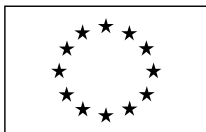


**Appendix E**

**ESAC Statement on the Validity of *In Vitro* Pyrogen Tests**

**ESAC Statement on the Validity of *In Vitro* Pyrogen Tests .....E-3**

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**EUROPEAN COMMISSION**  
DIRECTORATE GENERAL JRC  
JOINT RESEARCH CENTRE  
Institute for Health and Consumer Protection  
European Centre for the Validation of Alternative Methods (ECVAM)

## **STATEMENT ON THE VALIDITY OF IN-VITRO PYROGEN TESTS**

At its 24<sup>th</sup> meeting, held on 20-21 March 2006 at the European Centre for the validation of alternative methods (ECVAM), Ispra, Italy, the non-Commission members of the ECVAM Scientific Advisory Committee (ESAC)<sup>1</sup> unanimously endorsed the following statement:

Following a review of scientific reports and peer reviewed publications on the following range of in-vitro pyrogen tests:

1. Human Whole Blood IL-1,
2. Human Whole Blood IL-6,
3. PBMC IL-6,
4. MM6 IL-6, and
5. Human Cryopreserved Whole Blood IL-1,

it is concluded that these tests have been scientifically validated for the detection of pyrogenicity mediated by Gram-negative endotoxins, and quantification of this pyrogen, in materials currently evaluated and characterized by rabbit pyrogen tests.

These methods have the potential to satisfy regulatory requirements for the detection and quantification of these pyrogens in these materials subject to product-specific validation.

The test methods have the capacity of detecting pyrogenicity produced by a wider range of pyrogens, but the evidence compiled for, and considered within this peer review and validation process, is not sufficient to state that full scientific validation of this wider domain of applicability has been demonstrated and confirmed.

Thus, the above test methods can currently be considered as full replacements for the evaluation of materials or products where the objective is to identify and evaluate pyrogenicity produced by Gram-negative endotoxins, but not for other pyrogens.

This endorsement takes account of the dossiers prepared for peer review; the views of independent experts who evaluated the dossiers against defined validation criteria; supplementary submissions made by the Management Team; and the considered view of the Peer Review Panel appointed to oversee the process.

Thomas Hartung  
Head of Unit  
ECVAM  
Institute for Health & Consumer Protection  
Joint Research Centre  
European Commission  
Ispra

21 March 2006

1. The ESAC was established by the European Commission, and is composed of nominees from the EU Members States, industry, academia and animal welfare, together with representatives of the relevant Commission services.

This statement was endorsed by the following Members of the ESAC:

Prof Helmut Tritthart (Austria)  
Dr Dagmar Jírová (Czech Republic)  
Prof Elisabeth Knudsen (Denmark)  
Dr Timo Ylikomi (Finland)  
Prof André Guillouzo (France)  
Dr Manfred Liebsch (Germany)  
Dr Efstathios Nikolaidis (Greece)  
Dr Katalin Horvath (Hungary)  
Prof Michael Ryan (Ireland)  
Dr Annalaura Stammati (Italy)  
Dr Mykolas Maurica (Lithuania)  
Prof Eric Tschirhart (Luxembourg)  
Dr Jan van der Valk (The Netherlands)  
Dr Dariusz Sladowski (Poland)  
Prof Milan Pogačnik (Slovenia)  
Dr Argelia Castaño (Spain)  
Dr Patric Amcoff (Sweden)  
Dr Jon Richmond (UK)  
Dr Odile de Silva (COLIPA)  
Dr Julia Fentem (ECETOC)  
Dr Nathalie Alépée (EFPIA)  
Prof Robert Combes (ESTIV)  
Dr Maggy Jennings (Eurogroup for Animal Welfare)  
Mr Roman Kolar (Eurogroup for Animal Welfare)

The following Commission Services and Observer Organisations were involved in the consultation process, but not in the endorsement process itself.

Mr Thomas Hartung (ECVAM; chairman)  
Mr Jens Linge (ECVAM; ESAC secretary)  
Mr Juan Riego Sintes (ECB)  
Ms Beatrice Lucaroni (DG Research, Unit F.5)  
Mr Sylvain Bintein (DG Environment, Unit C.3)  
Mr Sigfried Breier (DG Enterprise, Unit F.3)  
Prof Dr Constantin Mircioiu (Romania)  
Dr William Stokes (NICEATM, USA)  
Prof Dr Vera Rogiers (ECOPA)

## Annex

The novel pyrogen tests are based on the human fever reaction. Monocytoid cells, either primary from human blood or as propagated cell lines, detect pyrogens of different chemical nature and respond by the release of inflammatory mediators such as cytokines. Since lipopolysaccharides from Gram-negative bacteria are the only type of proven pyrogen, for which an International reference material is available, the tests were standardised to detect the presence of significantly less than 0.5 Endotoxin Units of this preparation, which is considered to be the threshold level for fever induction in the most sensitive rabbit species according to pharmacopoeia test procedures.

The five tests which were sufficiently reproducible and exceeded the rabbit test with regard to sensitivity and specificity for the detection of lipopolysaccharide spiked samples, differ with regard to cell source and preparation, cryopreservation and cytokine measured. The tests have been described elsewhere (1-4). The concept of the validation study (5) and the international validation studies are available (6-7).

1. Poole, S., Thorpe, R., Meager, A., Hubbard, A.R., Gearing, A.J. (1988) Detection of pyrogen by cytokine release. *Lancet* 8577, 130.
2. Taktak, Y.S., Selkirk, S., Bristow, A.F., Carpenter, A., Ball, C., Rafferty, B., Poole, S. (1991) Assay of pyrogens by interleukin-6 release from monocytic cell lines. *J. Pharm. Pharmacol.* 43, 578.
3. Hartung, T., Wendel, A. (1996) Detection of pyrogens using human whole blood. *In Vitro Toxicol.* 9, 353.
4. Schindler S, Asmus S, von Aulock S, Wendel A, Hartung T and Fennrich S. (2004) Cryopreservation of human whole blood for pyrogenicity testing. *J. Immunol. Meth.* 294, 89-100.
5. Hartung, T., Aaberge, I., Berthold, S., Carlin, G., Charton, E., Coecke, S., Fennrich, S., Fischer, M., Gommer, M., Halder, M., Haslov, K., Jahnke, M., Montag-Lessing, T., Poole, S., Schechtman, L., Wendel, A., Werner-Felmayer, G. (2001) Novel pyrogen tests based on the human fever reaction. The report and recommendations of ECVAM Workshop 43. European Centre for the Validation of Alternative Methods. *Altern. Lab. Anim.* 29, 99.
6. Hoffmann S, Peterbauer A, Schindler S, Fennrich S, Poole S, Mistry Y, Montag-Lessing T, Spreitzer I, Loschner B, van Aalderen M, Bos R, Gommer M, Nibbeling R, Werner-Felmayer G, Loitzl P, Jungi T, Brcic M, Brugger P, Frey E, Bowe G, Casado J, Coecke S, de Lange J, Mogster B, Naess LM, Aaberge IS, Wendel A and Hartung T. (2005) International validation of novel pyrogen tests based on the human fever reaction. *J. Immunol. Meth.* 298, 161-173.
7. Schindler S, Spreitzer I, Loschner, Hoffmann S, Hennes K, Halder M, Brügger P, Frey E, Hartung T and Montag T. (2006) International validation of pyrogen tests based on cryopreserved human primary blood cells. *J. Immunol. Meth.* 316, 42-51.