Harmonisation of standards for Bacterial EndotoxinsTest

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Background

- The procedure for test bacterial endotoxins described in the European Pharmacopoeia (Ph.Eur. 2.6.14), U. S. Pharmacopeia (USP <85>) and Japanese Pharmacopoeia (JP 4.01) are fullyharmonized
- The procedure for test bacterial endotoxins described in the Chinese Pharmacopoeia (Ch.P. 1143) are largely the same as the harmonized procedure described in the Ph.Eur., USP and JP
  - The endotoxin standard to be used, however, is different inCh.P.
    - Ch.P. requires use of the NationalStandard of Endotoxin
    - Ph.Eur., USP and JP requires use of ReferenceStandard Endotoxin that has been calibrated against the InternationalStandard

Introduction to international endotoxin standards

- In the early 1990s as many as five different international endotoxin standards remained available:
  - WHO IS 84/650 (WHO 1st international standard)
  - EP BRP-2
  - JP standard
  - USP Lot F/EC-5
  - USP Lot G/EC-6
- In 1996 the 2nd International Standard for endotoxin was established
  - To harmonise the standards an international study was conducted
  - A candidate preparation was calibrated againstWHO IS (84/650); BRP (BRP-2); freeze-dried EC-5 and EC-6; and the JP standard
  - Based on the study candidate preparation 94/580 was recommended as WHO 2nd international standard
  - An equivalency of units (1 EU = 1 IU) was established, facilitating calibration among various endotoxin standards based on potency
  - Assigned potency of 10000IU/vial

ICH Q4

WHO 3rd International Standard

- 3 candidate preparations were produced in 2010
  - The candidate preparations were produced with same material and formulation as WHO 2nd International standard
  - Potency calibrated against WHO 2nd International standard (94/580)
  - Potency of the 3 candidate preparations can be considered equivalent
  - Candidate preparation 10/178 established as WHO 3rd International Standard with assigned potency: 10000 EU/vial
  - Candidate preparation 10/178 has been shared with EDQM
    - Adopted as Ph. Eur. Endotoxin Standard Biological Reference Preparation (BRP) batch 5
    - Assigned potency 10000 IU/vial
  - Candidate preparation 10/190 USP endotoxin RS (lot H0K354)
    - Assigned potency 10000 EU/vial
    - Will serve as US FDA Endotoxin Standard,EC-7
- The endotoxin used for WHO 3rd International Standard was isolated from Escherichia coli O113:H10:K(-)

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Reference Standard Endotoxin (RSE) and Control Standard Endotoxin (CSE)

- RSE is the primary calibration standard used for the bacterial endotoxins test.
  - E.g. USP endotoxin RS (lot H0K354)
- Definition of CSE:
  - Control Standard Endotoxin (CSE) is an endotoxin preparation other than the international or national reference standards that are traceable in their calibration to the international reference standard
- CSEs are working standards and can be secondary or tertiary
- Endotoxin used for CSEs can be extracted from various strains (e.g. E.coli O55:B5; E.coli O113:H10)

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The National Standard Endotoxin (NSE)

- Described in Chinese Pharmacopeia (Ch.P.):
  - Prepared and purified from *Escherichia coli*
  - Used for calibration of the Working Standard Endotoxin (WSE) etc.
- Ch.P. 1143: Endotoxin is expressed in Endotoxin Units (EU).
  1 Endotoxin Unit (EU) = 1 International Unit (IU) of endotoxin
- The endotoxin used for preparation of the 8th National Standard Endotoxin was isolated from Escherichia coli O111:B4

The National Standard Endotoxin (NSE) is prepared and purified from *Escherichia coli*. It is used for calibration of the working standard endotoxin (WSE). It also is used for

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Establishment of National Standard of Endotoxin

- The 7th National Standard of Endotoxin:
  - Potency calibrated against the 2nd International Standard (batch 94/580)
  - Assigned potency 10000 EU/vial (lot 1506000-200707)
  - Issued in 2007

- The 8th National Standard of Endotoxin: Potency calibrated against the 3rd International Standard (batch 10/178)[1]
- Assigned potency 9000 EU/vial (lot 150801-201601)

[1]"Establishment of the 8th National Standard of Endotoxin" Tong Cai, Yusheng Pei, Guolai Zhang, Chen Chen, Hua Gao*, *National Institute for Food and Drug Control, Beijing 100050, China*

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Summary and proposal for future harmonisation

- In the Chinese Pharmacopoeia The National Standard of Endotoxin corresponds to a Reference Standard Endotoxin since it has been calibrated against the international reference
  - NSE: Calibrated against WHO 3rd International standard
  - International endotoxin standards incl. WHO 3rd International Standard have been calibrated against WHO 2nd International Standard

- The fundamental basis of the standards described in Ch.P., JP, Ph.Eur. and USP are identical
  - No practical difference between the standards exists

- The difference in terminology of the standards described in Ch.P., JP, Ph.Eur. and USP can cause confusion
  - Harmonisation in terminology is recommended
  - Mutual recognition of use of the official endotoxin standards described in the pharmacopoeias is recommended

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Recombinant Factor C assay as alternative method for endotoxins test

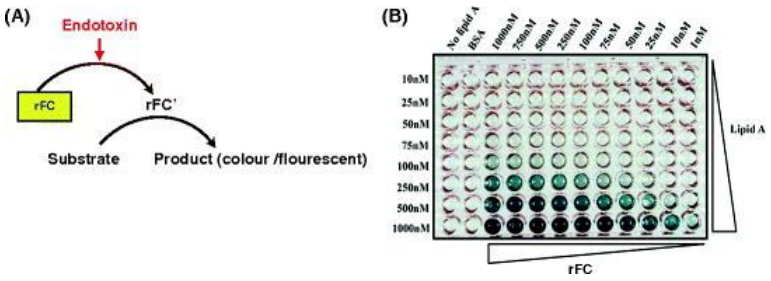

Illustration: Ding J.L., Ho B. (2010)

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Recombinant Factor C assayas alternative method for endotoxins test

- rFC: Recombinant Factor C
- Supply of the LAL and TAL tests is solely dependent on the horseshoe crab
- Demand for LAL/TAL testing is growing→ Increased demand on reagents
- Ethical: The LAL/TAL reagent is animal derived (3R principle: replace; reduce; refine),
- Batch-to-batch variation for LAL/TAL (variable reactivity)

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Recombinant Factor C for endotoxin detection

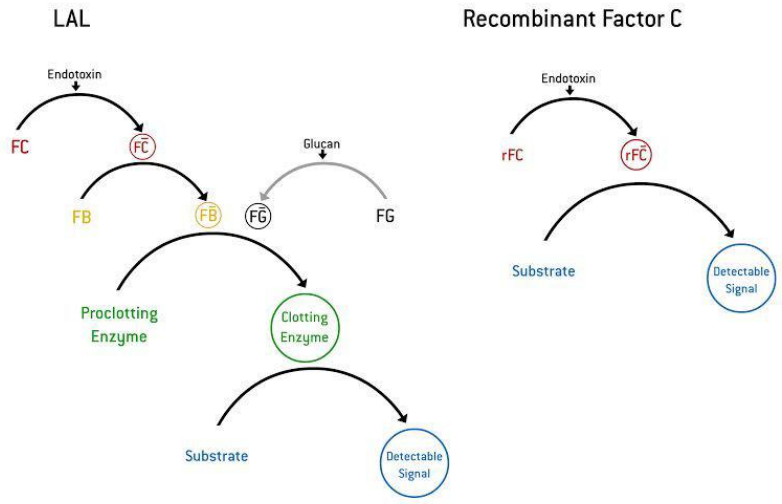

Illustration: Lonza (https://www.epmmagazine.com/opinion/testing-times/)

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Recombinant Factor C principle

- Factor C is the first component in the horseshoe crab clotting cascade
- Activated Factor C can transform a substrate into a detectable signal (e.g. a fluorescent product) that can be measured

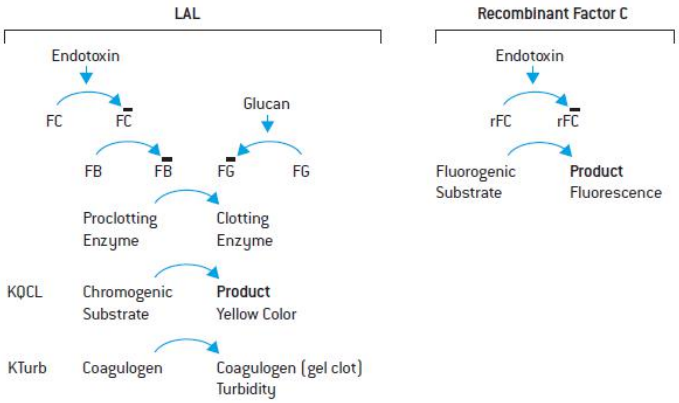

Illustration: Lonza product insert: PyroGene™ Recombinant Factor C Endotoxin Detection Assay

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Recombinant Factor C - advantages

- Specific & Sensitive:
  - 100 % endotoxin specificity - not activated byβ-glucans
  - Sensitivity down to 0.001 EU/ml
- Sustainable:
  - No use of horseshoe crab for production of reagent
- Standardised supply
  - Batch-to-batch consistency

Recombinant Factor C –Suppliers

- rFC assay solutions available from multiple vendors:
  - PyroGene™ (Lonza)
  - Haemotox rFC (Haemochrom Diagnostica)
  - ENDOZYME® (Biomérieux/Hyglos)

Recombinant Factor C – Regulatory Status

- FDA has currently approved a submission ofrFC as final release test for product (as alternative method)
- Validated and used by several pharmaceutical companies as alternative test method for test of water forendotoxin
- The rFC assay is considered an "Alternative Test", subject to the validation requirements of USP <1225> or ICHQ2B
- Methods based on rFC have recently been included in the European Pharmacopoeia guidelines (section 5.1.10) as valid alternatives to the LAL test

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Thank you for your attention

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