



Session 5.9 Biologicals: Progress and new approaches

Lecture

International validation of pyrogen tests based on fresh and cryopreserved human primary blood cells

*Stefanie Schindler*¹, *Sebastian Hoffmann**², *Kilian Hennes*³, *Ingo Spreitzer*⁴, *Marlies Halder*², *Peter Bruegger*⁵, *Esther Frey*⁵, *Thomas Montag-Lessing*⁴, *Bettina Loeschner*⁴, *Stephen Poole*⁶ and *Thomas Hartung*²

¹University of Konstanz, Institute of Biochemical Pharmacology, Konstanz, Germany; ²European Commission, JRC, ECVAM, Ispra, Italy; ³Qualis Laboratories, Konstanz, Germany; ⁴Paul Ehrlich Institut, Langen, Germany; ⁵Novartis Pharma AG, Biological Analytics, Basel, Switzerland; ⁶NIBSC, National Institute for Biological Standards and Control, Potters Bar, UK

Pyrogens as fever-inducing agents can be a major health hazard in parenterally applied drugs. For the control of these contaminants, pyrogen testing for batch release is required by Pharmacopoeias. This has been done either by the *in vivo* rabbit pyrogen test (since 1942) or the limulus amoebocyte lysate test (LAL), since 1976. A new approach are cell-based assays employing *in vitro* cultivation of human immune cells which respond e.g. with cytokine production (IL-1, IL-6) upon contact to pyrogens. 6 variants of these assays have recently been validated in a collaborative international study. From two of these methods, the development of successful cryopreservation meth-

ods promises to make standardised immunoreactive primary human blood cells available for widespread use. Furthermore, the pre-testing of donors for infectious agents such as HIV or hepatitis has made it possible to develop a safe and standardised reagent for pyrogen testing. Using altogether 13 drugs, we have validated here two pyrogen tests based on fresh and cryopreserved human whole blood or isolated PBMCs in four laboratories. The tests reached >90% sensitivity and specificity. In contrast to the LAL, the tests are capable of detecting non-endotoxin pyrogens derived from Gram-positive bacteria or fungi.