ICCVAM Recommendations on the Use of Five *In Vitro* Pyrogen Test Methods for Assessing the Potential Pyrogenicity of Pharmaceuticals and Other Products

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Pyrogen testing is a critical step towards ensuring the safety of parenteral pharmaceuticals. The U.S., European, and Japanese Pharmacopoeias currently recognize two pyrogen tests, both of which require animals (i.e., the rabbit pyrogen test [RPT] and the bacterial endotoxin test). Concerns for animal welfare have led researchers to develop alternative cell-based test methods that use human cells. ICCVAM recently evaluated the validation status of five *in vitro* test methods for assessing potential pyrogenicity of pharmaceuticals and other products as potential replacements for the RPT. These methods use IL-1 or IL-6 ELISAs to measure an increase in cytokines when human monocytoid cells (i.e., whole blood, isolated monocytes, or a Mono Mac 6 cell line) are exposed to Gram-negative endotoxin. The accuracy evaluation was based on 10 parenteral pharmaceuticals, each spiked with four concentrations of endotoxin. Accuracy ranged from 81% to 93%, false negative rates ranged from 1% to 27%, and false positive rates ranged from 3% to 23%. Quantitative and qualitative reliability analyses indicated that the test methods were generally reproducible within and among testing laboratories. Based on the results of these analyses, ICCVAM recommends that, while none of these five in vitro test methods should be considered as a complete replacement for the RPT, they could be considered to detect Gram-negative endotoxin in human parenteral drugs on a case-by-case basis, subject to product-specific validation by the appropriate regulatory agency. When used in this manner, these methods should further reduce the number of animals needed for pyrogenicity testing. ICCVAM recommends, consistent with U.S. Animal Welfare Regulations, that *in vitro* pyrogen tests must be considered prior to testing in animals and that an alternative test method be used when deemed appropriate.