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Endotoxin and pyrogen testing in biologic products

Allen L. Burgenson

Global SME – Testing Solutions

Today's presenters

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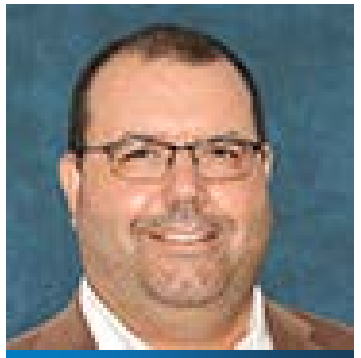
Speaker:

Allen L Burgenson

- Global Subject Matter Expert for Testing Solutions
- Over 30 years experience in industries regulated by the FDA
- Co-authored PDA Technical Reports regarding microbiological and endotoxin detection issues, as well as USP chapters on endotoxin and depyrogenation



Moderator:

Travis Wallace

- Scientific Support Specialist

Introduction

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What are biologic products?

How are biologic products tested for endotoxin and pyrogens?

Where in the process are such products tested?

What types of interferences may be encountered, and how are they overcome?

This presentation addresses considerations when testing biologic products and products made from them for pyrogenicity using LAL, rFC, or MAT assays

Biologic products defined

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- Biological products include a wide range of products such as vaccines, blood and blood components, allergenics, somatic cells, gene therapies, tissues, and recombinant therapeutic proteins

- Biologics can be composed of sugars, proteins, or nucleic acids or complex combinations of these substances, or may be living entities such as cells and tissues

- Biologics are isolated from a variety of natural sources – human, animal, or microorganism – and may be produced by biotechnology methods and other cutting-edge technologies

- Gene-based and cellular biologics, for example, often are at the forefront of biomedical research, and may be used to treat a variety of medical conditions for which no other treatments are available

How are biologics different from conventional drugs?

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- In contrast to most drugs that are chemically synthesized with a known chemical structure, most biologics are complex mixtures that are not easily identified or characterized

- Biological products, including those manufactured by biotechnology, tend to be heat sensitive and susceptible to microbial contamination necessitating the use of aseptic principles from initial manufacturing steps

- Biological products often represent the cutting-edge of biomedical research and, in time, may offer the most effective means to treat a variety of medical illnesses and conditions that presently have no other treatments

General testing requirements for biologics

Endotoxin and pyrogen testing

- Biologic preparations **are routinely tested for pyrogen content** before distribution to the public

- Test methods used for routine quality control of biologics are intended to monitor production consistency and to ensure comparability between commercial batches and those batches that were found to be safe and efficacious in clinical studies

- Inherent variability of *in-vivo* assays makes them less suitable to ensure potency and safety as compared to appropriately designed *in-vitro* assays

- This presentation outlines different methods to assess endotoxin and pyrogen quality attributes – from pilot scale to full-scale manufacturing with automation

Biologics belong to the injectable drugs category

How do pyrogens act?

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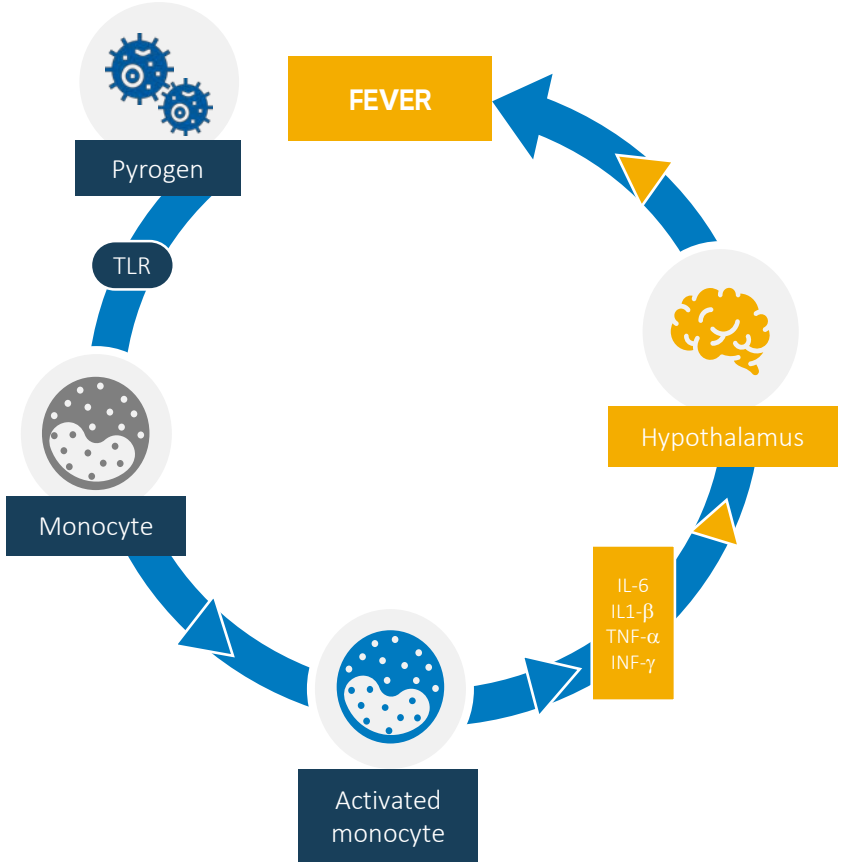
- Parenteral pharmaceutical products must be free of pyrogenic contaminants
- A pyrogen is linked to an immune defense mechanism and induces fever
- Pyrogens stimulate monocytes through toll-like receptors (TLR)
- Activated monocytes release pro-inflammatory cytokines that initiate the febrile response



Pyrogen		Ligand
Bacterial endotoxin	Lipopolysaccharide (LPS)	TLR 4
Non-endotoxin pyrogens (NEP)	Bacteria cell wall • Peptidoglycan • Lipopeptide, LTA Lipoproteins Flagellin	TLR1/ TLR2 TLR2 TLR5
	Mycoplasma Lipopeptide	TLR2/ TLR6
	Yeasts & Molds, e.g. Zymosan	TLR2/ TLR6
	Virus, e.g. ssRNA dsRNA CPG motif	TLR7/ TLR8 TLR3 TLR9
	Chemicals	Diverse

Biologics types

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01

Classical biologics such as blood-derived products and sera

02

Well-characterized proteins such as monoclonal antibodies

03

Antisera and anti venoms

04

Vaccines

05

Allergenics

06

Cell and gene therapies

Vaccine types

Three major production technologies for vaccines

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Pos.	Class	Description	Examples
1	Whole pathogen	• Entire pathogen that has been inactivated or weakened • Chimeric biologics contain genetic information and display biological properties of parent virus	• Weakened: measles, mumps, rubella • Inactivated: Hepatitis A • Chimeric: Zika
2	Subunit biologics	• Often include adjuvants to induce a long-term immunity • Include components or antigens that stimulate the immune system • May contain bacteria cellular components, outer membrane components, conjugated biologics • May use recombinant proteins • New: nanoparticle biologics (protein subunits, in development)	• Acellular: Pertussis • Conjugated: meningococcal, pneumococcal infections • Recombinant Protein: Hep B, HPV • Nanoparticles: universal flu, MERS coronavirus, RSV, EBV
3	Nucleic acid biologics	(In development, addressing recent outbreaks) • DNA plasmid biologics • mRNA biologics • Recombinant vector biologics	• DNA: SARS-CoV, H5N1, H1N1, Zika • mRNA: Zika • Recombinant: HIV, Zika and Ebola

Pyrogen testing for biologics

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- Biologics may contain lipopolysaccharide (LPS) and/or non-endotoxin pyrogens (NEP) by design, especially if they contain adjuvants

- Pyrogen content is one of the critical quality attributes impacting the safety of a biologic product

- It is particularly important, therefore, to separate unwanted and potentially dangerous levels of pyrogenicity coming from raw materials or the manufacturing environment

- There are now two suitable *in-vitro* tests for pyrogens, the Limulus Amebocyte Lysate (LAL) test and the Monocyte Activation Test (MAT) test

Sample injected into ear vein in the rabbit pyrogen test

Current compendial tests

Test specificities and limits of detection (LODs)

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	Test type	Detection mechanism	LOD EU/mL*	Identification
Pyrogens	 Rabbit Pyrogen Test (RPT) Ph. Eur. 2.6.8, USP <151>	*In-vivo:* measure raise in body temperature after injection of a drug	0.5	Bacterial endotoxins, non-endotoxin pyrogens (e.g. gram-positive bacteria, virus, yeasts & molds, chemicals
Pyrogens	 Monocyte Activation Test (MAT) Ph. Eur. 2.6.30, USP <1085>	*In-vitro:* measure cytokines released by monocytes from human blood	0.02	
Endotoxin	 Limulus Amebocyte Test (LAL) Ph. Eur. 2.6.14, USP <85>	*In-vitro:* measure initiation of a clotting cascade by horseshoe crab blood amebocytes	0.005	Gram-negative bacterial endotoxin
Endotoxin	 Recombinant Factor C Test (rFC) Ph. Eur. 2.6.32, USP <1085>	*In-vitro:* recombinant alternative to LAL, based on factor C (first component in LAL cascade)	0.005	

** Representative LODs*

Why are two tests for pyrogenicity recommended?

Non-endotoxin pyrogens (NEPs) cannot be detected with the bacterial endotoxins test (BET)

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Specific considerations when testing biologics for pyrogenicity:

- The BET is less complex, cheaper and faster for routine testing and, more importantly, process control
- However, the LAL test has certain limitations:
  - It will not detect non-endotoxin pyrogens (NEP) such as lipoproteins, peptidoglycan and lipoteichoic acids from gram-positive bacteria and is therefore an imperfect reflection of the human immune response
  - Evidence must be provided that especially adjuvanted vaccine formulations do not interfere with the LAL test
- Consequently, it is recommended to supplement the LAL test's ability to detect low levels of endotoxin with a specific test for NEPs
- The MAT assay is complementary to the BET and can be used at specific points in the manufacturing cycle

BET and MAT are complementary tests

Method	Targets	RPT	BET	MAT
Endotoxin	Gram-negative bacteria	♦♦	♦♦♦	♦♦
Non-endotoxin pyrogens	Human-specific NEP	–	–	♦♦
	Other bacteria	♦♦	–	♦♦♦
	Yeasts & molds	♦♦	–	♦♦♦
	Virus	♦	–	♦♦♦
	DNA/ RNA	♦	–	♦♦
	Chemicals	–	–	♦♦

Testing biologics for pyrogenicity

Regulatory chapters and the information that the tests provide

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- Biologics are regulated in the US under the authority of the Food, Drug, and Cosmetic Act by the U.S. Food and Drug Administration. The regulations may be found in Title 21 of the U.S. Code of Federal Regulations
- 21 CFR Sec. 211.167 Special testing requirements
  - (a) For each batch of drug product purporting to be sterile and/or pyrogen-free, there shall be appropriate laboratory testing to determine conformance to such requirements. The test procedures shall be in writing and shall be followed.
  - Animal vaccines are regulated In the US by the United States Department of Agriculture (USDA)

Other countries/world regions have similar regulations

Examples:

- Europe – European Medicines Agency (EMA)
- Great Britain – Medicines and Healthcare Products Regulatory Agency (MHRA)
- Japan – Pharmaceuticals and Medical Devices Agency (PMDA)
- Brazil – The Brazilian Health Regulatory Agency (ANVISA)
- Australia – Therapeutic Goods Administration (TGA)

Testing biologics for endotoxin, US regulations

Most biologics are exempted for RPT application

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- In the US, biologics are regulated as a biologic by FDA's Center for Biologics Evaluation and Research (CBER)
- **All biological products are required to be tested for pyrogenicity**, unless the product is specifically exempted by regulation
- Assay performed as per USP <151> Pyrogen Test

Number of experiments	Cumulative number of rabbits	Test sample passes if no rabbit shows	Test sample passes if aggregate rise is
1	3	> 0.5°C rise	
2	5	NMT 3 > 0.5°C rise	< 3.3°C rise

- If exempted, follow USP <85> Bacterial Endotoxins Test

Part 610. 13 General Biological Products Standards – Purity

(b) Test for pyrogenic substances. Each lot of final containers of any product intended for use by injection shall be tested for pyrogenic substances by **intravenous injection into rabbits** as provided in paragraphs (b) (1) and (2) of this section: Provided, That notwithstanding any other provision of Subchapter F of this chapter, **the test for pyrogenic substances is not required for the following products:** Products containing formed blood elements; Cryoprecipitate; Plasma; Source Plasma; Normal Horse Serum; **bacterial, viral, and rickettsial biologics and antigens;** toxoids; toxins; allergenic extracts; venoms; diagnostic substances and trivalent organic arsenicals.

Testing biologics for endotoxin, EU regulations

Biologic developers are encouraged to replace in vivo tests such as the RPT

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- EU Directive 2010/63 regulates the use of experimental animals for scientific and quality control purposes and asks for replacement of *in-vivo* tests for scientific and quality control purposes

- Chapter 5.2.14 (Guidance for substitution of *in-vivo* methods by *in-vitro* methods for quality control of biologics) of the Ph. Eur. outlines the importance to consider and establish *in-vitro* methods for quality control during vaccine development (human and veterinary use)

  The inherent variability of in-vivo assays can make them less suitable than appropriately designed in-vitro assays for monitoring the consistency of production and for assessing the potential impact of manufacturing changes.

European Medicines Agency (EMA)

- Committee for Medicinal Products for Human Use (CHMP)
- Committee for Medicinal Products for Veterinary Use (CVMP)

Alternatives to the RPT such as the MAT and BET shall be considered:

- Ph. Eur. 5.1.10 Guidelines for Using the Test for Bacterial Endotoxins
- Ph. Eur. 2.6.14 Bacterial Endotoxins
- Ph. Eur. 2.6.30 Monocyte Activation Test (MAT)
- Ph. Eur. 2.6.32 Test for Bacterial Endotoxins Using Recombinant Factor C

Testing biologics for endotoxin

Asia regulations examples

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Japan

- Minimum requirements for biological products
  - National Institute of Infectious Diseases, Japan, 2006
- Uses the pyrogen test – same principle as the USP <151> and same issues described by US 21 CFR 610.13 (many biologics are inherently pyrogenic, and may be exempted)

Number of experiments	Cumulative number of rabbits	Test sample passes if the summed response is	Test sample fails to pass if the summed response is
1	3	1.3°C or less	2.5°C or more
2	6	3.0°C or less	4.2°C or more
3	9	less than 5.0°C	5.0°C or more

- Follow chapter 4.01 bacterial endotoxins test

China

- Biologic testing requirements are found in Chinese Pharmacopoeia (ChP) Vol IV “General Requirements for Preparations/Injections”
- Parenteral drugs, in general, should be tested for endotoxin/pyrogen unless with special reasons (notice) for exemption
- The endotoxin level of the raw materials and excipients that are used in the manufacturing should also be tested and controlled

Testing biologics for pyrogenicity – summary

Endotoxin content and non-endotoxin pyrogenicity

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- Biologic products are routinely tested for endotoxin content using LAL before distribution to the public

- Viral vaccines need not be tested in rabbits for pyrogenicity if:
  - The vaccine may be incompatible with rabbits, causing the rabbit to die
  - Vaccines are meant to stimulate the immune system, and cause a fever in the animal unrelated to a pyrogen contamination

- In general, the RPT shall be replaced in EU following EU directives
  - The MAT is the *in-vitro* replacement for the RPT
  - Vaccine components may trigger the cells used in MAT to release cytokines – method C (batch comparison test) is used to test vaccine batches with reference lots that are clinically tested

Use *in-vitro* methods for pyrogen testing of biologics

RPT vs. MAT vs. LAL

Regulatory chapters and the information that the tests provide

- Both the MAT and the RPT provide information about the biological impact of the sample. They are essentially a form of dose-response assay
- The BET determines the quantifiable endotoxin concentration in a sample, not its direct activity although they are closely related
- Due to its speed and precision, the BET is additionally valuable as an in-process control
- The MAT mimics the overall human immune response most closely as it uses human cells

Test chapters	Pyrogen specificity	Measure	Time to result	Pre-clinical	End product	In-process test	Effect / presence
RPT USP <151> Ph. Eur. 2.6.8	Non-specific	Temperature increase in rabbits	> 1 day	Yes	If applicable	Uncommon	Effect
BET (rFC) USP <85>; Ph. Eur. 5.1.10, 2.6.14, 2.6.32,	Specific for endotoxin	Initial activation of factor C	< 2h	Yes	Yes	Yes	Presence
MAT Ph. Eur. 2.6.30	Non-specific	Release of pro-inflammatory cytokines	> 1 day	Yes	Yes	Process change	Effect

General clinical trial overview

Timing of phases for a typical clinical trial

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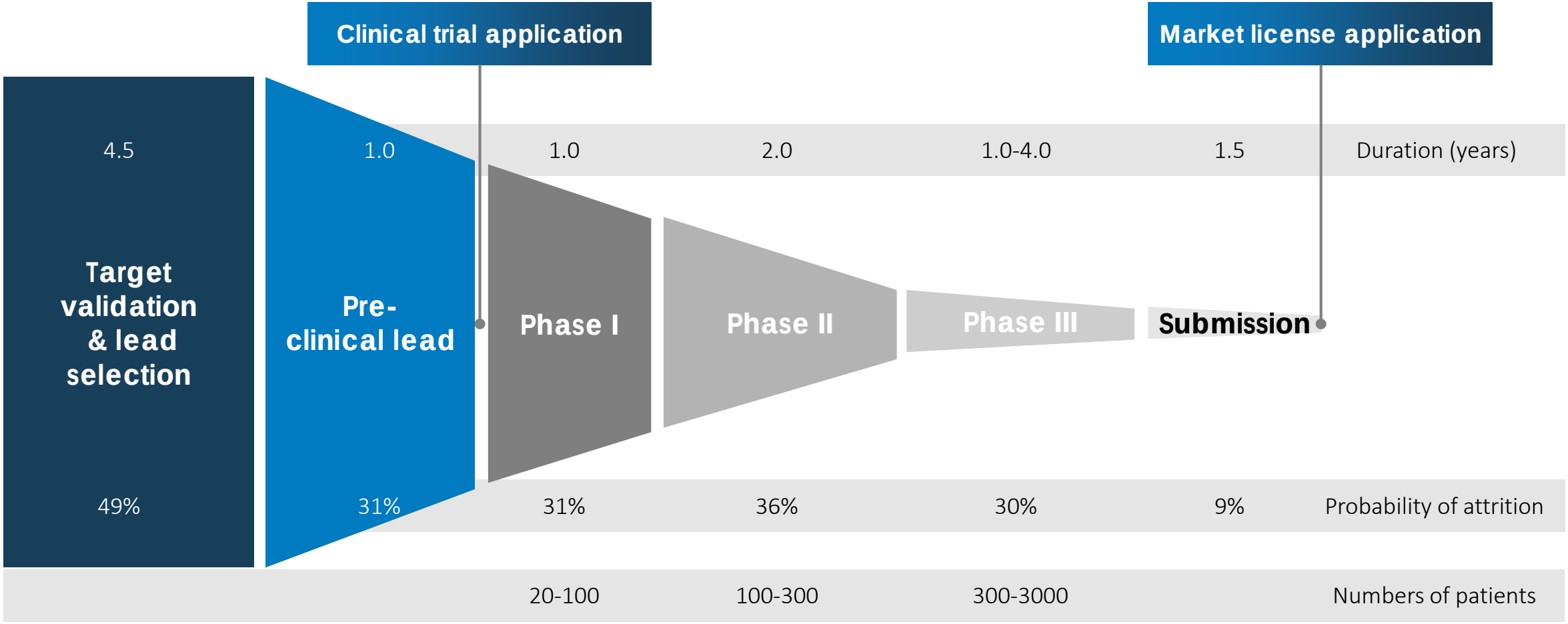

When to test for endotoxin during biologic product development?

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Pre-clinical lots

- Before administration to any animal used in clinical testing
  - BET (including rFC) and MAT
  - RPT or MAT before administration to humans

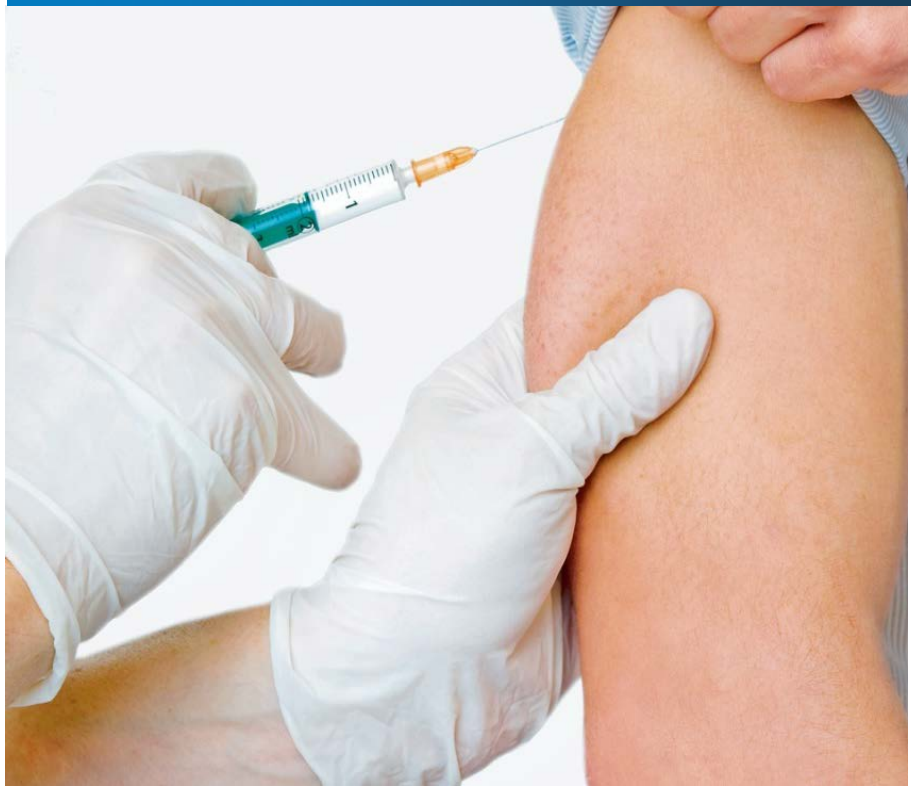
Clinical lots

- Phase I – Initial safety [BET (including rFC) and MAT]
- Phase II – Dose ranging and safety [BET and MAT if NEP contamination suspected]
- Phase III – Expanded general safety [BET and MAT if NEP contamination suspected]

Commercial lots

- All biologics in commerce shall be tested for pyrogenic substances as per regulations
- Most frequent contaminant is endotoxin from gram-negative bacteria
- BET (including rFC) and MAT if suspected NEP contamination

Trial vaccinations increase in size as phases progress



Testing of materials for biologic manufacture

Endotoxin contribution is the sum of the parts

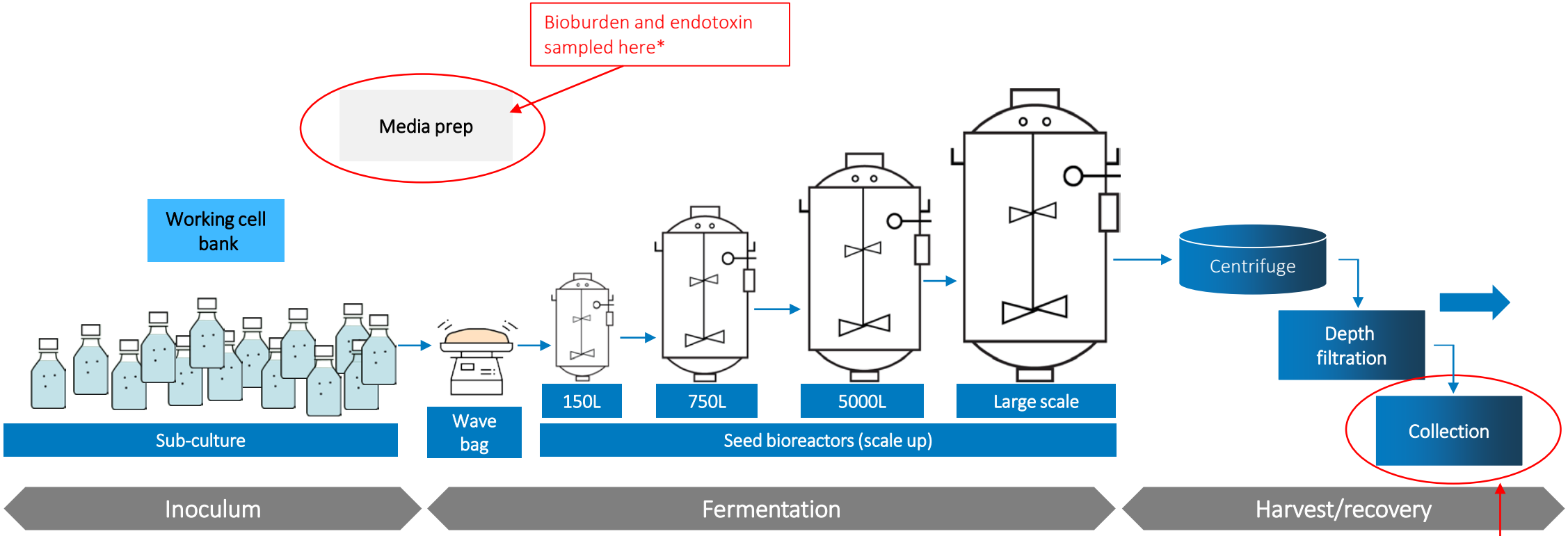
- In order to minimize the endotoxin contribution from various components and processes that are involved in manufacturing of a biologic product testing of the following is needed
  - Raw materials
  - Formulation water
  - In-process intermediates
  - Bulk formulation (drug substance)
  - Final vialed product (drug product)

- The overall endotoxin burden will be the composite of the individual contributions

Biologics must be targeted
and effective to be successful

Testing during the manufacturing process

Production scale up and harvest

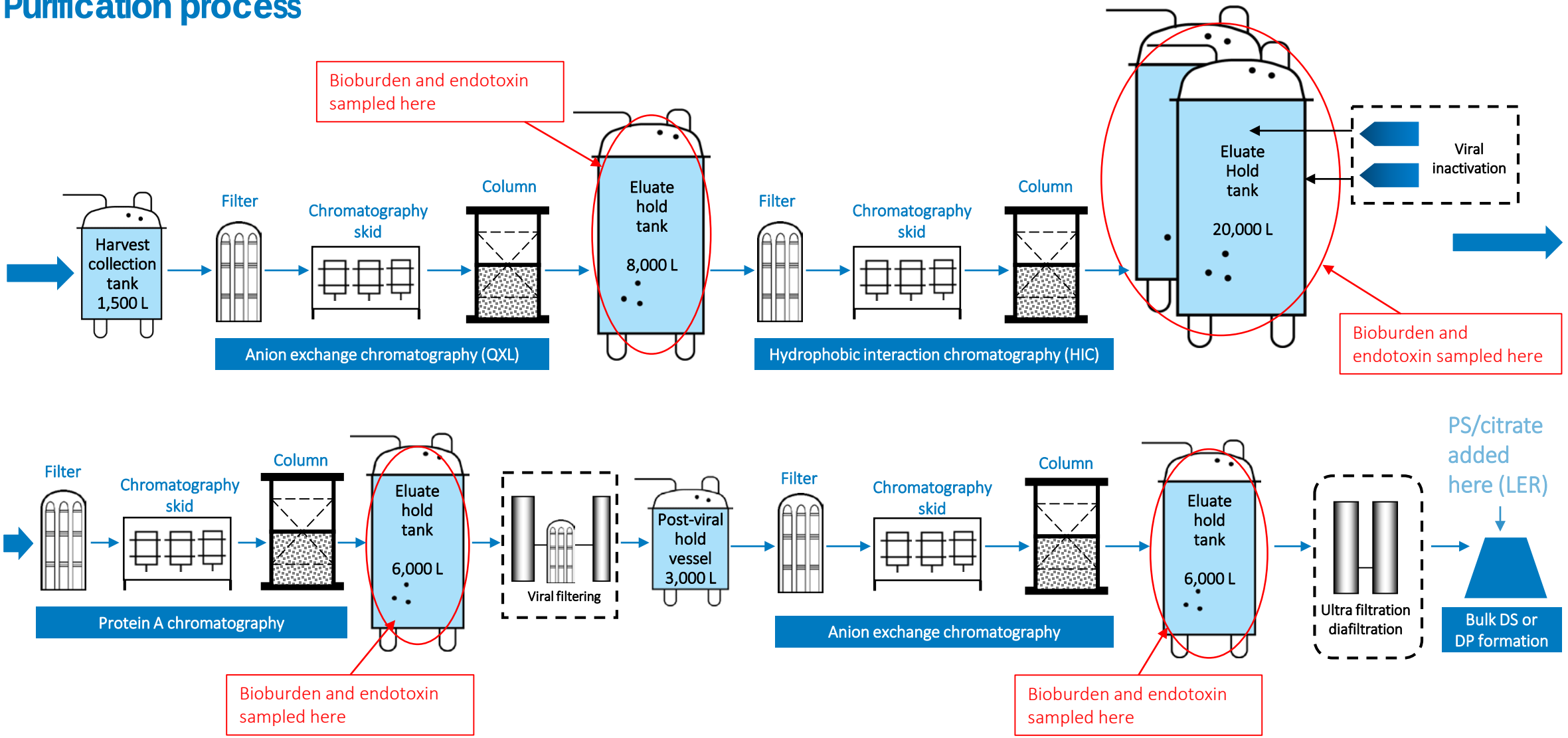


** Raw materials testing as indicated in previous slide*

Advisable to test for endotoxin and bioburden between transfers to new vessels

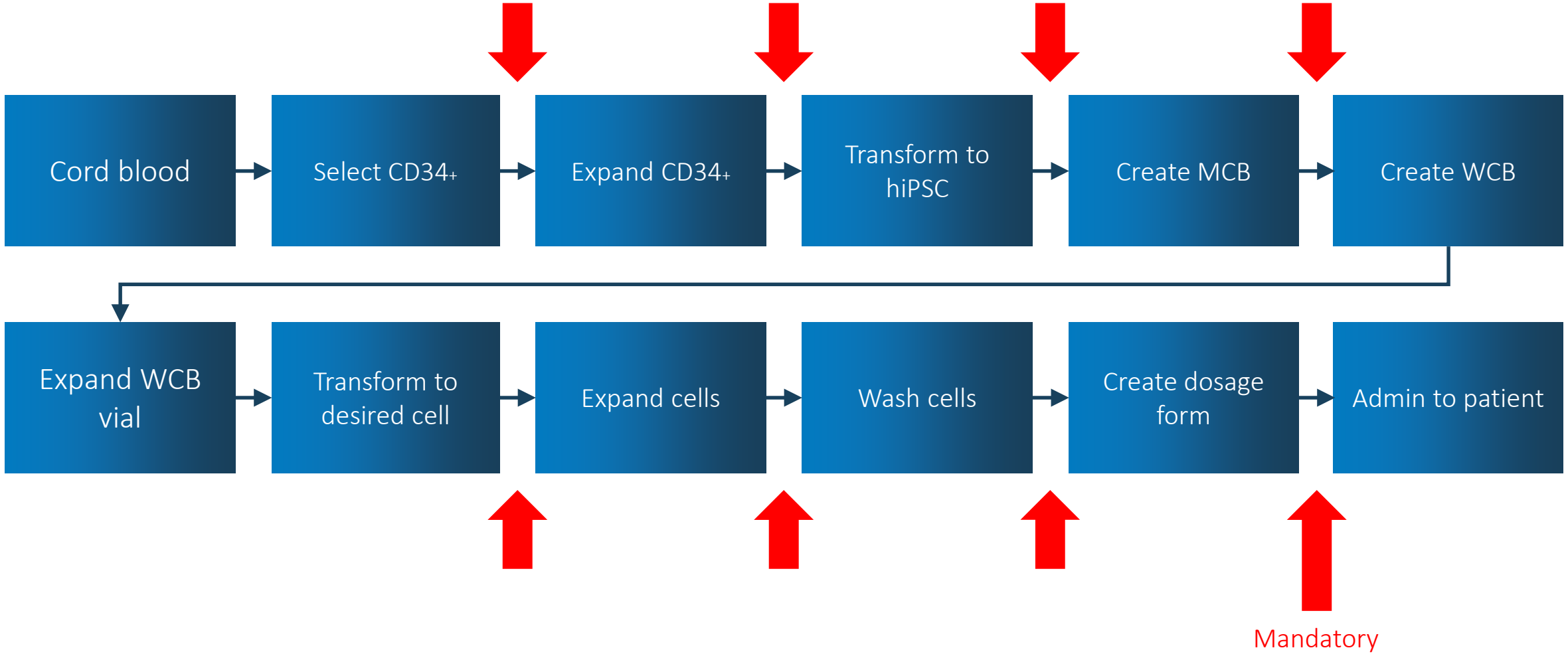
Testing during the manufacturing process

Purification process



Where to test cell therapy products

Cord blood to stem cell product



Steps to routine testing

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01 Get product information/ set specifications

02 Establish endotoxin release limit

03 Calculate Maximum Valid Dilution (MVD)

04 Perform inhibition/ enhancement testing

05 Product validation

06 Routine testing

Final product testing

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Setting specifications

Cell therapy small volume
- < 25 mL
  (syringe, direct injection)

Cell therapy large volume
- > 25 mL
  (blood bag, infusion)

Final product testing

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Cell therapy small volume

- < 25 mL (syringe, direct injection)

ERL = K/M

Where K = 5 EU/kg/hr for parenterals

= 0.2 EU/kg/hr for interthecals

M = Maximum total dose delivered in a single hour

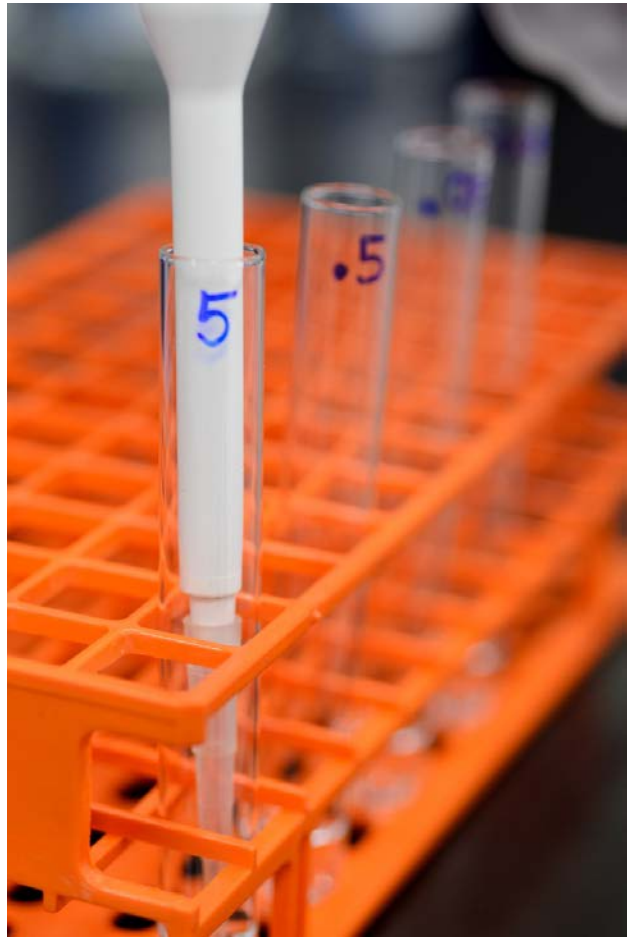
ERL = (5 EU/kg)(70 kg) / 2.5 mL

= 140 EU/mL

MVD = (ERL)(conc of soln)/ λ

= (140 EU/mL)(1) / 0.005)

= 28000



Final product testing

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Kinetic assay, lowest standard 0.005 EU/mL

Biologic small volume product

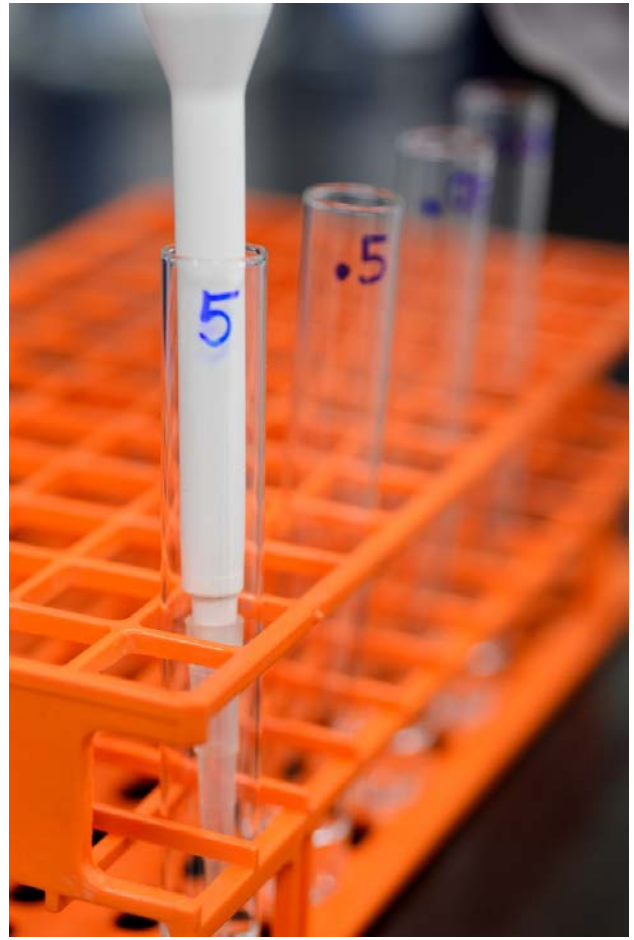
- 2.5 mL (syringe, direct injection)
- ERL = 140 EU/mL

$$MVD = \frac{ERL}{\lambda} = \frac{140\ {}^{EU}/_{mL}}{0.005\ {}^{EU}/_{mL}} = 28000$$

Biologic large volume

- 250 mL (blood bag, infusion)
- ERL = 1.4 EU/mL

$$MVD = \frac{ERL}{\lambda} = \frac{1.4\ {}^{EU}/_{mL}}{0.005\ {}^{EU}/_{mL}} = 280$$



What if… ?

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Biologic large volume example:

- MVD = 280
- BUT: product shows interference, can't be tested until 1:400

$$MVD = \frac{ERL}{\lambda}$$

Higher MVD

- More sensitive assay (λ ↓)
- Increase ERL by extending infusion time
  - t = 2 hours
  - 250 mL bag

$$ERL = \frac{K}{M} = \frac{5\ ^{EU}/_{kg \times h} \times 70\ kg \times \mathbf{2\ h}}{250\ mL} = 2.8\ ^{EU}/_{mL}$$

$$MVD = \frac{ERL}{\lambda} = \frac{2.8\ ^{EU}/_{mL}}{0.005\ ^{EU}/_{mL}} = 560$$

Interferences

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Validation of spike recovery

01 Determine Endotoxin Release Limit (ERL)

02 Determine Maximum Valid Dilution (MVD)

03 Perform dilution series to MVD

04 Spike each dilution with CSE to the middle of the standard curve

Typically, 5 EU/mL or 0.5 EU/mL

05 Valid recovery is 50 – 200% of spike

Interference testing

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Types of interference

- Inhibition
- Enhancement

Determined by judging recovery of a known endotoxin spike (positive product control, PPC)

- Dilution series up to MVD
- PPC in the middle of the standard curve (photometric methods)
- Special procedure for LAL gel clot

Specification: PPC recovery 50-200%

180-200%	Within assay error%
160-180%	Borderline
70-160%	Within normal assay parameters
60-70%	Borderline
50-60%	Within assay error %

Different levels of interference

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Endotoxin → **Enzyme cascade** → **Detection**

- Gel clot
- Turbidity
- Chromogenic assay
- Fluorescence

Restricted endotoxin availability

- Solubility
- Binding
- Aggregation
- Masking

Different levels of interference

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Endotoxin → **Enzyme cascade** → **Detection**

- Gel clot
- Turbidity
- Chromogenic assay
- Fluorescence

- pH
- Ionic strength
- Depletion of divalent cations
- Enzyme activity in the sample
- Enzyme inhibition

Different levels of interference

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Endotoxin → **Enzyme cascade** → **Detection**

- Gel clot
- Turbidity
- Chromogenic assay
- Fluorescence

- High background
- Precipitation
- Vibration
- Calculation errors

Treatments to overcome interference

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Treatment	Sample type	Notes
Dilution	Most samples	Up to MVD
pH adjustment	(Unbuffered) samples with pH outside range	pH 6-8 in sample/lysate mixture
Heat	Sample containing • active protease • proteins causing endotoxin masking	Variety of times / temperatures described in literature, e.g. 75°C/30 min
Surfactant/Dispersing Agent (Pyrosperse™)	Oily samples, components binding endotoxin	
Glucan blocker	Sample containing LAL-reactive material/glucans	Possible glucan sources: fungi, cellulose filters…
Divalent cation addition ($MgCl_2$)	Sample containing chelator (e.g. EDTA)	

Interferences

Validation of spike recovery

ERL = 14 EU/mL
MVD = 2800

Dilution	% Recovery
Und	20
10	33
50	51
100	75
500	99
1000	102
5000	99

Interferences

Validation of spike recovery

ERL = 14 EU/mL
MVD = 2800

Dilution	% Recovery
Und	20
10	33
50	51
100	75
500	99
1000	102
5000	99

Interferences

Validation of spike recovery

All treatments must be validated

Some treatments cause denaturation of proteins

- Locks up endotoxin

Spike → Treat → Test

Add pyrosperse → Spike → 70C 10 min → Test

Check spike recovery

Interferences

Validation of spike recovery

Validation of spike recovery – post treatment

ERL = 14 EU/mL
MVD = 2800

Dilution	% Recovery
Und	30
10	51
50	76
100	102
500	100
1000	99
5000	101

What is Low Endotoxin Recovery (LER)?

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Low Endotoxin Recovery (LER) is a controversial topic that has been circulating throughout the endotoxin detection community for several years

- Brought to regulator's attention by Dr. Joseph Chen (Genentech) in 2013

LER is theoretically described as:

- "A masking effect" manifested in the biophysical formation of a complex that blocks the ability of Factor C, the main component in LAL detection, to bind endotoxin"
- LER-subjected samples also show poor (MAT) to variable (RPT) recovery of endotoxin in mammalian-based tests

Low Endotoxin Recovery (LER): where does it occur?

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LER is currently primarily discussed in the context of **biological manufacturing**

A common biologic excipient, polysorbate combined with a chelator such as citrate or phosphate buffers, has been identified as the cause of the masking effect more commonly referred to as LER

Polysorbate is estimated to be used in more than 70% of protein formulations

There is also some evidence that histidine-containing formulations may occasionally be involved in LER

LER is considered to be distinct from interference commonly seen with the LAL assay that is normally overcome by dilution or other sample preparation methods

Low Endotoxin Recovery (LER) – basic facts

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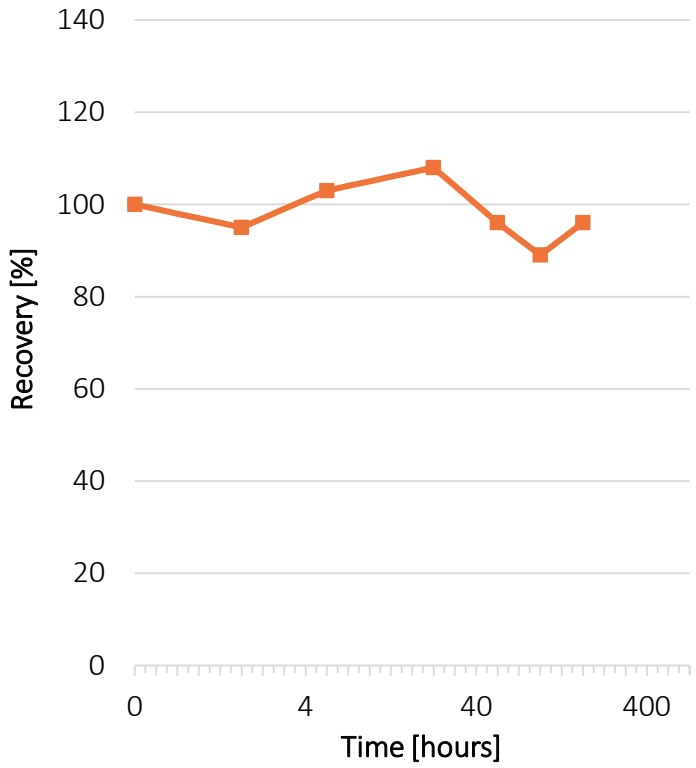
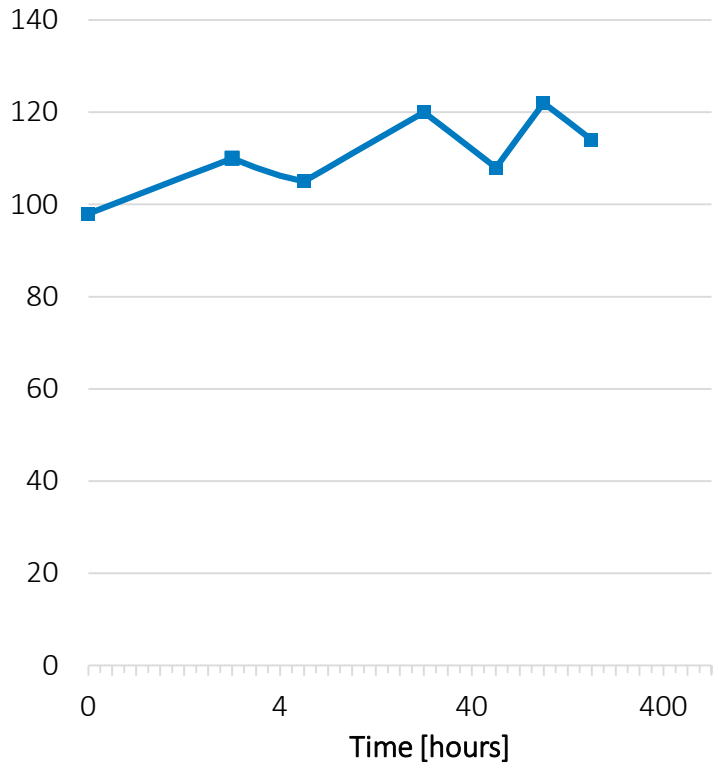
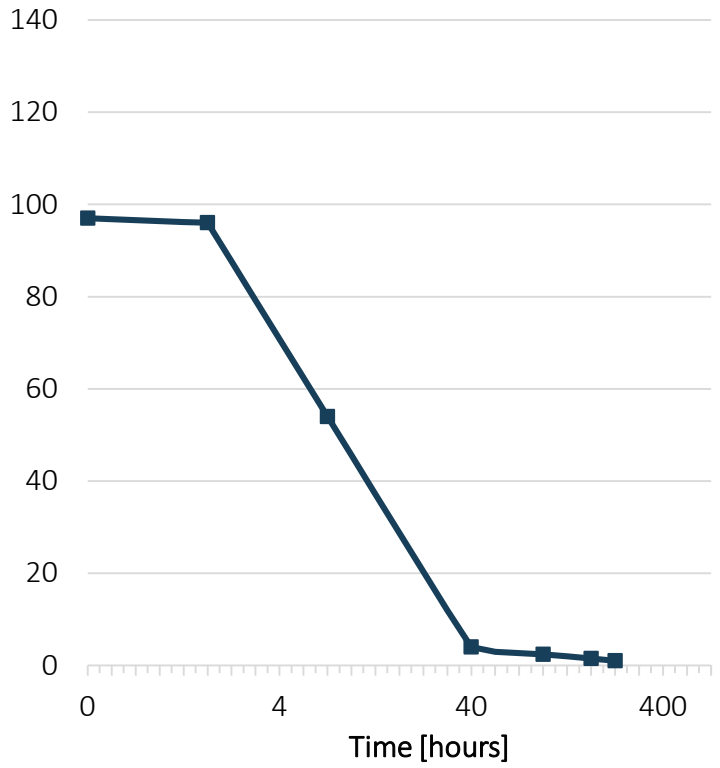
Surfactants and chelators are sufficient to induce LER (e. g. polysorbate & phosphate, often components of BIOLOGICALS)



Sodium citrate



Polysorbate 20



Polysorbate 20 + Sodium citrate

Low Endotoxin Recovery (LER) – basic facts

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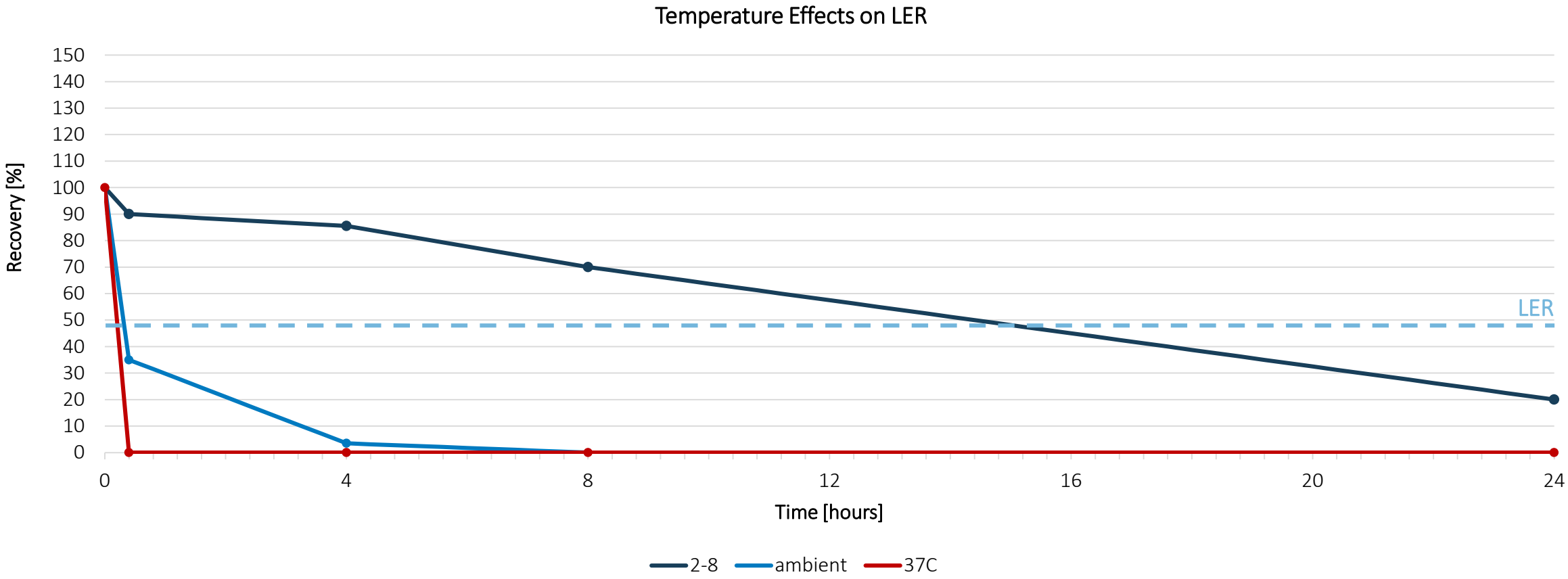
Occurrence of LER is time and temperature dependent



Low Endotoxin Recovery – basic facts

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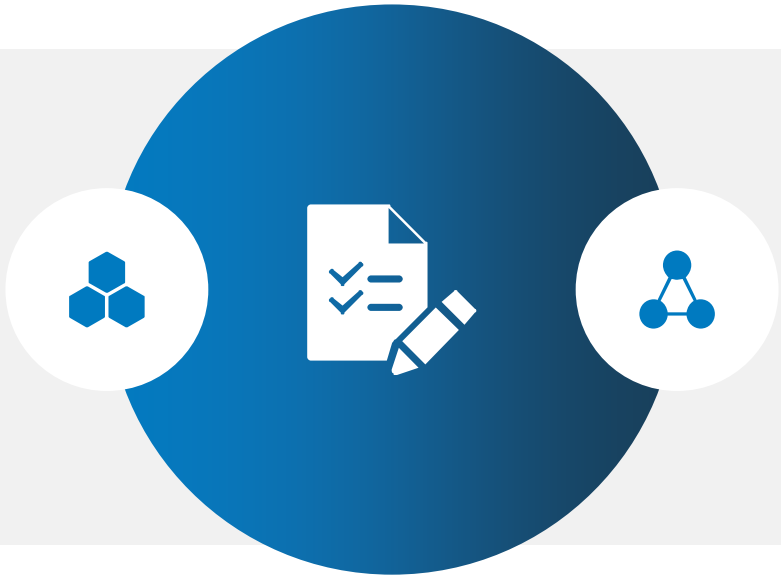
LER is **dilution independent** e. g. LER can not be overcome by sample dilution



Stable structure

Low Endotoxin Recovery (LER) – basic facts

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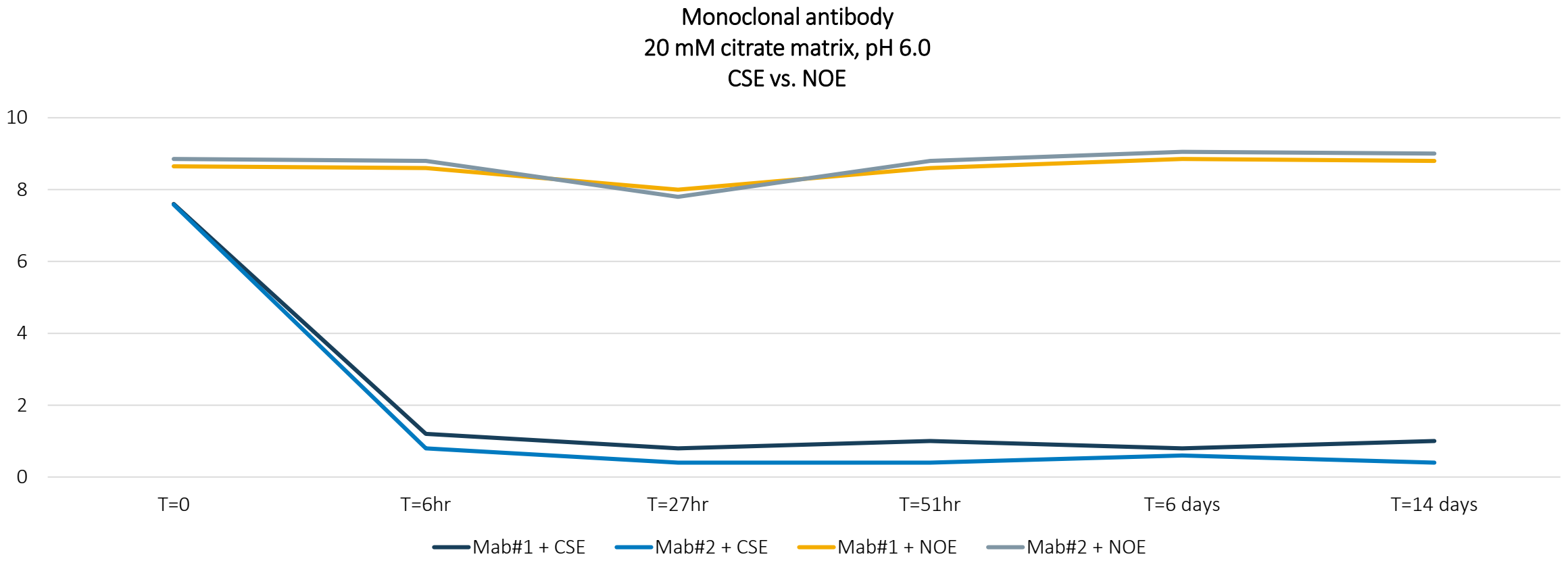
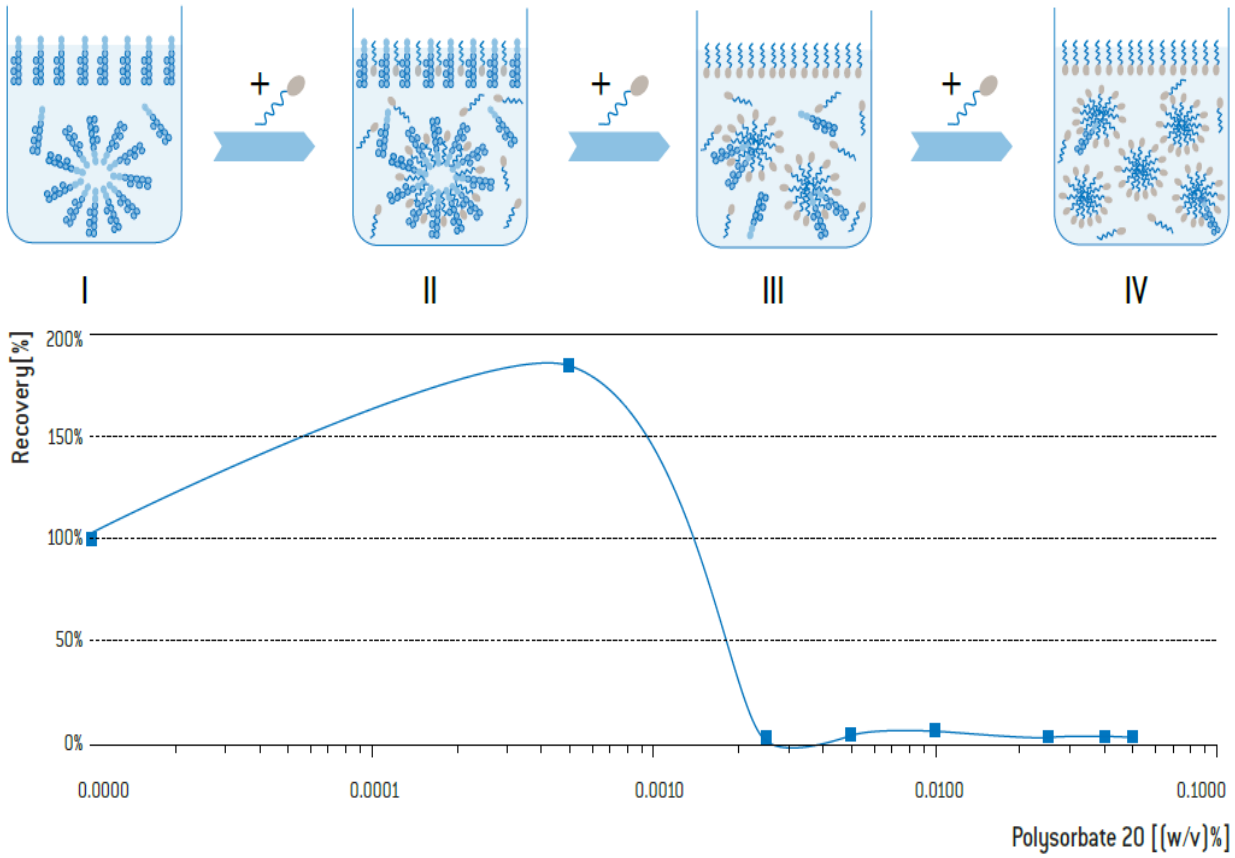
Endotoxins from different sources show different masking susceptibilities e.g. *E.coli (CSE)* vs. *E. cloacae (NOE)*



Endotoxin aggregation – LER mechanism (theory)



- I [LPS] pure
- II [LPS] + [surfactant] low
- III LPS] + [surfactant] medium
- IV [LPS] + [surfactant] high

Loss of spike recovery over time

LER mechanism (theory)

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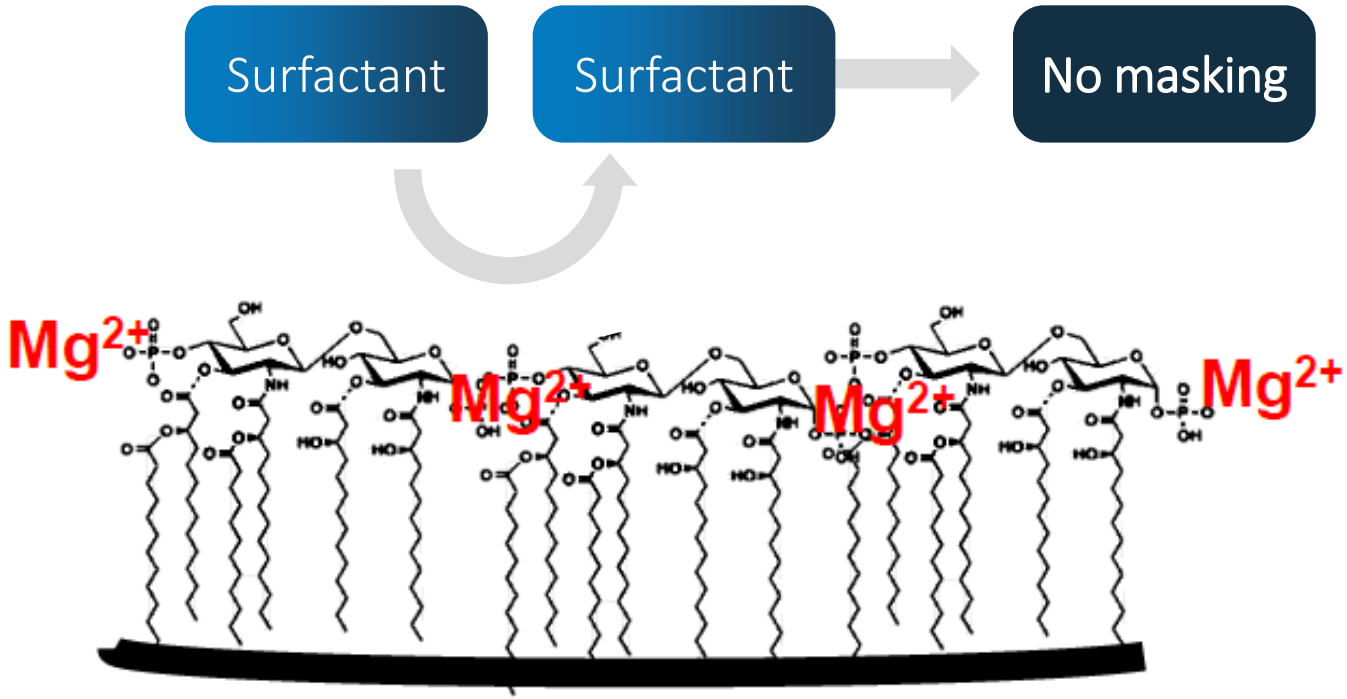
- LPS exhibits rigid supramolecular structure (stabilized by e.g.: Mg^{2+})
- Rigidity serves as protective shield for the bacteria
- Intercalation of non-ionic surfactants limited



Developed by Johannes Reich of Hyglos

LER mechanism Surfactant plus Chelator (theory)

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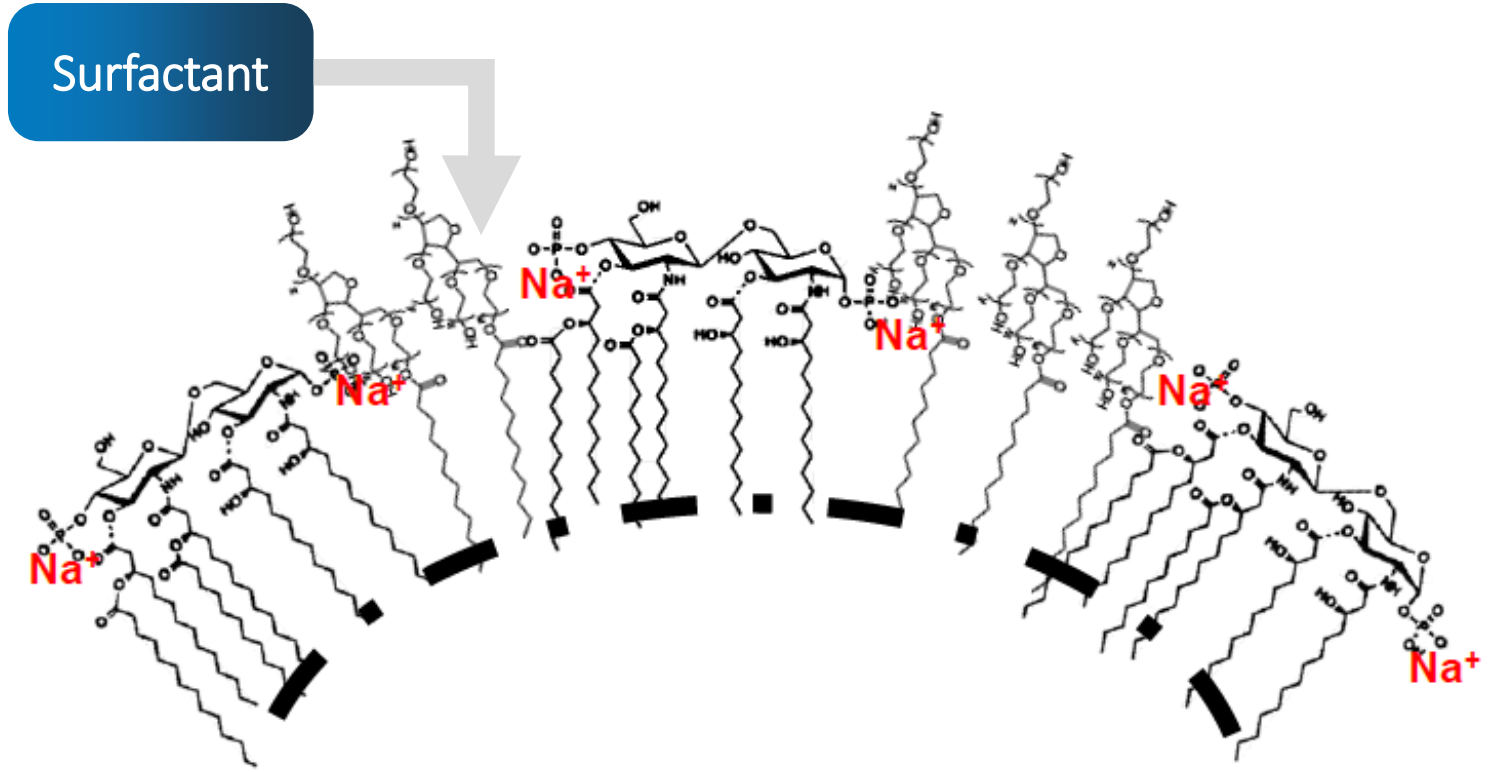
Change in supramolecular structure



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LER mechanism (theory)

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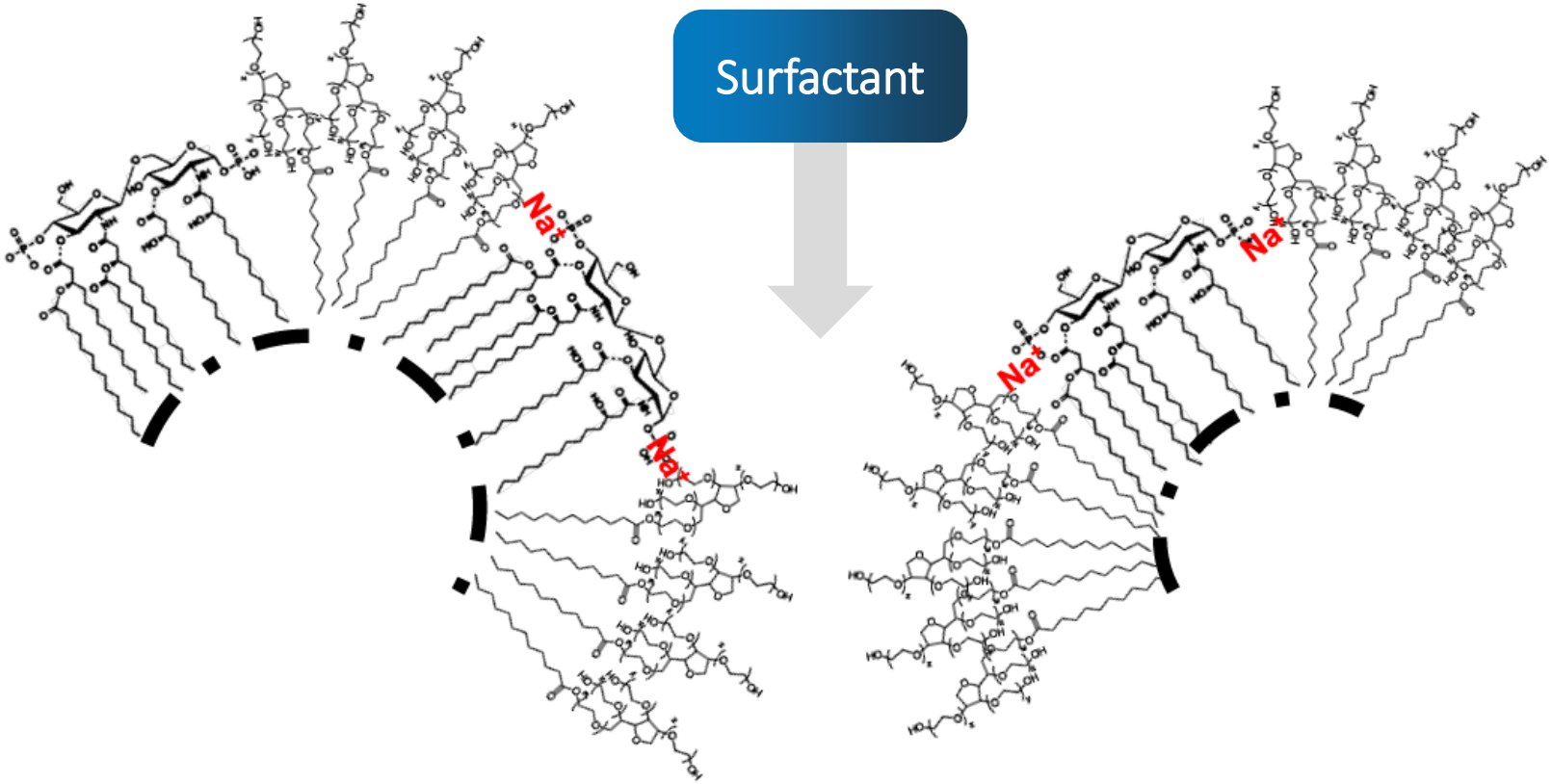
Change in supramolecular structure



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LER mechanism (theory)

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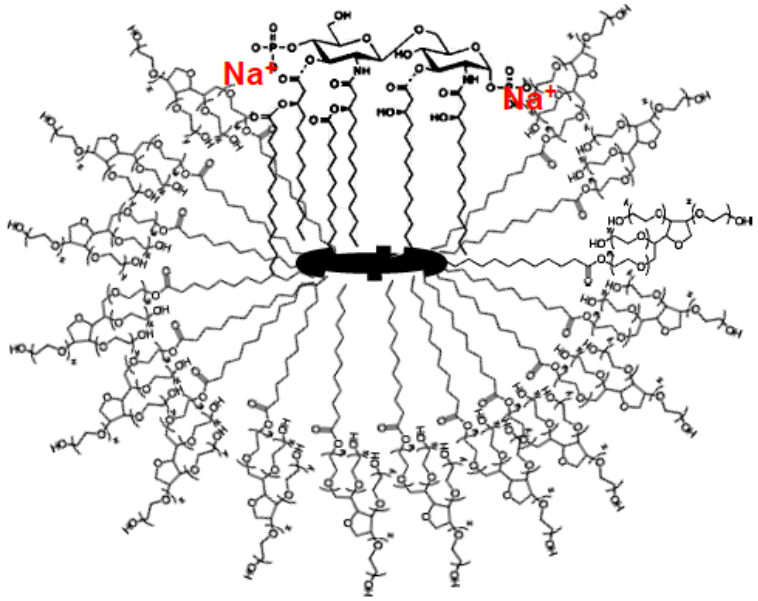
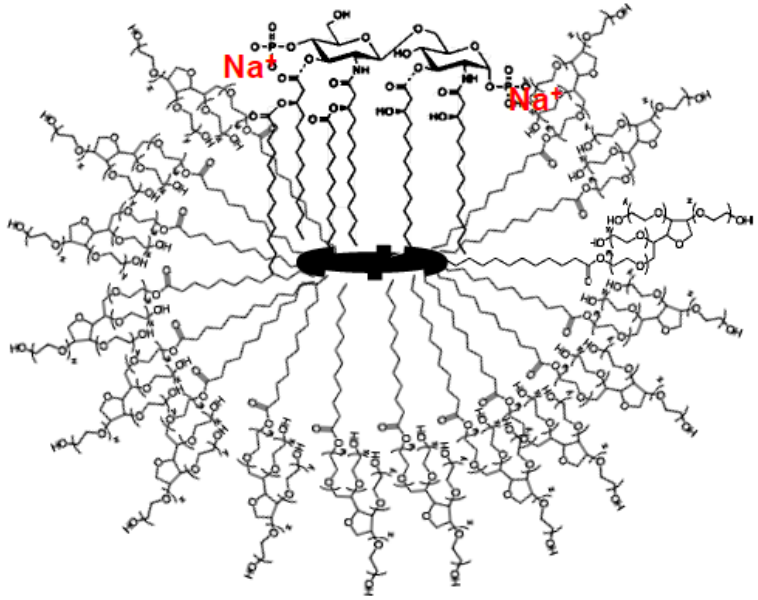
- Disaggregation of LPS
- Inadequate detection
- = LER





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What is the current regulatory stance?

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The FDA included a stability screen requirement in their 2012 Q&A Guidance for Industry Pyrogen and Endotoxins Testing

- Chen originally pointed to the phenomenon; hold-time studies are where users have subsequently seen the LER phenomenon

This guidance recommended that the industry performs hold-time studies to verify the amount of time a sample can be reasonably stored prior to final release testing

- This meant immediate assay vs. delayed assay
- Misinterpreted to mean spiked with LPS standard

Products showing LER that cannot be overcome must be tested by the Rabbit Pyrogen Test (USP <151>)

- In the EU companies cannot use the RPT

Why is the LER focus primarily associated with biologics?

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Regulators require a **Biologic License Application (BLA)** to be submitted for each new biological drug product before it is released to the public

- The BLA is a formal request for permission to introduce, or deliver for introduction, a biologic product into interstate commerce regulated under 21 CFR 601.20 – 680

- If the information provided meets FDA requirements, the application is approved and a license is issued, allowing the firm to market the product
  - Biological products include a wide range of products such as vaccines, blood and blood components, allergenics, somatic cells, gene therapy, tissues and recombinant therapeutic proteins

- These products are most likely to contain polysorbate and a chelator for stability

Why is the LER focus primarily associated with biologics?

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The rabbit pyrogen test (RPT) requirement applies to all of the BLA products regulated by CDER/CBER

For non-biologics (a.k.a. “small molecule”), equivalence is assumed between RPT and BET

In lieu of the RPT, a bacterial endotoxins test can be used if the method is shown to be equivalent

21 CFR 610.9 – Requires the applicant to provide evidence “demonstrating that the modification will provide assurances of the safety, purity, potency, and effectiveness of the biological product equal to or greater than the assurances provided by the method or processes specified in the general standards or additional standards for the biological product…”

FDA/CDER is currently requesting follow-up studies post-approval for drugs exhibiting LER

Prevalence of LER amongst BLAs submitted to US Food and Drug Administration (FDA)

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Formulation	Number of BLAs submitted	LER	Comments from regulators
Citrate w/polysorbate	6	6/6	One product requires Rabbit Pyrogen Test for release Two products unaffected in gel clot assay Two products showed significant assay variability
Phosphate w/polysorbate	3	1/3	Post-marketing commitment (PMC) to develop improved release method
Hisitidine w/polysorbate	7	2/7	Rabbit Pyrogen Test required for batch release
Acetate w/polysorbate	6	0/6	BET method used for release
Acetate w/o polysorbate	1	0/1	BET method used for release
Citrate w/HSA	1	1/1	Rabbit Pyrogen Test required for batch release

Patricia Hughes (FDA), PharmaLab Congress, Nov. 2015

Endotoxin aggregation – do monomers matter?

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To some, the LER issue seems to hinge on the "biological activity" of aggregated vs. disaggregated LPS (standard or natural endotoxin)

Endotoxin normally exists in an aggregated state and not as monomers

Biological inactivity of disaggregated LPS in LER solutions is easily demonstrated with LAL but is not so straightforward in mammals

- There is some evidence that LPS monomers may retain biological activity when administered to patients, i.e. detoxified LPS is sometimes used as an adjuvant during vaccine administration

How to recognize LER in a product?

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1

A simple screening test using CSE/RSE can demonstrate the presence or absence of LER for a given drug product, drug substance or in-process solution

2

Does the product contain polysorbate?

- This is the “trigger” for LER potential. Hold-time studies are being requested for all formulations containing Polysorbate surfactants.

3

Spike undiluted drug product, drug substance, or in-process solution with endotoxin

- CSE is recommended for LER screening as the standard seems to be more affected by LER than Naturally Occurring Endotoxin (NOE) preparations (worst-case)

4

Carry out hold-time study to determine whether initial spike is recoverable

- Container type, storage conditions and reasonable sample hold-time should be taken into consideration (=process knowledge)

How to recognize LER in a product?

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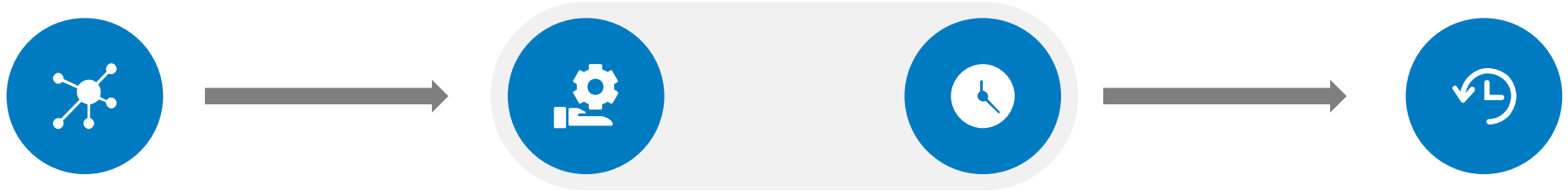
LER screening test/hold-time study



Direct spike into undiluted **drug product, drug substance or in-process solution** at a level < the specification.

Hold product before testing according to pre-determined parameters (temperature, container type, # days)

Product should be tested at specific time-points throughout hold period (ex. 4hr, 24hr, 72hr, day 5, day 7. Note day 7 is 8 days)

Determine **recovery** (%)* of spike compared to amount of endotoxin originally added to sample.

*Products exhibiting LER typically result in declining (≤ 50%) recovery at various or multiple time points throughout the hold period. Inability to recover CSE spike may occur in less than 4 hours.

Endotoxin and lipopolysaccharides

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Does RSE = CSE = NOE?

No

- RSE and CSE are highly purified entities and not found in nature
- NOE is what occurs in the environment
- The phenol extraction used to manufacture RSE and CSE strip away the associated proteins and cleave the O-specific side chains (Tsang et al. 1973)
- RSE and CSE behave differently from NOE in a product

Available support for biologics development and release

Testing solutions products for endotoxin testing

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Endotoxin and pyrogen testing

- PYROGENT® Gel Clot LAL Assays
- PYROGENT®-5000 Turbidimetric LAL Assays
- Kinetic-QCL® Chromogenic LAL Assays
- PyroGene® Recombinant Factor C (rFC) Assays
- *NEW* PyroCell® MAT System

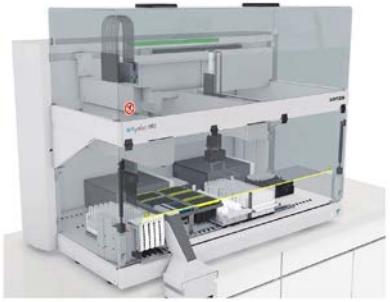
Along with…

- Reference standards
- Consumables
- Instrumentation and robotic solutions
- Informatics
- Services

Reagents

Consumables

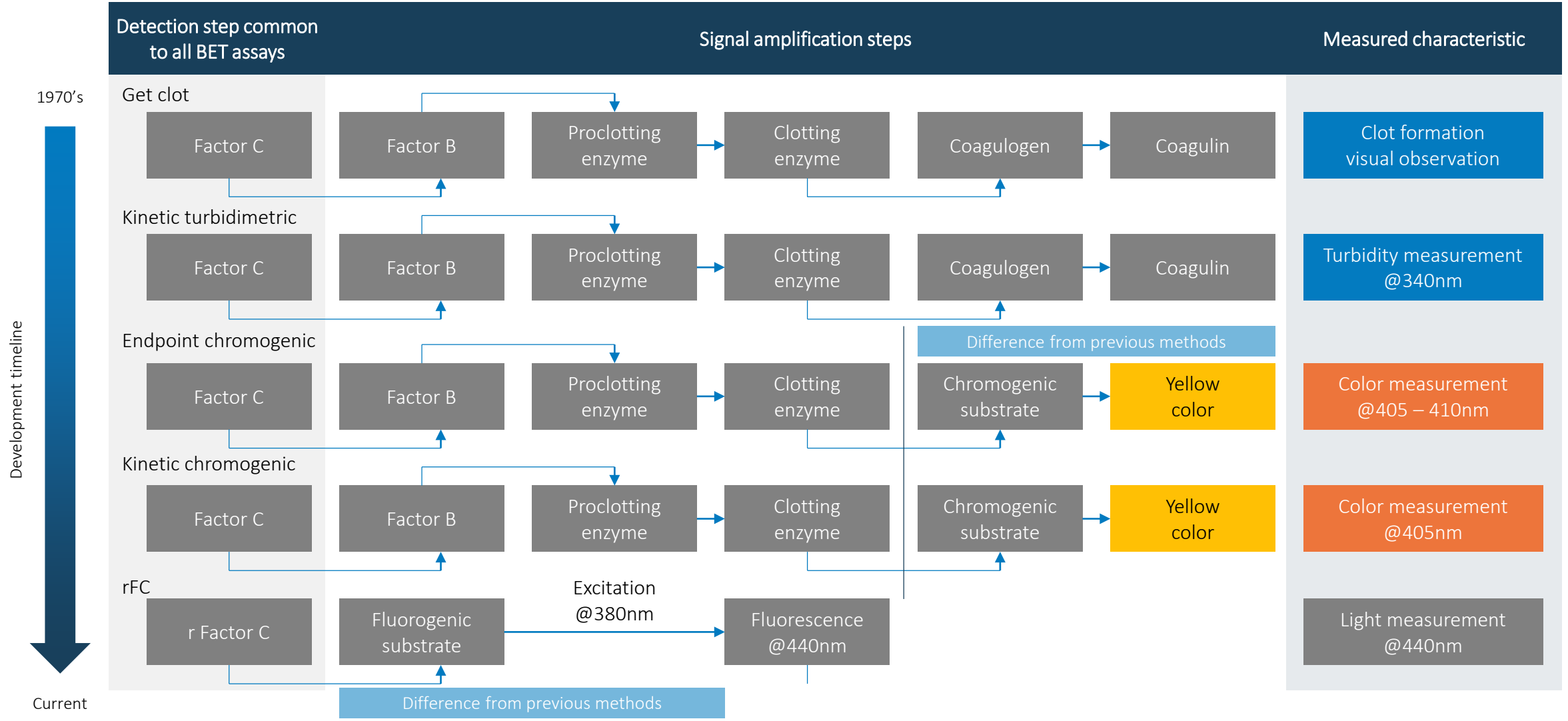
Instruments/robotics



Software

Endotoxin detection products

Comparison of amplification methods of bacterial endotoxin detection assays



Summary

- Biologics are a critical component in maintaining world-wide human health – the current pandemic and the speed of development of appropriate biologics requires careful quality control of technology platforms and manufactured batches
- Different compendial tests are available, however, inherent pyrogenicity and matrix complexity may not support current in-vivo testing methods
- Therefore, BET in process testing for bacterial endotoxin complemented by MAT testing for pyrogenicity are recommended
- While similar, regional requirements are not harmonized leading to complexity for market authorization

Current CoViD-19 pandemic

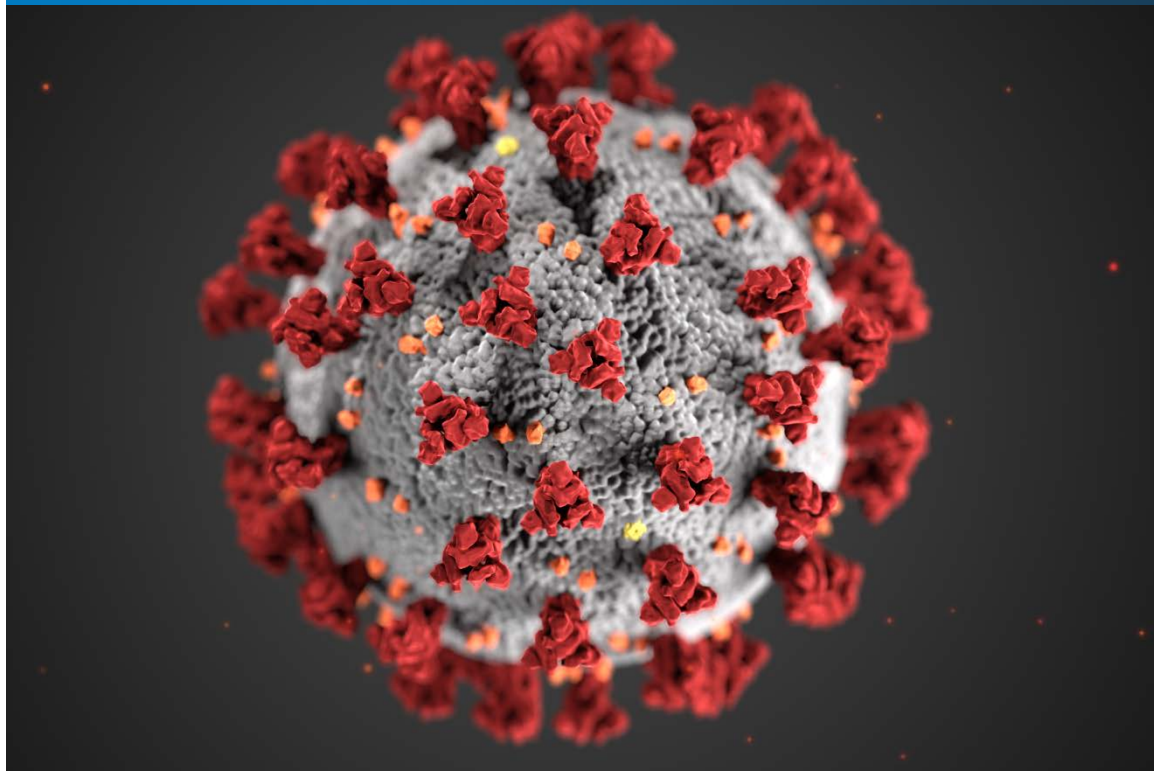


Image courtesy of the US Centers for Disease Control

Summary – biologics application

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What to test?

Routine testing of biologic preparations before public distribution

- As an exempted biological in US, viral biologics are not tested in rabbits
- The MAT is a suitable *in-vitro* replacement test if LAL is not applicable

Testing to be established through development

- Raw materials
- Formulation water
- In-process intermediates
- Bulk formulation (drug substance)
- Final vialed product (drug product)

When to test?

Pre-clinical lots

- Before administration to animals used in clinical testing

Clinical lots

- Phase I – Initial safety
- Phase II – Dose ranging and safety
- Phase III – Expanded general safety

Commercial lots

- All biologics in commerce shall be tested for pyrogenic substances

References and supporting documents

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Q&A session

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Any questions?

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Thank you