ICCVAM Recommendations for Five *In Vitro* Pyrogen Test Methods

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Test systems based on the *in vitro* activation of human blood cells have been developed for pyrogenicity testing. Such methods utilize human whole blood, isolated primary monocytes, or a monocyte cell line and are based on quantifying cytokine release to identify substances containing Gram-negative endotoxin. ICCVAM evaluated the validation status of five of these methods as potential replacements for the rabbit pyrogen test (RPT). In a multilaboratory validation study of 10 parenteral pharmaceuticals spiked with four concentrations of Gram-negative endotoxin, accuracy, false positive, and false negative rates ranged from 81-93%, 3-23%, and 1-27%, respectively. The methods were generally reproducible within and among testing laboratories. ICCVAM subsequently recommended that these *in vitro* test methods be considered to detect Gram-negative endotoxin in human parenteral drugs. Although none should be considered as a complete replacement for the RPT, these methods may be used 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 that *in vitro* pyrogen tests be considered prior to testing in animals and that an alternative test method be used when deemed appropriate. ICCVAM forwarded these recommendations to U.S. Federal agencies along with recommendations for research and development needed to further expand their usefulness. Agency responses are due by mid-2009 and will be summarized. The views expressed above do not necessarily represent the official position of any government agency.