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

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EXECUTIVE SUMMARY

This guideline lays down the requirements 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.

This guidance document specifically addresses the LAL-testing related issues, process related considerations, clinical considerations and regulatory aspects to be considered when replacing the test for pyrogens by an alternative test.

1. 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.

In a revision of the European Pharmacopoeia monographs for plasma derived medicinal products the use of alternative tests to the rabbit pyrogen test is encouraged.

2. SCOPE

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 medicinal products derived from human blood and human plasma (hereinafter called "plasma derived medicinal products").

3. LEGAL BASIS

Plasma derived medicinal products fall under the definition of Article 1(10) of Directive 2001/83/EC as follows: “Medicinal products based on blood constituents which are prepared industrially by public or private establishments, such medicinal products including, in particular, albumin, coagulating factors and immunoglobulins of human origin.” Furthermore, the pharmaceutical legislation also applies to plasma that is prepared by a method involving an industrial process (Article 3(6) of Directive 2001/83/EC). Solvent-detergent treated plasma is an example of this latter category.

This guideline also covers plasma derivatives used as:

- Excipients
- Ancillary substances in medical devices (Directive 2000/70/EC amending Directive 93/42/EEC)
- Investigational products as such or as excipients.

In accordance with article 3 (sections 1, 2 and 6) of Directive 2001/83/EC, the scope does not cover blood, blood components or medicinal products prepared on a small scale for individual patients in accordance with a medical prescription.

This guideline has to be read in conjunction with the Ph. Eur. method of analysis for Bacterial Endotoxins (2.6.14) and the accompanying guidance as well as the Ph. Eur. specific monographs for plasma derived medicinal products.

4. 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.

5. 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 (TNF-α) are natural constituents of human plasma and therefore also of plasma pools for fractionation. These cytokines are not detected by the endotoxin test. During the 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 TNF-α, should be followed. It should be validated that the finished products consistently contain non-pathophysiologically relevant concentrations of fever inducing cytokines.

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 contamination during the early steps of processing. In order to keep contamination as low as possible, and to identify possible steps which could contribute to contamination, 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 contamination is identified, if the manufacturer still considers the release of the batch, this should be based on a negative LAL, a careful risk assessment and also on the RPT.

If bioburden action limits are exceeded and gram-positive contaminations are identified on consecutive occasions although a root cause analysis and corrective actions have been performed, the Competent Authority should be informed.

6. 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.

7. REGULATORY ISSUES

Besides 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 a risk assessment for the respective plasma derived medicinal product considering the potential of a non-endotoxin pyrogen burden of the final product, 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 an additional 2 to 3 years of manufacture. During such intermediate periods, further experience is needed and the RPT and the endotoxin test are applied in parallel and batches cannot be released when the results of the two tests are discrepant. A product which failed validated bacterial endotoxin testing after approval of the change should not be released based on the RPT. Where there is extensive clinical experience with no indications of pyrogenic adverse events, an investigation for pro-inflammatory cytokines in plasma fractions and finished product would not be required as part of the justification to move to LAL testing.

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 with 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 defined and justified. A risk assessment for the respective plasma derived medicinal product considering the potential of a non-endotoxin pyrogen burden of the final product should be provided. Data on release testing of the finished product with the RPT and LAL tests on as many batches as possible should be provided with the application. If there are not enough data to license the new product with the alternative test for batch release without further parallel testing, then the time period and/or number of batches using the RPT and LAL test in parallel should be defined during the licensing procedure. At this time batch release should be based on the licensed test, the RPT. After the agreed time of parallel testing a variation for the change to an alternative test should be submitted.

8. 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 the release of fever inducing cytokines (usually in ELISA). 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.

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). The EMEA encourages every effort to further develop and establish this type of alternative test.

REFERENCES

- Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the community code relating to medicinal products for human use

- Directive 2000/70/EC of the European Parliament and of the Council of 16 November 2000 amending Council Directive 93/42/EEC as regards medical devices incorporating stable derivates of human blood or human plasma

- Ph. Eur. general chapter on Bacterial endotoxin test (2.6.14)

- Ph. Eur. general chapter on Pyrogens (2.6.8)

- Ph. Eur. specific monographs for plasma derived medicinal products