Implementing the In Vitro Pyrogen Test: One More Step Toward Replacing Animal Experimentation

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A pyrogen (from the Greek: pyros — fire, and genesis — generation) is a substance that induces fever upon blood contact in mammals. Deriving, for instance, from fragments of Gram-negative or Gram-positive bacteria, pyrogens can occur even in sterilised products, and have the capacity to pose a serious health threat to patients receiving parenteral treatments. The presence of such pyrogens can be assessed with an in vivo rabbit pyrogen test, which was officially introduced in the 1940s for the systematic risk assessment of infusion solutions. In this test, parenterals to be tested are injected into rabbits, and induce a rise in body temperature if they are contaminated with pyrogens. Another test system, which exclusively detects endotoxins from Gram-negative bacteria, is the LAL (Limulus amoebocyte lysate) test. The reaction of the lysate with the endotoxin can be measured chromogenically, or by turbidity assessment. The LAL test was a great innovation in the 1970s, but it does not detect the full range of pyrogens and cannot totally replace animal testing.

The in vitro Monocyte Activation Test (MAT) is a validated testing system that constitutes a complete replacement of the rabbit pyrogen procedure for the quality control of injectables. It comprises six variants of the same principle: the fact that cells produce cytokines upon contact with fever-inducing substances, so-called pyrogens. This reaction is (within a certain range) dose-dependent, and permits a quantitative determination of pyrogenic contents. One prominent feature of the MAT is that, unlike the LAL, it is not restricted to endotoxins. An in vitro pyrogen test, based on the MAT, is commercially available from Merck KGaA (Germany), under the name PyroDetect.

With the inclusion, from April 2010, of the Monocyte Activation Test (MAT) in the European Pharmacopoeia, an elementary step was taken toward the reduction of animal testing. Nevertheless, even after almost three years, the number of rabbits used for pyrogen testing still reaches approximately 100,000 animals per year in Europe alone. To counter this enormous use of animals, there are a number of qualified laboratories that perform the MAT as a service for customers from the pharmaceutical industry. The most important regulation for the pharmaceutical industry is GMP (Good Manufacturing Practice). Pharmaceutical products have to be manufactured and analysed according to these rules. Importantly, the MAT is applicable under GMP regulations, and the aforementioned commercial services can be offered with the necessary quality standard.

The testing of medical devices, however, is regulated separately (e.g. by ISO guidelines), where GLP (Good Laboratory Practice) rules are applied. Therefore, we are currently working on the introduction of PyroDetect for medical device testing in our existing GLP system. In this developmental work (which is being supported by Animalfree Research, Bern, Switzerland), the testing is performed in several steps. The medical devices are either eluated with pyrogen-free water and subsequently subjected to the whole blood assay (see Figure 1), or the devices are incubated directly with diluted human whole blood. Any pyrogenic content present in the eluate or on the devices leads to the secretion of the cytokine, IL-1β, by blood monocytes. In a final step, the cytokine secretion is quantified via an Enzyme-linked Immunosorbent Assay (ELISA).

After the accreditation of PyroDetect under GLP, we will be able to test medical devices for pyrogenic contamination according to these high standards. With this knowledge, we will continue to also set standards for other applications, such as the testing of air quality. It is notable that pyrogenic limits for medical devices and/or air quality (for working places) are restricted to endotoxins. With a species-specific system, based on human whole blood and for a wide range of pyrogens, more-relevant data are available to calculate health-related risks.
Figure 1: Incubation and ELISA procedure

1. Whole blood incubation

2. Interleukin-1β ELISA

3. Read-out and data analysis

Source: Merck KGaA, Darmstadt, Germany.

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