THE EUROPEAN DIRECTORATE FOR THE QUALITY OF MEDICINES & HEALTHCARE (EDQM)

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European Directorate for the Quality of Medicines & HealthCare | Direction européenne de la qualité du médicament & soins de santé

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Using recombinant factor C for bacterial endotoxin testing in the Ph. Eur. how far have we come, how far have we to go?

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Structure

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- Part V. Is chapter 2.6.32 legally binding?
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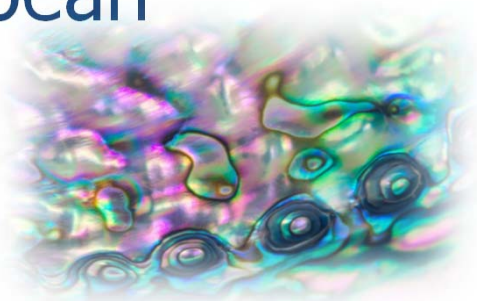
- Conclusion

how far have we come,
how far have we to go?

Part I. Council of Europe, EDQM and European Pharmacopoeia



The Council of Europe: the EDQM's parent organisation

- Founded in 1949
- Headquarters in Strasbourg, France
- 47 MEMBER STATES
  >820 million citizens
- The oldest pan-European organisation dedicated to fostering co-operation in Europe
  - Promotes DEMOCRACY
  - Protects THE RULE OF LAW
  - Protects HUMAN RIGHTS

European Directorate for the Quality of Medicines & HealthCare

- A Council of Europe Directorate, based on the Convention on the Elaboration of a European Pharmacopoeia (PA, 1964)

- Mission: to contribute to the basic human right of access to GOOD QUALITY MEDICINES AND HEALTHCARE

European Pharmacopoeia in 2021

- Protecting public health – one common compulsory quality standard
- Applied by all licencing authorities
- Legally binding for all medicinal products

- Mandatory on the same date for all Members

- 40 Members (39 Member States & EU)
- 30 Observers (5 European, 23 non-European countries, TFDA, WHO)
- 10th Edition (including Supplement 10.5): 2447 monographs, 378 general texts

Content and structure of the Ph. Eur.

General notices

- ✓ Alternative methods
- ✓ Validation of methods
- ✓ Implementation of methods
- ✓ Compliance with the pharmacopoeia

General chapters

- ✓ General methods & texts
- ✓ Editorial convenience
- ✓ Not mandatory "*per se*"
- ✓ Part of the standard when referred to in a monograph
- ✓ **Can be used** when there is no monograph → the method described requires product specific validation

General monographs

- ✓ Classes of substances and dosage forms
- ✓ Mandatory for all substances/preparations within the scope of the definition
- ✓ Not cross-referenced in individual monographs

Individual monographs

- ✓ Substance/product-based
- ✓ Specific
- ✓ Not stand-alone
- ✓ Take account of approved products

Part II. Tests for bacterial endotoxins

Test for bacterial endotoxins

- **Bacterial endotoxins (chapter *2.6.14*)**

  Uses a 'natural' reagent:

  LAL = Limulus amoebocyte lysate

  from the horseshoe crab

  Limulus polyphemus or *Tachypleus tridentatus*

  - 1979: first discussions on the topic
  - 1987: first publication of the chapter in the Ph. Eur: chapter *V.2.1.9 Bacterial endotoxins* (11 fascicule, 2nd Edition)
  - 1992: first discussions engaged towards International Harmonisation between USP, JP and Ph. Eur.
  - 2000: sign-off by PDG (coordinating pharmacopoeia: JP)

2.6.14 Test for bacterial endotoxins

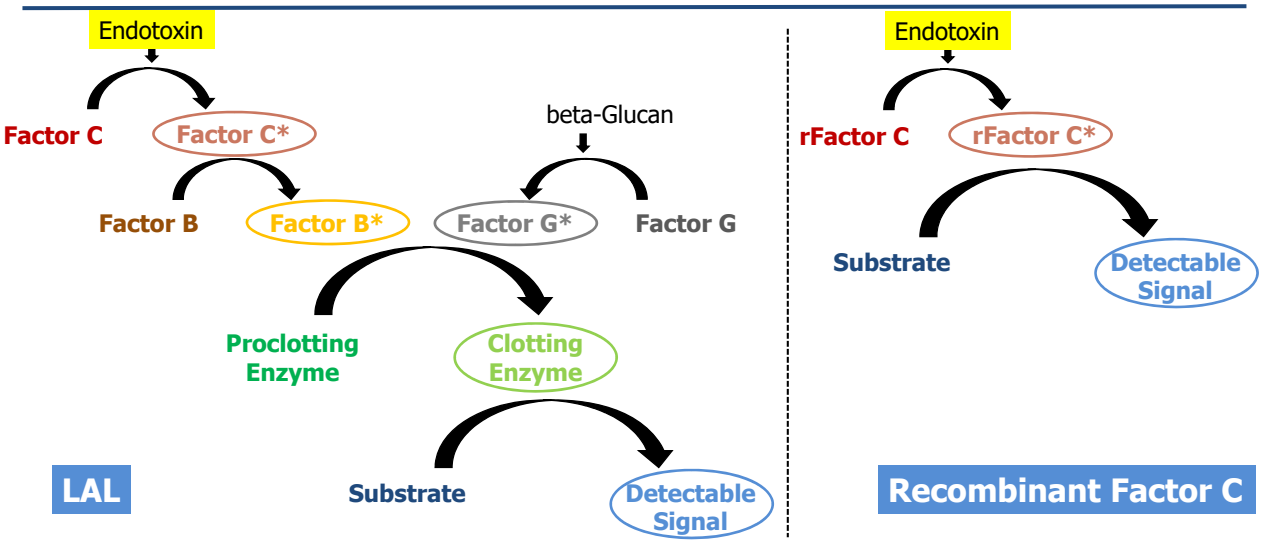
Method A. Gel-clot method: limit test
Method B. Gel-clot method: semi-quantitative test
Method C. Turbidimetric kinetic method
Method D. Chromogenic kinetic method
Method E. Chromogenic end-point method
Method F. Turbidimetric end-point method

Limulus amoebocyte lysate (LAL)

At least 4 suppliers

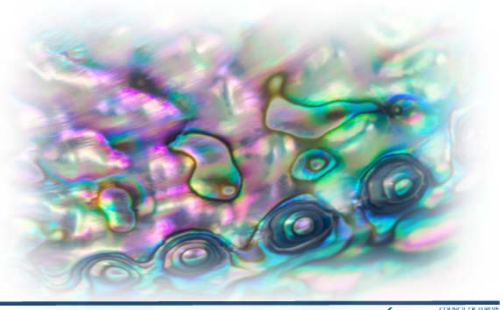
Many possible experimental configurations!

LAL … versus rFC



Part III. A brief history of Ph. Eur. Chapter 2.6.32

Chapter *2.6.32 Test for bacterial endotoxins using recombinant factor C*



Initial phase 2006-2013

- 2006: first brought to the attention of EDQM
- 2008: added to the work programme of the European Pharmacopoeia
- 2013: withdrawn from the work programme

➢ *only one rFC assay kit available*

➢ *more data on multi-product applications were needed*

Additional information required before engaging on this topic

July 2015: Viewpoint of the PDG

Ver2.1

Pharmacopoeial Discussion Group Meeting
30 June-1 July 2015

Meeting Highlights

PMDA office
Shin-Kasumigaseki Building, 3-3-2 Kasumigaseki, Chiyoda-ku,
Tokyo, JAPAN

3.2. Revision Proposals

3.2.1. Q-06 Bacterial Endotoxins (JP)

Following requests from users to include recombinant factor C reagent as an alternative to the animal-sourced Limulus Amoebocyte Lysate (LAL) reagent in the general chapter on bacterial endotoxins, PDG decided not to revise the chapter at this stage and limit its use strictly to LAL.

Additional information required before engaging on this topic

https://www.edqm.eu/sites/default/files/PDG062015.pdf

2016: Opening the door…

New sentence in chapter *5.1.10* Supplement 8.8 (07/2016):

"The use of alternative reagents such as recombinant factor C as a replacement to the amoebocyte lysate eliminates the use of a reagent extracted from live animals."

2017: The revival

- Project resumed in 2017, in light of new developments, including:
  - ✓rFC assay kits from several suppliers had become available (kits from 2 manufacturers in Europe)
  - ✓Increasing number and diversity of products on which validation had been performed
  - ✓Independent data published by JP (collaborative study results in Kikuchi, et al. 2017, comparison of 3 rFC and 3 LAL-based kits on 18 commercially available LPS types and 11 NOEs in water)
  - ✓Other publications with rFC/LAL comparability data e.g. articles from Eli Lilly (Bolden, et al. 2017, data on medicinal products)

2017: The revival

1. Is the information available sufficient for the chapter to be included in the Ph. Eur.?

- ➢Has the situation changed since September 2013, i.e. is the range of products on which validation has been performed sufficiently wide to start the elaboration of a Ph. Eur. general chapter

YES

- ➢Can the validation data be provided to the EDQM?

YES

- ➢Can the writing of such chapter be made independently from the use of a specific commercial reagent?

YES

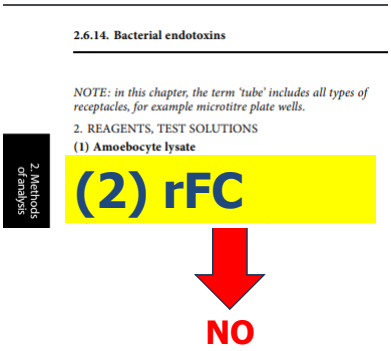
- ➢Are there any major feasibility issues anticipated?

NO

2017: The revival

2. What will be the impact on chapter *2.6.14* (Internationally Harmonised within the Pharmacopoeia Discussion Group)

- Addition of **rFC** as a **reagent** that can be used for the test?



2.6.14. Bacterial endotoxins

NOTE: in this chapter, the term 'tube' includes all types of receptacles, for example microtitre plate wells.

2. REAGENTS, TEST SOLUTIONS

(1) Amoebocyte lysate

2. Methods of analysis

(2) rFC

NO

- Addition of new METHOD G in *2.6.14*
- Needs approval from the PDG
- On hold due to impact on International Harmonisation

- **In the meantime… separate Ph. Eur. chapter**

Chapter *2.6.32*

Chapter *2.6.32*: now an official method of the Ph. Eur.

- Supplement 10.3 (Publication: 1 July 2020; implementation: January 2021)
- Recognised as an official method by the 39 member states of the Ph. Eur. and the EU
- Stand-alone chapter, not referenced in any monograph
- Describes a BET that uses a rFC based on the gene sequence of the horseshoe crab, and a fluorimetric end-point detection method

Chapter *5.1.10*: Guidelines for using the test for bacterial endotoxins

- Revised to reflect the adoption of chapter *2.6.32* and clarify requirements for the introduction of rFC assays by users of the Ph. Eur. (Publication in Suppl. 10.3)
- Implication for users: facilitated implementation

Press release 1 July 2020

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Recombinant factor C: new Ph. Eur. chapter available as of 1 July 2020

Back

EUROPEAN PHARMACOPOEIA GENERAL TEXT/CHAPTER | NEWS | 01 JULY 2020 | STRASBOURG, FRANCE

Published in European Pharmacopoeia (Ph. Eur.) Supplement 10.3, the new general chapter *2.6.32. Test for bacterial endotoxins using recombinant factor C* describes a test for bacterial endotoxins (BET) that can be used as an alternative to the classic limulus amoebocyte lysate (LAL)-based methods for the quantification of endotoxins from gram-negative bacteria. The method described involves the use of a recombinant factor C (rFC) based on the gene sequence of the horseshoe crab and fluorimetric detection, in keeping with the kits currently available on the European market.

Also published in Supplement 10.3 is the revised chapter *5.1.10. Guidelines for using the test for bacterial endotoxins*, which has been updated to reflect the new status of rFC-based methods and give prerequisites for their deployment by users of the pharmacopoeia.

With the introduction of general chapter *2.6.32*, Ph. Eur. users will have the possibility of using a standardised method, which will become official in the 39 signatory countries to the Ph. Eur. convention. A test for bacterial endotoxins using rFC can be used in the same way as LAL-based methods, after demonstration of its fitness for use for the specific substance or product. The use of rFC for BET testing does not need to be validated, thus facilitating the implementation of rFC-based methods by users. It should be noted however, that the replacement of an LAL-based method prescribed in a monograph by an rFC-based method is considered as the use of an *alternative method* as described in the Ph. Eur. General Notices.

For BET testing, the world currently relies on a single source of lysate, the horseshoe crab family, and more specifically, two species of the crab, *Limulus polyphemus* and *Tachypleus tridentatus* (both of which are known to be endangered). The publication of the new general chapter 2.6.32 therefore marks a significant step towards alleviating the need for these animal resources.

The EDQM's Director, Susanne Keitel, pointed out that "When used under appropriate conditions, rFC-based methods provide the same guarantee of a product's compliance with the test for bacterial endotoxins – and therefore, of its safety for use in patients – as LAL-based methods".

The new general chapter *2.6.32* and the revised general chapter *5.1.10* will become effective on 1 January 2021.

https://www.edqm.eu/en/news/recombinant-factor-c-new-ph-eur-chapter-available-1-july-2020

DIRECTOR

Susanne Keitel

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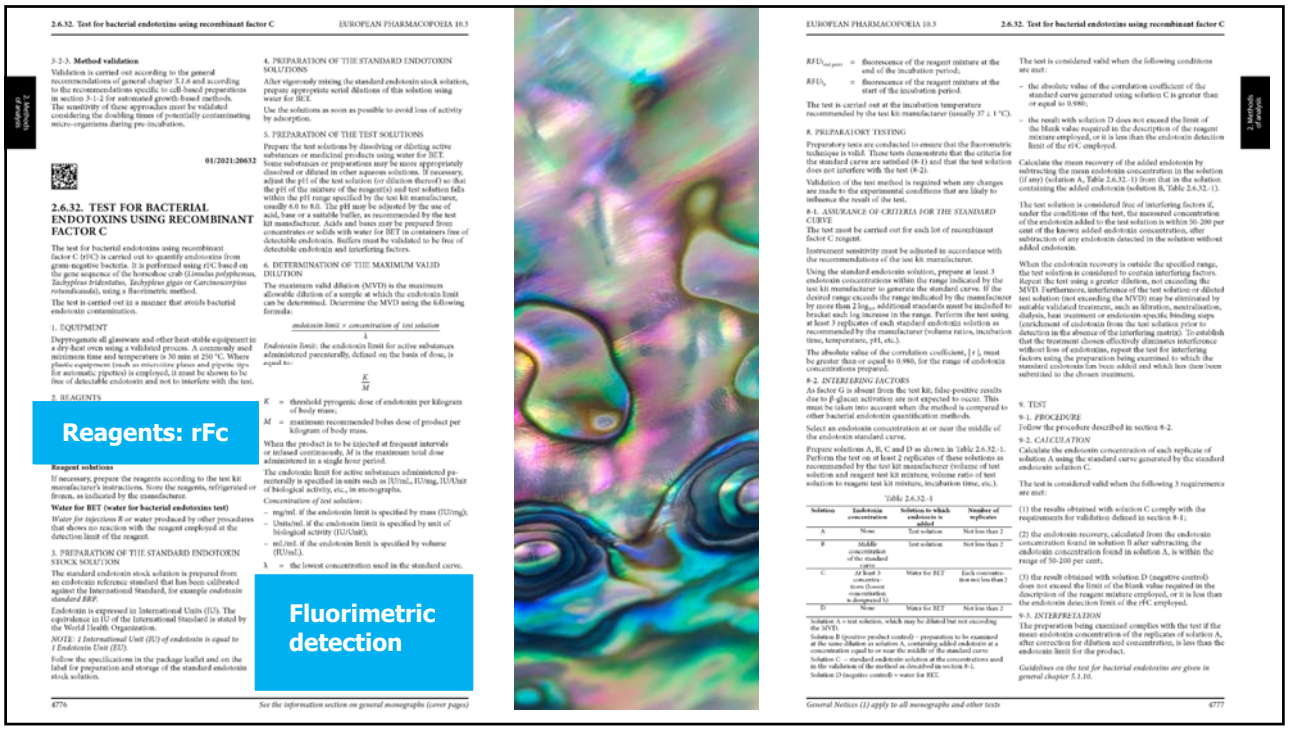
Part IV. Chapter *2.6.32:* What conditions need to be met, What needs to be verified, is validation required?



Ph. Eur. *General notices*

Validation of pharmacopoeial methods

The test methods given in monographs and general chapters have been validated in accordance with accepted scientific practice and current recommendations on analytical validation. Unless otherwise stated in the monograph or general chapter, validation of the test methods by the analyst is not required.

The « empty shell » concept

Test method given in a general chapter

Validation?

… applied to a specific article

Product-specific Validation

The 'empty shell' concept applied to the test for sterility

2.6.1

- ✓ Official pharmacopoeial method
- ✓ Culture media: sterility, growth promotion test

2.6.1 applied to a specific article

- ✓ 'Method suitability test'

Product-specific validation

The 'empty shell' concept applied to rFC

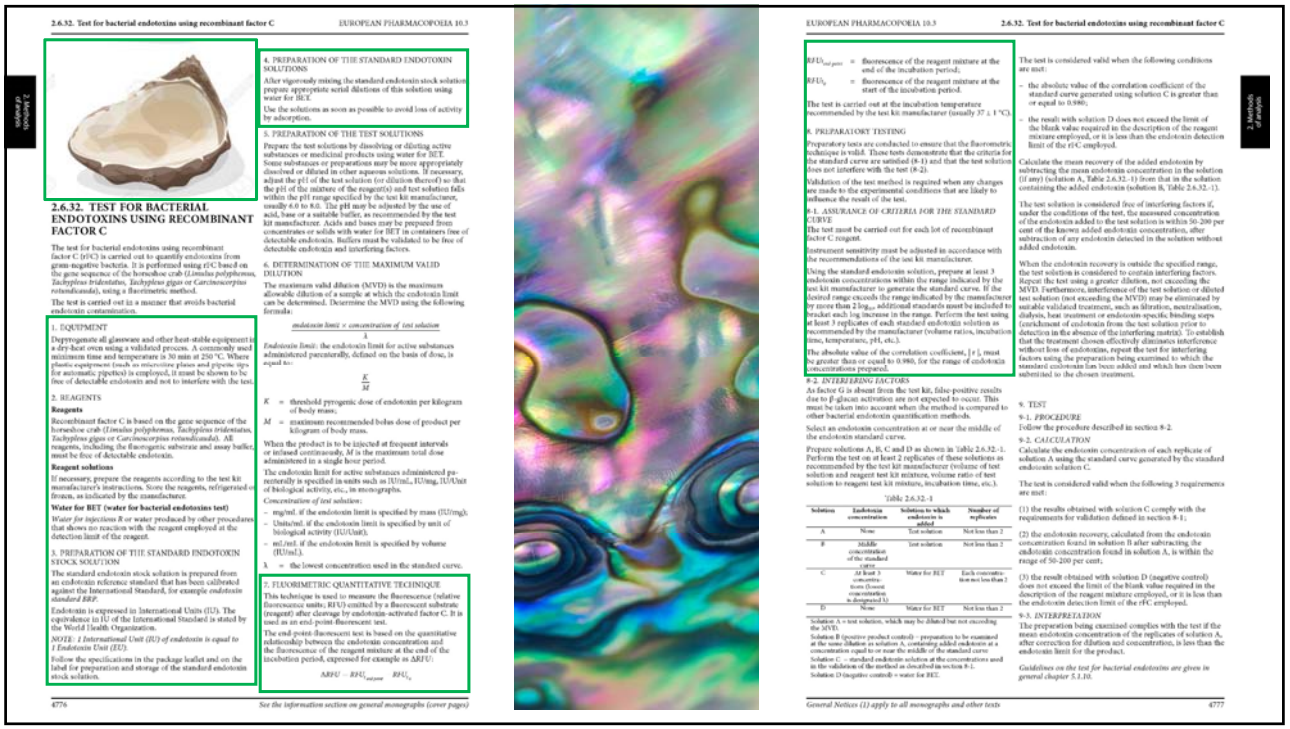
2.6.32

- ✓ **Official pharmacopoeial method**
- ✓ **Sections 1 to 4 and 7 and 8.1 Equipment, reagents, endotoxin solutions, standard curve**

***2.6.32* applied to a specific article**

- ✓ **Sections 5, 6 and 8.2 test solutions, preparatory testing interfering factors**

Product specific validation



2.6.32. Test for bacterial endotoxins using recombinant factor C — EUROPEAN PHARMACOPOEIA 10.3

2.6.32. TEST FOR BACTERIAL ENDOTOXINS USING RECOMBINANT FACTOR C

The test for bacterial endotoxins using recombinant factor C (rFC) is carried out to quantify endotoxins from gram-negative bacteria. It is performed using rFC based on the gene sequence of the horseshoe crab (*Limulus polyphemus*, *Tachypleus tridentatus*, *Tachypleus gigas* or *Carcinoscorpius rotundicauda*), using a fluorimetric method.

The test is carried out in a manner that avoids bacterial endotoxin contamination.

1. EQUIPMENT

Depyrogenate all glassware and other heat-stable equipment in a dry-heat oven using a validated process. A commonly used minimum time and temperature is 30 min at 250 °C. Where plastic equipment (such as microtitre plates and pipette tips for automatic pipettes) is employed, it must be shown to be free of detectable endotoxin and not to interfere with the test.

2. REAGENTS

Reagents

Recombinant factor C is based on the gene sequence of the horseshoe crab (*Limulus polyphemus*, *Tachypleus tridentatus*, *Tachypleus gigas* or *Carcinoscorpius rotundicauda*). All reagents, including the fluorogenic substrate and assay buffer, must be free of detectable endotoxin.

Reagent solutions

If necessary, prepare the reagents according to the test kit manufacturer's instructions. Store the reagents, refrigerated or frozen, as indicated by the manufacturer.

Water for BET (water for bacterial endotoxins test)

Water for injections R or water produced by other procedures that shows no reaction with the reagent employed at the detection limit of the reagent.

3. PREPARATION OF THE STANDARD ENDOTOXIN STOCK SOLUTION

The standard endotoxin stock solution is prepared from an endotoxin reference standard that has been calibrated against the International Standard, for example *endotoxin standard BRP*.

Endotoxin is expressed in International Units (IU). The equivalence in IU of the International Standard is stated by the World Health Organization.

NOTE: 1 International Unit (IU) of endotoxin is equal to 1 Endotoxin Unit (EU).

Follow the specifications in the package leaflet and on the label for preparation and storage of the standard endotoxin stock solution.

4. PREPARATION OF THE STANDARD ENDOTOXIN SOLUTIONS

After vigorously mixing the standard endotoxin stock solution prepare appropriate serial dilutions of this solution using water for BET.

Use the solutions as soon as possible to avoid loss of activity by adsorption.

5. PREPARATION OF THE TEST SOLUTIONS

Prepare the test solutions by dissolving or diluting active substances or medicinal products using water for BET. Some substances or preparations may be more appropriately dissolved or diluted in other aqueous solutions. If necessary, adjust the pH of the test solution (or dilution thereof) so that the pH of the mixture of the reagent(s) and test solution falls within the pH range specified by the test kit manufacturer, usually 6.0 to 8.0. The pH may be adjusted by the use of acid, base or a suitable buffer, as recommended by the test kit manufacturer. Acids and bases may be prepared from concentrates or solids with water for BET in containers free of detectable endotoxin. Buffers must be validated to be free of detectable endotoxin and interfering factors.

6. DETERMINATION OF THE MAXIMUM VALID DILUTION

The maximum valid dilution (MVD) is the maximum allowable dilution of a sample at which the endotoxin limit can be determined. Determine the MVD using the following formula:

$$\frac{\text{endotoxin limit} \times \text{concentration of test solution}}{\lambda}$$

Endotoxin limit: the endotoxin limit for active substances administered parenterally, defined on the basis of dose, is equal to:

$$\frac{K}{M}$$

K = threshold pyrogenic dose of endotoxin per kilogram of body mass;

M = maximum recommended bolus dose of product per kilogram of body mass.

When the product is to be injected at frequent intervals or infused continuously, M is the maximum total dose administered in a single hour period.

The endotoxin limit for active substances administered parenterally is specified in units such as IU/mL, IU/mg, IU/Unit of biological activity, etc., in monographs.

Concentration of test solution:

- mg/mL if the endotoxin limit is specified by mass (IU/mg);
- Units/mL if the endotoxin limit is specified by unit of biological activity (IU/Unit);
- mL/mL if the endotoxin limit is specified by volume (IU/mL).

λ = the lowest concentration used in the standard curve.

7. FLUORIMETRIC QUANTITATIVE TECHNIQUE

This technique is used to measure the fluorescence (relative fluorescence units; RFU) emitted by a fluorescent substrate (reagent) after cleavage by endotoxin-activated factor C. It is used as an end-point-fluorescent test.

The end-point-fluorescent test is based on the quantitative relationship between the endotoxin concentration and the fluorescence of the reagent mixture at the end of the incubation period, expressed for example as ΔRFU:

$$\Delta RFU = RFU_{\text{end point}} - RFU_{0}$$

4776 — *See the information section on general monographs (cover pages)*

EUROPEAN PHARMACOPOEIA 10.3 — 2.6.32. Test for bacterial endotoxins using recombinant factor C

$RFU_{\text{end point}}$ = fluorescence of the reagent mixture at the end of the incubation period;

RFU_{0} = fluorescence of the reagent mixture at the start of the incubation period.

The test is carried out at the incubation temperature recommended by the test kit manufacturer (usually 37 ± 1 °C).

8. PREPARATORY TESTING

Preparatory tests are conducted to ensure that the fluorometric technique is valid. These tests demonstrate that the criteria for the standard curve are satisfied (8-1) and that the test solution does not interfere with the test (8-2).

Validation of the test method is required when any changes are made to the experimental conditions that are likely to influence the result of the test.

8-1. ASSURANCE OF CRITERIA FOR THE STANDARD CURVE

The test must be carried out for each lot of recombinant factor C reagent.

Instrument sensitivity must be adjusted in accordance with the recommendations of the test kit manufacturer.

Using the standard endotoxin solution, prepare at least 3 endotoxin concentrations within the range indicated by the test kit manufacturer to generate the standard curve. If the desired range exceeds the range indicated by the manufacturer by more than 2 $\log_{10}$, additional standards must be included to bracket each log increase in the range. Perform the test using at least 3 replicates of each standard endotoxin solution as recommended by the manufacturer (volume ratios, incubation time, temperature, pH, etc.).

The absolute value of the correlation coefficient, $|r|$, must be greater than or equal to 0.980, for the range of endotoxin concentrations prepared.

8-2. INTERFERING FACTORS

As factor G is absent from the test kit, false-positive results due to β-glucan activation are not expected to occur. This must be taken into account when the method is compared to other bacterial endotoxin quantification methods.

Select an endotoxin concentration at or near the middle of the endotoxin standard curve.

Prepare solutions A, B, C and D as shown in Table 2.6.32.-1. Perform the test on at least 2 replicates of these solutions as recommended by the test kit manufacturer (volume of test solution and reagent test kit mixture, volume ratio of test solution to reagent test kit mixture, incubation time, etc.).

Table 2.6.32.-1

Solution	Endotoxin concentration	Solution to which endotoxin is added	Number of replicates
A	None	Test solution	Not less than 2
B	Middle concentration of the standard curve	Test solution	Not less than 2
C	At least 3 concentrations (lowest concentration is designated λ)	Water for BET	Each concentration not less than 2
D	None	Water for BET	Not less than 2

Solution A = test solution, which may be diluted but not exceeding the MVD.

Solution B (positive product control) = preparation to be examined at the same dilution as solution A, containing added endotoxin at a concentration equal to or near the middle of the standard curve.

Solution C = standard endotoxin solution at the concentrations used in the validation of the method as described in section 8-1.

Solution D (negative control) = water for BET.

The test is considered valid when the following conditions are met:

- the absolute value of the correlation coefficient of the standard curve generated using solution C is greater than or equal to 0.980;
- the result with solution D does not exceed the limit of the blank value required in the description of the reagent mixture employed, or it is less than the endotoxin detection limit of the rFC employed.

Calculate the mean recovery of the added endotoxin by subtracting the mean endotoxin concentration in the solution (if any) (solution A, Table 2.6.32.-1) from that in the solution containing the added endotoxin (solution B, Table 2.6.32.-1).

The test solution is considered free of interfering factors if, under the conditions of the test, the measured concentration of the endotoxin added to the test solution is within 50-200 per cent of the known added endotoxin concentration, after subtraction of any endotoxin detected in the solution without added endotoxin.

When the endotoxin recovery is outside the specified range, the test solution is considered to contain interfering factors. Repeat the test using a greater dilution, not exceeding the MVD. Furthermore, interference of the test solution or diluted test solution (not exceeding the MVD) may be eliminated by suitable validated treatment, such as filtration, neutralisation, dialysis, heat treatment or endotoxin-specific binding sorps (enrichment of endotoxin from the test solution prior to detection in the absence of the interfering matrix). To establish that the treatment chosen effectively diminishes interference without loss of endotoxins, repeat the test for interfering factors using the preparation being examined to which the standard endotoxin has been added and which has then been submitted to the chosen treatment.

9. TEST

9-1. PROCEDURE

Follow the procedure described in section 8-2.

9-2. CALCULATION

Calculate the endotoxin concentration of each replicate of solution A using the standard curve generated by the standard endotoxin solution C.

The test is considered valid when the following 3 requirements are met:

(1) the results obtained with solution C comply with the requirements for validation defined in section 8-1;

(2) the endotoxin recovery, calculated from the endotoxin concentration found in solution B after subtracting the endotoxin concentration found in solution A, is within the range of 50-200 per cent;

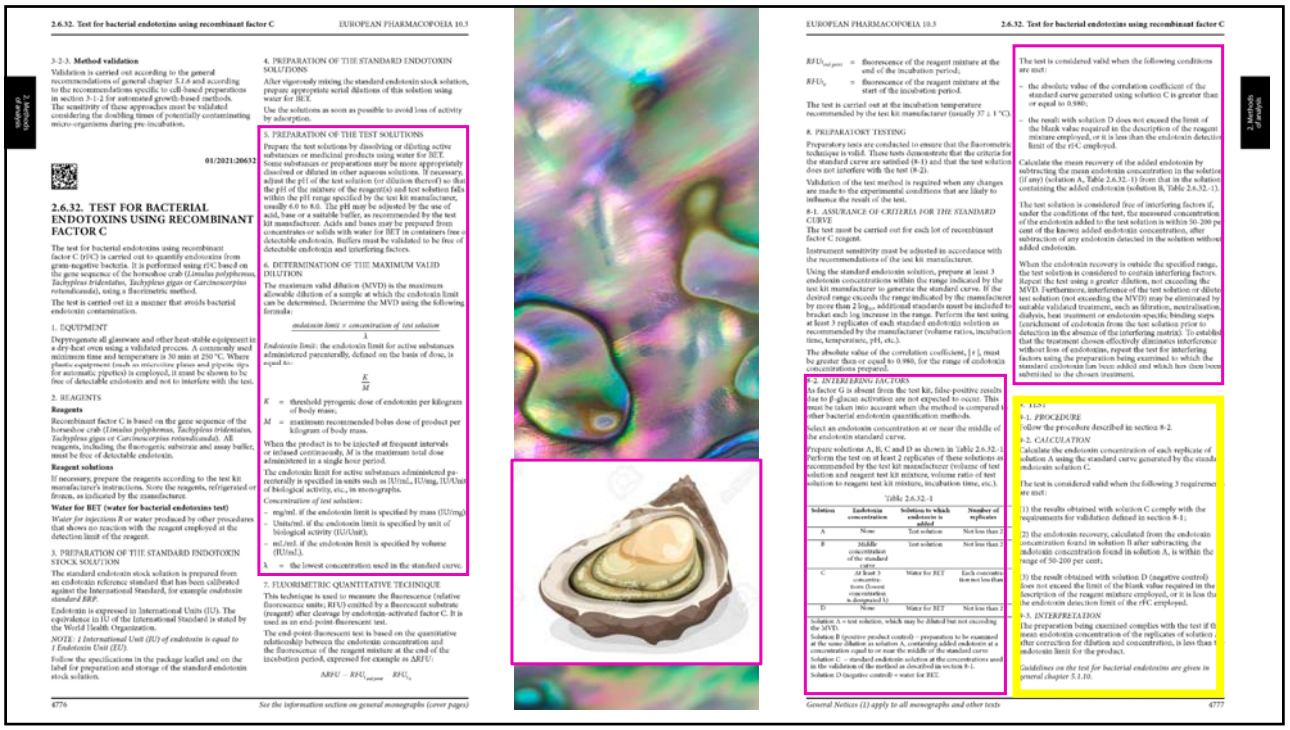
(3) the result obtained with solution D (negative control) does not exceed the limit of the blank value required in the description of the reagent mixture employed, or it is less than the endotoxin detection limit of the rFC employed.

9-3. INTERPRETATION

The preparation being examined complies with the test if the mean endotoxin concentration of the replicates of solution A, after correction for dilution and concentration, is less than the endotoxin limit for the product.

Guidelines on the test for bacterial endotoxins are given in general chapter 5.1.10.

General Notices (1) apply to all monographs and other texts — 4777



2.6.32. Test for bacterial endotoxins using recombinant factor C — EUROPEAN PHARMACOPOEIA 10.3

3-2-3. **Method validation**

Validation is carried out according to the general recommendations of general chapter 5.1.6 and according to the recommendations specific to cell-based preparations in section 3-1-2 for automated growth-based methods. The sensitivity of these approaches must be validated considering the doubling times of potentially contaminating micro-organisms during pre-incubation.

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$$\Delta RFU = RFU_{\text{end point}} - RFU_{t_0}$$

4776 — *See the information section on general monographs (cover pages)*

EUROPEAN PHARMACOPOEIA 10.3 — 2.6.32. Test for bacterial endotoxins using recombinant factor C

$RFU_{\text{end point}}$ = fluorescence of the reagent mixture at the end of the incubation period;

RFU_{t_0} = fluorescence of the reagent mixture at the start of the incubation period.

The test is carried out at the incubation temperature recommended by the test kit manufacturer (usually 37 ± 1 °C).

8. PREPARATORY TESTING

Preparatory tests are conducted to ensure that the fluorometric technique is valid. These tests demonstrate that the criteria for the standard curve are satisfied (8-1) and that the test solution does not interfere with the test (8-2).

Validation of the test method is required when any changes are made to the experimental conditions that are likely to influence the result of the test.

8-1. *ASSURANCE OF CRITERIA FOR THE STANDARD CURVE*

The test must be carried out for each lot of recombinant factor C reagent.

Instrument sensitivity must be adjusted in accordance with the recommendations of the test kit manufacturer.

Using the standard endotoxin solution, prepare at least 3 endotoxin concentrations within the range indicated by the test kit manufacturer to generate the standard curve. If the desired range exceeds the range indicated by the manufacturer by more than 2 $\log_{10}$, additional standards must be included to bracket each log increase in the range. Perform the test using at least 3 replicates of each standard endotoxin solution as recommended by the manufacturer (volume ratios, incubation time, temperature, pH, etc.).

The absolute value of the correlation coefficient, $|r|$, must be greater than or equal to 0.980, for the range of endotoxin concentrations prepared.

8-2. *INTERFERING FACTORS*

As factor G is absent from the test kit, false-positive results due to β-glucan activation are not expected to occur. This must be taken into account when the method is compared to other bacterial endotoxin quantification methods.

Select an endotoxin concentration at or near the middle of the endotoxin standard curve.

Prepare solutions A, B, C and D as shown in Table 2.6.32.-1. Perform the test on at least 2 replicates of these solutions as recommended by the test kit manufacturer (volume of test solution and reagent test kit mixture, volume ratio of test solution to reagent test kit mixture, incubation time, etc.).

Table 2.6.32.-1

Solution	Endotoxin concentration	Solution to which endotoxin is added	Number of replicates
A	None	Test solution	Not less than 2
B	Middle concentration of the standard curve	Test solution	Not less than 2
C	At least 3 concentrations (lowest concentration is designated λ)	Water for BET	Each concentration not less than 2
D	None	Water for BET	Not less than 2

Solution A = test solution, which may be diluted but not exceeding the MVD.

Solution B (positive product control) = preparation to be examined at the same dilution in solution A, containing added endotoxin at a concentration equal to or near the middle of the standard curve.

Solution C = standard endotoxin solution at the concentrations used in the validation of the method as described in section 8-1.

Solution D (negative control) = water for BET.

The test is considered valid when the following conditions are met:

- the absolute value of the correlation coefficient of the standard curve generated using solution C is greater than or equal to 0.980;
- the result with solution D does not exceed the limit of the blank value required in the description of the reagent mixture employed, or it is less than the endotoxin detection limit of the rFC employed.

Calculate the mean recovery of the added endotoxin by subtracting the mean endotoxin concentration in the solution (if any) (solution A, Table 2.6.32.-1) from that in the solution containing the added endotoxin (solution B, Table 2.6.32.-1).

The test solution is considered free of interfering factors if, under the conditions of the test, the measured concentration of the endotoxin added to the test solution is within 50-200 per cent of the known added endotoxin concentration, after subtraction of any endotoxin detected in the solution without added endotoxin.

When the endotoxin recovery is outside the specified range, the test solution is considered to contain interfering factors. Repeat the test using a greater dilution, not exceeding the MVD. Furthermore, interference of the test solution or dilute test solution (not exceeding the MVD) may be eliminated by suitable validated treatment, such as filtration, neutralisation, dialysis, heat treatment or endotoxin-specific binding steps (enrichment of endotoxin from the test solution prior to detection in the absence of the interfering matrix). To establish that the treatment chosen effectively diminishes interference without loss of endotoxins, repeat the test for interfering factors using the preparation being examined to which the standard endotoxin has been added and which has then been submitted to the chosen treatment.

9. TEST

9-1. *PROCEDURE*

Follow the procedure described in section 8-2.

9-2. *CALCULATION*

Calculate the endotoxin concentration of each replicate of solution A using the standard curve generated by the standard endotoxin solution C.

The test is considered valid when the following 3 requirements are met:

1) the results obtained with solution C comply with the requirements for validation defined in section 8-1;

2) the endotoxin recovery, calculated from the endotoxin concentration found in solution B after subtracting the endotoxin concentration found in solution A, is within the range of 50-200 per cent;

3) the result obtained with solution D (negative control) does not exceed the limit of the blank value required in the description of the reagent mixture employed, or it is less than the endotoxin detection limit of the rFC employed.

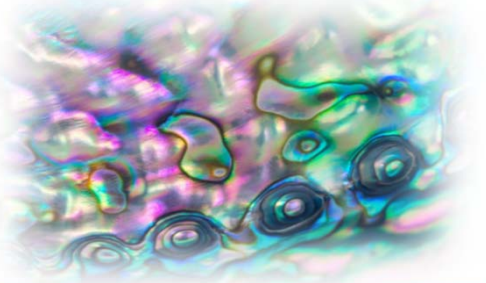
9-3. *INTERPRETATION*

The preparation being examined complies with the test if the mean endotoxin concentration of the replicates of solution A, after correction for dilution and concentration, is less than the endotoxin limit for the product.

Guidelines on the test for bacterial endotoxins are given in general chapter 5.1.10.

General Notices (1) apply to all monographs and other texts — 4777

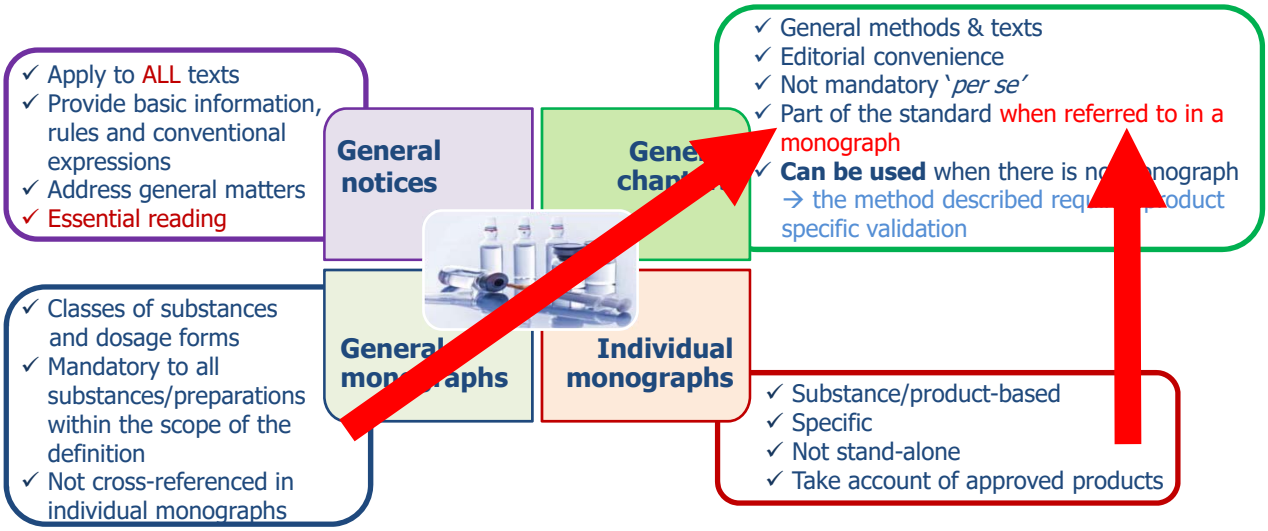
Part V. Is chapter *2.6.32* legally binding?



Ph. Eur. General Notices

- ✓ **Monographs**: mandatory requirements, unless otherwise indicated
- ✓ **General chapters**:
  - ➢ become mandatory when referred to in a monograph,

Content and structure of the Ph. Eur.



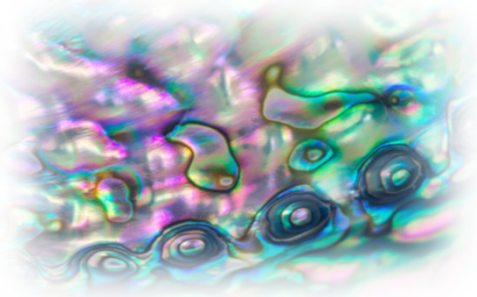
References to chapter *2.6.32* in other Ph. Eur. texts

- **General chapters**
  - Chapter *5.1.10*
- **Monographs**
  - Currently: None
  - Chapter *2.6.32* is currently not rendered legally binding by any of the Ph. Eur. monographs

Reference to 2.6.14 in the Ph. Eur.

- **Search: 527 hits (outside chapter 2.6.14)!**
- **For example:**
  - **General monographs, e.g.:**
    - Substances for pharmaceutical u
      Bacterial endotoxins (2.6.14). The
      endotoxins if it is labelled as
      parenteral preparation
      bacterial end
      ufacture of
      ure for the removal of
      ations for human use, if applicable after reconstitution or
      otoxins (2.6.14) or, where justified and authorised, with the test for
      the limits for bacterial endotoxins are given in general chapter 5.1.10.
  - **onographs, e.g:**
    - sulin (0838)
      Bacterial endotoxins (2.6.14): less than 10 IU/mg, if intended for use in the manufacture of parenteral preparations

2.6.14 replaced by 2.6.32 for articles within the scope of these monographs: to be considered as an **alternative method** *to 2.6.14*



Part VI. Alternative method

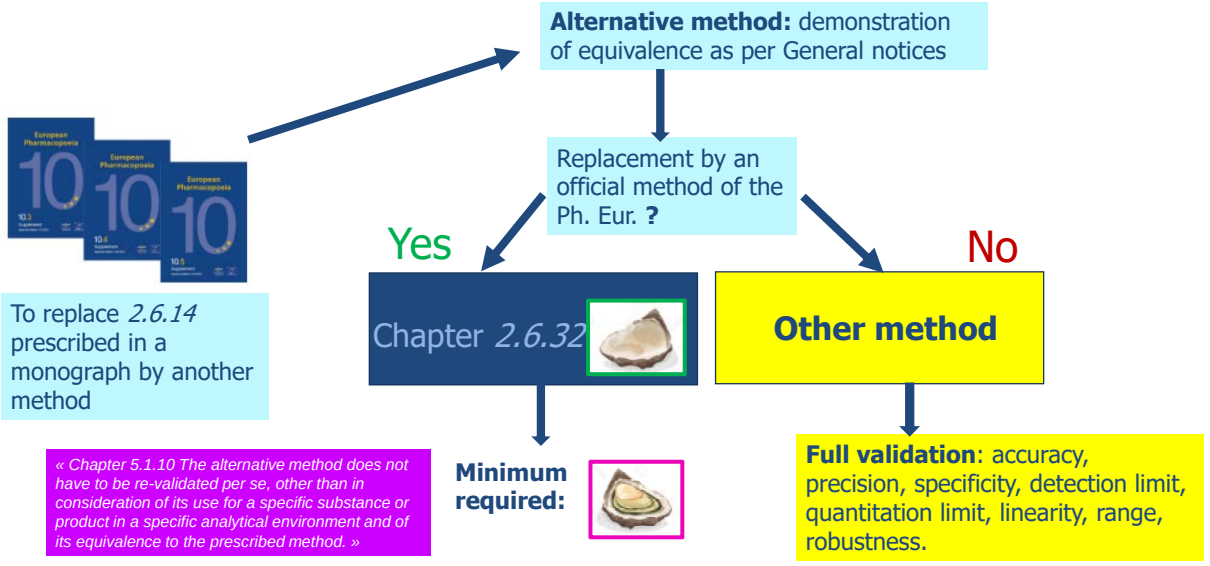
Ph. Eur. General Notices

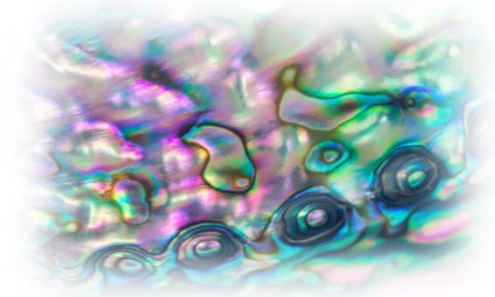
- **Alternative methods**
  - ➢ Equivalence as regards compliance with the standards of the monograph
  - ➢ Agreement of the competent authority
- When using rFC (chapter *2.6.32*) as an alternative to LAL (*2.6.14*), only the above statements apply
- *Validation requirements for the alternative method:*
  please refer to Part III.

Revised chapter *5.1.10*

'13. REPLACEMENT OF A METHOD PRESCRIBED IN A MONOGRAPH
13-1. REPLACEMENT BY ANOTHER METHOD DESCRIBED IN THE PH. EUR.
Replacement of a method prescribed in a monograph by another method described in the Ph. Eur. is to be regarded as the use of an alternative method in the replacement of a pharmacopoeial test, as described in the General Notices.
The analyst has to demonstrate that a valid test can be carried out on the substance or product concerned.
The alternative method does not have to be re-validated per se, other than in consideration of its use for a specific substance or product in a specific analytical environment and of its equivalence to the prescribed method.
13-2. REPLACEMENT BY A METHOD NOT DESCRIBED IN THE PH. EUR.
Replacement of a method prescribed in a monograph by a method not described in the Ph. Eur. is to be regarded as the use of an alternative method in the replacement of a pharmacopoeial test, as described in the General Notices. '

Use of chapter *2.6.32* as a replacement for *2.6.14*



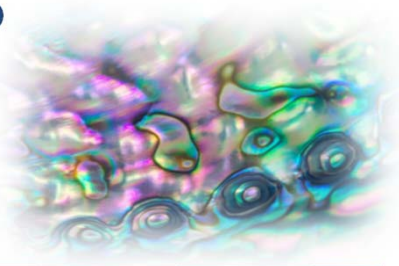


Part VII. What is next?

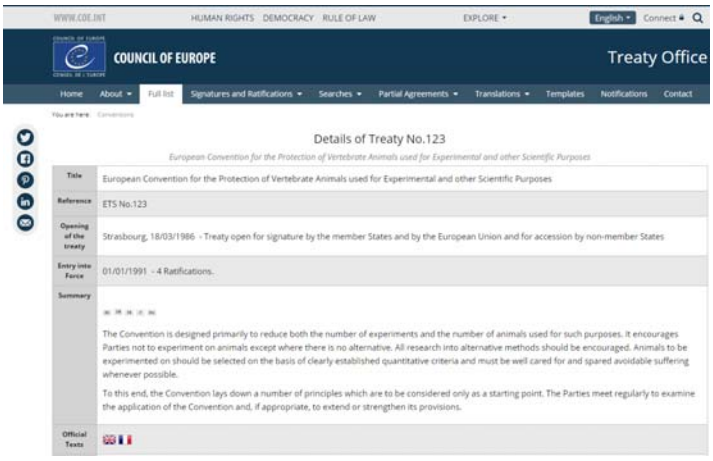
Next steps

- Revision of chapter *2.6.14* to include new method G?
- PDG to 'perform a gap analysis of the different approaches of the pharmacopoeias to better understand commonalities and differences' between the three pharmacopoeias.
- Discussion within the PDG is foreseen
- Two possible options:
  - rFC is included in the harmonised chapter => no longer a need for a stand-alone chapter in the Ph. Eur=> chapter *2.6.32* may be deleted
  - rFC is not included => there could be several options:
    - ✓ Direct reference to *2.6.32* in individual monographs or in general monographs might be considered by the Ph. Eur. Commission => stakeholders would be consulted via Pharmeuropa (revised texts)
    - ✓ Other options? To be discussed…

Part VIII: Does the use of rFC contribute to the 3Rs?



European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes



http://conventions.coe.int/Treaty/Commun/QueVoulezVous.asp?NT=123&CM=8&DF=29/07/2011&CL=ENG

Introduction to the Ph. Eur.

- **Use of animals**. In accordance with the *European Convention on the protection of animals used for experimental and other scientific purposes (1986)*, the Commission is committed to the reduction of animal usage wherever possible in pharmacopoeial testing, and encourages those associated with its work to seek alternative procedures. An animal test is included in a monograph only if it has clearly been demonstrated that it is necessary to achieve satisfactory control for pharmacopoeial purposes.

Recombinant factor C, a 3R achievement?

- Strictly speaking, does not come within the scope of the above mentioned Council of Europe Convention (the horseshoe crab is not used in pharmacopoeia testing)
- Nonetheless, rFC avoids the use of a reagent extracted from endangered species
- Significant technological progress
- Avoids potential shortage of supply and dependency on non-European countries

Conclusion

Acknowledgements

- **BET Working Party of the Ph. Eur.**
  - **Chair: Dr. Ingo Spreitzer, PEI**

  - **Scientific Programme Manager: Dr. Gwenaël Ciréfice, EDQM**

- **EDQM colleagues**

Dr. Mihaela Buda

Dr. Bruno Spieldenner

Empty shell

Empty shell

Platform procedure, multi-product procedure

Thank you for your attention

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