European Medicines Agency
Pre-authorisation Evaluation of Medicines for Human Use

Doc. Ref.: EMEA/CHMP/BWP/452081/2007
London, 5th December 2007

COMMITTEE FOR MEDICINAL PRODUCTS FOR HUMAN USE (CHMP)

DRAFT

NOTE FOR GUIDANCE ON PLASMA DERIVED MEDICINAL PRODUCTS (CPMP/BWP/269/05 rev. 3)

ADDENDUM ON THE REPLACEMENT OF RABBIT PYROGEN TESTING BY AN ALTERNATIVE TEST FOR PLASMA DERIVED MEDICINAL PRODUCTS (EMEA/CHMP/BWP/452081/2007)

DRAFT AGREED BY BIOLOGICS WORKING PARTY (BWP)	December 2007
ADOPTION BY CHMP FOR RELEASE FOR CONSULTATION	18th December 2007
END OF CONSULTATION (DEADLINE FOR COMMENTS)	30th June 2008

Comments should be provided using this template to alberto.ganan@emea.europa.eu
Fax +44 20 7418 8545

KEYWORDS	*Plasma-derived medicinal products, Rabbit pyrogen testing, bacterial endotoxin test, LAL test, Monocyte activation test*

Note: This addendum will be incorporated as a part of the Note for Guidance on plasma derived medicinal products which is currently under revision. This addendum has been released independently to the guideline in order to coordinate with complementary work that is being undertaken by the European Pharmacopoeia group 6B.

7 Westferry Circus, Canary Wharf, London, E14 4HB, UK
Tel. (44-20) 74 18 84 00 Fax (44-20) 74 18 85 45
E-mail: mail@emea.europa.eu http://www.emea.europa.eu

ADDENDUM ON THE REPLACEMENT OF RABBIT PYROGEN TESTING BY AN ALTERNATIVE TEST FOR PLASMA DERIVED MEDICINAL PRODUCTS

INTRODUCTION

Parenteral preparations have to be pyrogen-free because administration of pyrogens may induce fever, shock or even death. The severity of the adverse reaction depends on the concentration and biological activity of the respective pyrogen. There is a broad spectrum of pyrogens which are classified into endotoxin and non-endotoxin pyrogens.

Endotoxins, representing the lipopolysaccharides (LPS) of the cell wall of Gram-negative bacteria, are the best characterised and the most potent pyrogens. The structural identity of most non-endotoxin pyrogens has not yet been clarified. Examples of pyrogens from Gram-positive bacteria are the lipoteichoic acids and peptidoglycans which are constituents of the bacterial cell wall.

Currently, there are two tests described in the European Pharmacopoeia which are related to pyrogen testing of parenteral medicinal products:

- the rabbit pyrogen test (RPT) which is considered to detect most of the pyrogens, i.e. endotoxins and non-endotoxin pyrogens,
- and the bacterial endotoxin test, i.e.Limulus polyphemus amoebocyte Lysate- test (LAL-test) which is used to detect or quantify endotoxins of Gram-negative bacteria.

Because endotoxins are the most common and potent pyrogens the LAL testing has successfully replaced the rabbit pyrogen test for many products.

A revision of the European Pharmacopoeia monographs for plasma derived-medicinal products (e.g. human albumin, human normal immunoglobulin, human immunoglobulin for intravenous administration and human blood coagulation factor VIII) is currently on-going to encourage use of alternative tests to the rabbit pyrogen test. The proposed text for the revision is:
"It complies with the text for pyrogens or, preferably and where justified and authorised, a validated *in vitro* test, such as the bacterial endotoxins test. When the bacterial endotoxins test is used, the preparation to be examined contains less than ... IU/ml (or IU/IU)."

The purpose of this guidance is to highlight points to be addressed in any justification for use of a test for bacterial endotoxins as an alternative to a test for pyrogens for plasma-derived medicinal products. It should be read in conjunction with the Ph. Eur. method of analysis for Bacterial Endotoxins (2.6.14) and the accompanying guidance.

LAL-TEST RELATED ISSUES

Interference with endotoxin detection

Several plasma proteins, e.g. lipopolysaccharide binding protein (LBP), soluble CD14, high density lipoproteins (HDL), low density lipoproteins (LDL) and human serum albumin are able to bind endotoxins. This can lead to masking of endotoxins and impairment of their detection in the LAL-test. In addition, these endotoxins may detach from the carrier-protein in vivo and may induce adverse reactions. Given the seriousness of this issue, validation of the LAL test should take it into consideration as indicated in Chapter 2.6.14 of the European Pharmacopoeia.

Plasma fractions or plasma derived medicinal products may inherently contain constituents which may interfere with different LAL methods. Therefore, manufacturers should carefully validate the LAL test for a specific product according to Ph. Eur. 2.6.14.

PROCESS RELATED CONSIDERATIONS

Fever inducing cytokines in plasma derivatives

The main fever inducing cytokines interleukin-1ß (IL-1ß), interleukin-6 (IL-6) and tumour necrosis factor alpha (TNFα) are natural constituents of human plasma and therefore also of plasma pools for fractionation. These cytokines are not detected by the endotoxin test. During plasma fractionation process, the fever inducing cytokines could be enriched in certain fractions and injection of these cytokines as part of a medicinal product may cause pyrogenic adverse reactions in the recipient. Therefore, the content of plasma fractions and finished products for pro-inflammatory cytokines, especially of IL-1, IL-6 and TNFα, should be followed. It should be validated that the finished products consistently contain non-pathophysiologically relevant concentrations of fever inducing cytokines.

For marketed products, where there is extensive clinical experience with no indications of pyrogenic adverse events, an investigation for pro-inflammatory cytokines would not be required as part of the justification to move to LAL testing.

Role of contamination of manufacturing process by Gram-positive bacteria

Plasma derived medicinal products manufacture presents specific features with regard to microbiological contamination, due to the possibility of initial contamination of plasma units and due to the risk of introducing bacterial contaminations during the early steps of processing. In order to keep contamination as low as possible, and to identify possible steps which could contribute to contaminations, the manufacturer should demonstrate that the microbiological contamination level at different critical steps of the process is under control. For the purpose of process validation identification of bacteria species should be performed for relevant manufacturing steps. Bioburden limits should be set, using actual manufacturing data. These bioburden limits are part of the validation that should be performed in order to allow the switch from pyrogen testing to bacterial endotoxin testing.

In the case that bioburden action limits are exceeded after implementation of the LAL testing, a thorough root cause analysis should be carried out by the manufacturer. The microorganisms should be identified at the species level. In the case that gram-positive bacterial contaminations are identified, and after a careful risk assessment, if the manufacturer still considers the release of the batch, it should also be based on the RPT.

If bioburden action limits are exceeded and gram-positive bacterial contaminations are identified on more than one occasion, the applicability of the LAL-test as the replacement of the pyrogen test may not be possible any longer and it may be necessary to re-validate the process with respect to microbial contaminations. In this latter case, the Competent Authority should be informed.

CLINICAL CONSIDERATIONS

The manufacturer should review the adverse events reported with the product for any indications of clinical problems that could possibly be related to the presence of pyrogens in some batches that have not been detected by the LAL test. If there are indications of possible bacterial contamination, the root cause and the causative agent should be identified in order to avoid the contamination of future batches and to select appropriate testing for routine batch monitoring.

REGULATORY ISSUES

Beside showing suitability of the LAL test and appropriate validation of the assay according to Ph. Eur. (chapter 2.6.14) the additional points discussed above should be considered for each product and process separately. The endotoxin limit depends on the product and the route of administration. A

specification should be established for each product considering the calculation provided in Ph. Eur. 2.6.14, the results from LAL testing of a large number of batches and minimal requirements given in the product specific Ph. Eur. monographs.

If a manufacturer of an already licensed plasma derived medicinal product intends to change from the rabbit pyrogen test to an endotoxin test, a variation should be provided including the microbiological evaluation of the manufacturing process and specifications for in-process controls for bioburden at critical steps, validation of the LAL test in compliance with Ph. Eur. 2.6.14 together with data on parallel testing of the product with the RPT and the LAL-test. The product tested in parallel should reflect the current manufacturing process. Historical data may support the safety history of the manufacturing process (manufacturing site). If a batch was positive by the RPT but not tested by LAL and samples are still available, the manufacturer should test these samples by the LAL test to verify if the LAL is positive or not. The number of batches of a given product to be tested in parallel depends on the product's safety history, e.g. historical number of pyrogenic batches, number of RPT positive but LAL negative batches and reports on adverse drug reactions which might be related to pyrogens. Further parallel testing may be required after approval of the variation in cases where data on parallel testing on a significant number of batches cannot be obtained in an adequate time due to the small number of batches manufactured over a year (e.g. products for rare indications). In these cases the testing schedule should cover additional 2 to 3 years of manufacture.

A manufacturer developing a new medicinal product should be working towards use of an alternative to the pyrogen test from the beginning of its development. The manufacturer should establish a validated manufacturing process under special consideration of a bioburden programme. Considering product specific issues and the manufacturing consistency as well as information received from pyrogen testing and clinical studies, an appropriate endotoxin limit should be introduced and justified. Data on release testing of the finished product with the RPT and LAL tests on as many as possible batches should be provided with the application. After licensing, the manufacturer should perform parallel testing on a significant number of batches or for another 2 to 3 years depending on the data provided with the application, the number of batches manufactured over a year and the risk assessment.

All applications should provide a risk assessment for the respective plasma-derived medicinal product considering the potential of a non-endotoxin pyrogen burden of the final product. A product which failed validated bacterial endotoxin testing should not be released based on the rabbit pyrogen test.

ALTERNATIVE TEST

In the past ten years, a new principle in pyrogen testing has been developed, the so called Monocyte Activation Tests (MAT). These tests utilise human monocytes from different sources (human whole blood, human peripheral mononuclear cells, monocytic cell lines from human origin). All of them mimic the human fever reaction in vitro, i.e. the sample to be analysed is incubated with monocytes followed by determination of fever inducing cytokines (usually in ELISA). These tests are potentially able to detect both endotoxins and non-endotoxin pyrogens. In some cases, their application allowed detection of pyrogenicity in preparations which produced fever in patients although the RPT as well the LAL-test were negative.

Several Monocyte Activation Tests have been successfully examined in an international validation study organised by the European Centre for the Validation of Alternative Methods (ECVAM, Italy). This study included parenteralia for intravenous administration which have been artificially contaminated with endotoxin. Analysing the validation study, the ECVAM Scientific Advisory Board (ESAC) gave a positive assessment regarding the applicability of the involved MAT in endotoxin testing. The EDQM installed an Expert Group with the aim to prepare a monograph "Monocyte Activation Test" for the European Pharmacopoeia. Furthermore, the Interagency Coordinating Committee on Validation of Alternative Methods (ICCVAM, USA) is preparing a respective guideline for the application of MAT in USA. Due to the intrinsic variability of the biological reagents involved

in MAT, its application may require the same type of intra assay validation as in previous assays such as LAL.

This guidance does not specifically address justification needed for use of MAT tests as an alternative to a test for pyrogens for plasma-derived medicinal products. Nevertheless, a number of aspects of this guidance would also be relevant for such a justification (e.g. appropriate validation of the assay, parallel testing of the product).