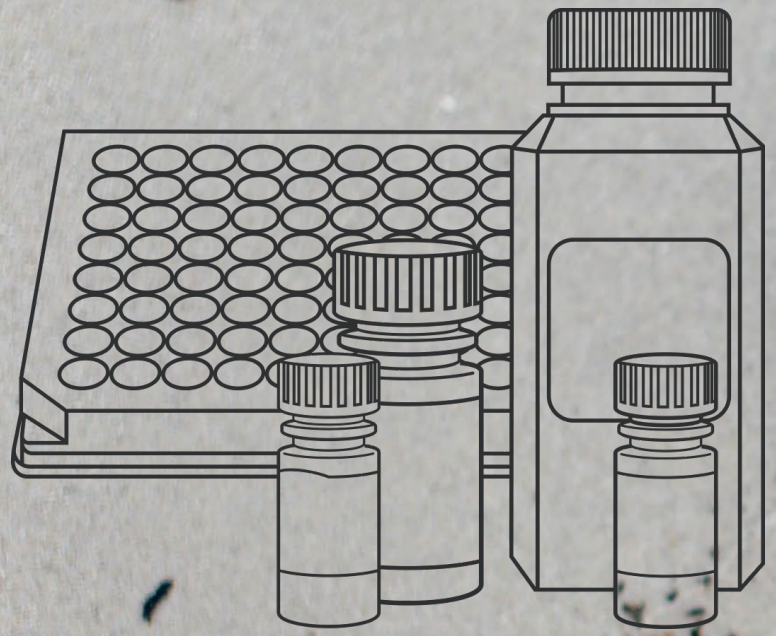
Sustainable Pharmaceutical Quality Control with Recombinant Factor C Technology

Ganesh Chaudhari



AGENDA

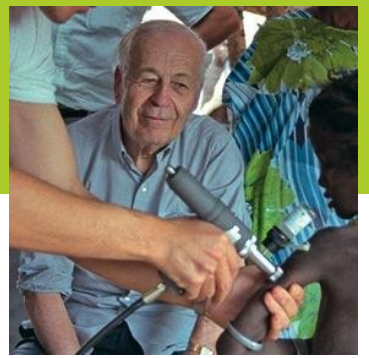
- Bacterial Endotoxin Testing – Challenges & Implications
- Introduction of Recombinant Factor C (rFC)
- The ENDONEXT™ Range – How to do thing differently?
- Summary and Q&A

AN HISTORIC FAMILY COMMITMENT TO MEDICINE AND PUBLIC HEALTH WORLDWIDE

- **A Pasteurian tradition:** Marcel Mérieux worked with Louis Pasteur in 1894.
- **A commitment that transcends four generations**: ever since the creation of Institut Mérieux, a generation has worked after another to expand its legacy.

Marcel Mérieux
1894 - Student of **Louis Pasteur**

Marcel Mérieux
1897 - Creation of Institut Mérieux

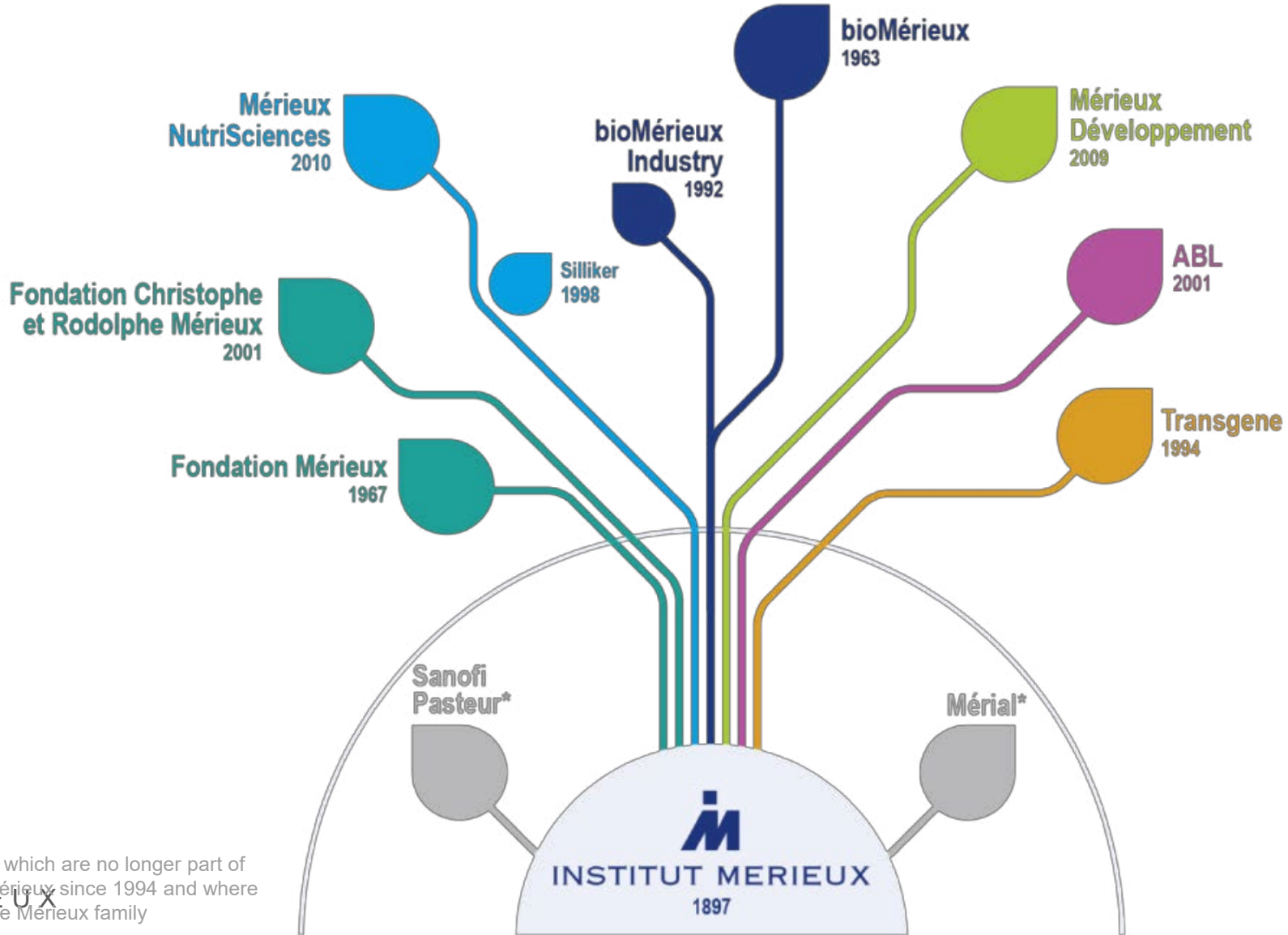


Dr Charles Mérieux
1937 - Dr Charles Mérieux took up the reins

Dr Alain Mérieux
1963 - Creation of bioMérieux

Alexandre Mérieux
2017 - Chairman & CEO of bioMérieux

INSTITUT MÉRIEUX: SERVING PUBLIC HEALTH FOR OVER A CENTURY



* Companies which are no longer part of the Institut Mérieux since 1994 and where created by the Mérieux family

DEVELOPMENT OF ENDOTOXIN TESTING

Limulus Amebocyte Lysate (**LAL**) Test

- Introduced in 1970s
- Detects endotoxins from GNB (in vitro)
- Principle: gelation, colour or turbidity change
- Requires bleeding protected horseshoe crabs

Monocyte Activation Test (**MAT**)

- Introduced in 2010 in Ph. Eur. 2.6.30
- Non-animal based in vitro pyrogen test
- Represents a full replacement of the rabbit test
- ELISA assay to measure cytokine release from human monocytes in response to pyrogens

Rabbit Pyrogen Test (**RPT**)

- Introduced in 1942, the original pyrogen test
- Detects endotoxin and non-endotoxin pyrogens (in vivo)
- Principle: rise in body temperature of rabbits following IV injection
- Not sustainable and has limitations

Recombinant Factor C (rFC) Test

- First registered in 2003 (PyroGene)
- Specific to endotoxin from GNB
- Principle: fluorescence signal
- Non-animal based & sustainable alternative to LAL/TAL

What's next?

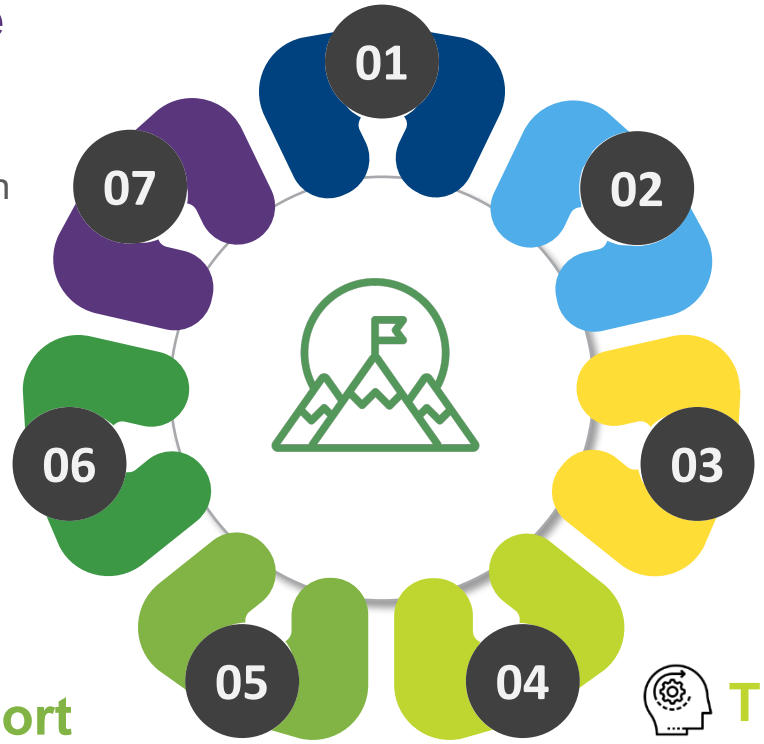
ENDOTOXIN TESTING CHALLENGES

Cost of Reagents & Equipment

Does the method fit in my budget? How do I do the testing without compromising product and patient safety?

Sustainability compliance

Does my method require animal based raw materials? Does it help with sustainability initiatives of my business? Are my supply chain practices sustainable?



Sample Throughput

How many samples can I run? How much time does it take? Is the method automated or manual?

Regulatory / Validation

Is it recognised by regulatory authorities? Is it compendial or alternative? Who does the PQ? Can I justify the spend?

Sample Matrix Interference

Does the method work for my samples? Do I need to use dilutions with water or buffers?

Vendor Support

Are they experts, can I rely on them? Do they have sufficient stock levels?

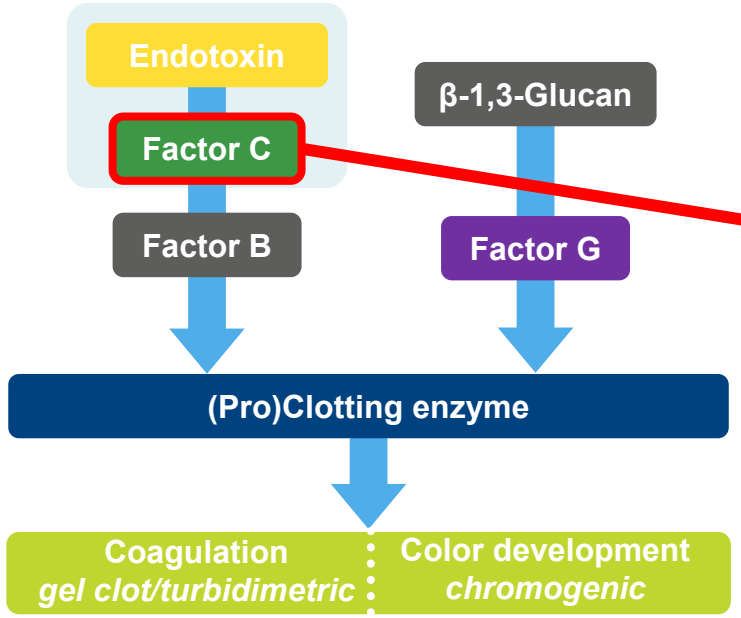
Training / Skills Required

How easy is it to train my operator? Do I need additional resources? What are the chances for errors?

INTRODUCTION OF RECOMBINANT FACTOR C

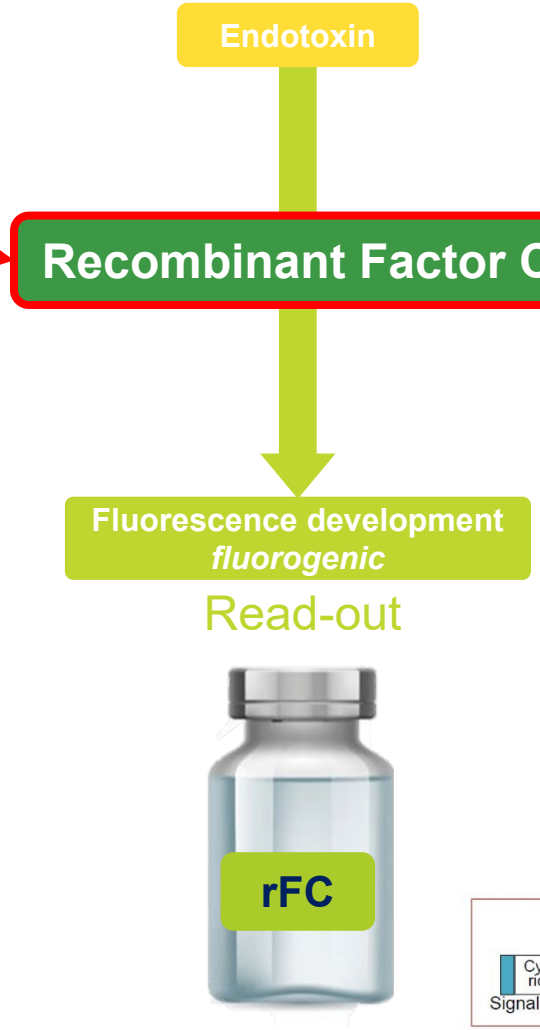
FACTOR C IS THE SPECIFIC SENSOR

LAL Cascade

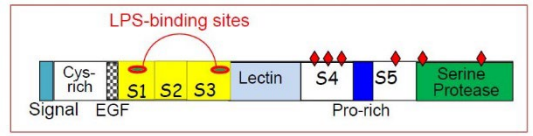


LAL is a biological enzyme system, and no 2 lots will react the same

Recombinant Factor C (rFC)



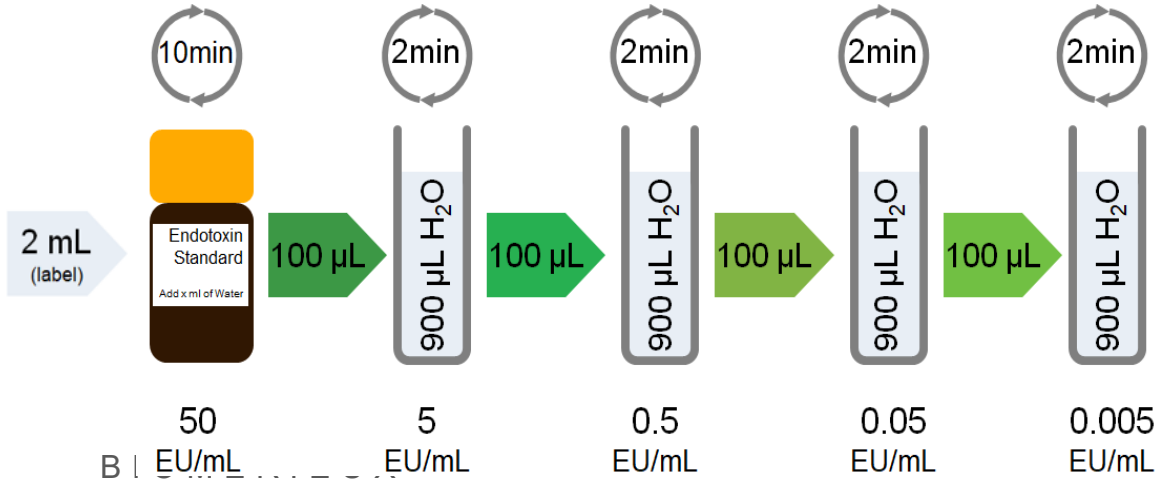
- **Exact copy of Factor C produced synthetically**
- **No extraction from the Limulus blood**
- **Simple Reaction without cascade**
- **No β–glucan / sugars interference**
- **rFC shows superior batch consistency**

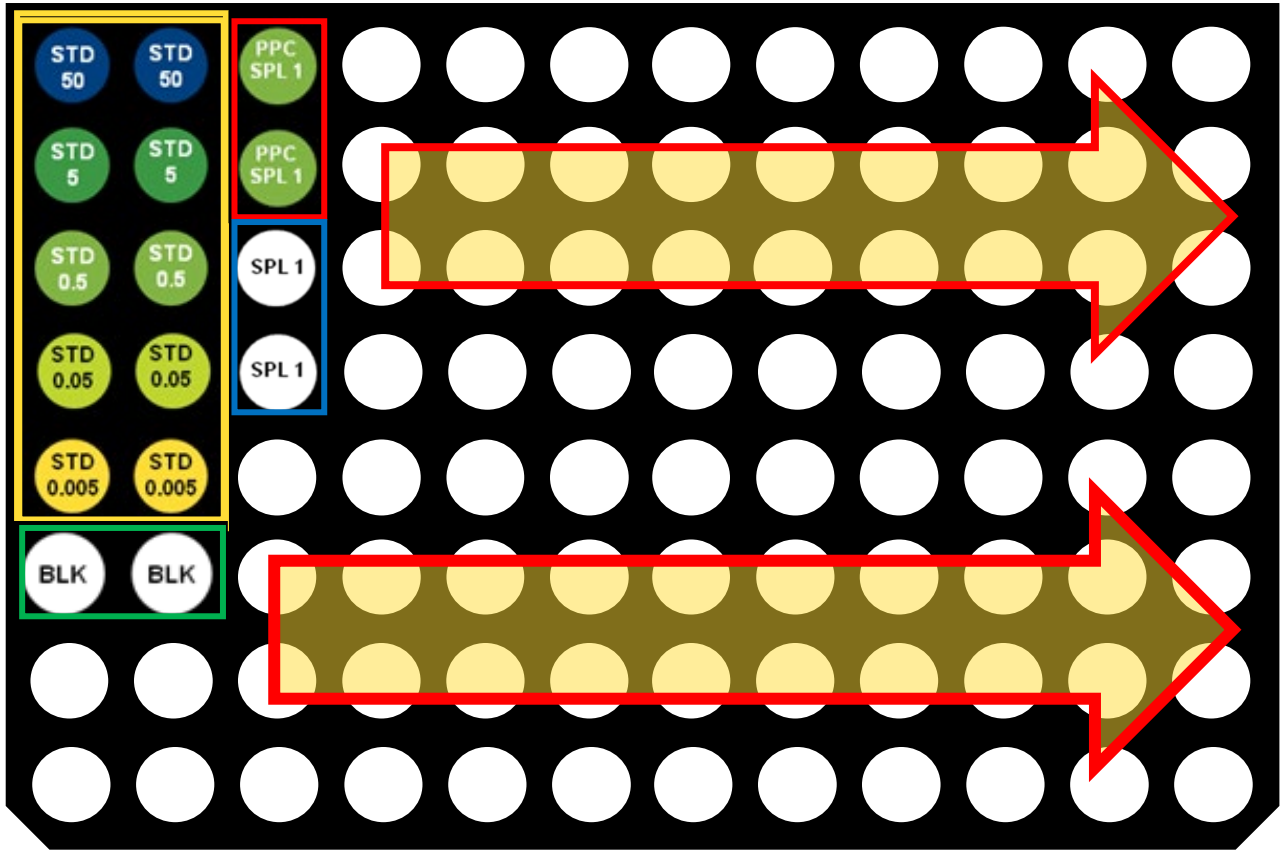


Ding & Ho (2001) Trends Biotech

rFC MEETS PHARMACOPEIA CONTROLS FOR BET

Solution	Endotoxin concentration	Solution to which endotoxin is added	Number of replicates
A	None	Test solution	Not less than 2
B	Middle concentration of the standard curve	Test solution	Not less than 2
C	At least 3 concentrations (lowest concentration is designated λ)	Water for BET	Each concentration not less than 2
D	None	Water for BET	Not less than 2



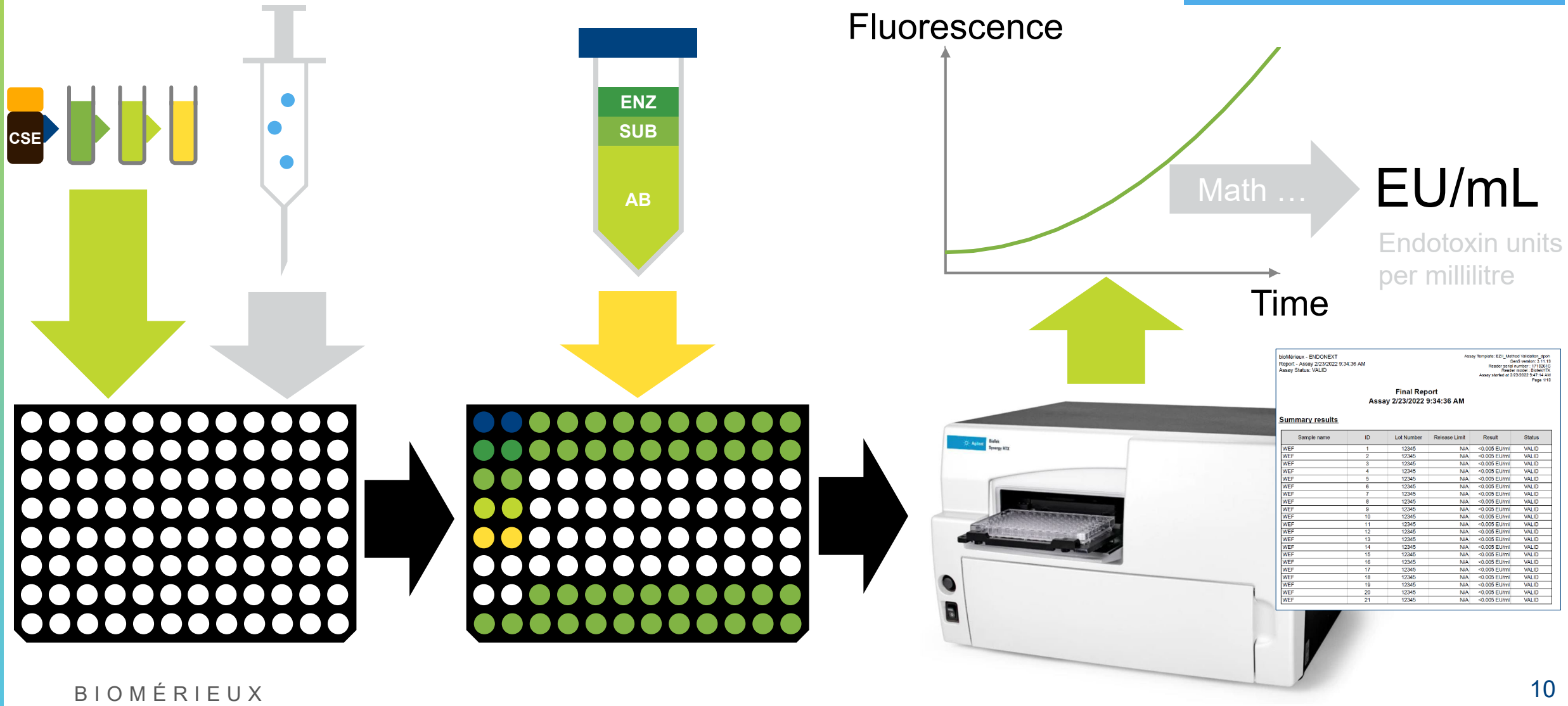


max 21 samples

TESTING WITH RFC IS JUST LIKE LAL

No surprise – same known requirements

Sensitivity:	Assay time:
0.05 EU/mL	20 min
0.005 EU/mL	60 min
0.001 EU/mL	120 min



THE ENDONEXT™ RANGE

HOW TO DO THING DIFFERENTLY?

THE ENDONEXT™ PORTFOLIO

REAGENTS

Wide applications; from rapid, high-throughput water testing (ENDOZYME II GO) to complex samples (ENDOLISA) & Low Endotoxin Recovery (ENDO-RS)

INSTRUMENT

BioTek Synergy HTX multimode reader (fluorescence & absorbance) with integrated incubation and shaking function, and a dual reagent injector module

SOFTWARE

ENDONEXT™ Software is an integrated, 21 CFR Part 11 compliant solution for data analysis, data management & full reporting

AUTOMATION

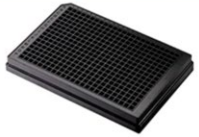
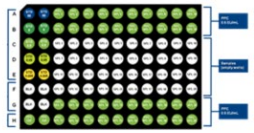
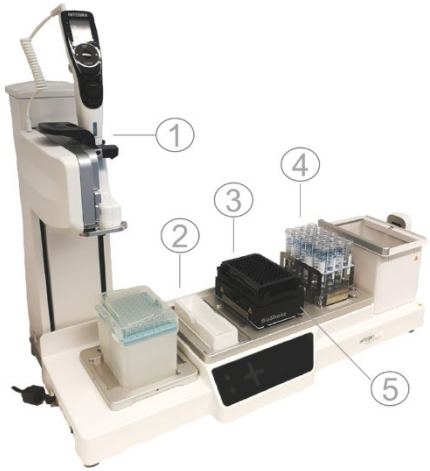
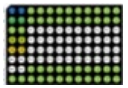
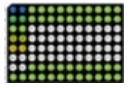
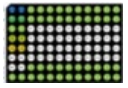
Semi- and fully-automated platforms for QC laboratories with higher throughput - in collaboration with INTEGRA & TECAN

ACCESSORIES

ENDOGRADE® certified Water & Glass Test Tubes

EZIIGO : FLEXIBILITY, SAVINGS & PERFORMANCE

Manual processing >> **Semi-Automation** >> **Full-Automation**

	Standard Microplate	ENDOZYME II GO	INTEGRA Productive Pipetting	TECAN
	Standard Reconstitution	Not needed		
	Prep. of Standard Curve + vortex + deposit	Not needed		
	Preparation of samples	Preparation of samples		
	Adding PPCs	Not needed		
	Filling of Plate	Filling of Plate		
				
	Reagent preparation	Reagent preparation		
	Addition of reagent	Addition of reagent		
	Successive Reads and Results	Successive Reads and Results		
Hands-on time				
Time to result (1 reader)	1 x  = 40 min		1 x  = 22 min	6 x  = 60 min

Reducing operator-related variability

RESULTS IN HALF THE TIME

25 MINUTES

ENDOZYME® II GO – The rapid endotoxin test

	1	2	3	4	5	6	7	8	9	10	11	12
A	STD 50	STD 50	PPC SPL 1	PPC SPL 2	PPC SPL 3	PPC SPL 4	PPC SPL 5	PPC SPL 6	PPC SPL 7	PPC SPL 8	PPC SPL 9	PPC SPL 10
B	STD 5	STD 5	PPC SPL 1	PPC SPL 2	PPC SPL 3	PPC SPL 4	PPC SPL 5	PPC SPL 6	PPC SPL 7	PPC SPL 8	PPC SPL 9	PPC SPL 10
C	STD 0.5	STD 0.5	SPL 1	SPL 2	SPL 3	SPL 4	SPL 5	SPL 6	SPL 7	SPL 8	SPL 9	SPL 10
D	STD 0.05	STD 0.05	SPL 1	SPL 2	SPL 3	SPL 4	SPL 5	SPL 6	SPL 7	SPL 8	SPL 9	SPL 10
E	STD 0.005	STD 0.005	SPL 11	SPL 12	SPL 33	SPL 14	SPL 15	SPL 16	SPL 17	SPL 18	SPL 19	SPL 20
F	BLK	BLK	SPL 11	SPL 12	SPL 33	SPL 14	SPL 15	SPL 16	SPL 17	SPL 18	SPL 19	SPL 20
G	BLK	BLK	PPC SPL 11	PPC SPL 12	PPC SPL 13	PPC SPL 14	PPC SPL 15	PPC SPL 16	PPC SPL 17	PPC SPL 18	PPC SPL 19	PPC SPL 20
H	PPC Control	PPC Control	PPC SPL 11	PPC SPL 12	PPC SPL 13	PPC SPL 14	PPC SPL 15	PPC SPL 16	PPC SPL 17	PPC SPL 18	PPC SPL 19	PPC SPL 20

CES Standards 50 - 0.005 EU/mL

Negative controls (Blank)

PPC Controls

PPC 0.5 EU/mL

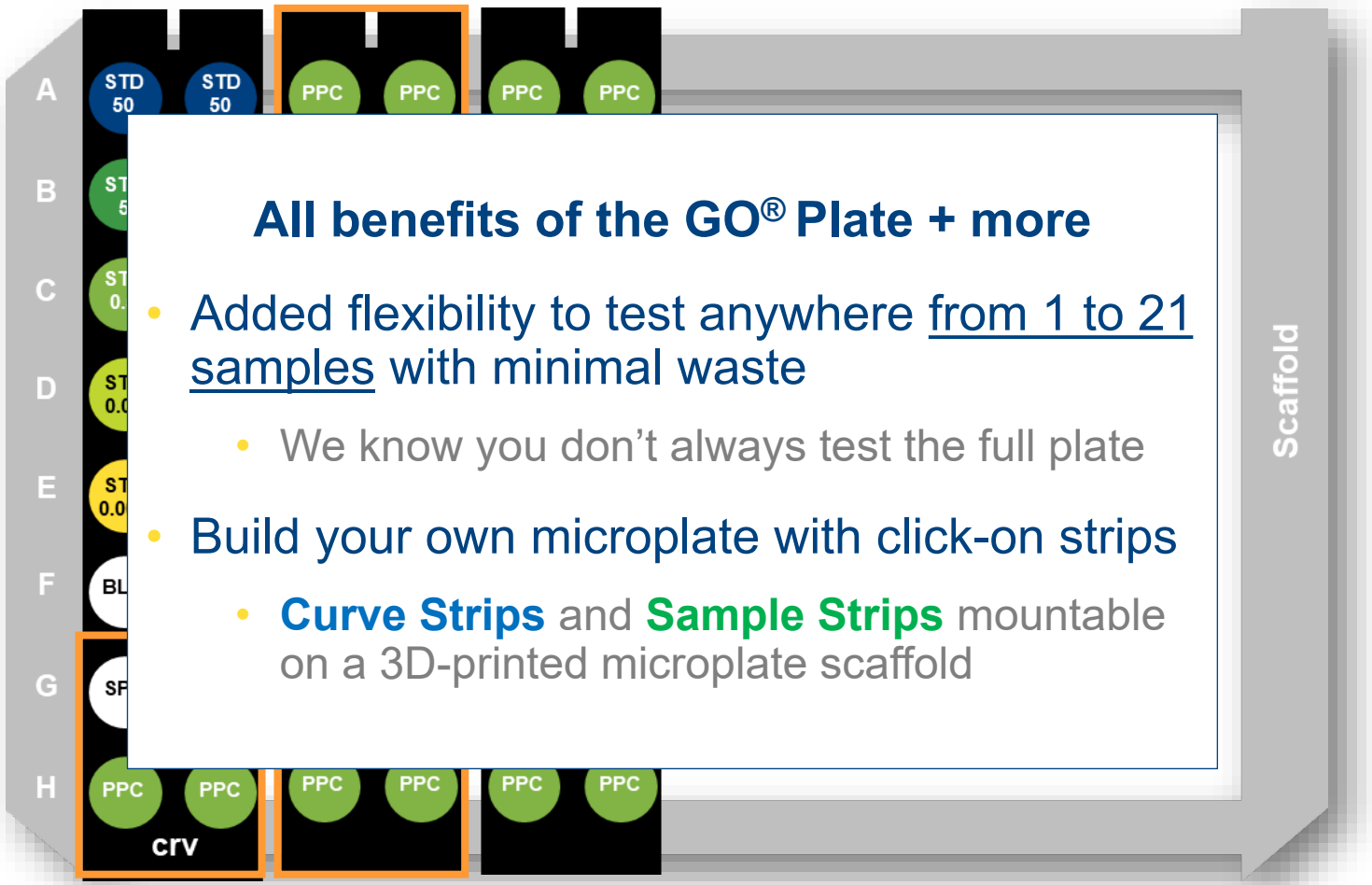
Samples (empty wells)

PPC 0.5 EU/mL

USE ENDOZYME® II GO STRIPS & REDUCE THE WASTE

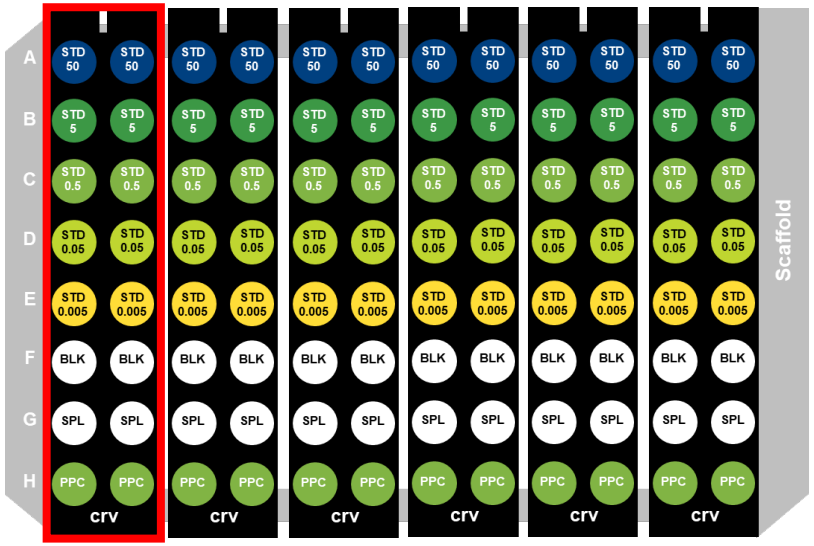
All benefits of the GO® Plate + more

- Added flexibility to test anywhere from 1 to 21 samples with minimal waste
  - We know you don't always test the full plate
- Build your own microplate with click-on strips
  - **Curve Strips** and **Sample Strips** mountable on a 3D-printed microplate scaffold

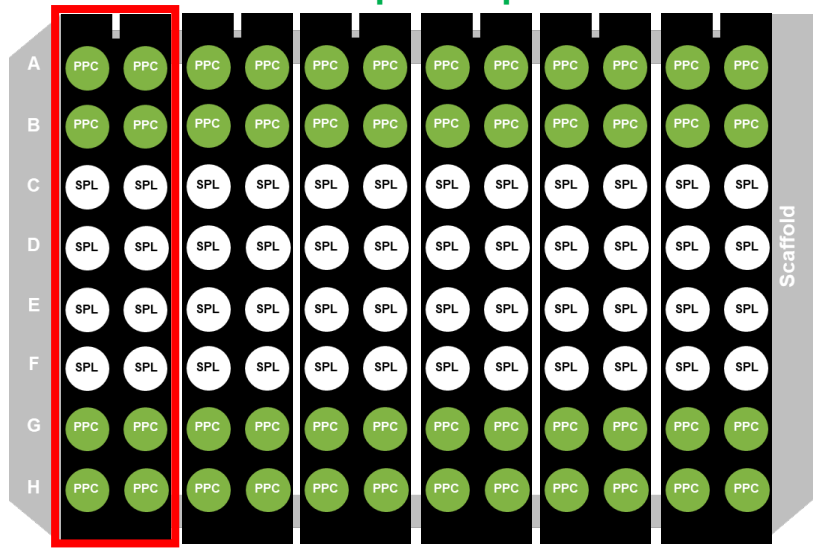


1 sample 2 - 5 samples 6 - 9 samples

Curve Strips



Sample Strips



ENDOLISA® – A SOLUTION FOR COMPLEX MATRICES

ENDOLISA® revolutionizes endotoxin testing of complex samples with a unique built-in sample preparation

Broad measurement range:
0.05 – 500 EU/ml

- **ELISA-like format** featuring a 96-well plate **pre-coated** with a specific **endotoxin-binding phage protein**
- **Overcomes limits** of traditional methods such as inhibition and enhancement
- Intended for **complex samples** with interfering substances present in the product matrix
- Important assay for **Endotoxin Demasking** in combination with ENDO-RS® (Low Endotoxin Recovery / Hold Time Studies)
- Described in the **European Pharmacopeia** as solution for removing interferences (Chapter 5.1.10, section 9)

REGULATORY STATUS OF RFC WORLDWIDE

REGULATORY LANDSCAPE OF rFC

US Pharmacopoeia

<86> Bacterial Endotoxins Test Using Recombinant Reagents

Compendial
PROPOSED

EU Pharmacopoeia

Ph. Eur. 2.6.32 Test for Bacterial Endotoxins with Recombinant Factor C (rFC)

Compendial 2021

Inclusion of rFC in the monographs:

- Water, purified (0008)
- Water for injections (0169)
- Gene Therapy & mRNA Products (proposed)

Korean Pharmacopoeia

General Information Test No. 23 Endotoxin Testing Method Using Recombinant Factor C

Alternative 2023

Japanese Pharmacopoeia

G4-4-180 Bacterial Endotoxins Test & Alternative Methods using Recombinant Protein-reagents for Endotoxin Assay

Alternative 2021

FDA

Guidance for Industry, Pyrogen and Endotoxins Testing: Q&A

MAPP 5310.7

Supports and accepts the use of rFC to replace LAL

British Pharmacopoeia

Added rFC to Appendix XIV C. Test for Bacterial Endotoxins

Compendial 2024

EAEU Pharmacopoeia

To add a chapter on Test for Bacterial Endotoxins with Recombinant Factor C (rFC) based on Ph. Eur. 2.6.32

Compendial
PROPOSED

Indian Pharmacopoeia

Guidelines on the BET Introduction of Alternate Test Methods

Alternative

Chinese Pharmacopoeia

G4-4-180 Bacterial Endotoxins ChP 9251 Guideline for BET Application (introduction of rFC)

Alternative 2020

SUMMARY & KEY TAKEAWAYS

EMBRACING RFC FOR LAB TRANSFORMATION

EFFICIENCY

Time and Cost Savings

- Decrease in retests & OOS investigations
- Standardized results (No variability due to animal variability)
- Reduce false positives (Not sensitive to Factor G)
- Consistent results batch-to-batch
- Easy staff training: more time for added value tasks in the lab

TRACEABILITY

Standardized Results for Data Integrity and fewer risks of error

- Reduced hands-on time
- Reduced manual pipetting-related errors
- Increased sample throughput
- Improved assay performance by better reproducibility & accuracy
- Tracking and trending of endotoxin results in a compliant software

SUSTAINABILITY

HSC-Free for Ethics & Compliance

- Reduction of waste & flexibility
- Meet 3R principles: animal free
- Supply chain sustainability:
  - no production risk linked to ecological matters
  - easy to ship around the world

PIONEERING DIAGNOSTICS