Alternative approaches to BET (LAL) test, reasons for a change

Dr. Ingo Spreitzer
Section INF 5 Microbiological Safety

Bundesinstitut für Impfstoffe und biomedizinische Arzneimittel
Federal Institute for Vaccines and Biomedicines

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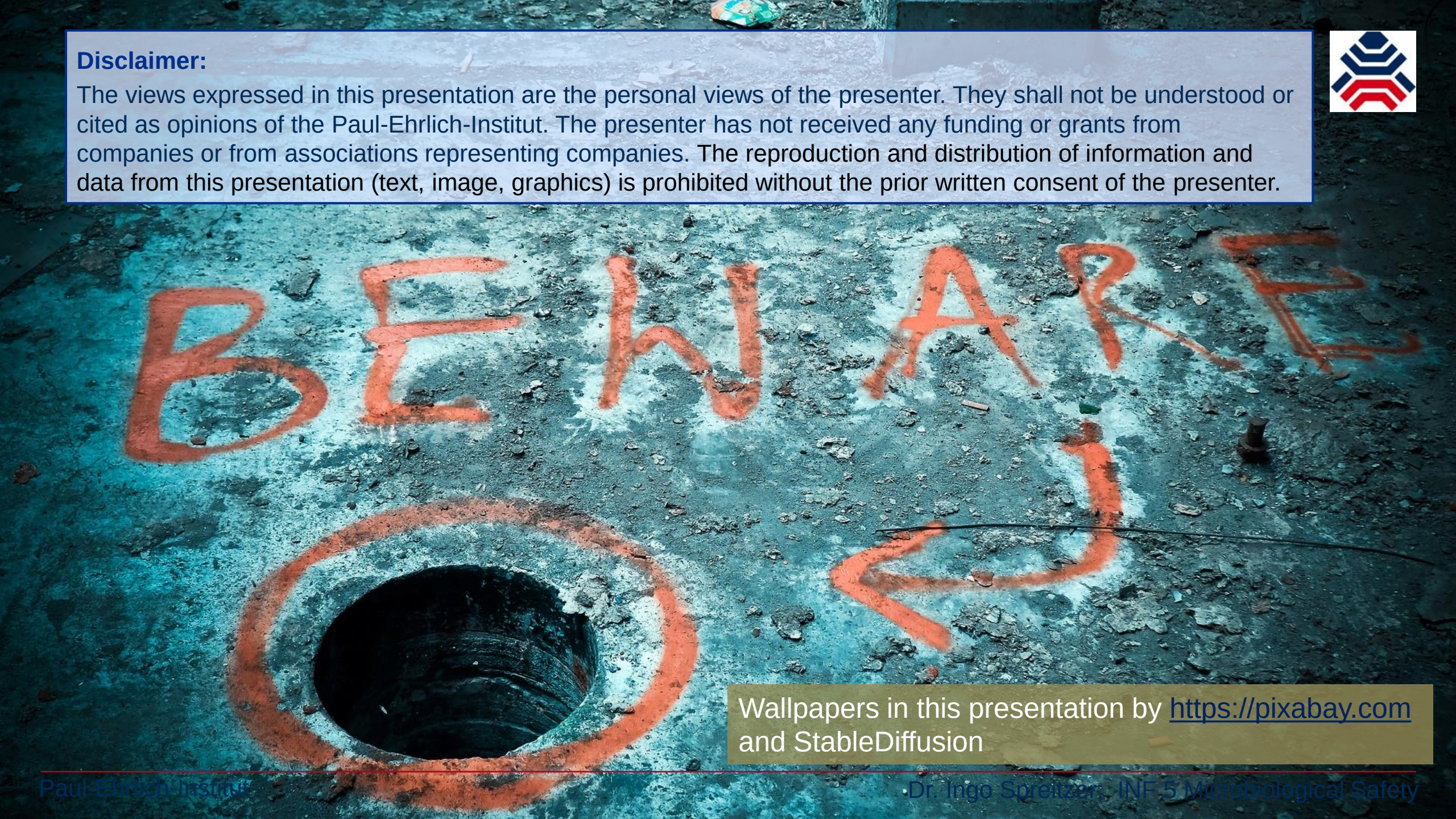

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HUMANE SOCIETY INTERNATIONAL INDIA

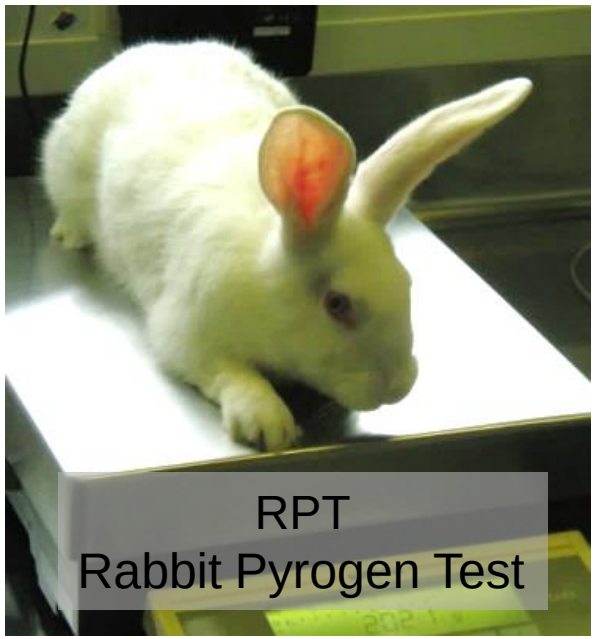
Joint webinar - HSI/India and USP India: Introduction to recombinant Factor C and its future in India

August 22nd 2024, 2:30-5:30 PM IST

Outlook

- current Pyrogen / Endotoxin tests
- how EU-Directive 2010/63 affected Pyrogen/Endotoxin testing
- The growing demand for BET (estimated CAGR 12.3%; + COVID), discussion on sustainability and climate change; Tachypleus **is endangered**
- the new pyrogenicity strategy in Europe and its consequences for drug import
- The global future of Pyrogen / Endotoxin testing

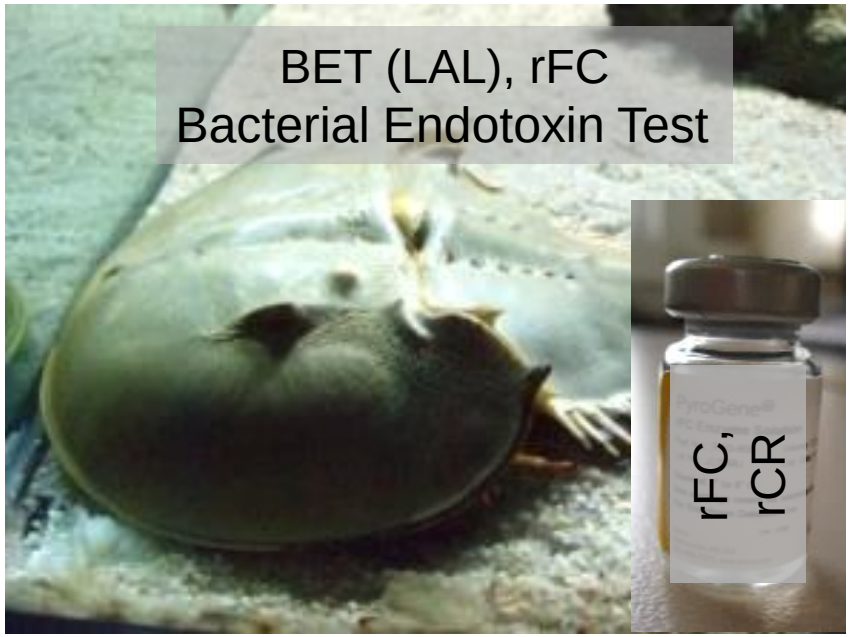
- Animal experiment
- no controls



RPT
Rabbit Pyrogen Test

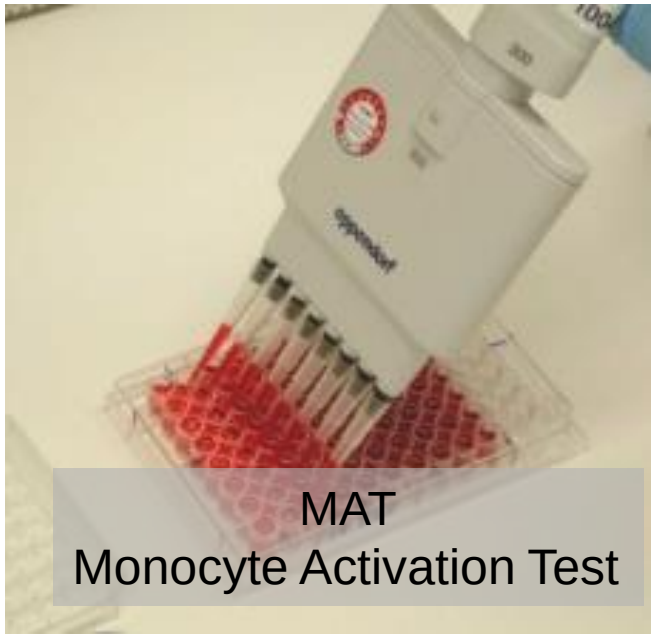
- **Sensitivity relatively unknown**, depends on Injection volume
- **no positive control**
- no standard curve
- no Spiking/Recovery

- No animal experiment (BET*)
- controls, Limits in IU / EE



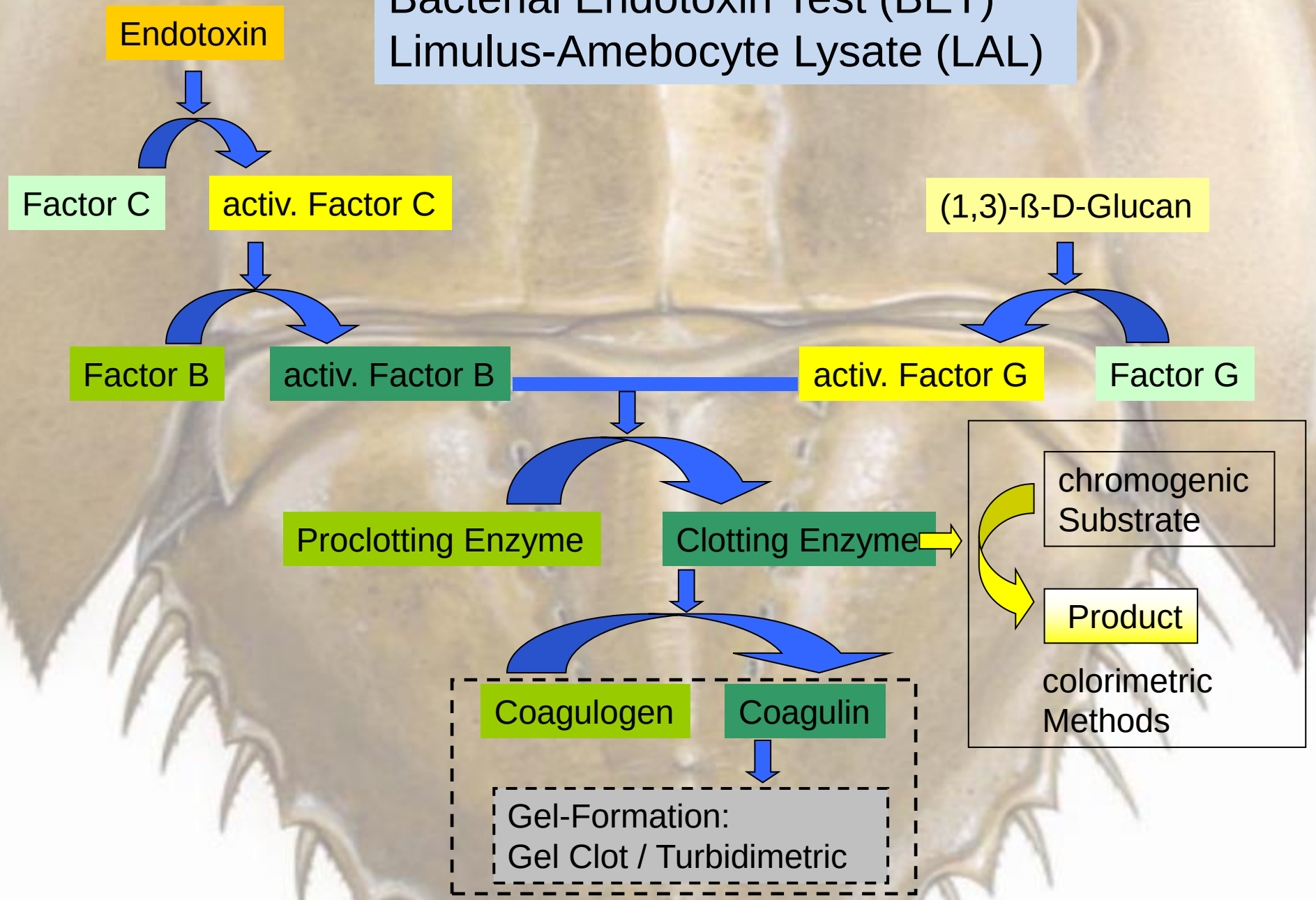
- sensitivity known (λ)
- Standard curve LPS
- Spiking/Recovery
- result in IU

- harmonised Limits in IU



- **sensitivity known**
- **Standard curve LPS**
- Spiking/Recovery
- NEP-controls
- Result in EE
- Limits in EE Ph. Eur.

Bacterial Endotoxin Test (BET)
Limulus-Amebocyte Lysate (LAL)



Directive 2010/63/EU on the protection of animals used for scientific purposes

- Directives have to be converted to national laws
  - “Instructions” for implementation

Choice of methods
1. Without prejudice to national legislation prohibiting certain types of methods, Member States shall ensure that a procedure is not carried out if another method or testing strategy for obtaining the result sought, not entailing the use of a live animal, is recognized under the legislation of the Union.

“FAQ”: if the Ph. Eur. prescribed an *in vitro* alternative to an animal test: would the “*alternative* “ method become the preferred method? YES

The new provisions of the German Animal Welfare Act based on Directive (EC) 2010/63/EU came into force in July 2013

The future of BET in Europe: Directive 2010/63
BET is no animal experiment,
Limulus is no vertebrate

3. This Directive shall apply to the following animals:

(a) live non-human vertebrate animals, including:

(i) independently feeding larval forms; and

(ii) foetal forms of mammals as from the last third of their normal development;

(b) live cephalopods.

Cephalopods mentioned (no vertebrates!)

DIRECTIVE 2010/63/EU OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL

of 22 September 2010

on the protection of animals used for scientific purposes

(16) It is necessary to ensure that the use of animals in procedures does not pose a threat to biodiversity. Therefore, the use of endangered species in procedures should be limited to a strict minimum.

biodiversity

CHAPTER I

GENERAL PROVISIONS

Article 1

Subject matter and scope

1. This Directive establishes measures for the protection of animals used for scientific or educational purposes.

To that end, it lays down rules on the following:

(a) the replacement and reduction of the use of animals in procedures and the refinement of the breeding, accommodation, care and use of animals in procedures;

(b) the origin, breeding, marking, care and accommodation and killing of animals;

Do not only see the experiment, include: breeding accomodation

>> 120.000 dead *Limulus* per year only for BET

Perils to Horseshoe crabs

- loss of breeding habitats (coastal development)
- Fishery (bait, food)
- Environmental pollution
- biomedical use (BET)

Impact

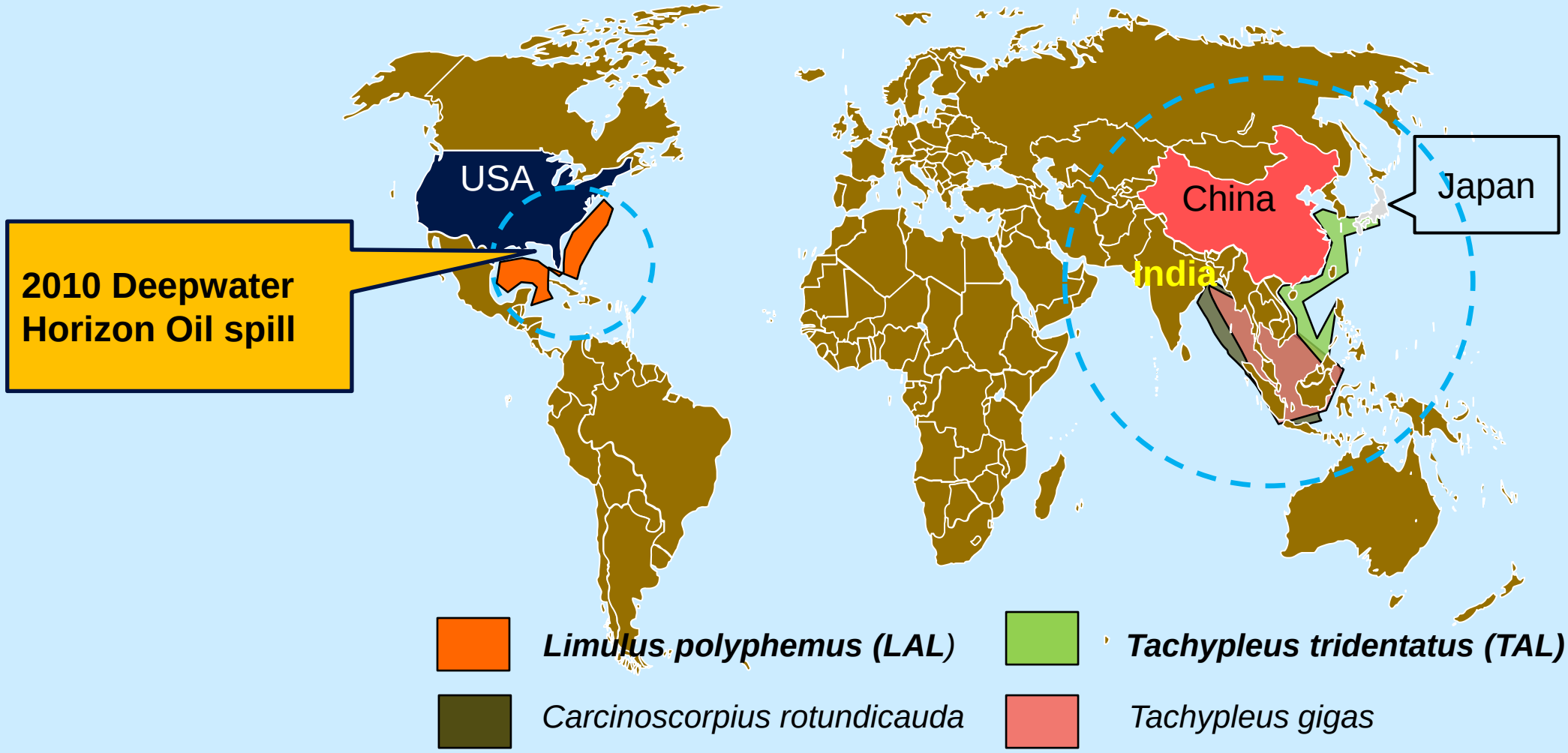
Risk for Drug safety

Lysate supply

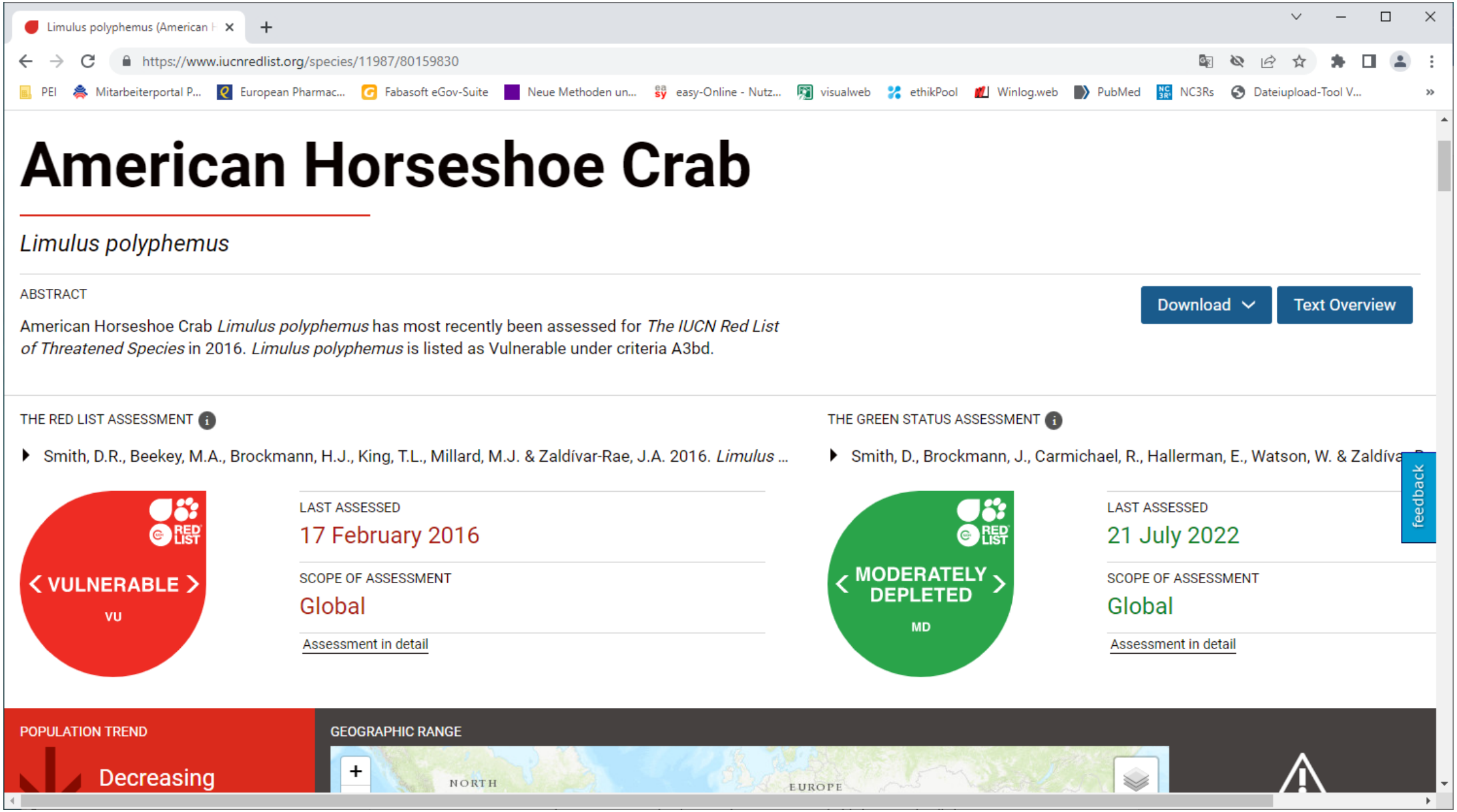
We have alternatives for Endotoxin / Pyrogen testing: **rFC + MAT**

Distribution and status of Horseshoe crab-species

No HSC and Lysate manufacturing in Europe!



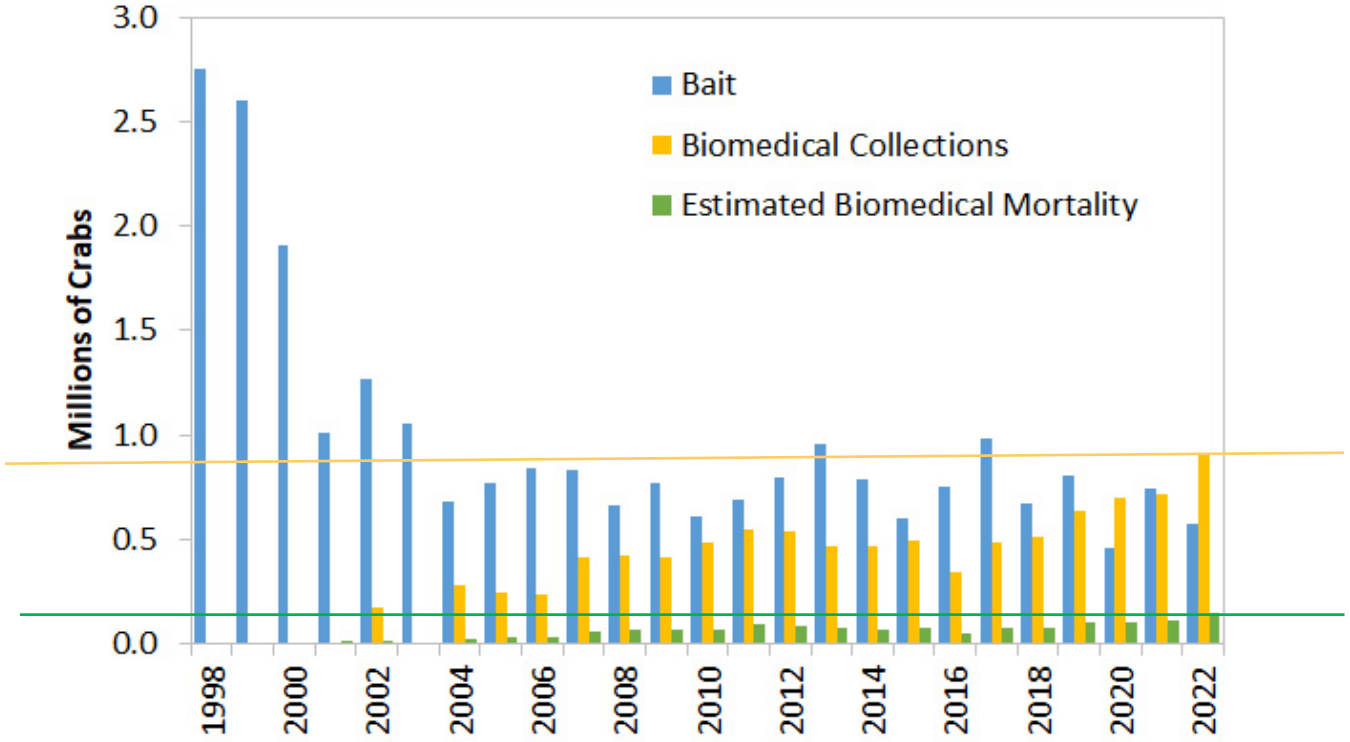
IUCN red list of threatened species


Limulus polyphemus (American
https://www.iucnredlist.org/species/11987/80159830
PEI
Mitarbeiterportal P...
European Pharmac...
Fabasoft eGov-Suite
Neue Methoden un...
easy-Online - Nutz...
visualweb
ethikPool
Winlog.web
PubMed
NC3Rs
Dateiupload-Tool V...
American Horseshoe Crab
Limulus polyphemus
ABSTRACT
American Horseshoe Crab Limulus polyphemus has most recently been assessed for The IUCN Red List of Threatened Species in 2016. Limulus polyphemus is listed as Vulnerable under criteria A3bd.
Download
Text Overview
THE RED LIST ASSESSMENT
Smith, D.R., Beekey, M.A., Brockmann, H.J., King, T.L., Millard, M.J. & Zaldívar-Rae, J.A. 2016. Limulus ...
VULNERABLE
VU
LAST ASSESSED
17 February 2016
SCOPE OF ASSESSMENT
Global
Assessment in detail
THE GREEN STATUS ASSESSMENT
Smith, D., Brockmann, J., Carmichael, R., Hallerman, E., Watson, W. & Zaldíva
MODERATELY DEPLETED
MD
LAST ASSESSED
21 July 2022
SCOPE OF ASSESSMENT
Global
Assessment in detail
feedback
POPULATION TREND
Decreasing
GEOGRAPHIC RANGE
NORTH
EUROPE

Biomedical use still rising…

Coastwide Horseshoe Crab Bait Landings & Biomedical Collections

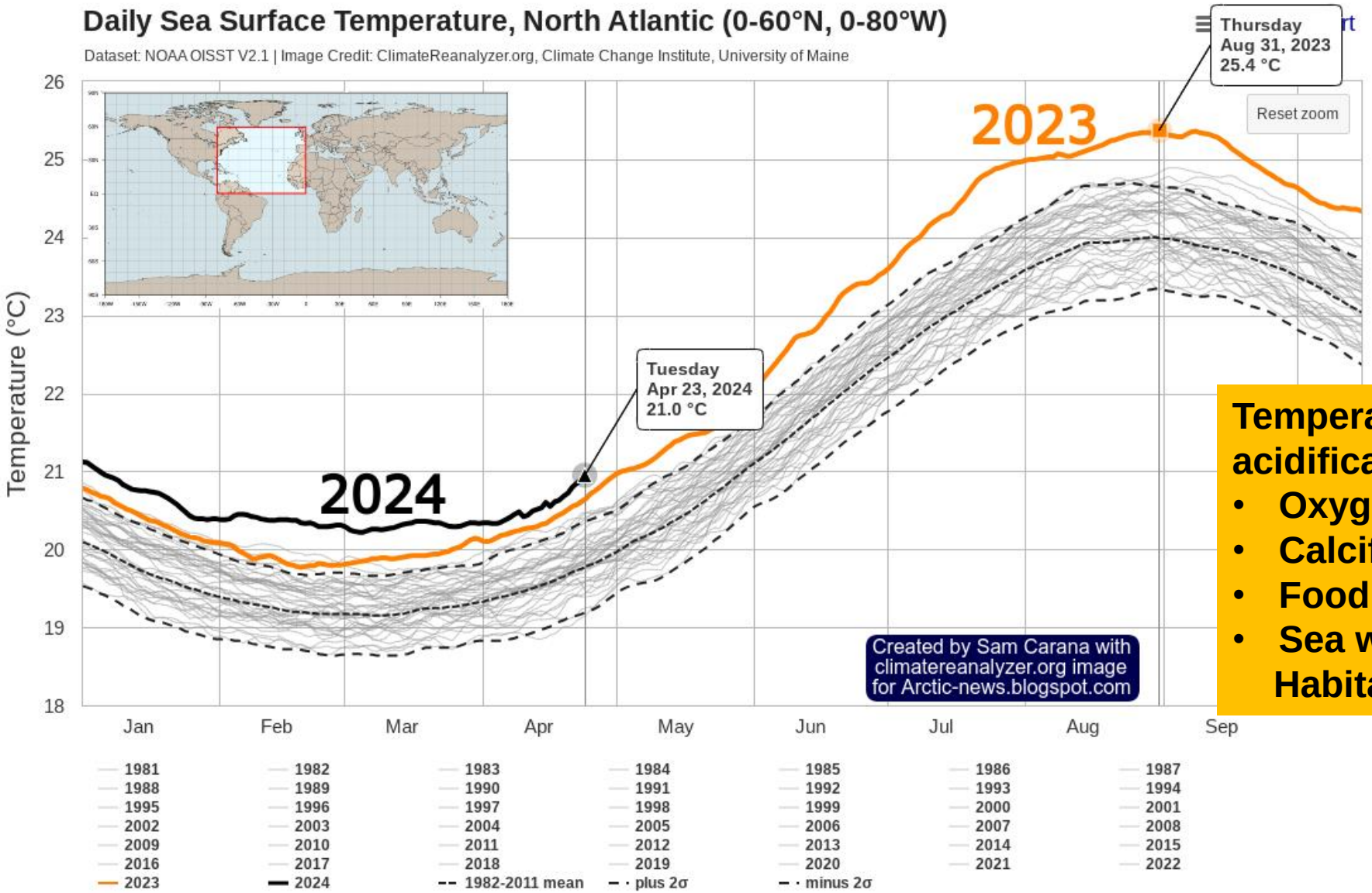
Source: ASMFC State Compliance Reports, 2022



Please note the following details regarding biomedical harvest numbers:

* Harvest numbers include all horseshoe crabs brought to bleeding facilities, including those that were harvested as bait and counted against state quotas.
* Most of the biomedical crabs harvested are returned to the water after bleeding; a 15% mortality rate is estimated for all bled crabs.

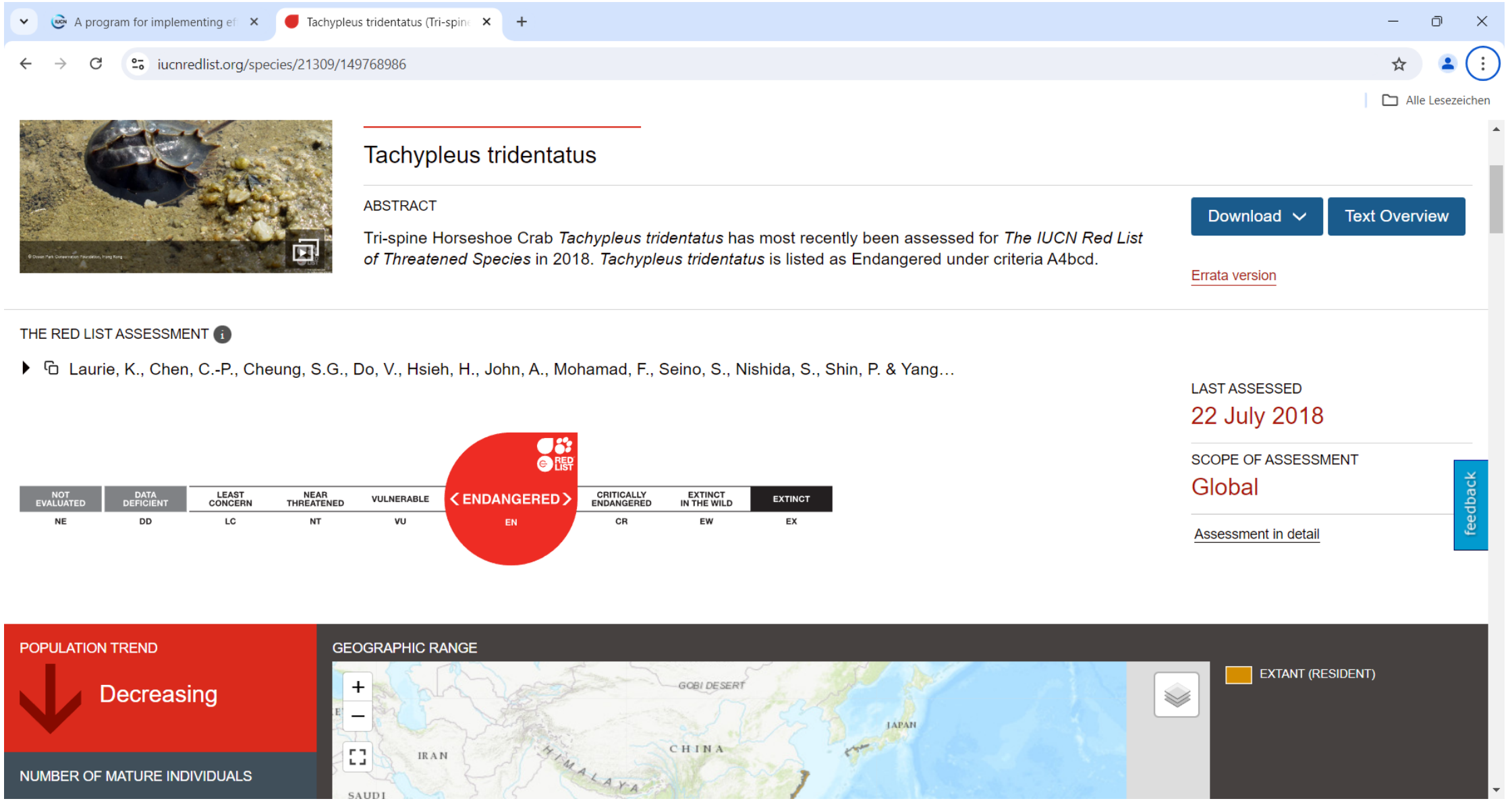
https://www.asmfc.org/species/horseshoe-crab



Temperature increase, acidification:

- **Oxygen content**
- **Calcification**
- **Food chain?**
- **Sea water level rising**

Habitat fully affected


A program for implementing ef
Tachypleus tridentatus (Tri-spin
iucnredlist.org/species/21309/149768986
Alle Lesezeichen
Tachypleus tridentatus
ABSTRACT
Tri-spine Horseshoe Crab Tachypleus tridentatus has most recently been assessed for The IUCN Red List of Threatened Species in 2018. Tachypleus tridentatus is listed as Endangered under criteria A4bcd.
Download
Text Overview
Errata version
THE RED LIST ASSESSMENT
Laurie, K., Chen, C.-P., Cheung, S.G., Do, V., Hsieh, H., John, A., Mohamad, F., Seino, S., Nishida, S., Shin, P. & Yang…
NOT EVALUATED NE
DATA DEFICIENT DD
LEAST CONCERN LC
NEAR THREATENED NT
VULNERABLE VU
ENDANGERED EN
CRITICALLY ENDANGERED CR
EXTINCT IN THE WILD EW
EXTINCT EX
LAST ASSESSED
22 July 2018
SCOPE OF ASSESSMENT
Global
Assessment in detail
feedback
POPULATION TREND
Decreasing
NUMBER OF MATURE INDIVIDUALS
GEOGRAPHIC RANGE
GOBI DESERT
CHINA
IRAN
HIMALAYA
JAPAN
SAUDI
EXTANT (RESIDENT)

PSCI PHARMACEUTICAL SUPPLY CHAIN INITIATIVE

Building responsible supply chains

The use of Horseshoe Crabs in the Pharmaceutical Sector

A position paper from the PSCI

22 May 2023

… ***Protect all endangered species – no further collection of TAL.*** The PSCI's members will end commercial pressure on the populations of *Tachypleus gigas* and *Tachypleus tridentatus*, by committing to no further collections from these species. PSCI members and first tier suppliers will no longer use TAL after existing supplies have been exhausted.

Minimize the requirements for naturally-derived testing materials. The PSCI recognizes that members will potentially require a range of endotoxin testing techniques, and the availability of rFC, other recombinant reagents, and microfluidics offers members a route to dramatically reduce the demand for LAL. Members are encouraged to explore and adopt alternatives, setting themselves internal goals to minimize the volume of LAL used in their own operations and first tier suppliers. …

https://pscinitiative.org/bulletin?bulletin=629

United Nations Sustainable Development Goals (SDGs)

History of rFC endorsement in Europe

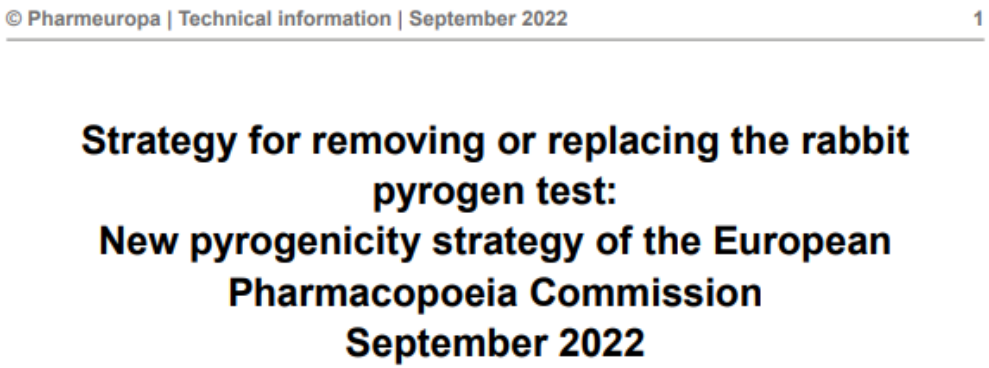
- First rFC released to the global market in **2003**
- Fist discussions to endorse rFC in PhEur were deemed premature (one vendor, few data)
- Next rFC-vendor appears, Patent dispute Lonza/Hyglos solved in 2013
- EU-Directive 2010/63 came into force 2013

- rising amount of validation data for rFC; PhEur approaches JP and USP on rFC without success,
  -> BET Working Party engages
- Active search for validation data packages, 2018 FDA approves rFC for mAB (Emgality)
- Controversial debate about quality of data and performance of rFC
- Publication PDA J Pharm Sci Technol. 2020 Sep-Oct;74(5):602-611.
  „Currently Available Recombinant Alternatives to Horseshoe Crab Blood Lysates:
  Are They Comparable for the Detection of Environmental Bacterial Endotoxins? A Review“
- Implementation of 2.6.32. rFC in 2020

- rCR released to global market first in **2021 (18 years later than rFC!)**
- 2022 PhEur New pyrogenicity strategy released

The new Pyrogenicity strategy in Europe

https://go.edqm.eu/NewPyrogenicityStrategy



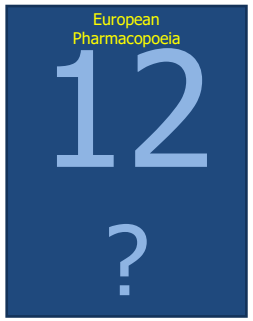
Strategy for removing or replacing the rabbit pyrogen test:
New pyrogenicity strategy of the European Pharmacopoeia Commission
September 2022

- RPT 2.6.8. (and BET-section) deleted in 60 texts including Substances for pharmaceutical use or Parenterals and replaced by citation of new Chapter 5.1.13. „Pyrogenicity“
- Chapter 5.1.13. Pyrogenicity guides the user in choosing the appropiate assay: MAT or/and BET/rFC

Replacement of chapter *2.6.8: proposed strategy*

Consolidated strategy approved by the European Pharmacopoeia Commission in June 2022

***2.6.8** in 60 texts of the PhEur*



***2.6.8** in 60 texts of the PhEur*

5.1.13
Pyrogenicity

New! Chapter *5.1.13.* Pyrogenicity

USER

Potential pyrogens other than endotoxins can be ruled out

Risk analysis, potential presence of non-endotoxin pyrogens Stage of manufacturing process

Exclusion of potential pyrogens other than endotoxins not possible

Chapters *2.6.14/ 2.6.32.* BET

or both

Chapter *2.6.30.* MAT

Decision on a testing strategy
Decision on the limits

slide by Dr. Emmanuelle Charton, EDQM

European Pharmacopeia

Replacing RPT 2.6.8. by Pyrogenicity 5.1.13.

Pyrogenicity
5.1.13.

Referring to:
2.6.14.
5.1.10.
2.6.32.
2.6.30.
2.6.40.

BET
2.6.14.
harmonised

MAT
2.6.30.

rFC
2.6.32.

BET-Guidelines
5.1.10.

~~RPT 2.6.8~~

RPT 2.6.8. replacement by 5.1.13. in 60 PhEur texts, including:
2.0.34. Substances for pharmaceutical use
5.0.20. Parenteral preparations

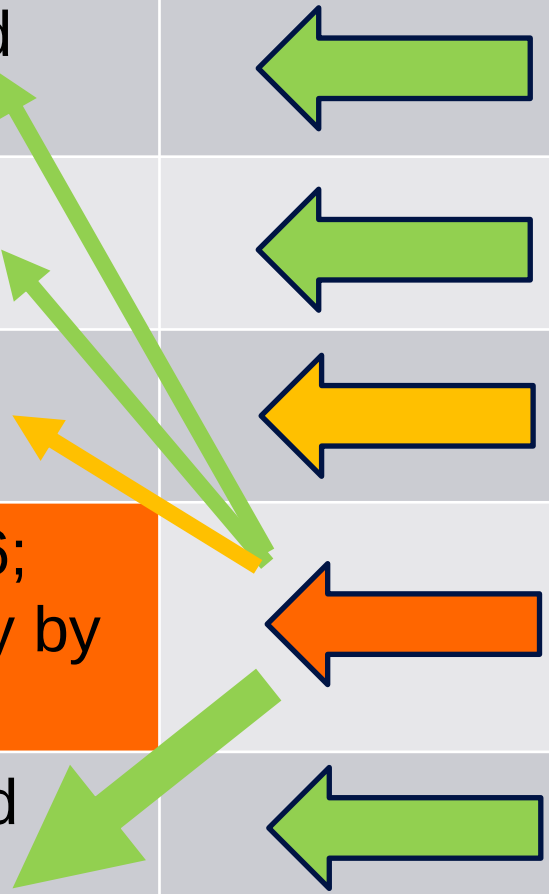
- MAT and rFC are **OFFICIAL** methods of the PhEur
- and are **COMPENDIAL** (if referenced in overarching Chapters or individual monographs) for certain products/groups

The revised monographs 0169 Water for injections and 0008 Water, purified allow the use of recombinant factor C method for the control of bacterial endotoxins with an implementation date on 1 April 2024; approx. 70-80% of BET-samples are water

Pharmeuropa 36.2.: rFC drafted into
5.32. CELL-BASED PREPARATIONS FOR HUMAN USE

5.36. mRNA VACCINES FOR HUMAN USE
5.37. RECOMBINANT VIRAL VECTORED VACCINES FOR HUMAN USE
5.39. mRNA SUBSTANCES FOR THE PRODUCTION OF mRNA VACCINES FOR HUMAN USE
5.40. DNA TEMPLATE FOR THE PREPARATION OF mRNA SUBSTANCES

Drug produced in EU	Import to EU = retest in EU; test fixed in market authorisation	Drug produced in Non-EU
BET official and compendial*		BET official and compendial*
rFC official and compendial*		rFC alternative
rCR alternative	Full method validation	rCR alternative
RPT ends 2026; replaced mainly by MAT		RPT official and compendial*
MAT official and compendial*		MAT alternative



*** if referenced**

History of rFC/rCR endorsement in USA and Japan

- First rFC released to the global market in **2003**
- USP opinion on rFC changed several times
- Both USP and JP see rFC/rCR still as alternative
- JP published 3 comparative studies between classical and recombinant BET-versions
- 2018 FDA approves rFC for mAB (Emgality)
- Controversial debate about quality of data and performance of rFC

- rCR released to global market first in **2021 (18 years later than rFC!)**
- 2022 USP removed the current Microbiological Expert commitee; restart in 2023 with new team

- 2024 USP <86> endorses (November 2024) both rfC and rCR (but still alternative)

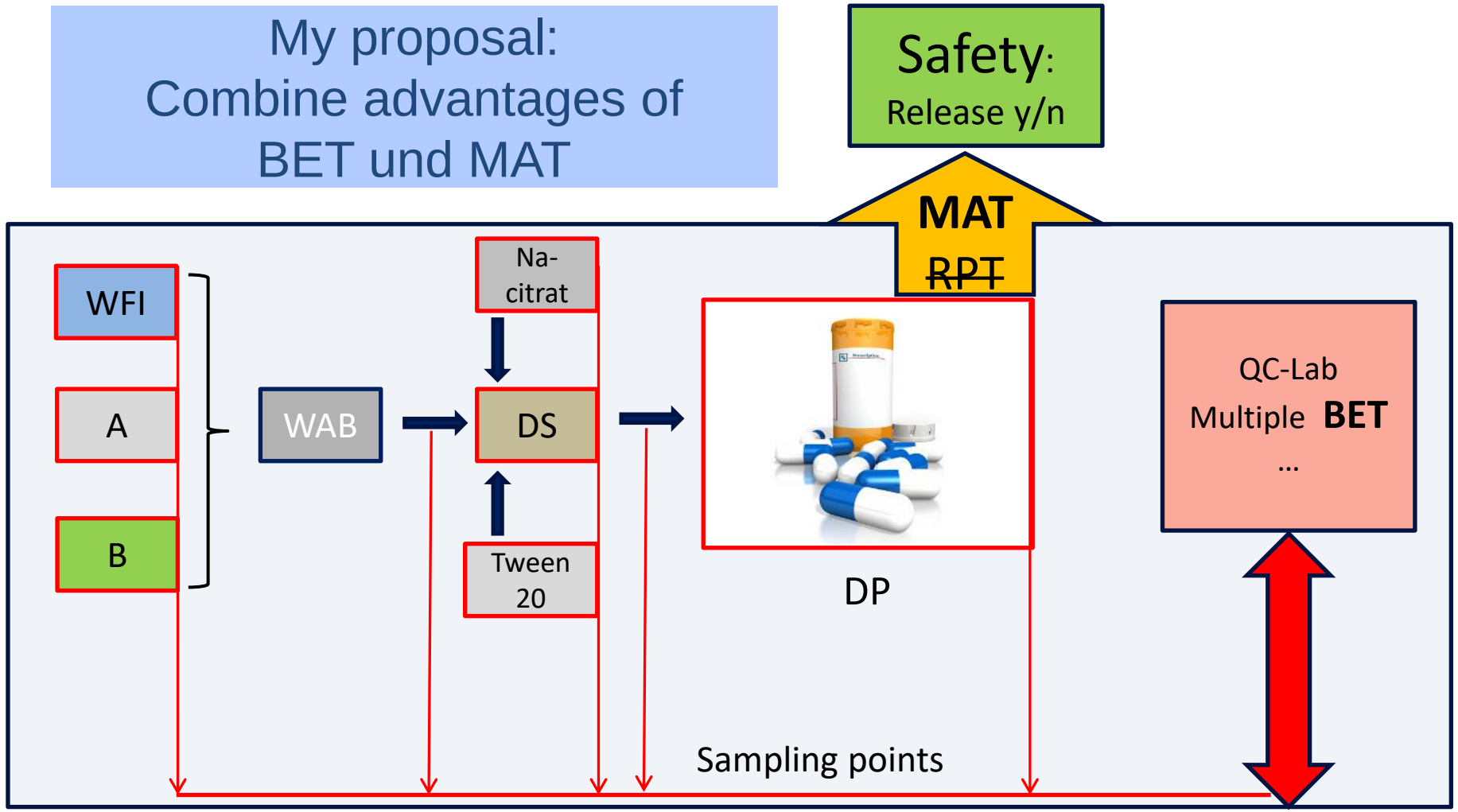
- Japan?

- Harmonisation? Indian Pharmacopeia as new PDG-member might encourage the others to harmonize

„Endotoxin-free“

„Pyrogen-free“

My proposal: Combine advantages of BET und MAT



Quality

Drug Production: hours to months

the majority of BET tests is done during production, only one on the final product

The future of BET and MAT

BET

1980

New BET-setups like PTS or Eclipse

rFC, (rCR)

2021

Sensor on a chip?; direct analytical detection techniques?

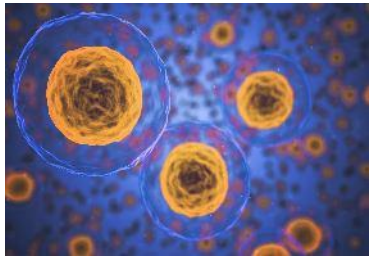
Time

RPT;
Prediction model for humans

1942

MAT;
In-vitro human model

2010



Optimized MAT

- *NEP-standards*
- *Dedicated Software*
- *Engineered cells (e.g. iPSC-derived cells)*
- *Direct readout*
- ***faster***

- animal test (RPT) replaced by superior *in-vitro* assay (MAT) predictive for humans

- rFC diminishes dependence on horseshoe crabs, thus improving drug supply chain resilience

Ingo.spreitzer@pei.de

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