safety assessment of medical devices. To address this issue, the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods and the PETA International Science Consortium Ltd. convened a workshop at the National Institutes of Health on September 18-19, 2018. Participants included representatives from MAT testing laboratories, medical device manufacturers, the U.S. Food and Drug Administration's Center for Devices and Radiologic Health (CDRH), the U.S. Pharmacopeia, the International Organization for Standardization, and experts in the development of MAT protocols. Discussions covered industry experiences with the MAT, remaining challenges, and how CDRH's Medical Device Development Tools (MDDT) Program, which qualifies tools for use in evaluating medical devices to streamline device development and regulatory evaluation, could be a pathway to qualify the use of MAT in place of the rabbit pyrogen test and the limulus amebocyte lysate test for medical device testing. Workshop outcomes and follow-up activities are discussed.

1 Introduction

Pyrogens are a diverse group of substances that produce fever when parenterally introduced to the body. Pyrogens may be classified as endogenous (produced in the body) or exogenous (from outside sources). Exogenous pyrogens include bacterial endotoxins, such as lipopolysaccharides, the most prevalent and potent pyrogens, which are unique to Gram-negative bacteria. Exogenous microbial non-endotoxin pyrogens originate from yeast, mold, viruses, and other organisms beyond Gram-negative bacteria, such as lipoteichoic acid, a cell wall polymer found in Gram-positive bacteria. A loosely-defined third class of non-endotoxin exogenous pyrogens called material-mediated pyrogens (MMPs) – chemicals which may leach from medical devices during use and directly initiate a pyrogenic response – are discussed below.

Medical products that come in contact with the cardiovascular system, cerebrospinal fluid, have ophthalmic contact or are implanted or injected, and any devices labeled "non-pyrogenic" should meet pyrogen limit specifications before they can be marketed. Two animal-based pyrogen tests are typically used for evaluation of pyrogenicity: the rabbit pyrogen test (RPT) and the limulus amebocyte lysate test (LAL), which are performed using rabbits or hemolymph derived from horseshoe crabs, respectively. However, non-animal replacements, including monocyte activation tests (MAT), are available. The MAT methods have been described in detail elsewhere and will not be reviewed here (Hartung et al., 2001; Hasiwa et al., 2013; Hartung, 2015; Fennrich et al., 2016).

Pyrogen tests continue to account for a significant number of animals used in laboratory procedures despite the availability of MAT, with annual usage of rabbits for this endpoint remaining relatively constant over the past several years. In 2017, the most recent year for which these figures are available, 6,716 rabbits were used in pyrogen tests in Germany[1] and the UK[2] alone. The LAL (also known as the bacterial endotoxin test or BET), which is based on the premise that the blood of horseshoe crabs clots in the presence of bacteria (Levin and Bang, 1964, 1968), was suggested as a replacement for the RPT in 1971 (Cooper et al., 1971). Today, the vast majority of pyrogen testing is conducted using this method (Hartung, 2015), and its use has been associated with a decline in the horseshoe crab population (Anderson et al., 2013).

The first MAT papers were published in 1982 using peripheral blood mononuclear cells (PBMCs; Duff and Atkins, 1982) and in 1995 using whole blood (Hartung and Wendel, 1995). The MAT can be conducted using whole blood, cryopreserved blood, PBMCs, or monocytoid cell lines, and protocols are available for each of these approaches. Based on replicating the initiating events of the human fever response, in the MAT, pyrogens are recognized by pattern recognition receptors, including the toll-like receptors (TLRs) expressed on monocytic cells. When

[1] BMEL (2017). Data on the use of laboratory animals in 2017. https://www.bmel.de/SharedDocs/Downloads/DE/_Tiere/Tierschutz/Tierversuche/Versuchstierdaten2017.pdf?__blob=publicationFile&v=2 (accessed 25.11.2020).

[2] UK Home Office (2018). Annual Statistics of Scientific Procedures on Living Animals Great Britain 2017. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/724611/annual-statistics-scientific-procedures-living-animals-2017.pdf (accessed 25.11.2020).

Tab. 1: Workshop speakers and presentation titles

Workshop speaker	Presentation title
Thomas Hartung, Johns Hopkins Center for Alternatives to Animal Testing	Introduction and overview: Monocyte activation tests
Chandramallika (Molly) Ghosh, FDA/CDRH	The FDA MDDT program and considerations for MAT testing of medical devices
Anita Sawyer, International Organization for Standardization Technical Committee 194, Biological and clinical evaluation of medical devices	Material-mediated pyrogenicity and ISO TC 194 standards
Radhakrishna Tirumalai, United States Pharmacopoeia	Alternate pyrogen tests
Kelly Coleman, Medtronic	Material-mediated pyrogens in medical devices

All presentation slides are accessible at https://www.piscltd.org.uk/medical-device-pyrogen/.

stimulated by pyrogens, monocytes produce cytokines (e.g., interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF)-α, and interferon (IFN)-γ), which are measured via ELISA as a marker of the fever response. Standardized ELISA kits are available from several providers, including Merck Millipore (PyroDetect and PyroMAT™ System), CTL-MAT (MAT Kit), Sanquin (MAT Cell Set), Haemochrom Diagnostica (HaemoMAT), and MAT Research (MAT). The only remaining restriction on the MAT is a U.S. patent that applies to the use of cryopreserved blood, which expires in 2020. Several MAT studies have been conducted with medical devices to detect pyrogenic contamination from Gram-negative and Gram-positive bacterial and fungal sources (Hasiwa et al., 2007; Mazzotti et al., 2007).

2 Regulatory landscape for pyrogen testing guidance and standards

The MAT was validated in 2004 (Hoffmann et al., 2005; Schindler et al., 2006), and in 2006 and 2007. The European Centre for the Validation of Alternative Methods and the Interagency Coordinating Committee on the Validation of Alternative Methods endorsed the MAT for identifying Gram-negative endotoxins and recognized its capacity to detect a wider range of pyrogens. In 2009, the U.S. Food and Drug Administration (FDA) acknowledged that the MAT may be used after product-specific validation for parenteral drugs.[3] Information about FDA's acceptance of the MAT can be found in "Guidance for Industry: Pyrogen and Endotoxins Testing: Questions and Answers".[4] Specifically, the 2012 guidance allows for the MAT assay to be used as a replacement for the LAL for medical device testing using product-specific validation. In 2010, the MAT was integrated into general chapter 2.6.30 ("Monocyte Activation Test") in the European Pharmacopoeia, stating that the MAT could be used as a full replacement for the RPT following product-specific validation (EDQM, 2010). The U.S. Pharmacopeia (USP) General Chapter <151> ("Pyrogens") allows use of a "*validated, equivalent* in vitro *pyrogen or bacterial endotoxin test*" in place of the RPT, where appropriate.[5] ISO 10993-1:2018 gives preference to *in vitro* models when they yield equally relevant information (ISO, 2018), and additional information on pyrogen testing can be found in ISO 10993-11:2017 (ISO, 2017). In December 2018, ISO Technical Committee (TC) 194 ("Biological and clinical evaluation of medical devices") Working Group (WG) 16 ("Pyrogenicity") met to discuss its draft technical report 21582 on "Principle and method for pyrogen testing of medical devices". This report notes that in some cases the MAT can be a useful alternative to traditional pyrogenicity test methods (rabbit and LAL); however, "*the rabbit test will need to be retained for detection of pyrogens not detected by the MAT, including material-mediated pyrogens*". This is because, as the report states, "*material-mediated pyrogens are chemical agents that do not operate through the cytokine network to induce a febrile reaction and most likely will not be detected on the MAT*". ISO/TC 194 WG 16 recommends further studies to validate the detection of known MMPs by the MAT assay and thereby show that MMPs that induce a pyrogenic response through mechanisms other than the TLR signaling pathway are detected.

3 Workshop discussion

This workshop was convened by the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) and the PETA International Science Consortium Ltd. with a goal of fostering discussions

[3] US FDA (2009). Response from Dr Norris Alderson, Associate Commissioner for Science. https://ntp.niehs.nih.gov/iccvam/methods/pyrogen/transmitnov08/fda-response.pdf (accessed 20.04.2020).

[4] US FDA (2012). Guidance for industry pyrogen and endotoxins testing: Questions and answers. https://www.fda.gov/media/83477/download (accessed 20.04.2020).

[5] United States Pharmacopeia (2017). General Chapter 151 "Pyrogens." http://www.pharmacopeia.cn/v29240/usp29nf24s0_c151.html (accessed 20.04.2020).

between key stakeholders at MAT testing laboratories, medical device manufacturers, standards-making organizations, and the FDA's Center for Devices and Radiologic Health (CDRH) on the use of the MAT. Presentations (Tab. 1) were followed by a lively discussion. The topics below reflect the workshop discussions, which were focused by questions posed by CDRH in preparation for the workshop.

4 CDRH medical device development tools program

A central element of this workshop was exploring the use of the CDRH Medical Device Development Tools (MDDT) program[6] to allow companies to provide data demonstrating that MAT assays could routinely be used as a replacement for the LAL for all medical devices and to explore whether the MAT is capable of detecting MMPs such that the RPT for medical devices also could be replaced. This program facilitates collaboration where individuals, stakeholders, consortia, or companies may work together to expedite development, validation and use of *in vitro* methods as non-clinical assessment models for a defined context of use as a replacement for animal-based tests without the need to reconfirm the suitability and utility of the tool when used in medical device submissions to CDRH. Once qualified under this program, and when used within the qualified context of use, the qualified MDDT can be used by multiple sponsors across multiple medical device development programs. The MDDT program is actively seeking new submissions with a strong potential to meet a public health need, thus the MAT is a good candidate for review under this program. Proposals submitted to the program, designed to be relatively short, should include a description of the development tool with sufficient detail for the agency to understand how the tool works for the proposed context of use, a discussion of how this tool meets a public health need, with no need to submit data during the proposal stage as the data is reviewed in the subsequent phases of the MDDT process. Once an MDDT is accepted into the program, this initiates collaboration between the tool submitter and CDRH to aid in the development of this tool by providing feedback on a plan to collect evidence to support qualification of the tool.

There was consensus on the need and interest in submitting a MDDT proposal for the MAT. Drivers for the use of the MAT included the desire for: (1) a quantitative, sensitive, and reliable *in vitro* test that can detect both endotoxin and material-mediated pyrogenic response, (2) an extract method that requires a smaller amount of test article for smaller devices and/or a direct contact test that adequately assesses both small and large devices, and (3) a test that is not affected by the stress level or physiological state of an animal. Furthermore, qualification of the MAT through the MDDT program would streamline product review. Workshop participants discussed the process of developing a MDDT to validate the MAT as a replacement for the RPT and/or LAL with input from CDRH attendees on the agency's critical needs.

4.1 Addressing material-mediated pyrogens in a MDDT proposal

Borton and Coleman (2018) proposed the following definition for MMPs: "*any exogenous non-biological substance known to cause a febrile response. This definition excludes substances such as endogenous chemicals (i.e., cytokines and prostaglandins), fungi, yeast, viruses, bacteria, and parasites*." The ISO TC 194 WG 11 Task Force developed a list of known MMPs, which have generated a pyrogenic response in rabbits in testing performed by medical device companies, that is included as Annex G of ISO-10993-11 (2017). The list, which was reaffirmed recently by ISO/TC 194 WG 16, includes:

- prostaglandin;
- inducers (e.g., polyadenylic, polyuridylic, polybionosinic and polyribocytidylic acids);
- substances disrupting the function of thermoregulatory centers (e.g., LSD, cocaine, morphine);
- uncoupling agents of oxidative phosphorylation (e.g., 4, 6-dinitro-o-cresol, dinitrophenol, picric acid);
- N-phenyl-β-naphthylamine and aldo-α-naphthylamine (the febrile mechanism is unknown);
- neurotransmitters (e.g., noradrenaline, serotonin); and
- metals such as nickel salts, in some instances.

However, the relevance of MMPs in pyrogen testing of medical devices, and whether any of the pyrogen tests can detect these substances, is disputed (Borton and Coleman, 2018). The relevance of material-mediated pyrogenicity is debatable as the list of chemicals includes substances that should not be found in many medical devices. Whether the MAT could detect MMPs was debated, because the listed substances do not signal via a pattern recognition receptor (PRR) pathway. To address this question, there was a recommendation to evaluate the ISO-defined MMPs in the MAT to see if these substances are detectable. One important step in completing this exercise will be acquiring pure test substances that can be used as positive controls.

The MMP biocompatibility evaluation (currently the RPT) typically only needs to be performed once on the final, finished device. Whether the MAT could also be used in the nearer term to perform product release testing (i.e., to replace the LAL) was also discussed. For both types of pyrogenicity assessments, appropriate positive controls would need to be selected to demonstrate that the MAT can detect endotoxins as well as MMPs and to be made available as reference preparations. CDRH expressed a need to understand if there are any pyrogens that the RPT would detect that the MAT would not (for example, whether any pyrogens of concern act through mechanisms other than PRRs and whether these would be detectable in the MAT). It

[6] US FDA (2017). MDDT program final guidance, qualification of medical device development tools – Guidance for industry, tool developers, and Food and Drug Administration staff. https://www.fda.gov/MedicalDevices/ScienceandResearch/MedicalDeviceDevelopmentToolsMDDT/default.htm (accessed 20.04.2020).

also needs to be shown whether there is evidence of any specific substances present in medical devices that would inhibit the MAT response.

It was noted that companies would be more likely to use the MAT if it were approved for detection of all pyrogens and not only endotoxins, especially considering that, while the MAT and RPT costs are similar, the LAL is generally cheaper. It was also agreed that standardized protocols that produce consistent results in multiple laboratories would be essential.

4.2 Sample preparation for the MAT

The RPT is conducted with a saline extract of the finished device that is injected at 100% concentration. LAL testing is conducted using extracts of the device in depyrogenated water at up to 100% concentration (when there is no interference with the assay) in the test system. For MAT testing, dilution of the extract may be needed for use in its cell system; how this dilution could affect sensitivity and consistency across test methods must be considered. Direct contact with the device would preclude the need for a separate extraction procedure. This would be applicable to small devices as a separate extraction and subsequent dilution are not needed if they can be completely immersed (and cytokine release is adjusted to the volume of the media used). However, performing assays using direct contact also has limitations that will need to be addressed and validated.

4.3 Standardization of the MAT

There is a need to standardize the cell number used per surface area of the device (i.e., the number of cells will vary depending on the size of the device, but cell density should remain consistent). This also includes establishing an optimal ratio for producing reliable and reproducible results. For the whole blood and cryopreserved blood assays, donor variability and standardization of blood cells need to be considered (e.g., with blood pooled from several donors). This parameter is easier to control with a monocytoid cell line. However, care should be taken to validate steps to standardize measurements for cell reactivity as well as concentration, as this will also highly affect the assay's readout.

Inhibition or enhancement information should be included as part of the test procedures. Assay interference testing will verify that a test article does not interfere with the cell system or with the cytokine-specific ELISA; however, published studies have indicated that the interference observed in the LAL is not an issue for the MAT (Correa et al., 2017; Schwarz et al., 2017).

4.4 Correlation of RPT, LAL, and MAT results

There was a discussion around how to correlate the temperature results from the RPT, the endotoxin units per device in the LAL, the ELISA results in the MAT, and the human response. The importance of understanding how cytokine levels produced in monocyte cultures relate to fever in humans was emphasized as was the need for a standardized positive control. A number of potential non-endotoxin standards (e.g., prostaglandins, metals, inducers) were discussed and merit additional consideration. Other points to understand and explain in an MDDT application include whether cytokine measurement alone is sufficient for assessing the fever response irrespective of the mechanism of action of pyrogens and which cytokine(s) need to be measured to predict what will happen *in vivo* (Davila et al., 2014; Lee et al., 2014).

4.5 Grouping of devices for a MDDT proposal

It was acknowledged that different types of devices may require the use of distinct test protocols, and one suggestion was to start by optimizing the testing protocol for a representative group of small devices that fit into a 10 mL tube and can come in complete contact with small volumes of whole blood/PBMCs/monocytic cell line. In an MDDT proposal, it is critical to outline the proposed context of use and why it is appropriate. For example, CDRH would need to know with what types of devices the proposed MAT protocol could be used (e.g., durable/absorbable devices that include polymers, ceramics, metals, biologics, hydrogels, liquids, nanoparticles) and what test substances would be incompatible with the test system. The protocol would also need to define whether any device-specific method optimizations are needed; for example, whether the protocol can be used with large and small surface area devices, with device extracts, or by direct testing of the device itself.

If direct testing is conducted on the device, the MDDT would need to describe if the test is limited to detecting surface-bound pyrogens only and, if so, whether this is sufficient. The protocol would also need to state whether there are any differences if the test is conducted under static versus dynamic incubation conditions and consider optimization of the incubation period to increase test sensitivity. Engaging USP, the National Institute of Standards and Technology, or other organizations in the development of reference standards was recommended.

4.6 Next steps for organizing the development of a MDDT project proposal

The workshop attendees agreed that next steps should include publication of a brief workshop report and MDDT proposal development. Each MDDT proposal would briefly describe a proposed context of use, a description of the MAT test methods, an overview of the proposed evidence plan that will be used to qualify the tool, including a timeline and a description of how the tool will meet a public health need. Once prepared, the tool submitter assembles information for the agency via the FDA Q-Submission Program as an Informal Meeting, including a cover sheet indicating this is an MDDT proposal. Once submitted to the agency, CDRH notifies tool submitters within 60 days whether the proposal has been accepted into the MDDT Program.

[7] http://www.usp.org/events-training/workshops/future-of-endotoxins-and-pyrogen-testing

One workshop recommendation was to submit a two-part MDDT with one part focused on biocompatibility and the other on batch-release testing. The reason for this is that, as we learn more about MMPs and whether they can be assessed using the MAT or require an additional detection test process, acceptance of the MAT for batch-release testing in place of the LAL in a broad context is likely to be a nearer-term goal than replacing the RPT for biocompatibility testing of new products. In the meantime, another action item was to test the ISO 10993-11:2017 Appendix G list of MMPs with the MAT. This process will involve identifying: (1) companies interested in participating in a MDDT application; (2) avenues for gaining access to existing data; and (3) funding opportunities for prospective MAT testing to include in the MDDT submission.

Training and education on the MAT were also highlighted as critical activities to facilitate its adoption. To help address this issue, the USP held a workshop on June 10-11, 2019 in Rockville, Maryland, USA on the "Future of Endotoxins and Pyrogen Testing: Standards and Procedures".[7]

5 Conclusions

The formation of public-private partnerships and training opportunities will be critical to accomplish the workshop recommendations. NICEATM and the PETA International Science Consortium Ltd. will collaborate with companies and CDRH to facilitate ongoing activities, including MDDT development and organizing training for reviewers and companies concerning the use of the MDDT in medical device regulatory submissions.

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Disclaimer

The views expressed in this article are those of the authors and do not necessarily reflect the views or policies of the U.S. Food and Drug Administration.

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Meeting Report

Virtual Class on Alternative Methods: Ethics & Science

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The international Virtual Class on *Alternative Methods: Ethics & Science* (http://ames.lakecomoschool.org), aimed at young scientists, was focused on alternative methods and the 3Rs (Reduce, Refine, Replace animal use), from the ethical and scientific points of view. The purpose was to provide an overview, through an ethical, scientific, and philosophical approach, to illustrate the history, the application, and the future of alternative methods. The class was chaired by Francesca Caloni, Università degli Studi di Milano, Department of Environmental Science and Policy, and was attended by 27 participants from all over the world with backgrounds ranging from agronomy, biology, environmental science, veterinary medicine, to economy, and computer and social sciences.

Francesca Caloni started the class with a brief overview entitled "Alternative methods: Educational experience". The main aspects underlined during the presentation were related to the importance of an "inclusive" education on the 3Rs, not only focused on scientific aspects but promoting and developing a mental attitude through a multidisciplinary 3Rs educational approach, answering the increasingly tangible needs of a complex knowledge. An educational experience of the 3Rs, merging different disciplines through a well-structured pathway and a multistep approach, aimed at undergraduates to early career researchers would address the future requirements of a global society.

Marco Pedrazzi, Università degli Studi di Milano, Department of International, Legal, Historical and Political Studies, presented a lecture entitled "Ethics and science: Which role for research integrity?" Research integrity (RI) has become a central topic worldwide, particularly in Europe. The presentation defined RI and looked at some of the main themes of RI, such as the duties existing within a research group and towards external participants and society as a whole, plagiarism, and self-plagiarism.

Isabella De Angelis, Environment and Health (ISS), spoke on "Alternative methods move towards new approach methodologies: Reflections and perspectives". Since the early 2000's, an impressive acceleration in efforts to develop non-animal approaches for investigating hazardous properties of chemical substances and drugs has taken place. These efforts have gone hand in hand with the new, exciting possibilities offered by human biology-based innovative technologies and approaches. For this reason, researchers and legislators now prefer to address their attention to new approach methodologies (NAMs) instead of "sim-