ENDONEXT™

A Evolução no Teste de Endotoxina

Associação Brasileira dos Produtores de Soluções Parenterais

Philippe Gadal Ph.D General Manager Hyglos GmbH a bioMérieux Company

PIONEERING DIAGNOSTICS

CONTENTS

- **Endotoxin:** Definition and importance
- **History:** Pyrogen and Endotoxin testing
- **LAL versus rFC** – What are the main differences
- **Regulation :** status of rFC
- **Validation**: What is required?
- **Latest generation rFC :** ENDOZYME™II GO –
- **Conclusion**

ENDOTOXIN - DEFINITION

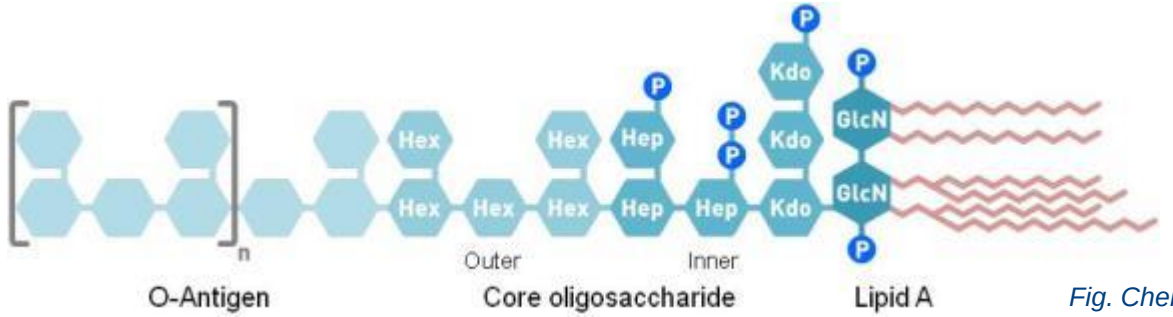


Fig. Chemical structure of endotoxin - modified from Retschel, 1994.

- **Pyrogen**

A substance that causes fever; pyrogenic substances include endotoxin.

- **Endotoxin**

Toxic part of Gram-negative bacteria in the outer part of the cell membrane.

- **Lipopolysaccharide (LPS)**

Chemical and structural components of endotoxin. Covers approximately 75% of the outer membrane.

- **Lipid A**

Lipid moiety of LPS molecule which has biological activity.

ENDOTOXIN - IMPORTANCE

- **Why is it important to test for endotoxins?**
  Endotoxins are pyrogenic (fever-inducing) substances, toxic to humans at nano gram level by injectable route, can trigger severe infection and lead to death. Pharmaceutical processes and equipment are at risk; hazards stem from human handling, dust, packaging, contaminated rinse water and microbial growth can all contaminate components with endotoxin.

- **Endoxin testing is mandatory according to regulation for release of:**
  - All **injectable products**; pharmaceuticals and medical solutions
  - **Medical devices** in contact with the bloodstream, cerebrospinal fluid and ocular systems

CHRONOLOGY OF ENDOTOXIN TESTING

1925 RBT Rabbit pyrogen test (Seibert)

1964 LAL test based on the blood from Limulus (Bang and Levin)

1974 TAL test based on the blood from Tachypleus (Kobayashi); Asia horseshoecrab.

1981 Iwanaga describes the alternative activation pathway through β-1,3-D-glucans in the LAL/TAL cascade

2003 rFC introduced by Cambrex → rFC derived from ***Carcinoscorpius rotundicauda*** (patent Ding)

2010 MAT test based on activation of Human Monocytes by Pyrogens generating cytokines / Interleukines that are detected in an immunological assay

2011 EndoLISA® with endotoxin-specific phage protein for capture and rFC for detection rFC derived from ***Tachypleus tridentatus*** (Iwanaga, Muta et al.)

2012 FDA issues Guidance for Industry Pyrogen and Endotoxins . Use of rFC is accepted by the FDA as per USP General Chapter <1225> Validation of Compendial Method

2013 EndoZYME rFC Recombinant Factor C introduced by Hyglos

2014 Endo-RS ® Endotoxin Recovery Kit is a sample preparation method addressing LER in biologics.

2016 European Pharmacopeia Revised Chapter 5.1.10 Supplement 8.8 include rFC as an alternative method

2017 Kikuchi and al of PMDA part of Japanese Pharmacopeia publish a comparison study of 3 LAL tests and 3 rFC tests showing equivalence.

2018 EndoZYME II GO introduced by bioMérieux rFC endotoxin testing made significantly easier and faster

ENDOTOXIN - TEST METHODS

ANIMAL UTILIZATION

Rabbit Pyrogen Test
EP 2.6.8 - USP151
Approved in 1941

Limulus amebocyte lysate (LAL)
EP 2.6.14, USP 85
Approved in 1980

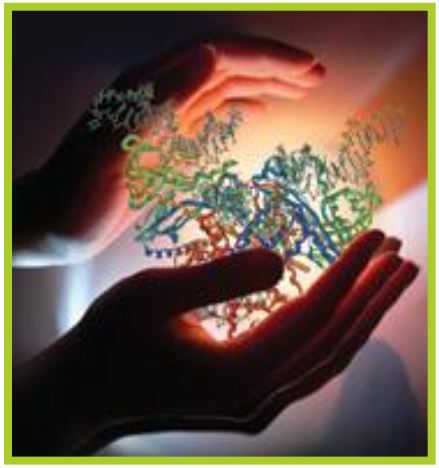
Pyrogen Testing

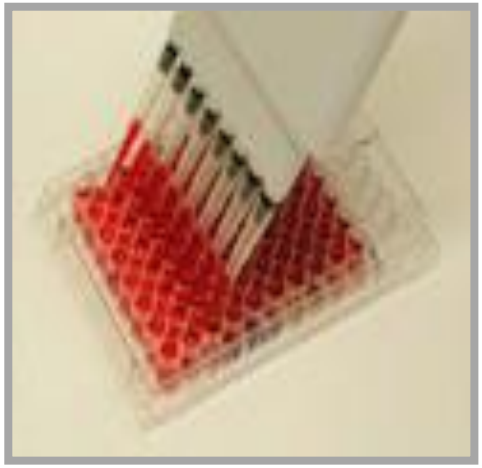
EndotoxinTesting

NO ANIMAL UTILIZATION

Recombinant Horseshoe Crab Factor C (rFC)
EP 5.1.10
Approved Alternative Method in 2016

Monocyte Activation Test (MAT)
EP 2.6.30
Approved in 2016

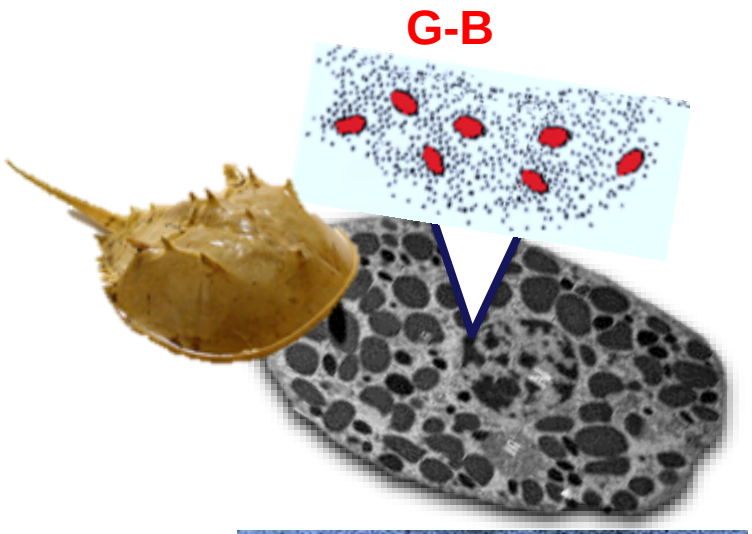


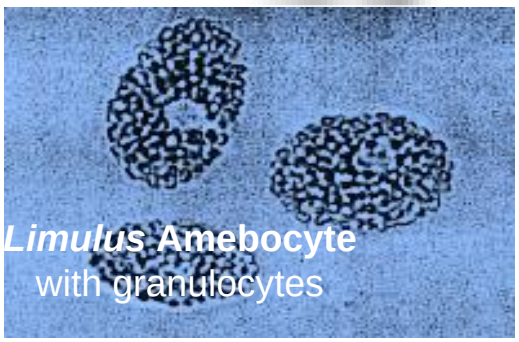


Pyrogen Testing

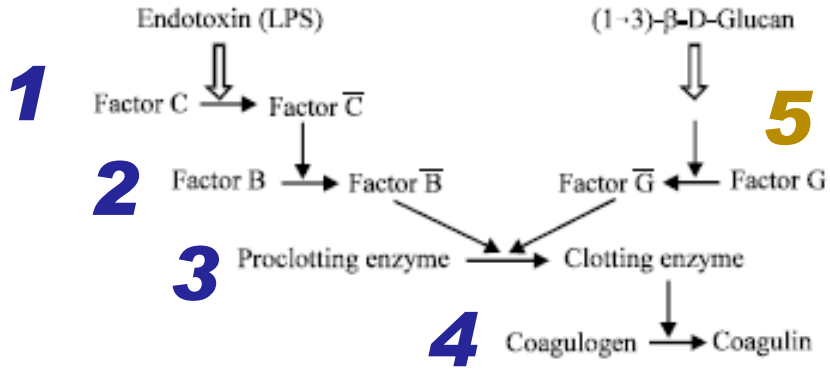
LAL VERSUS rFC: TECHNOLOGY

FC is the ONLY endotoxin biosensor in the HSC serine protease detection cascade.

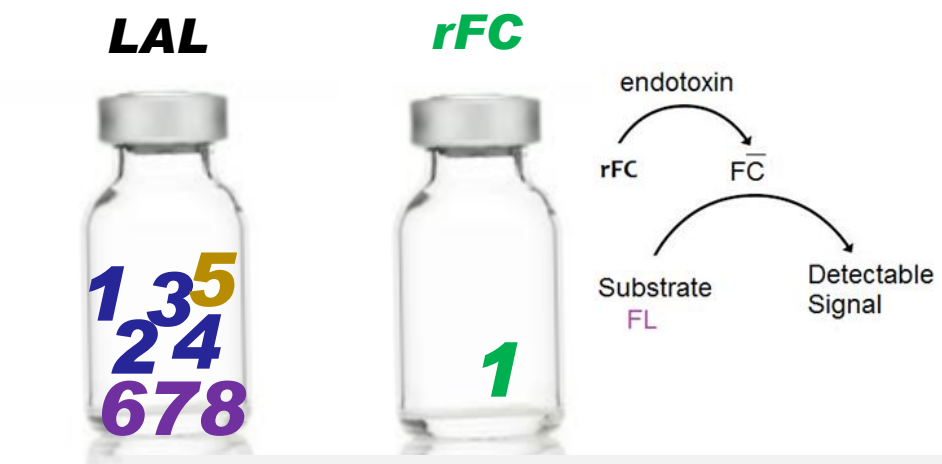




Blood Cell Histology of Horseshoe Crab, *Tachypleus gigas*
[1]Sh. Shakibazadeh, [1]A. Christianus and [2]P. Hajeb



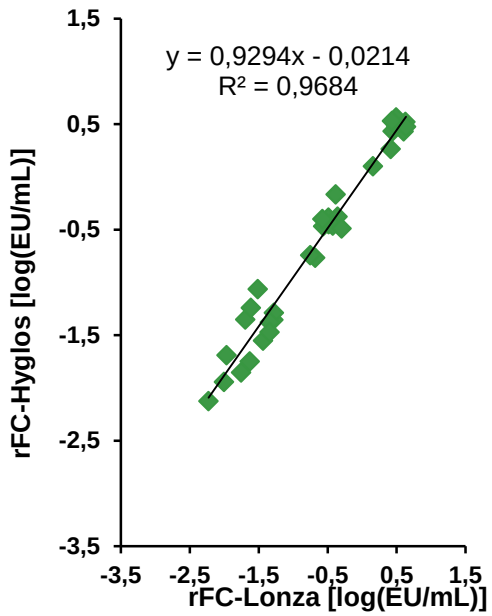
6, 7, 8 Coagulation inhibitor proteins



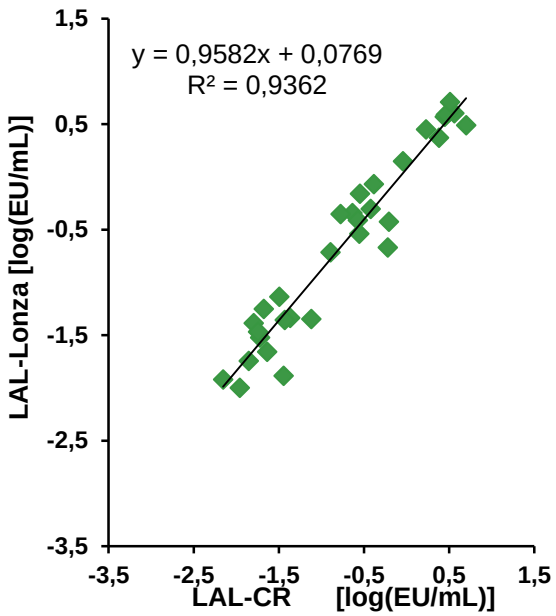
Reduce complexity of the test milieu.

PERFORMANCE rFC VS LAL

- **Correlation between rFC-tests and between LAL-tests:**



Tested endotoxin preparations
E. coli O26:B6
E. coli O128:B12
E. coli EH100 (Ra mutant)
E. coli F583 (Rd mutant)
S. enterica serotype minnesota
S. enterica Re 595 (Re mutant)
S. enterica serotype enteritidis
S. enterica serotype abortus equi
S. enterica serotype typhimurium
Klebsiella pneumoniae
P. aeruginosa serotype 10
E. coli J5 (Rc mutant)



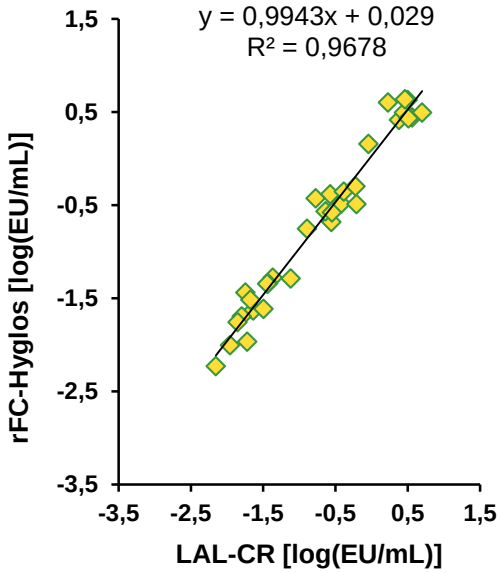
96.8% correlation between rFC tests from two different manufacturers

93.6% correlation between LAL tests of two different manufacturers

➔Alternative method correlation higher than intercompendial method correlation

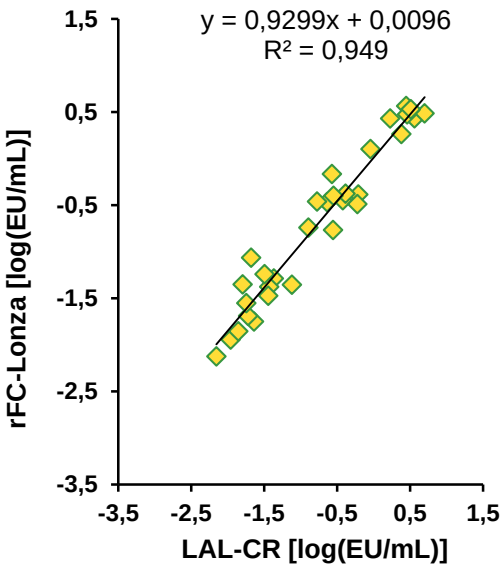
PERFORMANCE rFC VS LAL

Correlation between rFC and LAL:



96.8% correlation between rFC-Hyglos and LAL-CR.

Tested endotoxin preparations
E. coli O26:B6
E. coli O128:B12
E. coli EH100 (Ra mutant)
E. coli F583 (Rd mutant)
S. enterica serotype minnesota
S. enterica Re 595 (Re mutant)
S. enterica serotype enteritidis
S. enterica serotype abortus equi
S. enterica serotype typhimurium
Klebsiella pneumoniae
P. aeruginosa serotype 10
E. coli J5 (Rc mutant)



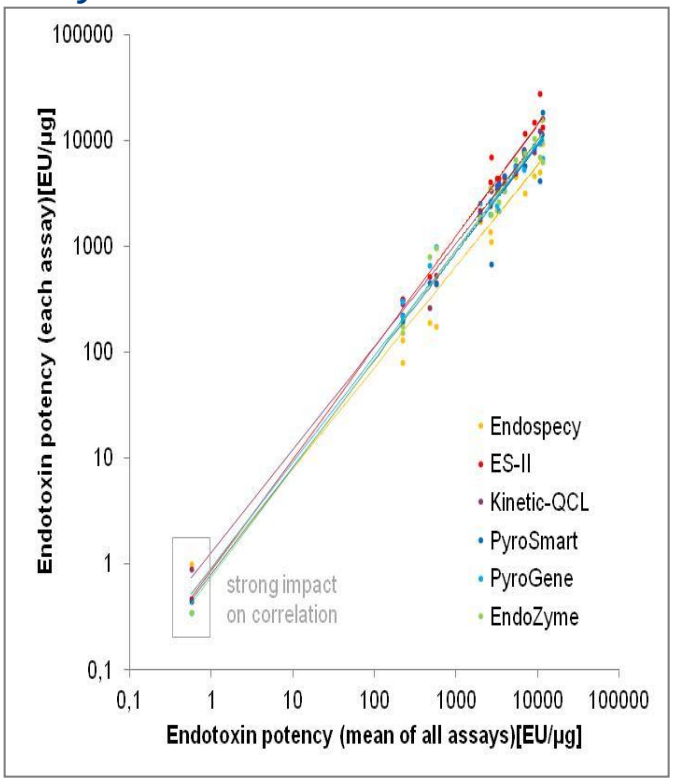
94.9% correlation between rFC-Lonza and LAL-CR.

➔ rFC tests demonstrate full correlation with LAL.

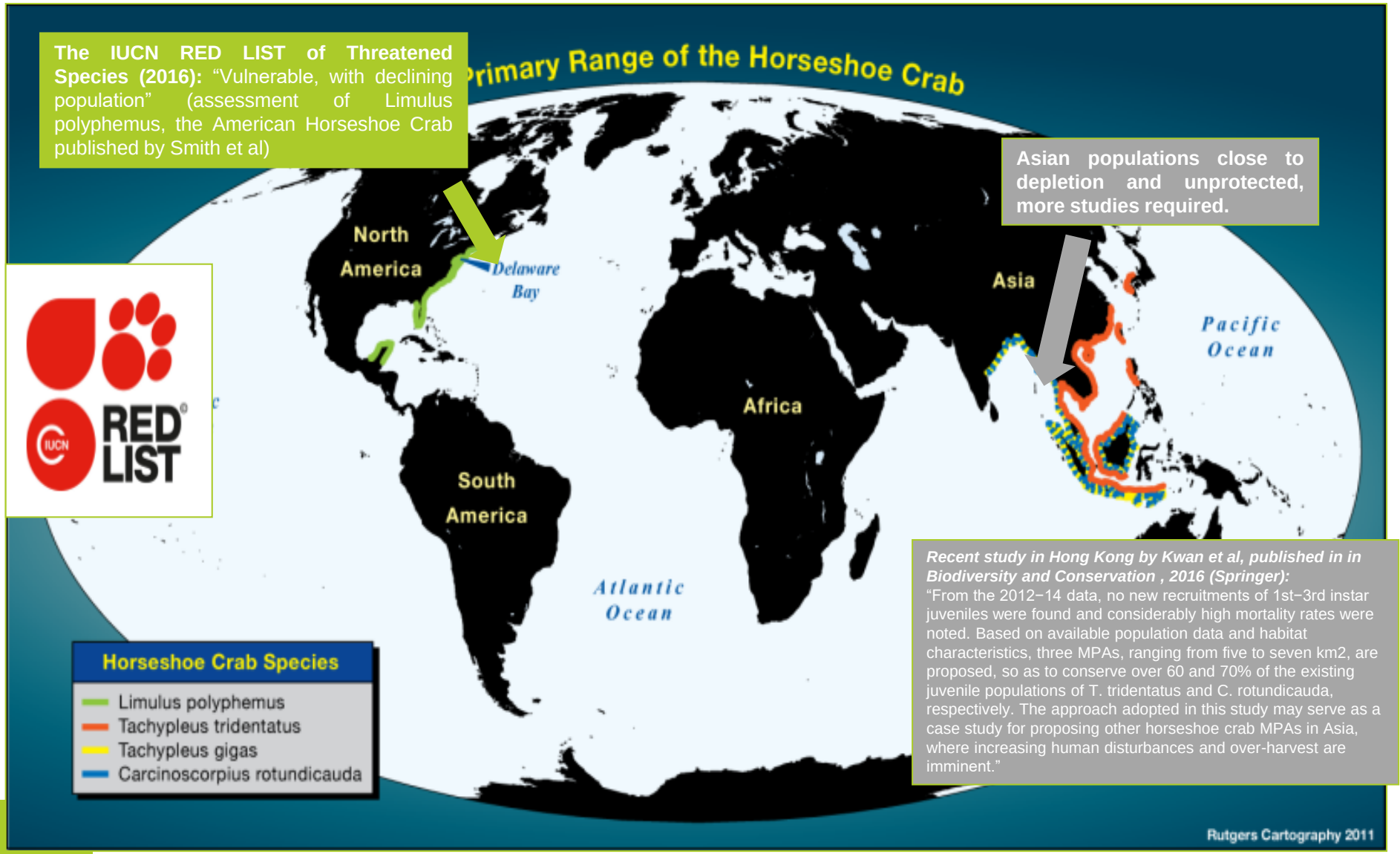
PERFORMANCE - JAPANESE PHARMACOPOEIA

- Kikuchi et al. National Institute of Health Science (NIHS) and Pharmaceutical and Medical Device Regulatory Science Society of Japan (PMRJ) **- *Collaborative Study on the Bacterial Endotoxins Test Using Recombinant Factor C-based Procedure for Detection of Lipopolysaccharides*** - published in Pharmaceutical and Medical Device Regulatory Science, Vol. 48 No. 4., May, 2017**.**

  - Equivalence demonstrated between three LAL tests (Wako, Lonza and Seikagaku) and three rFC tests (Hyglos, Lonza and Seikagaku) with commercially available endotoxins (purified and NOE:s):

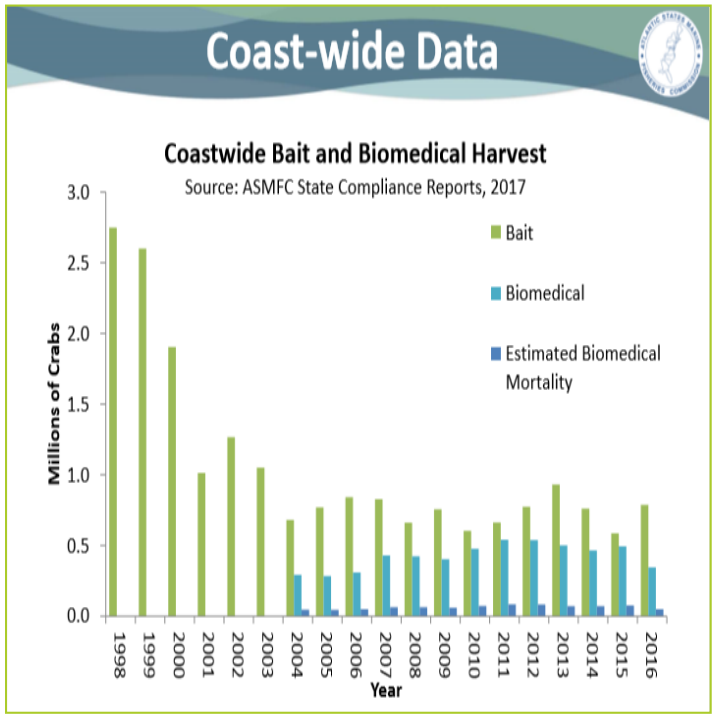


LAL VERSUS rFC: ENVIRONMENTAL ISSUES

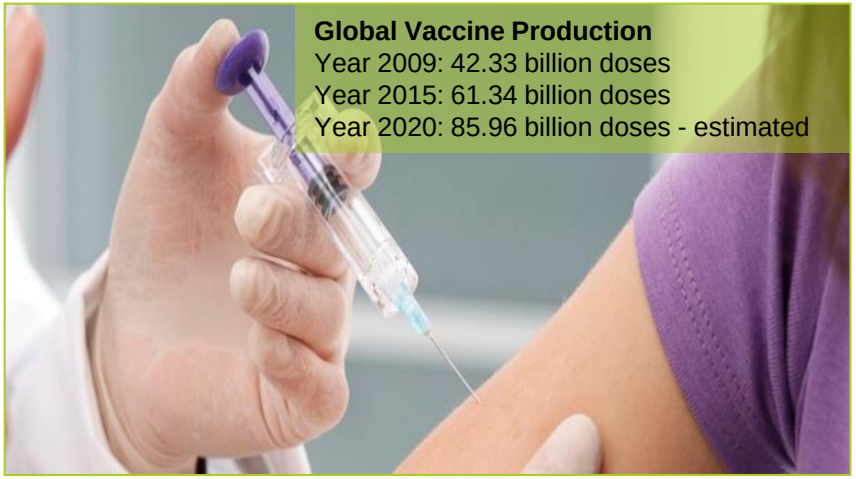


GLOBAL ASSESSMENT

- **Harvest restrictions in the USA and close to depletion in Asia, LAL manufacturers still claiming in their advertisement that bleeding is “sustainable”:**



- **But can harvesting of horseshoe crabs really follow the demand?**
  - Growing demand of endotoxin testing, doubling within 15 years
  - Limited and vulnerable horseshoe crab populations



REGULATORY

Pharmacopoeial status rFC

LAL VERSUS rFC: REGULATION

FDA/USP recombinant Factor C (rFC) accepted as alternative method since 2012

Guide for Industry: Pyrogen and Endotoxins Testing (2012), rFC methods validated according to USP chapters <1225> and <85>

European Pharmacopoeia (Ph. Eur.) Chapter 5.1.10 supplement 8.8, revision from 2016:

- rFC is accepted and listed as alternative method to LAL
- rFC meets the European directive 2010/63/EU to reduce animal use for scientific purpose.
- ENDOLISA technology described for removing interfering substances - section 9

***New chapter for rFC in process*:**

- Monograph Ph. Eur. chapter for rFC in preparation - publication in Pharmeuropa expected for end of 2018

Japanese Pharmacopoeia (JP) - Concluded first comparison study of three rFC methods (Hyglos-bioMérieux, Lonza, Seikagaku) with LAL showing equivalence - published by Kikuchi et al in May 2017

Collaborative Study on the Bacterial Endotoxins Test Using Recombinant Factor C-based Procedure for Detection of Lipopolysaccharides - in Pharmaceutical and Medical Device Regulatory Science, Vol. 48 No. 4.

RECENT FDA APPROVAL BASED ON rFC TESTING

Emgality™ (galcanezumab-gnlm) 120 mg injection

09/27/2018

Lilly

Lilly's Emgality™ (galcanezumab-gnlm) Receives U.S. FDA Approval for the Preventive Treatment of Migraine in Adults

Eli Lilly has now received U.S. FDA approval for Emgality™ (galcanezumab-gnlm), including the use of recombinant Horseshoe Crab Factor C (rFC) test for bacterial endotoxin. The rFC test replaces the Limulus amebocyte lysate (LAL), thereby eliminating the use of a reagent extracted from live animals, and has been validated according to USP chapters <1225> and <85>,

VALIDATION

what is required?

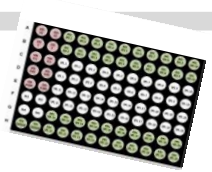
VALIDATION FOR NEW ENDOTOXIN TESTING

- **Validation of rFC methods - a Quantitative Analytical Procedure for the Detection of Impurities:**

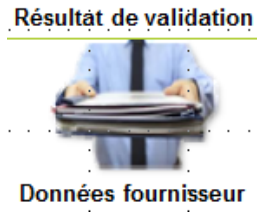
SUPPLIER	USER		
Pre-phase **General parameters** Supplier method validation data - supporting specific data elements 	**1** **IQ / OQ** **Available to perform from Supplier** • Draft SOPs • Validate equipment • Train users • Draft PQ1, PQ2 **FDA Q&A** Expects to review and comment prior to PQ Performance	**2** **PQ1** **Method Validation Water - Non-interfering <1225>** • Accuracy, precision, LOQ • Ruggedness / robustness: 2 users, 2 days, 2 reagent lots • Collect summarize in report	**3** **PQ2** **Method Suitability Specific product <85>** • NIC test on 3 product lots • I/E test on 3 product lots • Compare to compendial expectations • [Endogenous samples]

Full validation protocol provided by Supplier

CHANGE OF SUPPLIER rFC OR LAL



LAL Method	New LAL	New rFC
	Justification for use	Justification for use
Initial Qualification	Initial Qualification	Initial Qualification
		Parameters Verification PQ1
Research of interference	Research of interference	Research of interference
	Comparability / LAL with customer's product	Comparability / LAL with customer's product
Validation Dossier	Validation Dossier	Validation Dossier
Routine Test	Routine Test	Routine Test



ENDONEXT™

The latest generation of Endotoxin Testing

Easier and faster

ENDOZYME® II GO THIRD GENERATION rFC

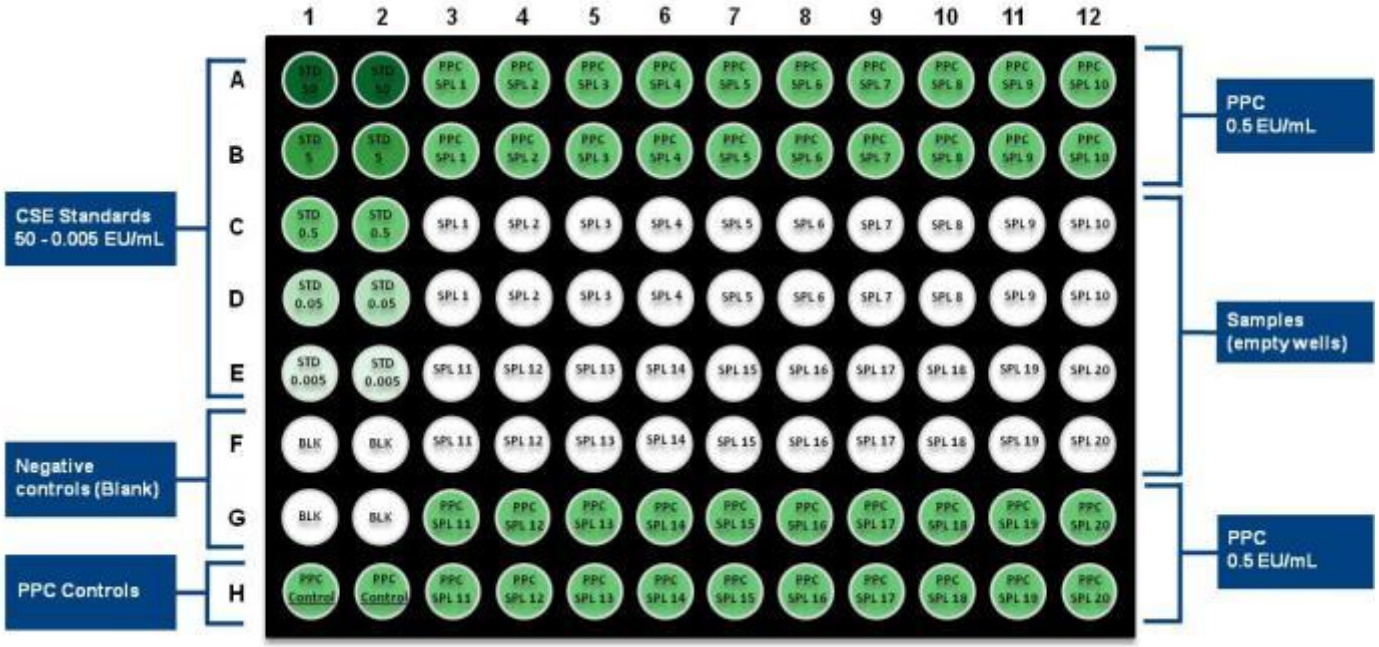
- **The rapid GO version of ENDOZYME® II features the GOPLATE™ - a microplate pre-filled with required standard curve and positive product control concentrations (PPC):**

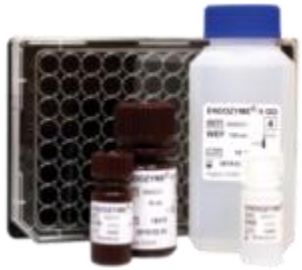
- Eliminates manual preparation of standard dilutions & PPCs
- Over 50% reduction in handling time compared to conventional microplate assays
- Significantly reduces risk of human error during preparation steps
- Higher precision and rate of valid results
- Easy automation
- Ideal for in-process control of water and raw materials as well as product release testing

ENDOZYME® II GO

Key differentiator, the GOPLATE™

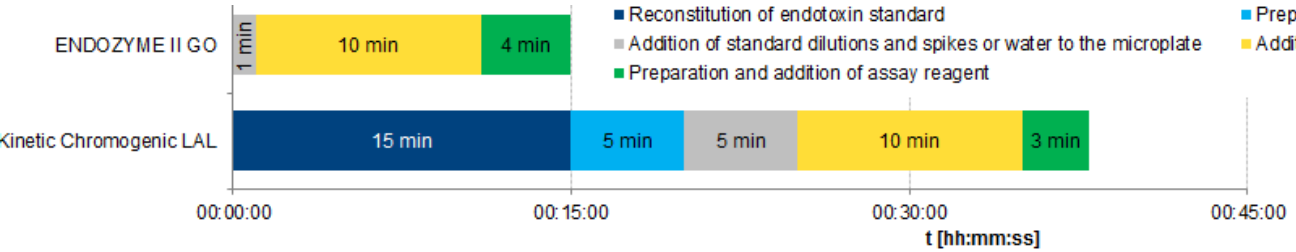
- Pre-filled with dried Control Standard Endotoxin for the standard curve 0.005 - 50 EU/mL and Positive Product Controls 0.5 EU/mL, all in duplicate replicates fulfilling pharmacopoeial standard curve requirements:





MAIN BENEFITS OF ENDOZYME® II GO

GAIN OF TIME: 50% SAVING ON PREPARATION TIME



- On Average **1 hour SAVING per Kit**
- **Cost of Technician per hour: between 180 to 220 €**

REDUCE INVALID RESULTS

- Customers shows reduction of Invalid Results
  - Between 2 to 3 % for Water Testing
  - Much more for Final products!
- **Cost of an Invalid Result between 180 to 700 €**

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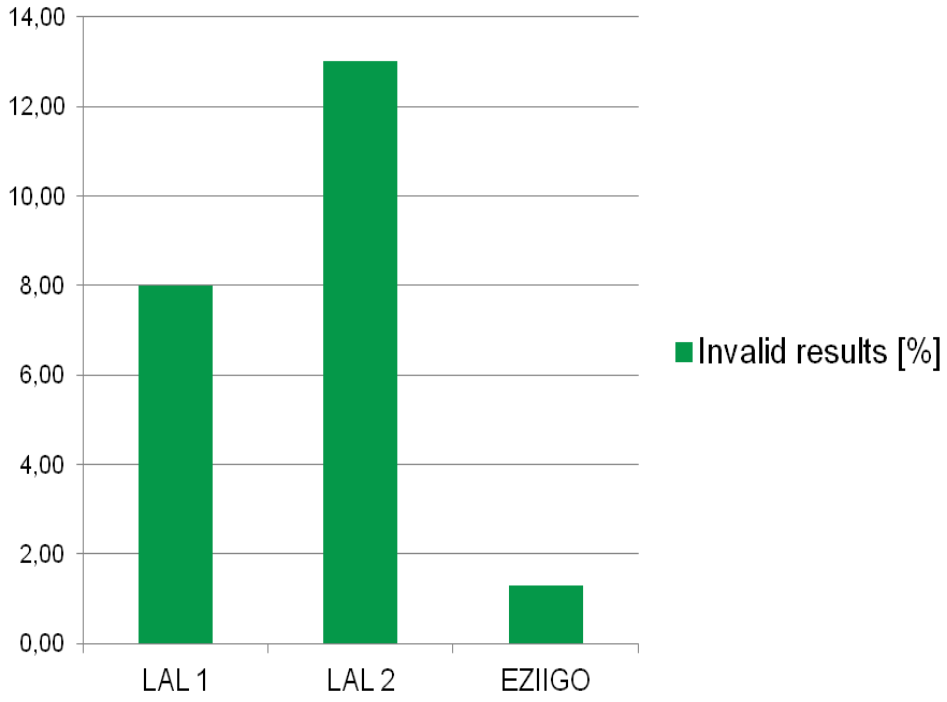
ENDOZYME® II GO

- **A significant reduction in error rate, saving both time and money otherwise spent on test repetition and investigations:**



(source: Marius, M., Sanofi Pasteur 2018)

CONCLUSION

A revolutionary method

Combines 21st century technology with the horseshoe crab's endotoxin sensitive protein: natural evolution of the LAL test

Improvement of LAL testing & Simplified workflow facilitating automation

Ethical

Prevention of animal sources: use of recombinant proteins

Meets official regulations including 3Rs principles

Financial

Lower rate of invalid results
Reducing the operator risk

&

On time reduction & shorter TTR

Resulting in Saving

Sustainable

Front. Mar. Sci., 05 June 2018 | https://doi.org/10.3389/fmars.2018.00185

The Role of Horseshoe Crabs in the Biomedical Industry and Recent Trends Impacting Species Sustainability

Limitless source

Ideal solution to ensure the sustainability of the endotoxin detection test

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QUESTIONS??