Quantification of endotoxins from gram-negative bacteria using recombinant factor C assay

Commonly used acronym: *rFC*

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Organisation

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SCOPE OF THE METHOD

The Method relates to	Human health
The Method is situated in	Regulatory use - Routine production
Type of method	Other: The test is based on the gene sequence of the horseshoe crab (instead of the blood of horseshoe crab itself)

DESCRIPTION

Method keywords

LAL

rFC

recombinant factor c

endotoxins

pyrogens

european pharmacopoeia

Scientific area keywords

EDQM

OCABR

OMCL

quality control

vaccine

analytical chemistry

Method description

Endotoxins are lipopolysaccharides anchored at the outer membrane of gram-negative bacteria. These pyrogenic compounds can be introduced in pharmaceutical products during their manufacture and could induce severe physiological reactions in humans. This is why endotoxins are dosed, in order to assure quality and safety of products. Testing of endotoxins is well described in the Ph. Eur. (Chapters 2.6.14 & 5.1.10) and several detection methods exist, such as animal-derived Limulus amoebocyte lysate (LAL) assays, which are widely used. However, these have limitations such as the use of animals, a high lot to lot variability and interference of complex components such as beta-glucans. Since 2021, assay using recombinant factor C (rFC) is considered as alternative method in the Ph. Eur. (Chapter 2.6.32.). This method inspired by the LAL assay has the advantages to be animal free and to avoid interference of beta-glucans. It is based on the gene sequence of the horseshoe crab, using a fluorimetric method. This is an end-point detection method (correlation log/log between delta relative fluoresence unit RFU and the concentration of endotoxins). It is performed thanks to a kit bought from a supplier, that includes the microplate and reagents to perform the tests.

Lab equipment

fluorimeter

Method status

Internally validated

Validated by an external party (e.g. OECD, EURL ECVAM,…)

REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION