PyroDex

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# Non-endotoxin Pyrogens Jeopardize Life-saving Drugs and Clinical Trials: Automated MAT Offers Fast, Reliable Detection

Popular endotoxin detection kits based on the horseshoe crab blood cell lysates fail to address the detrimental impact of non-endotoxin pyrogens or fever-causing substances on the effects of parenteral medical drugs in their different phases of clinical applications. However, new detection methods can identify all pyrogenic contaminants in injectable medicines and implantable medical devices, and help reduce or eliminate the risk of fever, septic shock, organ failure or death associated with the administration of new drugs or compounds.

Despite the reassuring negative results provided by endotoxin, Mycoplasma and sterility tests, current gene and cell therapies have shown adverse events or side effects of variable severity, particularly in cancer patients. Clinical trials may have been compromised, in some cases stopped, due to unsuspected non-endotoxin pyrogens. It is possible that investigational drugs of high pharmacological quality have been abandoned for this reason after several years of investment, a significant blow to startups or established organizations as well as patients. As

interest in immunotherapy for the treatment of cancers and other diseases increases, this class of non-endotoxin pyrogens remains a major problem that the current commercially available LAL and Mycoplasma kits do not address.

**Compendial methods for the quality control and safety of injectable and implantable therapeutic products:**

1. *The 2- to 3-day **sterility test** determines the presence or absence of live bacteria or fungi (USP <71>).*
2. *The 28- to 35-day **Mycoplasma test**, USP <63>, detects live Mycoplasma.*
3. *The faster, 1- to 2-hour **LAL-based kits** uses various formulations (gel clot, turbidimetric, and kinetic chromogenic tests) to detect endotoxins (USP <85>).*

Toxicity associated with immunotherapies has been reported [1-3]; cytokine release syndrome (CRS) has been identified as the most significant and life-threatening toxicity. Teachey and his collaborators showed that the severity of CRS is linked to predictors such as cytokines interferon gamma (IFN$\gamma$) and interleukin-6 (IL-6), whose levels peak in the first month after infusion [4].

In their 2015 review article, Jeffrey Weber and his colleagues reported that toxicities are a common theme in current immunotherapies, including cancer vaccines, cytokine treatment, adoptive cell therapy and checkpoint protein inhibitors [3]. Their comparative overview of symptoms identifies fever as a component of all reported side effects. Despite stringent manufacturing processes, these reports suggest that the cytokines, biologics, or cell-based drugs used in various immunotherapies may carry with them pyrogenic contaminants that elude current microbial detection methods.

The monocyte activation test (MAT), adopted in 2010 in the European Pharmacopeia (EP 2.6.30), has become a compendial method since 2016 [5]. All member countries of the European Union and United Kingdom agreed to enforce use of this pyrogen detection method in replacement of the rabbit pyrogen test. In the United States, the FDA, in its guidance for industry for pyrogen and endotoxin testing, recognized the MAT as an alternative method [6]. Still, current commercial MAT kits, whether based on whole human blood [7, 8], isolated white blood cells (peripheral blood mononuclear cells or PBMC [9], or a cell line (MonoMac6), are tedious and time-consuming and require skilled operators. Furthermore, their sensitivity levels based on the reference standard endotoxin are variable, and significantly lower than that of the LAL-based kinetic chromogenic method.

## Principle of the Monocyte Activation Test

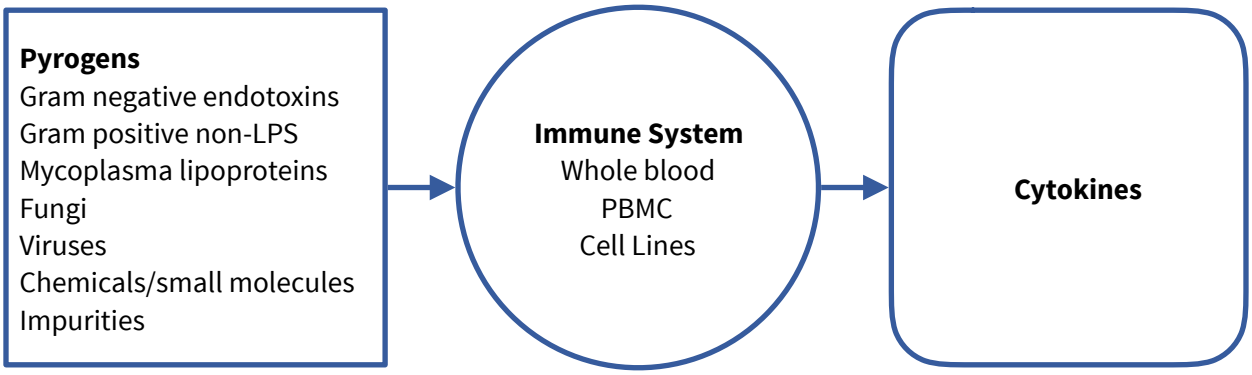


**Figure 1.** Principle of monocyte activation test (MAT). Activation of white blood cells from the whole blood, isolated peripheral blood mononuclear cells (PBMC), or engineered Mono Mac6 cells, triggers the expression and release of cytokines detectable and measurable by enzyme-linked immunosorbent assay (ELISA). Pyrogen levels are determined using serial dilutions of reference standards and interpolation from a 4-parametric logistic regression function.

However, recent investigations using automated technology have produced a proprietary formulated monocyte activation test that is 2 to 30 times more sensitive than commercially available endotoxin- specific detection kits. The test provided by PyroDex, a Baltimore-based startup, offers rapid, comprehensive detection of pyrogens or fever-causing contaminants in injectable or implantable medical drugs and devices, using isolated human leukocytes coupled with integrated technologies that eliminate variability between runs, instruments, and operators. Test results that fully assess pyrogenic contaminants including impurities originating from manufacturing processes, allow quality control and assurance managers, as well as practitioners involved in clinical trials, to focus on the efficacy of the therapeutic products. Just as important, results from the automated MAT are available within 2 to 5 days, enabling clients to quickly release safe, reliable products to accelerate clinical applications and their expected beneficial outcome for the patients.

The cost of failed clinical trials is enormous, and may be associated with non-endotoxin pyrogens not detected by rabbit pyrogen test or LAL-based endotoxin detection methods. The perspective of mitigating such potential losses, using an enhanced, comprehensive monocyte activation test along with a fast return of test results, is a giant step forward in the manufacture of safe, reliable implantable and injectable therapeutic products.

## About the Author

Djikolngar Maouyo, PhD, Managing Director and President at PyroDex LLC, where he leads the research and development program for alternative methods to current pyrogen detection methods. Prior to that he was Senior Scientist at Lonza Walkersville, where he developed kits and evaluated budding technologies for microbial detection in the US and Europe for 9 years. Dr. Maouyo is also an external member of the Institutional Biosafety Committee of Johns Hopkins University. Previously, he held faculty positions at the Johns Hopkins University School of Medicine and the University of Maryland Baltimore.

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