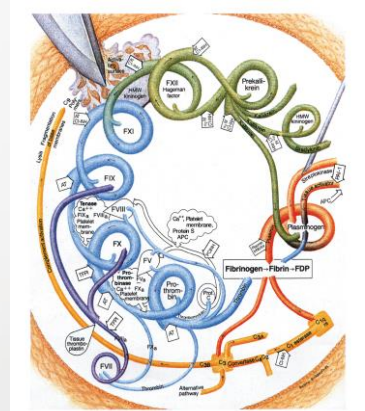


Haemochrom Diagnostica

Competence in Haemostasis Diagnostics and Endotoxin Analytics

Endotoxin Detection Methods



Haemochrom
Diagnostica

Haemochrom Diagnostica
www.haemochrom.de
[info\[at\]haemochrom.de](mailto:info[at]haemochrom.de)
+49 201 843 770

Haemochrom Diagnostica
www.haemochrom.se
[info\[at\]haemochrom.se](mailto:info[at]haemochrom.se)
+46 31 706 2070

Endotoxin Detection Methods

Pharmaceutical Production

» Biopharmaceuticals

- Antibodies, immunoglobulins, infusions
- Vaccines, proteins, cell therapy products

» Buffers, raw materials, water

» Medical devices, environmental samples

» Sources of contamination

- Active ingredients (API`s), excipients, water
- Primary packaging materials
- Staff



Endotoxin Detection Methods

Evolution of measuring methods



Haemotox® rFC 2014

Technologische Entwicklung - Zeit



Rabbit Test 1912

LAL 1977

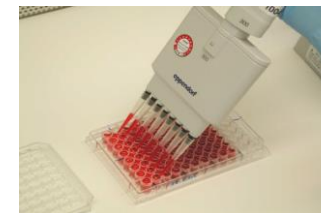


rFC 2003

EndoLISA 2011

Endozyme 2013

MAT Test 2010



HaemoMAT® 2018





Endotoxin Detection Methods

Test Systems Overview

Biological test systems:

- » Rabbit test (RPT)
- » Monocyte Activation Test (MAT)

Biochemical test systems:

- » Limulus amebocyte lysate test (LAL)
 - Gel Clot Test
 - Kinetic turbidimetric assay / turbidimetric endpoint assay
 - Kinetic chromogenic assay / chromogenic endpoint assay
- » Recombinant factor C methods

Endotoxin Detection Methods

Biochemical Test Systems

Limulus Amebocyte Lysate Test (LAL): EP 2.6.14/USP 85/JP 4.01

“Classical LAL Tests”

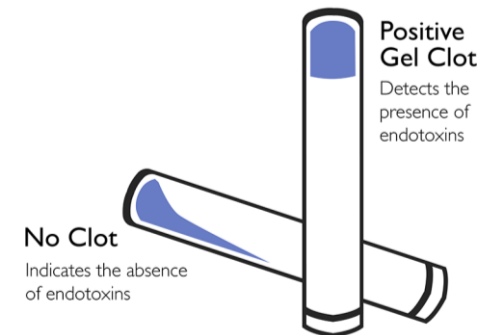
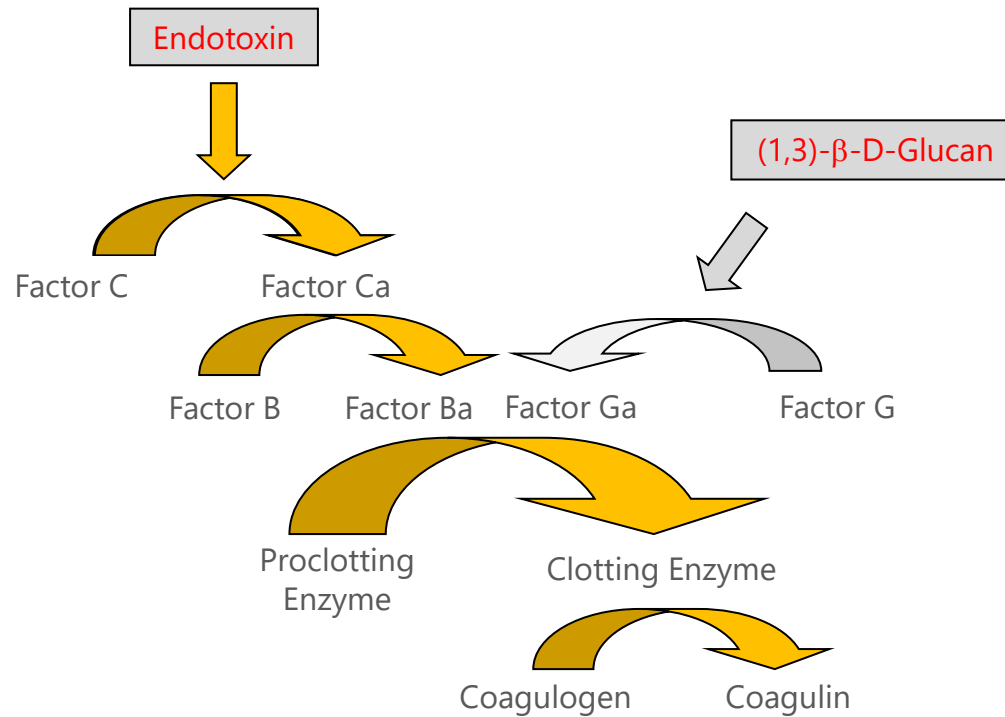
- » Method A: Gel Clot Limit Test
- » Method B: Gel Clot Quantitative Test
- » Method C: Kinetic turbidimetric assay
- » Method D: Kinetic chromogenic assay
- » Method E: Chromogenic endpoint assay
- » Method F: Turbidimetric endpoint assay



Source: ECA, Flyer for LER Course

Endotoxin Detection Methods

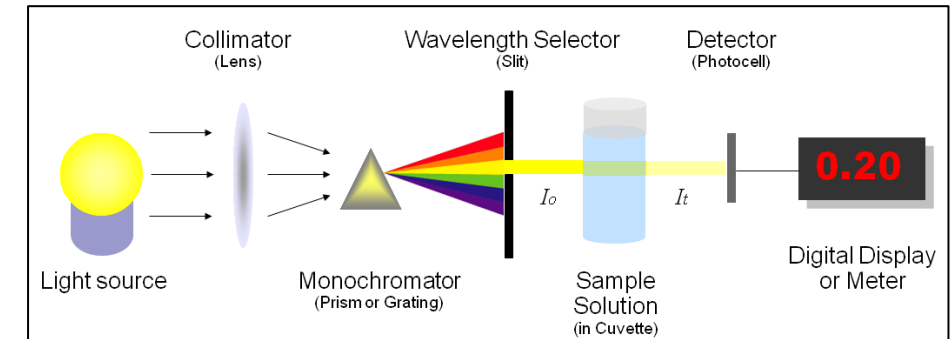
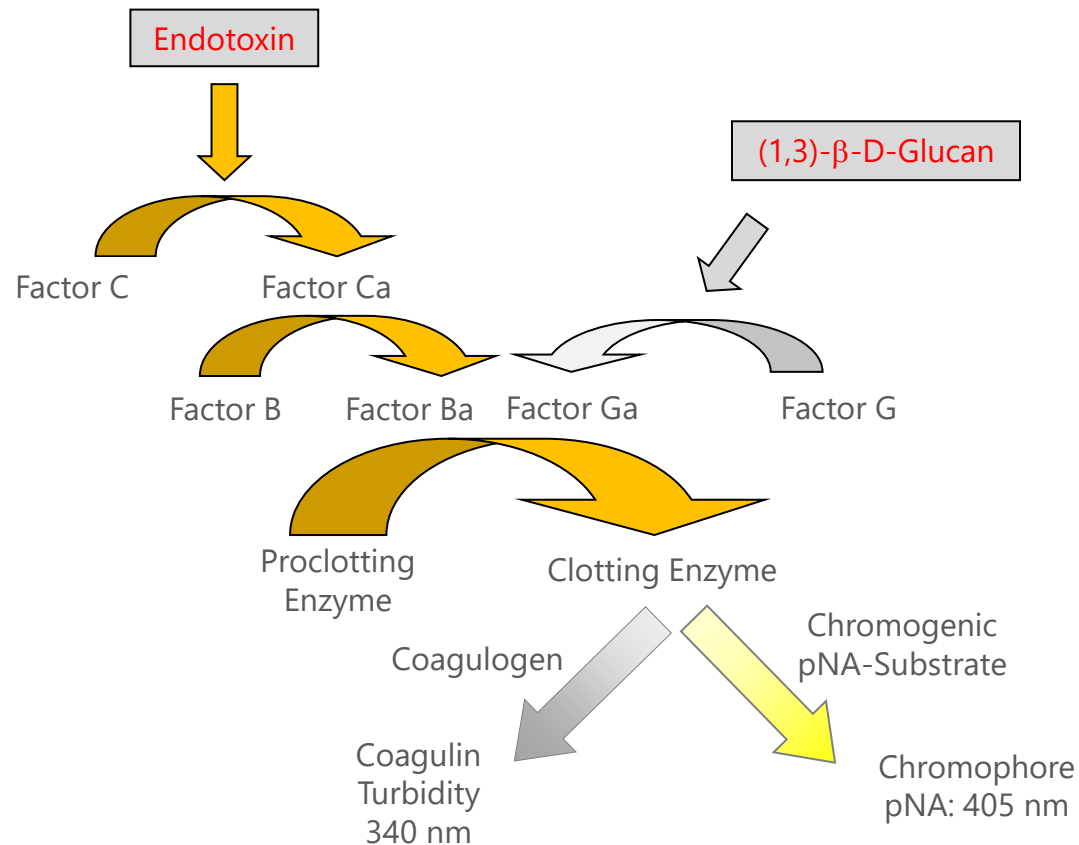
Cascade of Reactions (Gel Clot)



Source: www.kepleybiosystems.com

Endotoxin Detection Methods

Cascade of Reactions (Photometric Methods)



Source: www.edusanjalbiochemist.blogspot.com



Endotoxin Detection Methods

Biochemical Test Systems

LAL-Test Evaluation

» Method C and D: Kinetic assay

- » Quantitative relationship between Endotoxin concentration and time to onset OD or
- » Quantitative relationship between Endotoxin concentration and rate of turbidity/color development in OD/min

» Method E and F: Endpoint assay

- » Quantitative relationship between Endotoxin concentration and turbidity/release of pNA at the end of an incubation period

Endotoxin Detection Methods

Biochemical Test Systems

Recombinant Factor C Method

- » Use of recombinant Factor C instead of LAL
- » Fluorometric Endpoint Method to quantify Endotoxins
 - » Quantitative relationship between Endotoxin concentration and fluorescence of the reaction mixture



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Endotoxin Detection Methods

Biochemical Test Systems

Recombinant Factor C Method

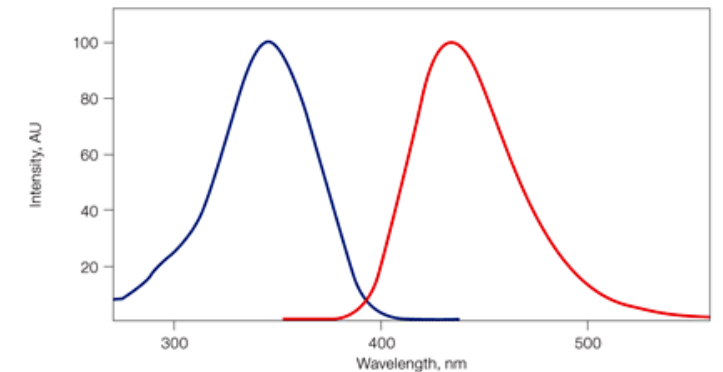
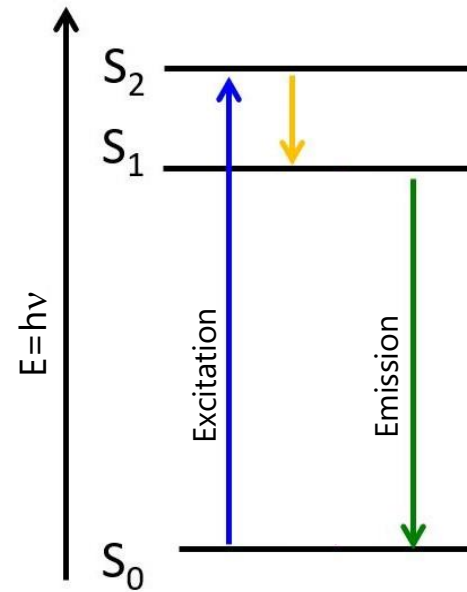
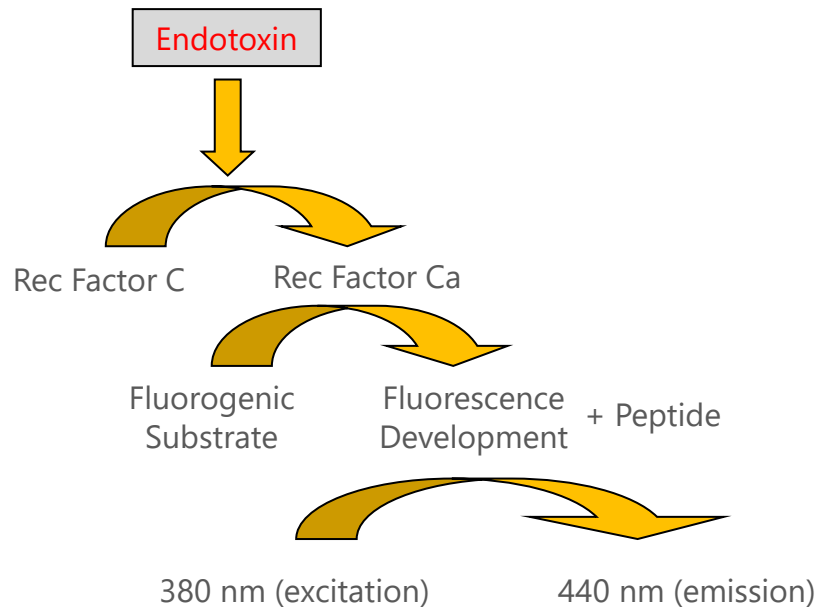
- » EP 2.6.32, „Test for Bacterial Endotoxins using Recombinant Factor C“
 - » Effective as of 01.01.2021
 - » Published in Ph. Eur. Suppl. 10.3.
- » EP 5.1.10: rFC accepted as alternative method since 2014
- » USP 85: adoption of rFC method is under discussion
- » JP: rFC as alternative method: draft published



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Endotoxin Detection Methods

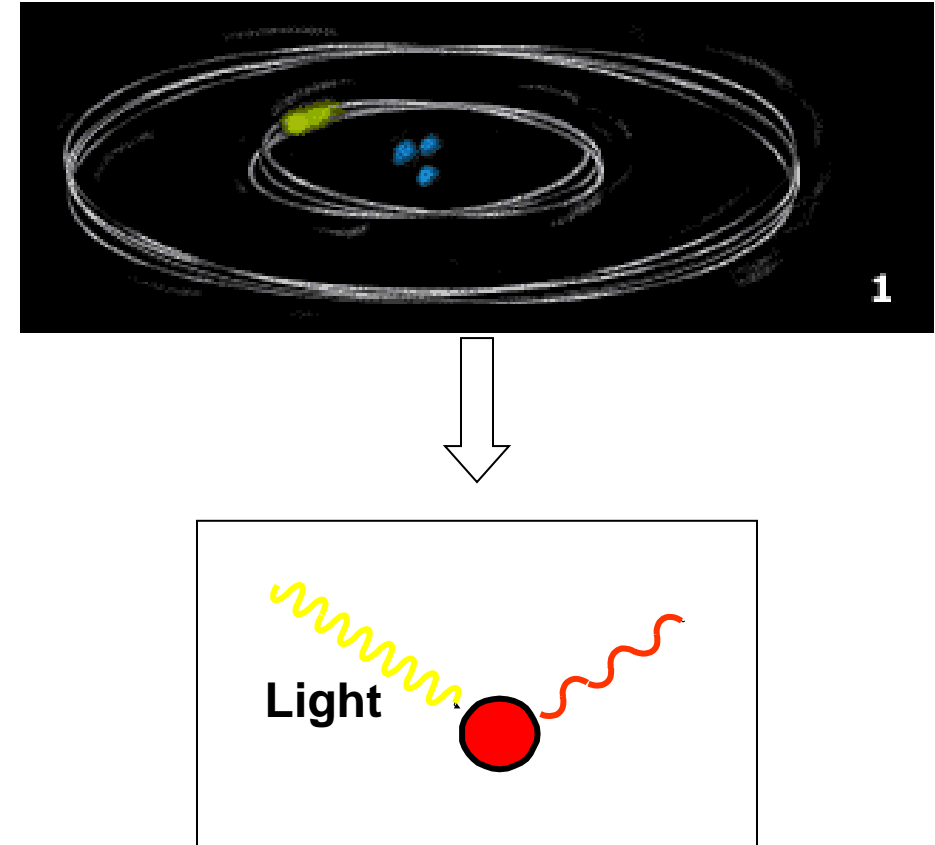
Cascade of Reactions (rFC Methods)



Endotoxin Detection Methods

Principle of Fluorescence

- » Discovery in 1852 by George Gabriel Stokes
- » Name of the fluorescent mineral "Fluorite" (Fluorspar, Calcium fluoride CaF_2)
- » Short-term, spontaneous emission of light
- » Transition of an excited stage (through absorbed light or other electromagnetic radiation) back to lower ground stage
- » Emitted light has less energy than the previously absorbed light

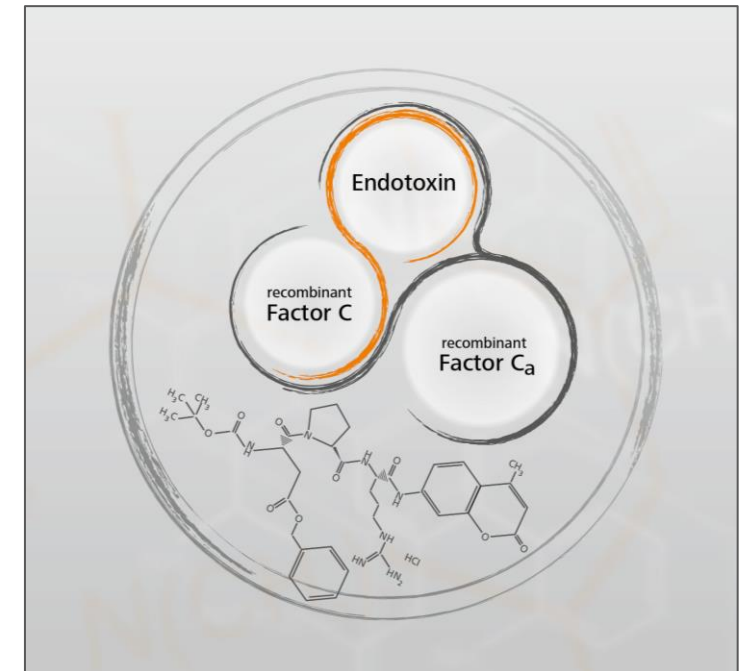


Endotoxin Detection Methods

Biochemical Test System

Recombinant Factor C Test

- » Homogeneous end point assay for the quantitative determination of endotoxins (LPS)
- » The main receptor is recombinant factor C (rFC) instead of factor C from the horseshoe crab
- » Activated enzyme (rFCa) directly cleaves a fluorogenic substrate
 - » No signal amplification by intermediary steps (Signal amplification is performed via the fluorescence reader)
 - » Factor B, Proclotting Enzyme and Coagulogen are absent (No interference by β -D-Glucans)

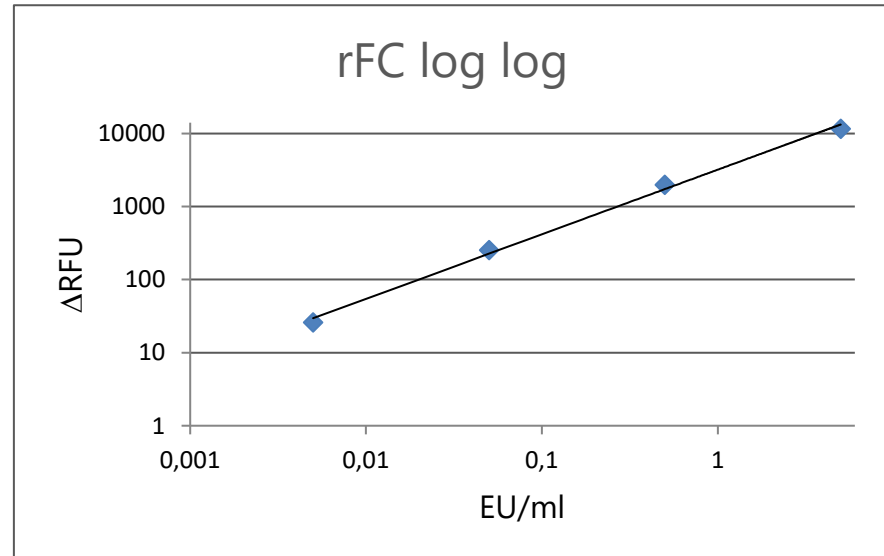


Endotoxin Detection Methods

Biochemical Test System

Recombinant Factor C Test

Standard Curve (rFC)



Regulatory requirement: $r \geq 0,980$

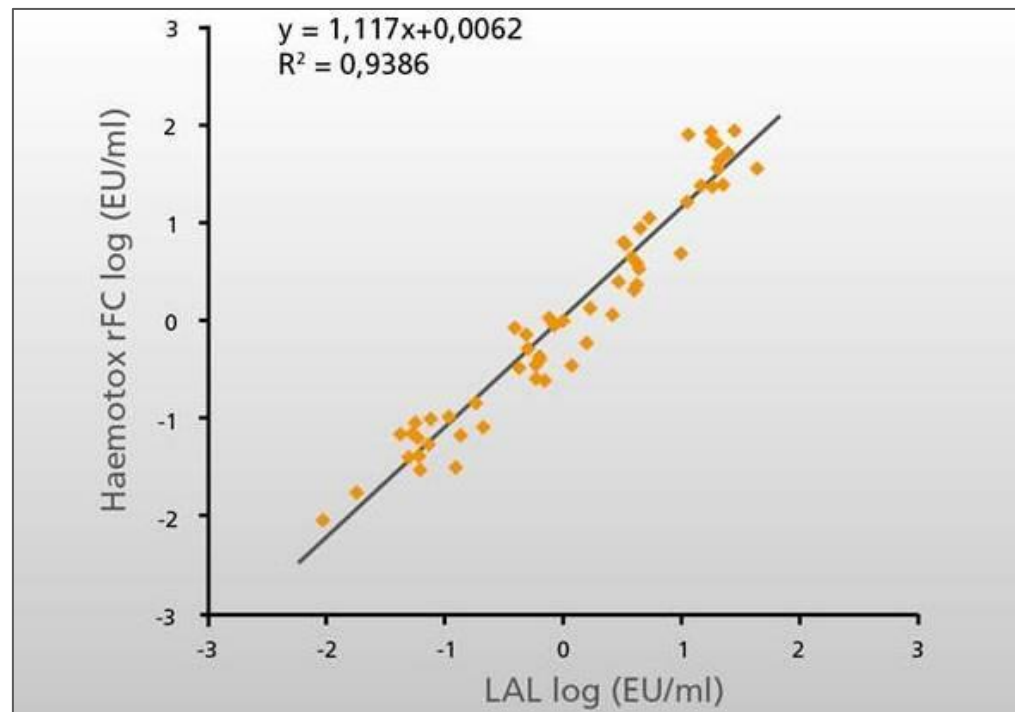
rFC Endpoint	
EU/ml	ΔRFU
0,005	26
0,05	255
0,5	1994
5	11617



Endotoxin Detection Methods

Biochemical Test System

Comparison LAL - rFC



Ph. Eur. 2.6.32:

„As factor G is absent from the test kit, false positive β -Glucan activation is not possible. This must be taken into account when the method is compared to other bacterial endotoxin quantification methods.”

Endotoxin Detection Methods

Conclusion

- » Endotoxin is parameter for IPC and release testing
- » Different LAL methods are available and accepted by regulatory authorities (EP 2.6.14/USP 85/JP 4.01)
- » Recombinant Factor C method: chapter 2.6.32 is adopted to Ph. Eur.
- » USP 85: adoption is under discussion
- » JP: draft for rFC and recombinant cascade as alternative method
- » Factor C is biosensor for LPS/Endotoxin detection in all methods

