BMB
Reports

BMB Rep. 2025; 58(3): 105-115
www.bmbreports.org

Invited Mini Review

Monocyte activation test (MAT) as an ethical alternative to animal testing

Yeram Jeon[1,#], Soyoung Jeon[1,#], Ji-Youn Lim[2,#], Hyungjung Koh[3], Chan Woong Choi[3], Su Kyoung Seong[3], Boksik Cha[2,] & Wantae Kim[1,*]*

[1]Department of Life Science, University of Seoul, Seoul 02504, [2]New Drug Development Center, Daegu Gyeongbuk Medical Innovation Foundation, Daegu 41061, [3]Biopharmaceutical and Herbal Medicine Evaluation Department, National Institute of Food and Drug Safety Evaluation, Chungju 28159, Korea

Ethical considerations surrounding the utilization of animals in scientific research have prompted a widespread search for alternative methodologies. This review explores the historical context and ethical dilemmas associated with traditional animal testing methods, before introducing the Monocyte Activation Test (MAT) as a promising alternative, and outlining its basic principles, historical development, and advantages over conventional animal testing. The role of monocytes in the immune system and the activation pathways utilized in MAT are elucidated, while regulatory acceptance and guidelines for MAT validation are introduced, alongside case studies proving its reliability and reproducibility. The applications of MAT in pharmaceutical and medical device testing are summarized, together with its potential future uses. Although the MAT faces limitations and challenges, the global perspective to reduce unnecessary animal tests has become a general concept in animal welfare and scientific research. [BMB Reports 2025; 58(3): 105-115]

BACKGROUND ON THE USE OF ANIMALS IN TRADITIONAL TESTING METHODS

Animal models for research in biopharmaceuticals have played an important role in advancing scientific knowledge. Animal experiments have been of great help in understanding physiological processes and developing medical treatments. For example, animal models have been used to study immune diseases, cancer, and neurodegeneration, helping to understand their underlying mechanisms and develop treatment strategies. Animal research models have also been crucial in assessing the safety and effectiveness of pharmaceuticals, chemicals, cosmetics, and medical devices, all of which directly affect human health (1). Animal testing has been instrumental in determining the safety of these products and assessing potential side effects or allergenic risks, prior to clinical application in humans.

Examination of animals in various research and medical endeavors has been conducted for a considerable duration, originating from ancient Greece, through medieval Islam, to the present. Throughout this extensive history, significant advancements in methodology and its application have been developed. Many national regulatory agencies currently require specific examinations on animals to ensure safety and effectiveness before human use. These assessments aim to evaluate the potential toxicity, side effects, and allergic reactions, and also include methods for detecting the presence of pyrogens (fever-inducing substances) used in the manufacturing processes of drugs and medical devices (2-4).

Ethical concerns and limitations regarding the animal research model

Traditional experimental methods using animals are continually challenged by ethical, scientific, and pragmatic concerns, such as animal welfare issues, the restricted applicability of animal-derived data to human, as well as the substantial expense and time investment associated with animal-based research (5, 6). The ethical issue of animal welfare has been a longstanding concern. In 1822, the first animal protection law was enacted by the British Parliament, while the American Society for the Prevention of Cruelty to Animals (ASPCA) was established in the United States, further emphasizing the growing awareness of animal welfare issues. The Animal Welfare Act was passed, leading to arguments asserting the need to oppose the use of animal other than human in experiments, and to ethically protect animal welfare. Based on these laws, people who value animal welfare are demanding an end to animal testing, arguing that it is inhumane behavior that causes pain, fear, or

*Corresponding authors. Boksik Cha, Tel: +82-53-790-5210; Fax: +82-53-790-5219; E-mail: boksik-cha@kmedihub.re.kr; Wantae Kim, Tel: +82-2-6490-2669; Fax: +82-2-6490-2664; E-mail: wantaekim@uos.ac.kr
#These authors contributed equally to this work.

https://doi.org/10.5483/BMBRep.2024-0089

**Received 4 June 2024, Revised 8 July 2024,
Accepted 29 August 2024, Published online 4 December 2024**

Keywords: Alternative animal test, Endotoxin, Monocyte activation test, Non-endotoxin pyrogen, Rabbit pyrogen test

ISSN: 1976-670X (electronic edition)

death for the benefit of humans (7, 8). Furthermore, in 2013, the European Union enacted legislation prohibiting animal testing for cosmetics and the sale of cosmetics tested on animals, and many countries have joined in adopting such similar laws (9).

There are limitations in applying research findings obtained from experiments using animal to humans. Due to physiological differences between animals and humans, the accuracy and reliability of experimental results may be reduced. Large-scale experiments requiring a significant number of animals can take considerable time to obtain results. However, for some research, there are still no alternative systems or methods that can replace animal models. Therefore, ethical approval and supervision are essential to ensure that animal experiments are conducted with the utmost ethical and moral consideration. To accomplish this, the 3R principles, initially proposed by Russell and Burch, encompass Replacement, Reduction, and Refinement. Replacement seeks alternative methods to fulfill research goals, without resorting to animal experiments; Reduction aims to minimize the number of animals used in experiments, while maximizing the information obtained; and Refinement focuses on alleviating pain and stress for animals and enhancing their welfare, when alternatives to animal testing are unavailable (10-12). Here, we provide a concise review of pyrogen testing methodologies, with a particular focus on alternative approaches that serve as substitutes for animal models.

ALTERNATIVE METHODS TO ANIMAL MODEL-BASED PYROGEN TESTS

Pyrogen tests typically necessitate animals, and some may even require a substantial number of experimental subjects, raising concerns about animal reproducibility. Thus, the development and adoption of alternative methods for these tests can reduce the number of animals needed. Pyrogens are substances, including proteins or LPS, sourced from bacteria, viruses, fungi, or other microorganisms, that can induce fever through inflammatory responses (13-17). Pyrogen testing is the process of detecting the presence of potential fever-inducing agents used in the manufacturing of drugs or medical devices. As products containing pyrogens can result in severe human side effects, like fever, hypotension, or blood clotting, the pyrogen test serves a vital function in evaluating product safety and safeguarding human health. Previously, the Rabbit Pyrogen Test (RPT) was widely conducted as a routine pyrogen test, aiming to identify fever-inducing agents. It was initially documented in the British Pharmacopoeia in 1912, and subsequently included in the United States Pharmacopeia in 1942 (Fig. 1). However, due to its repetitive and inefficient nature, as well as ethical concerns, alternative methods have been considered (18, 19).

To address these issues, the Limulus Amoebocyte Lysate (LAL) test was proposed as an alternative to the RPT, developed in the 1970s. The LAL test utilizes the hemolymph clotting system of the Atlantic horseshoe crab (Limulus polyphemus) to detect pyrogens *in vitro*. This method is sensitive, rapid, and reproducible, making it relatively efficient in terms of both time and cost (20-27). It has become widely used as a golden standard method in the pharmaceutical and medical device manufacturing industries. Nevertheless, this method solely detects endotoxins, and is unable to detect non-endotoxin pyrogens (NEPs). Moreover, its applicability is limited to liquid samples, raising limitations. Ethical concerns persist, due to its reliance on animal-based testing approaches. Additionally, recent concerns have emerged regarding the decline in the Atlantic horseshoe crab population. To address these challenges, a proposed solution involves utilizing recombinant factor C protein recombination for pyrogen test (28-30).

Given that the widely used RPT and LAL tests rely on animal-based methods, they cannot be exempt from ethical concerns. Moreover, they are limited in mimicking human physiological responses to test substances, and comparing them to responses in other animal species. To overcome these issues, an alternative method, known as the Monocyte Activation Test (MAT), has recently been proposed. Originating in 1988 with Poole, this approach utilizes human-derived monocytes, resulting in test outcomes that closely resemble human physiological responses, and addressing ethical concerns related to animal welfare. Initially endorsed by the European Pharmacopoeia (Ph. Eur.) in 2010, MAT has been acknowledged as a replacement for RPT in various countries, including Europe. By utilizing human whole blood or human cell lines, it accurately mimics the fever response to pyrogens in a manner relevant to human physiology, making it the preferred choice for depicting reactions occurring within the human body following drug administration (31-34).

OVERVIEW AND HISTORICAL BACKGROUND OF THE MAT

The MAT has emerged as a crucial experimental method for detecting and assessing pyrogens. It utilizes monocytes isolated from human blood to detect pyrogens, effectively mimicking the fever response observed in humans (35-38), When monocytes are cultured with samples, those exposed to pyrogens triggering inflammation become activated through Toll-like receptors (TLRs) on the cell surface, initiating innate immune responses. Subsequently, activated monocytes release cytokines and signaling molecules, which serve as indicators of the presence and concentration of pyrogens in the sample (39, 40).

As mentioned earlier, Poole's MAT proposal involves initially evaluating recombinant human growth hormone through LAL and RPT tests. However, when cultured with human peripheral blood mononuclear cells, the release of cytokines from white blood cells triggered a fever response, indicating that these methods fall short of fully replicating human physiological reactions. Based on these findings, a novel pyrogen test was proposed, relying on the release of IL-1 and TNF from human monocytes (41, 42). Subsequently, a new *in vitro* system was developed to detect and quantify pyrogens. Using Enzyme-

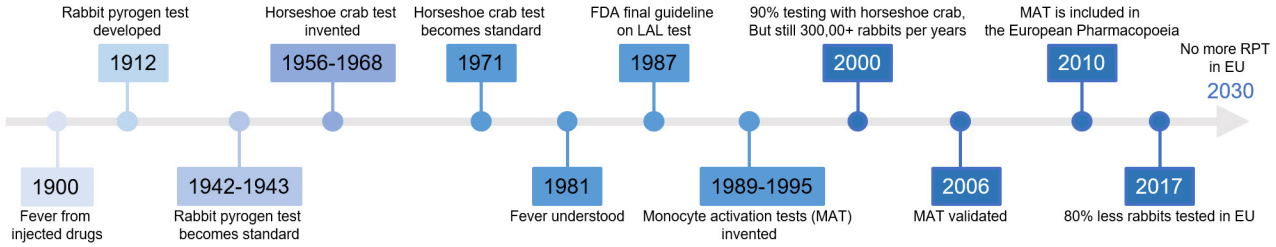

Fig. 1. Timeline of pyrogen test development. In the early 1900s, scientists observed that certain drugs caused patients to develop high fevers, and some even died. In 1912, the rabbit fever test was developed to screen for this dangerous effect of injected drugs. By 1942, the Rabbit Pyrogen Test (RPT) had become the standard pyrogen test. In 1956, a new test using horseshoe crabs was developed to replace the RPT. In 1987, the Limulus Amebocyte Lysate (LAL) test was approved by the FDA. The Monocyte Activation Test (MAT) was developed in 1989, and was approved by the European Pharmacopoeia in 2010.

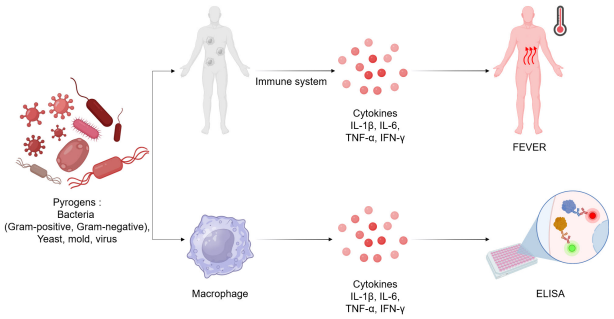

Fig. 2. Principle of the monocyte activation test. Exogenous pyrogens trigger the release of cytokines by the human immune system, resulting in fever. By mimicking this *in vivo* response, human-derived monocytes secrete cytokines in response to pyrogens. These cytokines are then detected and measured by ELISA.

linked immunosorbent assay (ELISA), the secretion of the fever-inducing cytokine IL-6 from samples and monocytes was identified (43). A significant aspect of this study was the detection of pyrogenic contamination in three therapeutic human serum albumin samples that triggered adverse reactions, a phenomenon overlooked by RPT or LAL tests. Consequently, MAT demonstrated a superior ability to mimic human physiological responses. In 1996, a highly sensitive endotoxin-monitoring system was proposed, based on measuring cytokines from human cell lines of myelomonocyte origin, aiming to develop an *in vitro* test system to detect pyrogens (44). Subsequently, research compared TNF secretion measured using human monocyte cell lines to LAL and RPT methods to obtain valid *in vitro* test results for the relatively accurate determination of endotoxin in commercial products. In 2010, MAT was first adopted by the Ph. Eur., and recognized in various countries as an alternative to RPT (9, 45).

In 2017, the NF-kB-mediated transcriptional control of innate mediators in monocyte activation led to the development of an NF-kB reporter analysis based on monoMAC-6 cells to measure organismal responses to endotoxin, thereby confirming the suitability of MAT (46, 47). Two years later, concerns were raised regarding the suitability of RPT and LAL tests for pyrogenic products, such as Bexsero (48, 49). PRT exhibited variability and led to erroneous results, while the LAL assay was unsuitable for monitoring the stability of samples containing both endotoxin and NEPs (45, 50). Consequently, MAT was generally accepted as the most effective method for analyzing samples, similar to Bexero. In 2022, similar experiments using NEPs (*Staphylococcus aureus*, R848, and lipoteichoic acid) confirmed that MAT detects endotoxin and NEPs sensitively, while also identifying contamination by various pyrogens in non-oral drugs (51). Recent research further solidifies the MAT as the leading pyrogen test to mimic human fever responses and detect NEPs, surpassing RPT and LAL tests (Fig. 2).

Advantages and limitations of MAT

MAT is a highly reproducible assay that faithfully mimics the immune response in humans, because it uniquely reflects the human immune system *in vitro* using human-derived monocytes. MAT can be performed relatively quickly and at an efficient cost, and has the advantage of using independent biological markers as a method of measuring the activation status of cells, such as inflammatory cytokines (31, 52, 53). Moreover, MAT possesses the ability to detect all types of pyrogens, including both endotoxins (Gram-negative) and NEPs (derived from Gram-positive bacteria, yeast, molds, and viruses) (54). Compared to traditional tests like the RPT, MAT warrants higher accuracy and a broader detection range. Furthermore, unlike the RPT and LAL tests, MAT can detect all pyrogens present in a sample, including those that are uncharacterized and NEPs, irrespective of the sample's composition (55). Its most significant advantage lies in the ability to detect pyrogens without animal use. Unfortunately, compared to the LAL test, there are limitations, in that the sensitivity is low, and the time to obtain results is long. Additionally, there is variation in analysis sensitivity depending on the blood donor, and other types of cells exist in human blood in addition to monocytes, which may affect the results, so interpreting MAT results requires a careful evaluation of how similar the results are to reactions in the

human body. As the culture environment for monocytes fails to precisely replicate physiological conditions, MAT experiments are subject to limitations, and warrant further development (Table 1).

MECHANISMS ON MONOCYTE ACTIVATION

Living organisms have an immune system that protects them against invading pathogens or harmful substances. The immune system is a complex network of organs, cells, and proteins that defend against invasion and quickly eliminate invading foreign substances to maintain life (56, 57). Immunity can be mainly classified into two categories. Innate immunity, the first line of defense, is inherent, and present from birth (58). On the other hand, Adaptive immunity is a response triggered by the immune system's memory upon exposure to harmful pathogens or substances, enabling a subsequent response upon re-encounter. Monocytes, a type of white blood cell, play a vital role in the innate immune system. Serving as a source for crucial immune components, like macrophages and dendritic cells, they are predominantly generated in the bone marrow, and during circulation, migrate to various tissues. Their primary function involves participating in both inflammatory and anti-inflammatory processes during immune responses (59-62). Inflammatory reactions prompt monocytes to respond to external invaders, such as inflammatory chemicals, foreign bacteria, or pathogens, by engaging in phagocytosis to capture and digest them. They also present antigens and secrete chemokines to either neutralize or eliminate the invaders, and aid in tissue destruction, when necessary. Additionally, monocytes contribute to tissue regeneration and healing in cases of inflammation or injury. Importantly, they help maintain immune system balance by regulating excessive immune responses (63).

Activation pathways and markers used in MAT

During the inflammatory process, the activation of monocytes by invading foreign substances prompts the secretion of chemokines and cytokines, leading to a fever response. MAT evaluates the levels of monocyte activation, and can be utilized to assess the safety of pharmaceuticals, medical devices, and other medicinal products by examining and evaluating key markers linked to the activation pathway. Pyrogens that invade the living organism stimulate phagocytic cells or systemic endothelial cells through TLR (64-66), and the activated immune cells generate and release pro-inflammatory cytokines. Secreted pro-inflammatory cytokines reach the central nervous system through the bloodstream, and are recognized by the Cytokine receptor of the Organum vasculosum laminae terminalis (OVLT) (42, 67) which synthesizes PGE-2 (68), which stimulates cAMP in the hypothalamus. As a result, the set point of the temperature regulator is elevated, leading to heat generation in the body (69, 70).

To perform the MAT, human monocytes are isolated from blood, and cultured. These monocytes are then exposed to test samples to activate them. If pyrogens are present, they trigger an immune response by activating TLR on the surface of the monocytes, leading to the secretion of pro-inflammatory cytokines. The levels of these cytokines can be measured using techniques such as ELISA. Data analysis involves comparing the measurements from activated monocytes with those from inactive ones, serving as a positive control to assess the presence of pyrogens and evaluate the sample's safety (Fig. 3) (39, 71).

VALIDATION OF THE MAT

Regulatory acceptance and guidelines for MAT validation

MAT is currently recommended as the primary test for detecting

Table 1. Comparison of various pyrogen tests

Analytical technique		RPT (Ph.Eur.2.6.8)	LAL (Ph.Eur.2.6.14)	rFC (Ph.Eur.2.6.32)	MAT (Ph.Eur.2.6.30)
Assay type		*In vivo*	*Ex vivo*	*In vitro*	*In vitro*
Sensitivity (Limit of Detection: LoD)		0.05 EU/ml	0.005 EU/ml	0.005 EU/ml	0.004-0.002 EU/ml
Endotoxin	LPS	+	+	+	+
Non-endotoxin	LTA	+	−	−	+
	Yeast	+	−	−	+
	Virus	+/−	−	−	+
Application	Pharmaceuticals	+	+	+	+
	Biologicals	+	+/−	+	+
	Medical devices	+	+/−	+	+
	Cell therapeutics	−	+/−	+	+

The Rabbit Pyrogen Test (RPT) boasts high sensitivity, and is capable of detecting most non-endotoxins. It is versatile, and can be used for all applications, except cell therapeutics. However, it relies on the use of animals. The Limulus Amebocyte Lysate (LAL) test, another animal-based method, has lower sensitivity compared to RPT, and cannot detect non-endotoxins. Its applicability varies, depending on the type of sample being tested. The recombinant Factor C (rFC) assay is an *in vitro* method with sensitivity similar to LAL, but like LAL, it cannot detect non-endotoxins. Nevertheless, rFC is suitable for a wider range of applications than LAL. The Monocyte Activation Test (MAT) has the lowest sensitivity among these tests, yet it uniquely detects both endotoxins and non-endotoxins, making it suitable for all types of applications.

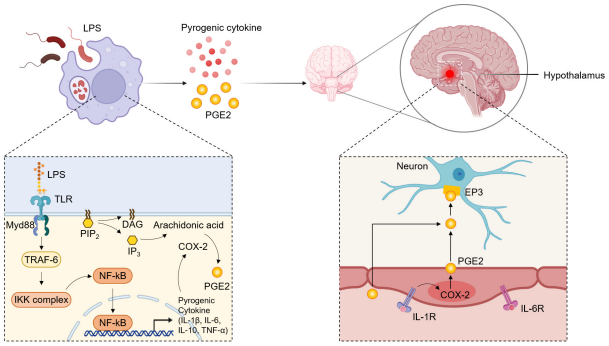

Fig. 3. Mechanisms of endogenous pyrogen production and fever. Exotoxins, such as lipopolysaccharides (LPS), bind to Toll-like receptors (TLRs) on the surface of immune cells, like macrophages, monocytes, and neutrophils. This interaction triggers the release of pyrogenic cytokines, which induce the enzyme COX−2 to synthesize prostaglandin E2 (PGE2). Additionally, cytokines secreted into the bloodstream by immune cells can travel to the hypothalamus in the brain. There, they stimulate the hypothalamus to produce PGE2. The synthesized PGE2 is then transported to the hypothalamus, where it binds to EP3 receptors on neurons. This binding raises the set point of the hypothalamic thermostat, leading to an increase in body temperature, and causing fever.

pyrogens, and regulatory guidelines and standards for conducting and validating such tests are crucial. In the 1990s, regulatory agencies, such as the European Medicines Agency (EMA) and the U.S. Food and Drug Administration (FDA), recognized MAT as an important method for detecting pyrogens, with official guidelines released in 2009 (72). The European Center for the Validation of Alternative Methods (ECVAM) and the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) approved MAT to identify Gram–negative endotoxins in 2006 and 2008, respectively. The FDA also approved MAT usage post-product validation testing in 2009, extending to regulated products like medical devices by 2012. The Ph. Eur. integrated MAT into General Chapter 2.6.30 as an *in vitro* alternative to the RPT, further supported by the United States Pharmacopeia (USP)'s General Chapter <151> in 2018, allowing validated *in vitro* tests as RPT alternatives. Moreover, the International Organization for Standardization (ISO), which develops and provides international standards related to pharmaceutical production, has provided regulatory guidelines for standardizing tests like MAT since 2009 (73, 74).

APPLICATIONS OF THE MAT

Testing of pharmaceuticals

To ensure safety, pharmaceuticals primarily administered directly to the body or in contact with blood must be pyrogen-free. They typically enter through the skin, mucous membranes, or via injections. These products must pass pyrogen tests to be approved as medicines. Vaccines, for example, are pharmaceuticals that enhance immunity against specific diseases or pathogens. They contain components resembling disease-causing microorganisms, along with antigens, stabilizers, adjuvants, and sometimes antibiotics, which may include free endotoxins or other inflammatory substances. Some free-endotoxins can act as adjuvants, stimulating the immune system, or the antigens themselves may cause inflammation. While the RPT is still used for some vaccines, the Bacterial Endotoxin Test (BET) has been introduced for others. However, in the case of BET, certain vaccine additives, like aluminum hydroxide, can interfere with the test; whereas, in whole blood MAT, they do not act as interfering factors, suggesting that it may be a more suitable model for assessing a vaccine's safety (75).

Recently, there has been a significant effort to evaluate and introduce various vaccines using MAT. *Neisseria meningitidis*, a bacterium recognized for its role in causing meningitis, produces and releases potent LPS. One of the vaccine candidates targeting these characteristics is based on native outer membrane vesicles (NOMVs) of group B N. *meningitidis*, containing LPS. Pyrogen tests and safety evaluations carried out using LAL and RPT demonstrated notably lower sensitivity in detecting and inducing cytokines, compared to MAT. Additionally, MAT using Human Whole Blood and peripheral blood mononuclear cells (PBMCs) showed differences in distinguishing vaccines with various LPS structures. These results demonstrate the high sensitivity of the Human Whole Blood MAT system to endotoxin variants, and the usefulness of using human tissues to predict toxicity in humans. In Brazil, from 2017 to 2018, due to the rapid spread of yellow fever virus, the health department launched an emergency yellow fever vaccination campaign. As part of this emergency strategy, the immunobiology technology institute massively produced the 17DD-YF vaccine for domestic supply. This increased production required efficient manufacturing and rigorous quality control processes to meet demand. This study confirmed the applicability of MAT for 17DD-YFV, and demonstrated its effective detection of pyrogen contamination by proving its correlation with LAL test. These findings support the utility of MAT as a valuable tool for biological quality control research (76).

Other studies have validated the suitability of MAT kits using hyperimmune sera (73). Additionally, both MAT and RPT for *Shigella sonnei* vaccines containing generalized membrane antigens (GMMA) showed lower pyrogen activity, compared to modified LPS (77). For vaccines, including the Tick-borne encephalitis virus vaccine, commonly administered intramuscularly, it has been proposed to use sensitivity (the lowest endotoxin value on the standard curve), rather than detection limit values, to define Method A (quantitative) and Method B (semi-quantitative) in current MAT protocols (78). Furthermore, recent assessments of three batches of Bexsero suggested that MAT could serve as an alternative to RPT for monitoring fever-inducing vaccines, highlighting its potential usefulness in various vaccine development fields. 30448065 Efforts to ensure vaccine and medication safety through MAT continue, and are expected to grow. Recent studies found (1,3)–beta–glucan con-

tamination in all batches of Human serum albumin (HAS), disrupting the conventional LAL test. Endotoxin-specific LAL test was ineffective for HSA due to detection limitations. Likewise, combining MAT with polymyxin B revealed endotoxin contamination in batches with heating elements, missed by endotoxin-specific LAL test. (1,3)−beta−glucan increased MAT/IL-6 response, indicating potential safety concerns. MAT shows a strong correlation with the RPT, enhancing safety in pyrogen testing of HSA and other therapeutic proteins (79-81).

Testing of medical devices

Medical devices for blood or human tissue transplantation may carry pyrogens on their surfaces that cause inflammation and lower compatibility in the body. Therefore, rigorous pyrogen test is crucial for these devices. The traditional RPT involved injecting the sample solution into rabbits or directly transplanting the sample, and measuring body temperature changes to examine pyrogen levels. However, this method does not accurately show how much pyrogen comes from the actual device, and by diluting the pyrogens, interferes with clear detection. Moreover, it turns out that this method does not accurately represent the original concentration of pyrogens detected, casting doubt on its usefulness. Additionally, directly transplanting samples into rabbits can result in tissue damage rather than pyrogen-induced effects, making it difficult to consider as a reliable pyrogen test. Therefore, various *in vitro* pyrogen tests, called MAT, are now the preferred alternatives (45, 82).

Monocytes are recognized as the initial cells to reach the transplant site, where they perform various functions (83, 84). Therefore, analyzing monocytes for pyrogen test of transplant devices has emerged as an attractive alternative. Pyrogen test of transplant devices was carried out by measuring the secretion of IL-1B from fresh or refrigerated human whole blood *in vitro*. This method circumvents the need for washing procedures, and proves effective, as the test device directly interacts with blood cells, demonstrating notably high sensitivity, and broadening the detectable range of pyrogen contamination (85).

Mohanan *et al*. evaluated polymer gelatin materials for pharmaceutical capsules, and directly compared the RPT, LAL, and MAT tests (86). All five gel materials tested were contaminated with endotoxins, showing significant pyrogenic responses in all three analyses. Werner *et al*. also reported on the effectiveness of MAT and LAL in detecting pyrogenicity in intraocular lenses (IOLs). These studies provide evidence that MAT consistently detects pyrogenicity in medical devices, indicating the potential for improvement in MAT (87). Hasiwa *et al*. introduced a method to test the pyrogenicity of medical devices using a 15-compartment stainless steel culture chamber. By modifying the chamber, they could screen medical device samples for pyrogenicity in separate wells, enabling high-throughput screening. Meanwhile, Stang *et al*. proposed a dynamic culture method to examine pyrogenicity for large medical devices. This method allows the entire device surface to directly contact blood cells, facilitating the detection of material-induced pyrogenicity. However, since ongoing verification of material pyrogenicity is required for regulatory approval of medical devices, the MAT could serve as an alternative to detect such material-induced pyrogenicity (55, 88).

Other applications and potential future uses of MAT

Different microorganisms found in both indoor and outdoor air can lead to infections, allergies, or immune reactions. Therefore, it is essential to evaluate air quality by sampling and analysis to understand the potential risks to people. Metal processing and manufacturing produce various aerosols that carry microorganisms and bacteria. However, the LAL test mainly detects endotoxins from Gram−negative bacteria, neglecting the full spectrum of airborne microorganisms. Additionally, fungal glucans and DNA may interfere with endotoxin detection in the LAL test, limiting its reliability (89). To address the issue of lack of standardized methods and acceptable thresholds, Kindinger *et al*. suggested using reliable tests that reflect the human body response. They employed a MAT to measure heat-generating substances in the air, and collected microbial contaminants through air samples passed through a filter. These filters were then combined with diluted human whole blood in a collection device, left to incubate overnight, and analyzed for IL-1β release (90). For this purpose, air sampling devices, such as the styreneacrylnitrile monitor system equipped with a polytetrafluoroethylene (PTFE) membrane filter, have been developed. Verena Liebers *et al*. developed an Electrostatic Dust Collector (EDC) for the long-term collection of dust in both household and industrial air environments. Using this device, they explored the possibility of assessing endotoxin levels and pyrogenic activity. Their findings highlighted that monocyte chemotactic protein−1 (MCP-1) could function as a secondary inflammatory indicator, assessed via whole blood analysis. This affirmed its suitability for evaluating dust deposition from airborne particles during prolonged exposure periods (38, 91, 92).

Moreover, metalworking fluid (MWF), commonly used in metal processing, can provide an environment for the growth of various bacteria and microorganisms, leading to the generation of biological contaminants. This environment can be harmful to workers, so to evaluate the safety of the work environment, the MAT can examine the level of contamination in MWF, and measure the resulting immune responses. Therefore, MAT serves as a crucial tool in identifying potential risks in the work environment by validating the biological activity of MWF (93, 94).

Cell therapy involves injecting healthy living cells into patients to restore cell and tissue function. It is divided into somatic cell therapeutics and stem cell therapeutics. There are two main approaches: one uses stem cell transplantation to replace damaged tissues, while the second approach involves aiding the self-healing of damaged tissues through the secretion effects of cytokines, chemokines, and growth factors produced by specific cells. In this method, transplanted cells are rela-

tively short-lived, and after a period of time, eventually die. This involves a variety of cells, like cartilage, stem cells, bone marrow, and blood cells, such as lymphocytes, erythrocytes, and platelets. Cells stored at room temperature are at higher risk of extensive bacterial proliferation. Transfusion reactions can elicit a variety of inflammatory responses, such as seizures, fever, chills, and sepsis. Specifically, febrile reactions associated with transfusions prompt various inflammatory responses in recipients. Since these febrile reactions may go undetected below the detection threshold during experiments, there is a demand for tests with greater sensitivity than current stability tests, necessitating the development of a new MAT for this purpose (50).

CURRENT CHALLENGES AND FUTURE DIRECTIONS

Limitations of MAT and improvement

Compared with other tests, the MAT exhibits superior detection capabilities concerning toxin spectrum, sensitivity, detectability, and experimental significance. However, despite these advantages, the MAT still faces many unresolved issues that need to be addressed (72).

The duration of conducting the MAT is a critical factor to consider. MAT involves three cell groups in experiments: whole blood samples, monocytes, and monocytic cell lines. These cells are cultured alongside various stimuli for approximately (2-24) h to detect cytokines like IL-6, IL-1B, and TNF–α. Typically, analysis results are obtained (8 to 48) h afterward, with the duration potentially varying based on the sample and cell type used for MAT (95). Prolonged analysis time for the MAT can lead to delays in obtaining crucial information for quality management and decision-making concerning pharmaceuticals and products. Furthermore, it could lead to increased expenditures on reagents, equipment, and labor, ultimately compromising operational efficiency. In particular, when performing febrile tests on large volumes of pharmaceuticals or products, the process may require significantly more time. Therefore, expediting the analysis time of MAT is essential for both commercial and technical purposes.

Next, the extensive application of MAT largely depends on obtaining fresh human blood or monocytes. However, acquiring and storing these cell sources present significant challenges. Due to strict ethical regulations and complex procedures, obtaining human blood and monocytes is difficult. Moreover, these resources are limited, and require proper storage and handling. Maintaining their freshness obviously requires suitable storage and transportation.

Lastly, MAT has limitations in its applicability, particularly in terms of reliable positive controls for pyrogen testing beyond LPS. ISO 10993-11 guidelines have raised questions about whether certain substances in medical devices can induce fever reactions. Trinitrophenol's positive reactions in Whole Blood–MAT have hindered its use as a pyrogen test for chemical substances, highlighting the challenges in applying MAT to diverse fever-inducing substances, and the requirement to broaden its scope (74).

An extensive variety of developments and efforts are being made to overcome the limitations of MAT. Researchers are exploring different MAT formats using human whole blood, isolated PBMCs, and monocytic cell lines to shorten the analysis time and improve sensitivity. In particular, utilizing isolated PBMCs with propanol during stimulation has shown promising results, reducing analysis time to less than 24 h. Adjusting culture temperatures effectively accelerates MAT, facilitating the detection of endotoxin and NEPs alike. This modified setup is proposed as the fastest and most efficient MAT format (96-99). Frozen PBMCs have been effectively used in MAT for vaccine testing, especially for endotoxin-related cases. These PBMCs remain stable for (2 to 12) months in liquid nitrogen, facilitating convenient access from leukocyte filters processed at medical facilities or blood donation centers (100).

Recent technical updates of MAT and related studies

Recently, various methods have been developed to enhance the detection of febrile reactions using the Monocyte Activation Test (MAT). The conventional MAT relies on ELISA to measure a single type of cytokine, IL-6, secreted by monocytes in response to pyrogens. However, during fever, a range of cytokines, including IL-1β and TNF-α, are also secreted (101, 102). Thus, measuring only one cytokine may not fully capture the fever response. To address this, Vollenbroich and colleagues developed a digital PCR (dPCR) system using nucleic acid amplification technology (NAT). This system simultaneously measures multiple cytokines and housekeeping genes, providing a more comprehensive reflection of the pyrogenic response and ensuring data accuracy. This system detects pyrogenic contaminants and ensures drug stability, and has been evaluated as a reliable alternative method without the need for animal testing using the human lymphoblast cell line HL-60 (103).

Additionally, a new approach to the Monocyte Activation Test (MAT) has been devised that differs from the conventional method of measuring secreted inflammatory cytokines as pyrogenic markers. Numerous cytokines are secreted by the immune response, and the specific cytokines secreted depend on the immune signaling pathway involved (104-106). Most of these pathways involve the activation of NF-κB, which plays a crucial role in the mechanism by which pyrogens stimulate the secretion of inflammatory factors in humans (107). He *et al.* developed a transgenic human monocyte cell line, THP-1, stably transfected with a luciferase reporter gene vector containing the DNA response element of NF-κB. Their study demonstrated that this proposed test could serve as a novel alternative for pyrogen detection (108).

SUMMARY

In this review, we have considered the development process, advantages, and limitations of MAT as a method for replacing

animal experiments, as well as its future direction. Compared to RPT and LAL, MAT has a significant ethical impact on animal welfare, and is a suitable alternative for pyrogen test, due to its ability to detect endotoxins and NEPs more sensitively. Efforts to advance MAT are accelerating through international regulation and experiment development, primarily led by OECD countries. However, the limitations of MAT are evident, requiring diverse efforts and developments to overcome them. Moreover, collaboration among various countries and organizations is necessary to regulate and apply new technologies to MAT, making it a more attractive alternative.

The impact of MAT on both animal welfare and scientific research is significant and multifaceted. MAT provides a promising alternative to traditional animal research, especially in pyrogen test, where it offers clear advantages. By utilizing human cells instead of animals, MAT reduces the need for animal trials, thereby alleviating animal suffering and promoting animal welfare. Embracing such non-animal testing methods aligns with ethical considerations and societal expectations, contributing to the reduction and replacement of animal experiments. Moreover, MAT provides scientific researchers with a more reliable and relevant means of assessing the safety of pharmaceuticals and medical devices. By using human cell lines, MAT predicts human responses more accurately than animal models, enabling a more precise evaluation of potential risks associated with these products. This increased accuracy enhances patient safety by ensuring the use of only safe products. MAT also contributes to refining scientific research practices by providing standardized and reproducible methods for fever evaluation, improving the reliability of research findings. Furthermore, MAT promotes the use of scientifically validated methods in regulatory decision-making. Overall, the application of MAT has significantly advanced the safety evaluations of pharmaceuticals and medical devices through a more humane, ethical, and scientifically rigorous approach, positively impacting both human health and animal welfare.

ACKNOWLEDGEMENTS

This research was supported by the 2024 grant (23142MFDS153) from the Ministry of Food and Drug Safety to Boksik Cha and the 2024 Research Fund of the University of Seoul to Wantae Kim.

CONFLICTS OF INTEREST

The authors have no conflicting interests.

REFERENCES

1. Hajar R (2011) Animal testing and medicine. Heart Views 12, 42
2. Mukherjee P, Roy S, Ghosh D and Nandi SK (2022) Role of animal models in biomedical research: a review. Lab Anim Res 38, 18
3. de Kanter AJ, Jongsma KR, Verhaar MC and Bredenoord AL (2023) The ethical implications of tissue engineering for regenerative purposes: a systematic review. Tissue Eng Part B Rev 29, 167-187
4. Hunckler MD and Levine AD (2022) Navigating ethical challenges in the development and translation of biomaterials research. Front Bioeng Biotechnol 10, 949280
5. Nordgren A (2004) Moral imagination in tissue engineering research on animal models. Biomaterials 25, 1723-1734
6. Combrisson H (2017) [Animal experiment, can we replace?]. Transfus Clin Biol 24, 93-95 [French]
7. Kiani AK, Pheby D, Henehan G et al (2022) Ethical considerations regarding animal experimentation. J Prev Med Hyg 63, E255-E266
8. Brown MJ, Symonowicz C, Medina LV et al (2018) Culture of care: organizational responsibilities; in management of animal care and use programs in research, education, and testing, Weichbrod RH, Thompson GA and Norton JN (eds.), 11-26, CRC Press/Taylor & Francis © 2018 by Taylor & Francis Group, LLC., Boca Raton (FL)
9. Fennrich S, Hennig U, Toliashvili L, Schlensak C, Wendel HP and Stoppelkamp S (2016) More than 70 years of pyrogen detection: current state and future perspectives. Altern Lab Anim 44, 239-253
10. Daneshian M, Akbarsha MA, Blaauboer B et al (2011) A framework program for the teaching of alternative methods (replacement, reduction, refinement) to animal experimentation. Altex 28, 341-352
11. Curzer HJ, Perry G, Wallace MC and Perry D (2016) The Three Rs of animal research: what they mean for the institutional animal care and use committee and why. Sci Eng Ethics 22, 549-565
12. Grabow L, Hein A, Hendrikx B, Thiel W and Schilling E (1986) [Equivalence of oral and intramuscular premedication. III. Effect of premedication on anesthesia and postoperative pain]. Anasth Intensivther Notfallmed 21, 181-186 [German]
13. Henderson B and Wilson M (1996) Cytokine induction by bacteria: beyond lipopolysaccharide. Cytokine 8, 269-282
14. Hort EC and Penfold WJ (1912) Microorganisms and their relation to fever: preliminary communication. J Hyg (Lond) 12, 361-390
15. No author listed (1886) Discussion on micro-organisms in relation to puerperal fever. Trans Edinb Obstet Soc 11, 9-27
16. Zubova SV, Melzer M and Prokhorenko IR (2005) Effect of environmental factors on the composition of lipopolysaccharides released from the Rhodobacter capsulatus cell wall. Izv Akad Nauk Ser Biol 2, 168-173
17. Kulp A and Kuehn MJ (2010) Biological functions and biogenesis of secreted bacterial outer membrane vesicles. Annu Rev Microbiol 64, 163-184
18. Grant R and Hirsch JD (1950) Pyrogen fever in rabbits: effects of adrenalectomy. Am J Physiol 161, 528-533
19. van Dijck P and van de Voorde H (1977) Factors affecting pyrogen testing in rabbits. Dev Biol Stand 34, 57-63
20. Levin J and Bang FB (1964) A description of cellular coagulation in the limulus. Bull Johns Hopkins Hosp 115, 337-345

21. Solano G, Gómez A and León G (2015) Assessing endotoxins in equine-derived snake antivenoms: comparison of the USP pyrogen test and the Limulus Amoebocyte Lysate assay (LAL). Toxicon 105, 13-18
22. Bolden JS, Warburton RE, Phelan R et al (2016) Endotoxin recovery using limulus amebocyte lysate (LAL) assay. Biologicals 44, 434-440
23. Levin J and Bang FB (1964) The role of endotoxin in the extracellular coagulation of Limulus blood. Bull Johns Hopkins Hosp 115, 265-274
24. Neun BW and Dobrovolskaia MA (2019) Detection of endotoxin in nano-formulations using limulus amoebocyte lysate (LAL) Assays. J Vis Exp 143, e58830
25. Iwanaga S, Morita T, Harada T et al (1978) Chromogenic substrates for horseshoe crab clotting enzyme. Its application for the assay of bacterial endotoxins. Haemostasis 7, 183-188
26. Nakamura S, Iwanaga S, Harada T and Niwa M (1976) A clottable protein (coagulogen) from amoebocyte lysate of Japanese horseshoe crab (Tachypleus tridentatus). Its isolation and biochemical properties. J Biochem 80, 1011-1021
27. Nakamura S, Takagi T, Iwanaga S, Niwa M and Takahashi K (1976) A clottable protein (coagulogen) of horseshoe crab hemocytes. Structural change of its polypeptide chain during gel formation. J Biochem 80, 649-652
28. Ding JL and Ho B (2010) Endotoxin detection–from limulus amebocyte lysate to recombinant factor C. Subcell Biochem 53, 187-208
29. Marius M, Vacher F and Bonnevay T (2020) Comparison of Limulus amoebocyte lysate and recombinant factor c assays for endotoxin detection in four human vaccines with complex matrices. PDA J Pharm Sci Technol 74, 394-407
30. Dubczak J, Reid N and Tsuchiya M (2021) Evaluation of limulus amebocyte lysate and recombinant endotoxin alternative assays for an assessment of endotoxin detection specificity. Eur J Pharm Sci 159, 105716
31. Hartung T and Wendel A (1995) Detection of Pyrogens using human whole blood. Altex 12, 70-75
32. Daneshian M, von Aulock S and Hartung T (2009) Assessment of pyrogenic contaminations with validated human whole-blood assay. Nat Protoc 4, 1709-1721
33. Hermann C, von Aulock S, Graf K and Hartung T (2003) A model of human whole blood lymphokine release for *in vitro* and *ex vivo* use. J Immunol Methods 275, 69-79
34. Weigandt F, Lexa P and Sonntag HG (1998) A new test for the detection of pyrogens in pharmaceutical products. Examinations for the validation of the human whole blood assay. Altex 15, 13-17
35. Schindler S, Fennrich S, Crameri R, Jungi TW, Montag T and Hartung T (2007) Fever in the test tube–towards a human(e) pyrogen test. Altex 24, 60-62
36. Fennrich S, Wendel A and Hartung T (1999) New applications of the human whole blood pyrogen assay (PyroCheck). Altex 16, 146-149
37. Fennrich S, Fischer M, Hartung T et al (1998) Evaluation and further development of a pyrogenicity assay based on human whole blood. Altex 15, 123-128
38. Stang K, Fennrich S, Krajewski S et al (2014) Highly sensitive pyrogen detection on medical devices by the monocyte activation test. J Mater Sci Mater Med 25, 1065-1075
39. Kikkert R, de Groot ER and Aarden LA (2008) Cytokine induction by pyrogens: comparison of whole blood, mononuclear cells, and TLR-transfectants. J Immunol Methods 336, 45-55
40. Lekshmi N, Geetha CS and Mohanan PV (2012) Detection of interleukin-1β from isolated human lymphocyte in response to lipopolysaccharide and lipoteichoic acid. Indian J Pharmacol 44, 726-731
41. Dinarello CA (1991) Interleukin-1 and interleukin-1 antagonism. Blood 77, 1627-1652
42. Dinarello CA (1991) Inflammatory cytokines: interleukin-1 and tumor necrosis factor as effector molecules in autoimmune diseases. Curr Opin Immunol 3, 941-948
43. François C, Neveu J, Sauvaire D, Bonnet PA and Tissier MH (2006) *In vitro* pyrogenicity of Gram-positive bacteria- -validation of the kit using fresh human whole blood. Pharmeur Sci Notes 2006, 17-21
44. He Q, Gao H, Xu LM et al (2018) Analysis of IL-6 and IL-1β release in cryopreserved pooled human whole blood stimulated with endotoxin. Innate Immun 24, 316-322
45. Hartung T (2015) The human whole blood pyrogen test - lessons learned in twenty years. Altex 32, 79-100
46. Moesby L, Jensen S, Hansen EW and Christensen JD (1999) A comparative study of Mono Mac 6 cells, isolated mononuclear cells and Limulus amoebocyte lysate assay in pyrogen testing. Int J Pharm 191, 141-149
47. Palma L, Rossetti F, Dominici S et al (2017) Determination of interference during *in vitro* pyrogen detection: development and characterization of a cell-based assay. Assay Drug Dev Technol 15, 64-76
48. Studholme L, Sutherland J, Desai T et al (2019) Evaluation of the monocyte activation test for the safety testing of meningococcal B vaccine Bexsero: a collaborative study. Vaccine 37, 3761-3769
49. Valentini S, Santoro G, Baffetta F et al (2019) Monocyte-activation test to reliably measure the pyrogenic content of a vaccine: an *in vitro* pyrogen test to overcome *in vivo* limitations. Vaccine 37, 3754-3760
50. Hartung T (2021) Pyrogen testing revisited on occasion of the 25th anniversary of the whole blood monocyte activation test. Altex 38, 3-19
51. Solati S, Zhang T and Timman S (2022) The monocyte activation test detects potentiated cytokine release resulting from the synergistic effect of endotoxin and non-endotoxin pyrogens. Innate Immun 28, 130-137
52. Fennrich S, Fischer M, Hartung T et al (1999) Detection of endotoxins and other pyrogens using human whole blood. Dev Biol Stand 101, 131-139
53. Hartung T (2002) Comparison and validation of novel pyrogen tests based on the human fever reaction. Altern Lab Anim 30 Suppl 2, 49-51
54. Akira S, Uematsu S and Takeuchi O (2006) Pathogen recognition and innate immunity. Cell 124, 783-801
55. Hasiwa N, Daneshian M, Bruegger P et al (2013) Evidence for the detection of non-endotoxin pyrogens by

the whole blood monocyte activation test. Altex 30, 169-208
56. Warrington R, Watson W, Kim HL and Antonetti FR (2011) An introduction to immunology and immunopathology. Allergy Asthma Clin Immunol 7 Suppl 1, S1
57. Marshall JS, Warrington R, Watson W and Kim HL (2018) An introduction to immunology and immunopathology. Allergy Asthma Clin Immunol 14, 49
58. Litman GW, Cannon JP and Dishaw LJ (2005) Reconstructing immune phylogeny: new perspectives. Nat Rev Immunol 5, 866-879
59. Pancer Z and Cooper MD (2006) The evolution of adaptive immunity. Annu Rev Immunol 24, 497-518
60. Kawai T and Akira S (2006) Innate immune recognition of viral infection. Nat Immunol 7, 131-137
61. Miller SB (2006) Prostaglandins in health and disease: an overview. Semin Arthritis Rheum 36, 37-49
62. Ogawa Y and Calhoun WJ (2006) The role of leukotrienes in airway inflammation. J Allergy Clin Immunol 118, 789- 798; quiz 799-800
63. Ma WT, Gao F, Gu K and Chen DK (2019) The role of monocytes and macrophages in autoimmune diseases: a comprehensive review. Front Immunol 10, 1140
64. Beutler BA (2009) TLRs and innate immunity. Blood 113, 1399-1407
65. Kawai T and Akira S (2011) Toll-like receptors and their crosstalk with other innate receptors in infection and immunity. Immunity 34, 637-650
66. Takeuchi O, Sato S, Horiuchi T et al (2002) Cutting edge: role of Toll-like receptor 1 in mediating immune response to microbial lipoproteins. J Immunol 169, 10-14
67. Dinarello CA, Gatti S and Bartfai T (1999) Fever: links with an ancient receptor. Curr Biol 9, R147-R150
68. Wunderlich C, Schumacher S and Kietzmann M (2015) Prostaglandin E2 as a read out for endotoxin detection in a bovine whole blood assay. J Vet Pharmacol Ther 38, 196-198
69. Bennett IL Jr. and Beeson PB (1953) Studies on the pathogenesis of fever. II. Characterization of fever-producing substances from polymorphonuclear leukocytes and from the fluid of sterile exudates. J Exp Med 98, 493-508
70. Dinarello CA (2004) Infection, fever, and exogenous and endogenous pyrogens: some concepts have changed. J Endotoxin Res 10, 201-222
71. Pool EJ, Johaar G, James S, Petersen I and Bouic P (1998) The detection of pyrogens in blood products using an *ex vivo* whole blood culture assay. J Immunoassay 19, 95-111
72. Schindler S, von Aulock S, Daneshian M and Hartung T (2009) Development, validation and applications of the monocyte activation test for pyrogens based on human whole blood. Altex 26, 265-277
73. da Silva CC, Presgrave OA, Hartung T, de Moraes AM and Delgado IF (2016) Applicability of the Monocyte Activation Test (MAT) for hyperimmune sera in the routine of the quality control laboratory: comparison with the Rabbit Pyrogen Test (RPT). Toxicol In Vitro 32, 70-75
74. Brown J, Clippinger AJ, Fritz Briglia C et al (2021) Using the monocyte activation test as a stand-alone release test for medical devices. Altex 38, 151-156
75. Carlin G and Viitanen E (2005) *In vitro* pyrogenicity of the diphtheria, tetanus and acellular pertussis components of a trivalent vaccine. Vaccine 23, 3709-3715
76. de Mattos KA, Navega ECA, Silva VF et al (2018) Applicability of the Monocyte Activation Test (MAT) in the quality control of the 17DD yellow fever vaccine. Altern Lab Anim 46, 23-37
77. Gerke C, Colucci AM, Giannelli C et al (2015) Production of a Shigella sonnei vaccine based on Generalized Modules for Membrane Antigens (GMMA), 1790GAHB. PLoS One 10, e0134478
78. Etna MP, Giacomini E, Rizzo F et al (2020) Optimization of the monocyte activation test for evaluating pyrogenicity of tick-borne encephalitis virus vaccine. Altex 37, 532-544
79. Neun BW, Cedrone E, Potter TM, Crist RM and Dobrovolskaia MA (2020) Detection of beta-glucan contamination in nanotechnology-based formulations. Molecules 25, 3367
80. Buchacher A, Krause D, Wiry G and Weinberger J (2010) Elevated endotoxin levels in human intravenous immunoglobulin concentrates caused by (1->3)-{beta}-D-glucans. PDA J Pharm Sci Technol 64, 536-544
81. Perdomo-Morales R, Pardo-Ruiz Z, Spreitzer I, Lagarto A and Montag T (2011) Monocyte activation test (MAT) reliably detects pyrogens in parenteral formulations of human serum albumin. Altex 28, 227-235
82. Martis L, Patel M, Giertych J et al (2005) Aseptic peritonitis due to peptidoglycan contamination of pharmacopoeia standard dialysis solution. Lancet 365, 588-594
83. Anderson JM and Miller KM (1984) Biomaterial biocompatibility and the macrophage. Biomaterials 5, 5-10
84. Ziats NP, Miller KM and Anderson JM (1988) *In vitro* and *in vivo* interactions of cells with biomaterials. Biomaterials 9, 5-13
85. Mazzotti F, Beuttler J, Zeller R et al (2007) *In vitro* pyrogen test–A new test method for solid medical devices. J Biomed Mater Res A 80, 276-282
86. Mohanan PV, Banerjee S and Geetha CS (2011) Detection of pyrogenicity on medical grade polymer materials using rabbit pyrogen, LAL and ELISA method. J Pharm Biomed Anal 55, 1170-1174
87. Werner L, Tetz M, Mentak K, Aldred M and Zwisler W (2009) Detection of pyrogens adsorbed to intraocular lenses: evaluation of limulus amoebocyte lysate and *in vitro* pyrogen tests. J Cataract Refract Surg 35, 1273-1280
88. Hasiwa M, Kullmann K, von Aulock S, Klein C and Hartung T (2007) An *in vitro* pyrogen safety test for immune-stimulating components on surfaces. Biomaterials 28, 1367-1375
89. Tamura H, Reich J and Nagaoka I (2021) Outstanding contributions of LAL technology to pharmaceutical and medical science: review of methods, progress, challenges, and future perspectives in early detection and management of bacterial infections and invasive fungal diseases. Biomedicines 9, 536
90. Kindinger I, Daneshian M, Baur H et al (2005) A new method to measure air-borne pyrogens based on human

whole blood cytokine response. J Immunol Methods 298, 143-153
91. Liebers V, van Kampen V, Bünger J et al (2012) Assessment of airborne exposure to endotoxin and pyrogenic active dust using electrostatic dustfall collectors (EDCs). J Toxicol Environ Health A 75, 501-507
92. Punsmann S, Liebers V, Stubel H, Brüning T and Raulf-Heimsoth M (2013) Determination of inflammatory responses to Aspergillus versicolor and endotoxin with human cryo-preserved blood as a suitable tool. Int J Hyg Environ Health 216, 402-407
93. Borton LK and Coleman KP (2018) Material-mediated pyrogens in medical devices: applicability of the *in vitro* Monocyte Activation Test. Altex 35, 453-463
94. Thorne PS, Bartlett KH, Phipps J and Kulhankova K (2003) Evaluation of five extraction protocols for quantification of endotoxin in metalworking fluid aerosol. Ann Occup Hyg 47, 31-36
95. Gaines Das RE, Brügger P, Patel M, Mistry Y and Poole S (2004) Monocyte activation test for pro-inflammatory and pyrogenic contaminants of parenteral drugs: test design and data analysis. J Immunol Methods 288, 165-177
96. Koryakina A, Frey E and Bruegger P (2014) Cryopreservation of human monocytes for pharmacopeial monocyte activation test. J Immunol Methods 405, 181-191
97. Wunderlich C, Schumacher S and Kietzmann M (2014) Pyrogen detection methods: comparison of bovine whole blood assay (bWBA) and monocyte activation test (MAT). BMC Pharmacol Toxicol 15, 50
98. Chanput W, Mes JJ and Wichers HJ (2014) THP-1 cell line: an *in vitro* cell model for immune modulation approach. Int Immunopharmacol 23, 37-45
99. Stoppelkamp S, Würschum N, Stang K et al (2017) Speeding up pyrogenicity testing: identification of suitable cell components and readout parameters for an accelerated monocyte activation test (MAT). Drug Test Anal 9, 260-273
100. Solati S, Aarden L, Zeerleder S and Wouters D (2015) An improved monocyte activation test using cryopreserved pooled human mononuclear cells. Innate Immun 21, 677-684
101. Nakagawa Y, Maeda H and Murai T (2002) Evaluation of the *in vitro* pyrogen test system based on proinflammatory cytokine release from human monocytes: comparison with a human whole blood culture test system and with the rabbit pyrogen test. Clin Diagn Lab Immunol 9, 588-597
102. Martínez V, Mitjans M and Vinardell MP (2004) TNF alpha measurement in rat and human whole blood as an *in vitro* method to assay pyrogens and its inhibition by dexamethasone and erythromycin. J Pharm Sci 93, 2718-2723
103. Vollenbroich D, Bage D, Kussatz S, Michalski J and Paland N (2024) Development of a monocyte activation test based on digital pcr - nat-mat. Cytotherapy 26, S122
104. Vipond C, Sutherland J, Nordgren K et al (2019) Development and validation of a monocyte activation test for the control/safety testing of an OMV-based meningococcal B vaccine. Vaccine 37, 3747-3753
105. Cot M, Ray A, Gilleron M et al (2011) Lipoteichoic acid in Streptomyces hygroscopicus: structural model and immunomodulatory activities. PLoS One 6, e26316
106. Gnauck A, Lentle RG and Kruger MC (2016) The characteristics and function of bacterial lipopolysaccharides and their endotoxic potential in humans. Int Rev Immunol 35, 189-218
107. Yu H, Lin L, Zhang Z, Zhang H and Hu H (2020) Targeting NF-κB pathway for the therapy of diseases: mechanism and clinical study. Signal Transduct Target Ther 5, 209
108. He Q, Yu CF, Wu G et al (2024) A novel alternative for pyrogen detection based on a transgenic cell line. Signal Transduct Target Ther 9, 33