Monocyte Activation Test: A Relevant Alternative Tool for Medical Device Development

Djikolngar Maouyo, PhD

Founder, Managing Director and President

(443) 459-1374; Email: dmaouyo@pyrodextesting.com

PyroDex, LLC

Sinai Hospital BioIncubator, Schapiro Bldg, Ste 203

2401 W. Belvedere Ave, Baltimore, Maryland 21215, USA

https://pyrodextesting.com

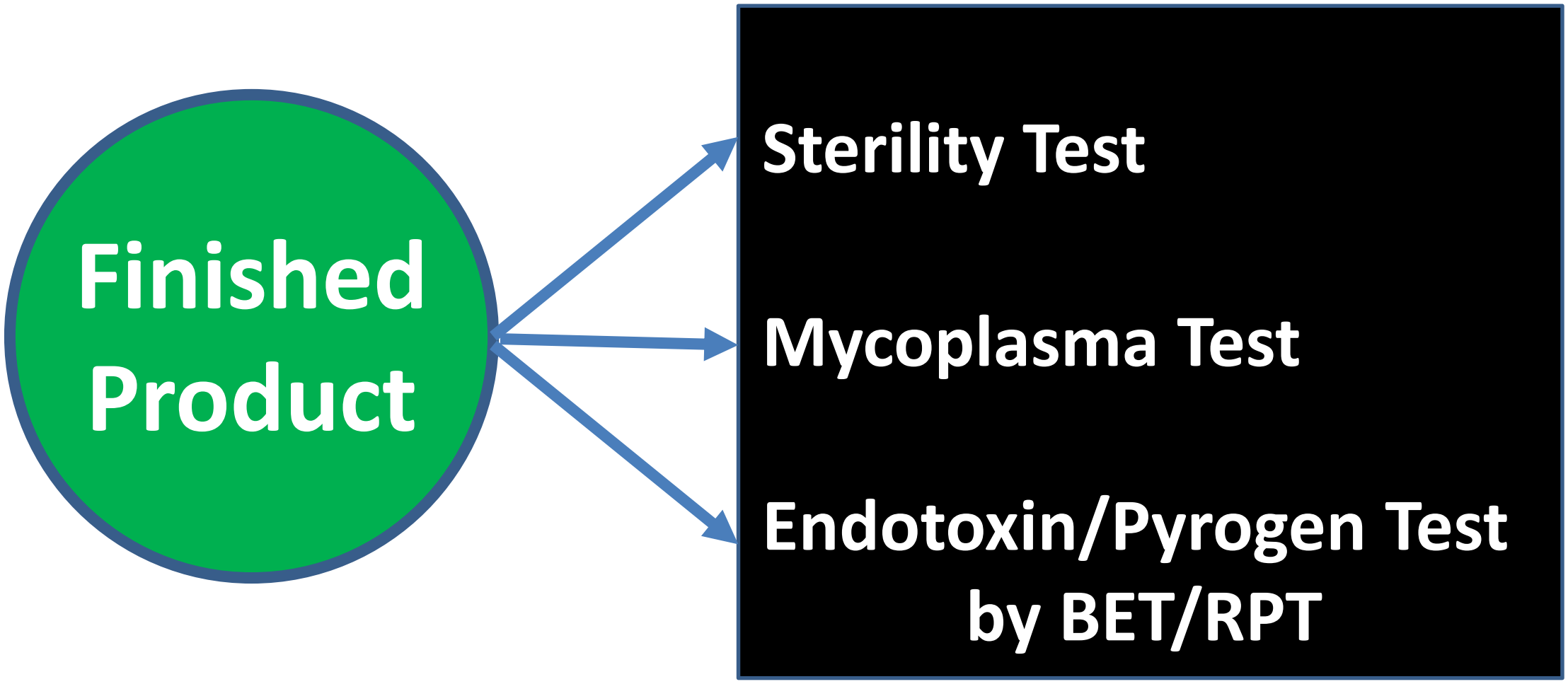
Public Health Policy: One Fundamental Question

Does the medical device at hand have the potential to cause a damage to the human health?

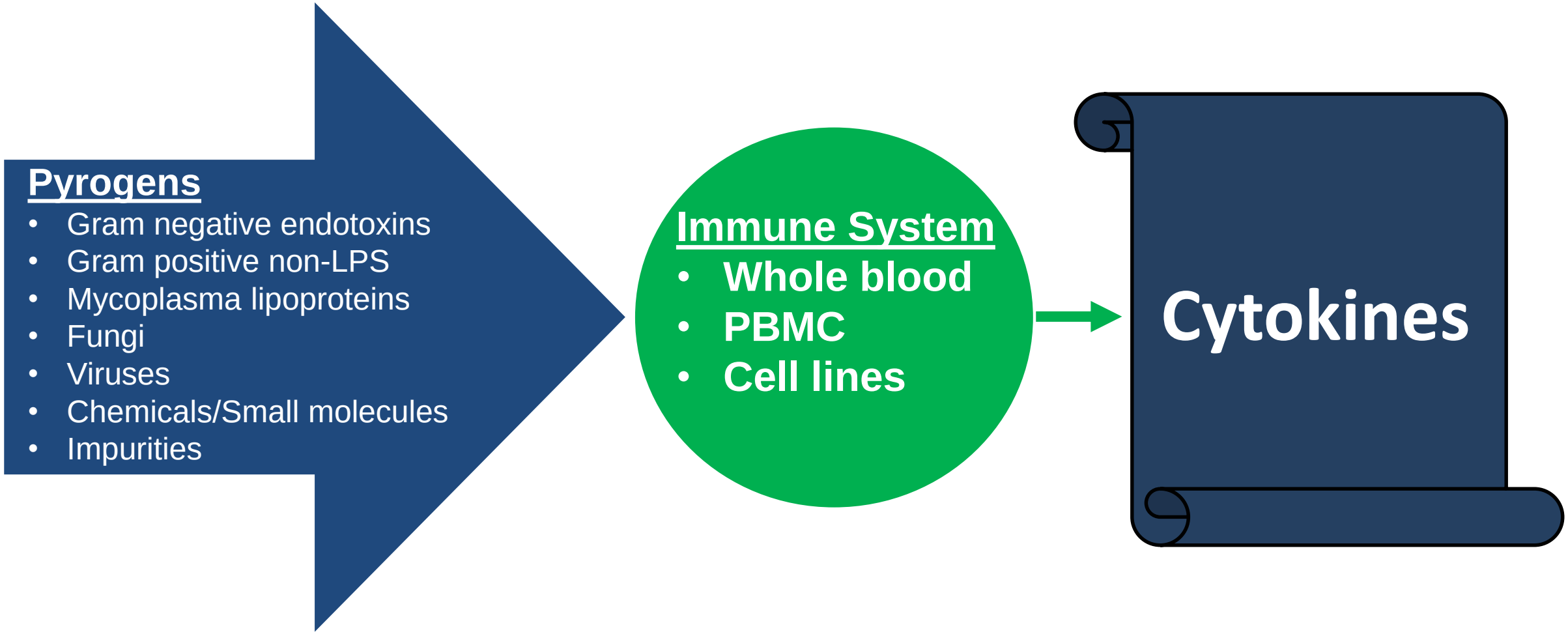
Some Facets of This Question

- *Does the medical device have the potential to negatively impact the biological functions of human bodies?*
- *Does the medical device carry disease-causing germs or microbes?*
- *Does the medical device carry other molecular entities that can trigger inflammation in human subjects who are already ill and therefore vulnerable?*

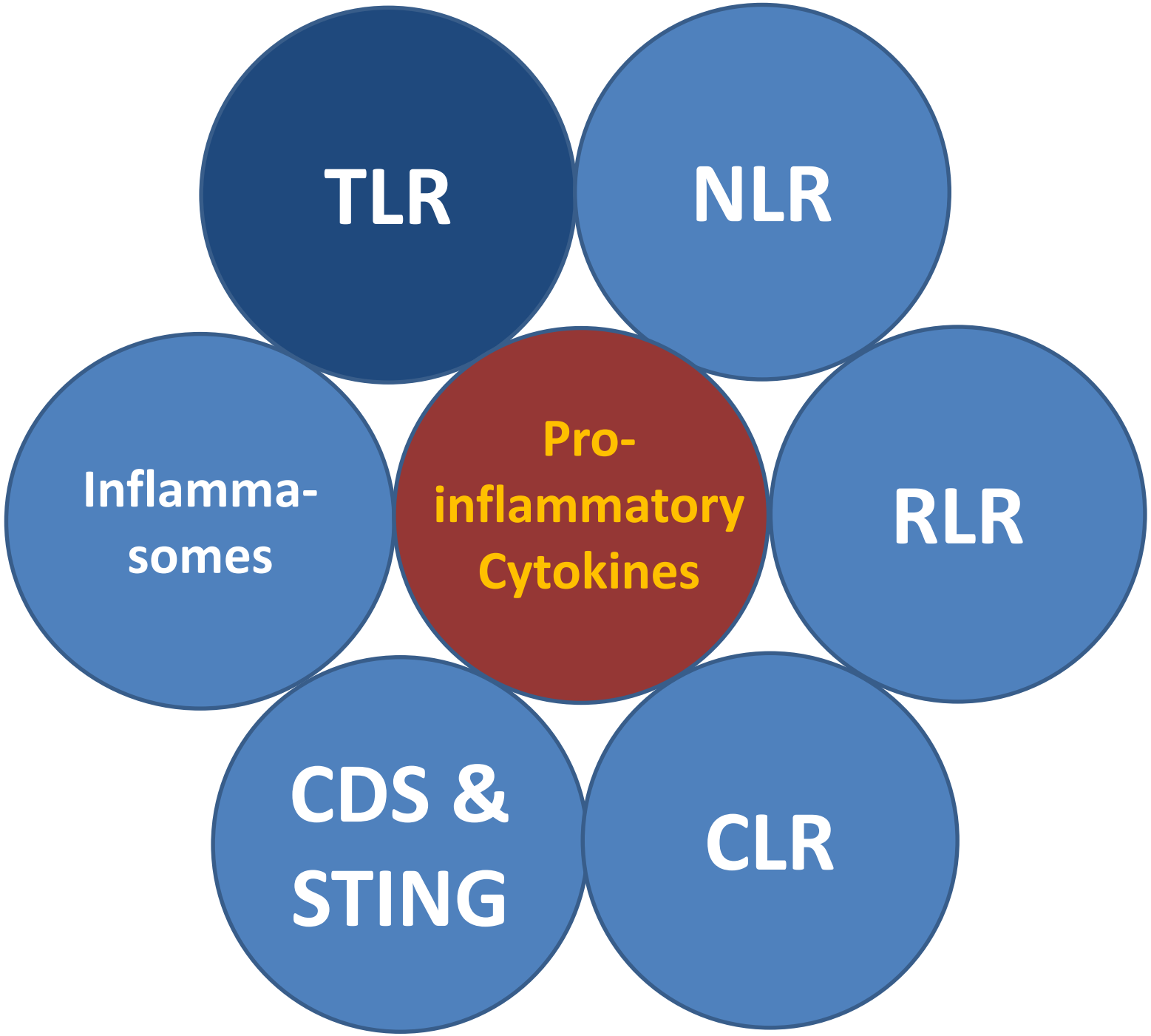
Quality Control Tests of Therapeutic Products



Principle of the Monocyte Activation Test



Detection and quantification of cytokines by ELISA


TLR
NLR
Inflamma-somes
Pro-inflammatory Cytokines
RLR
CDS & STING
CLR

PyroDex MAT and TLR Ligands

Pattern Recognition Receptor (PRR)	Ligand	Limit of detection
TLR1	Pam3CSK4	3 ng/mL*
TLR2	HKCA	100 cfu/mL
TLR2	HKLM	100 cfu/mL
TLR3	Poly(I:C) HMW	31 ng/mL
TLR3	Poly(I:CP LMW	31 ng/mL
TLR4	USP Reference Standard Endotoxin	0.002 EU/mL
TLR5	Flagellin (FLA-ST)	63 ng/mL
TLR6	FSL-1	156 ng/mL*
TLR7	Imiquimod (R837)	78 ng/mL
TLR8	ssRNA40/LyoVec	78 ng/mL
TLR9	ODN 2006 (ODN 7909)	0.156 µM

*Low dilution tested

PyroDex MAT and NLR and RLR Ligands

NOD-like receptors (NLR)		
Pathogen Recognition Receptor	**Ligand**	**Limit of detection**
NOD1	Ei-DAP	78 ng/mL
NOD2	MDP	10 ng/mL
NOD2	Murabutide	195 ng/mL
NOD1/2	PGN-ECndi Ultrapure	78 ng/mL
NOD1/2	PGN-SAndi Ultrapure	10 ng/mL
RIGI-like receptors (RLR)		
RLR	3pppdsRNA	10 ng/mL
	3p-hpRNA	10 ng/mL

PyroDex MAT and CLR and CDS Ligands

C-type lectin receptors (CLR)		
Pattern Recognition Receptor	**Ligand**	**Limit of detection**
CLR	Dectin-1/beta glucan peptide	781 ng/mL
CLR	Dectin-2/Furfurman	195 ng/mL
CLR	Mincle/TDM	391 ng/mL
Cytosolic DNA Sensors (CDS)		
CDS-CDN	dsDNA HSV-60	781 ng/mL
CDS-STING canonical	3’3’-cGAMP	10 ng/mL
CDS-STING noncanonical	2’3’-c-di-AM(PS,PS)	625 µg/mL

MAT as a Replacement for RPT/BET

- **MAT is a comprehensive test**; it includes all known virulence factors (PAMP and DAMP)
  - Endotoxins
  - Non-endotoxin pyrogens
- **MAT is biologically relevant**
  - Human blood cells reflect the human innate immune response to medical device-associated pyrogens in their intended biological environment
- **MAT is quantitative**
  - Highly sensitive
  - Large range: 0.002 – 200 EEU/mL
- **MAT is practically adaptable to various targets and scalable**
  - The structure of medical devices to be tested is not a limiting factor

MAT and Direct Incubation of Medical Devices

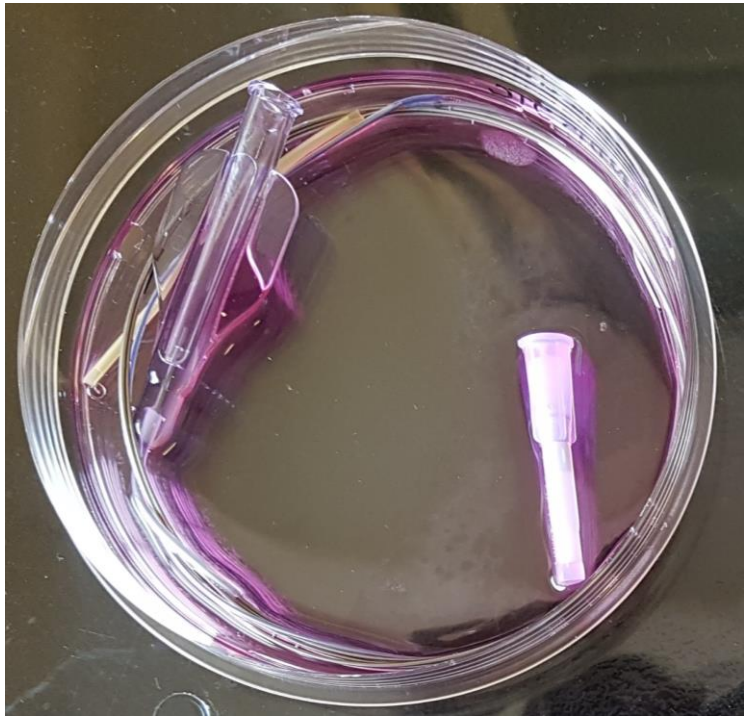
- Prior exposure to depyrogenated water for extraction of pyrogens is not needed
- Direct incubation with culture medium in a volume sufficient to cover the medical device in an adequate culture receptacle

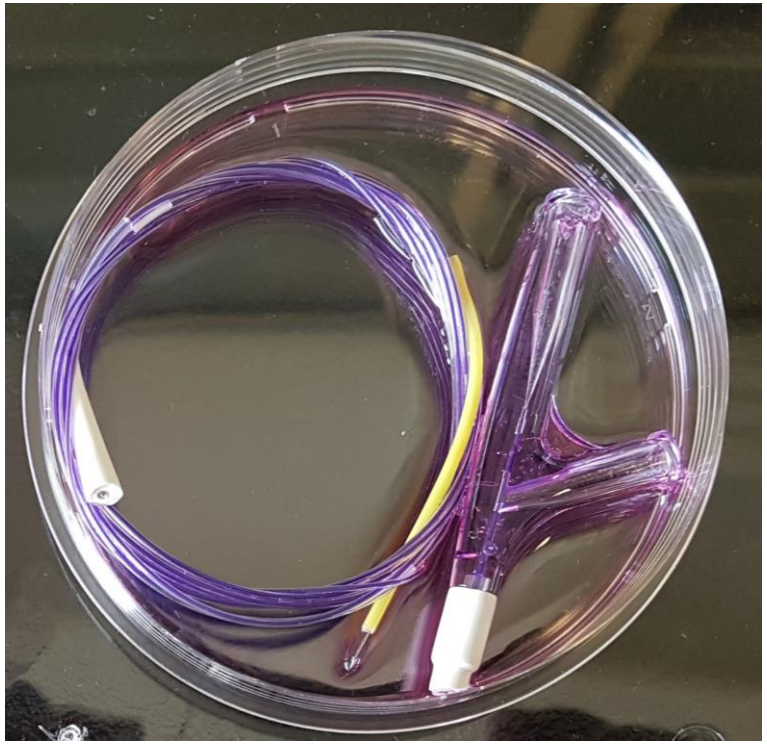
Methodology alteration

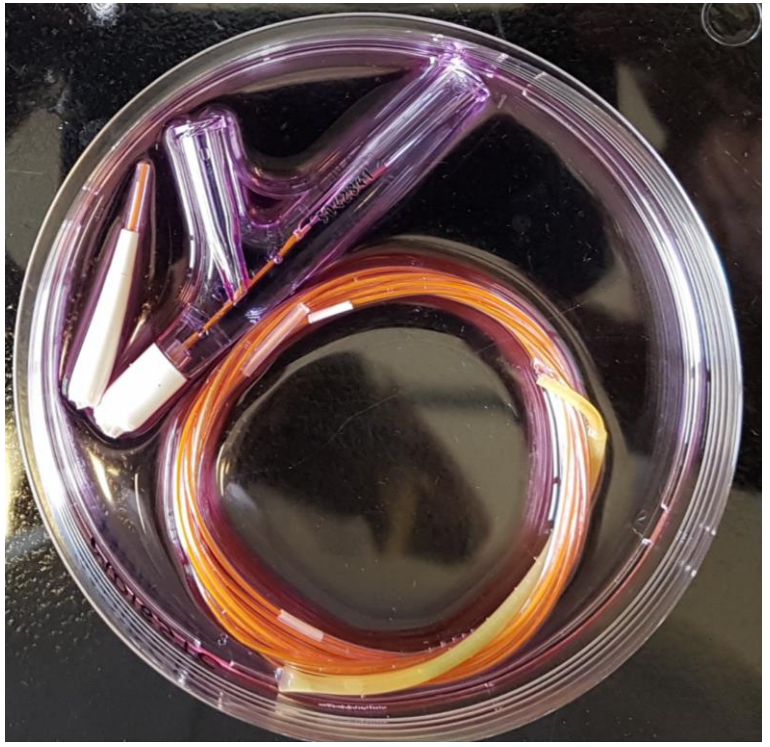
- Not currently needed, but there room for improvement
- Incubation for 20 hours is optimal in the current state-of-the art
- ELISA test configuration is flexible, with several dilutions

Device Preparation for Use with the MAT

- No major preparation is needed
- Small- or large-size medical devices
  - Check device dimensions per manufacturer’s specifications
  - Identify appropriate recipient/cell culture dish
  - Determine volume of cell culture medium required to cover the material
  - Maintain the optimized cell density







Stent Systems

Immune Response to Medical Device Exposure

Inhibited	Basic	Enhanced
Physiological inhibition • Lower cytokine • No significant change in cell viability **Toxicity** • Low or no cytokine • Significant cell death (Viability <50%)	**Reference** • Basic cytokine • Viable cells: Viability >80%	**Pyrogen presence** • Higher cytokine • No significant change in cell viability

Inhibition/Enhancement Information

- In LAL-based endotoxin test, a Positive Product Control (PPC) is included in the test
- In MAT, PPC is neither needed nor recommended
  - Three false assumptions
    - Homogeneity of receptors (in reality, PRR are a class of 5 families of receptors)
    - Additive effects (consider also synergistic effects)
    - The cytokine release is a linear function
  - Difference between a biological, multifactorial response vs kinetic reaction, more reductionist
- Significance of inhibition in MAT
  - Physiological inhibition: biomarker level below the untreated/unexposed cells (blank or negative control), without significant reduction of cell viability
  - Toxicity-related inhibition: lower level of biomarker and significant cell death
- Potential chemical inhibitors of MAT
  - DMSO used for cryopreservation
  - Preservatives

Cytokine Release Syndrome (CRS)

Characteristic Cytokines (7/39)

- IFN-g
- IL-5
- IL-6
- IL-10
- FLT-3L
- Fracktalkine
- GM-CSF

CRS Groups

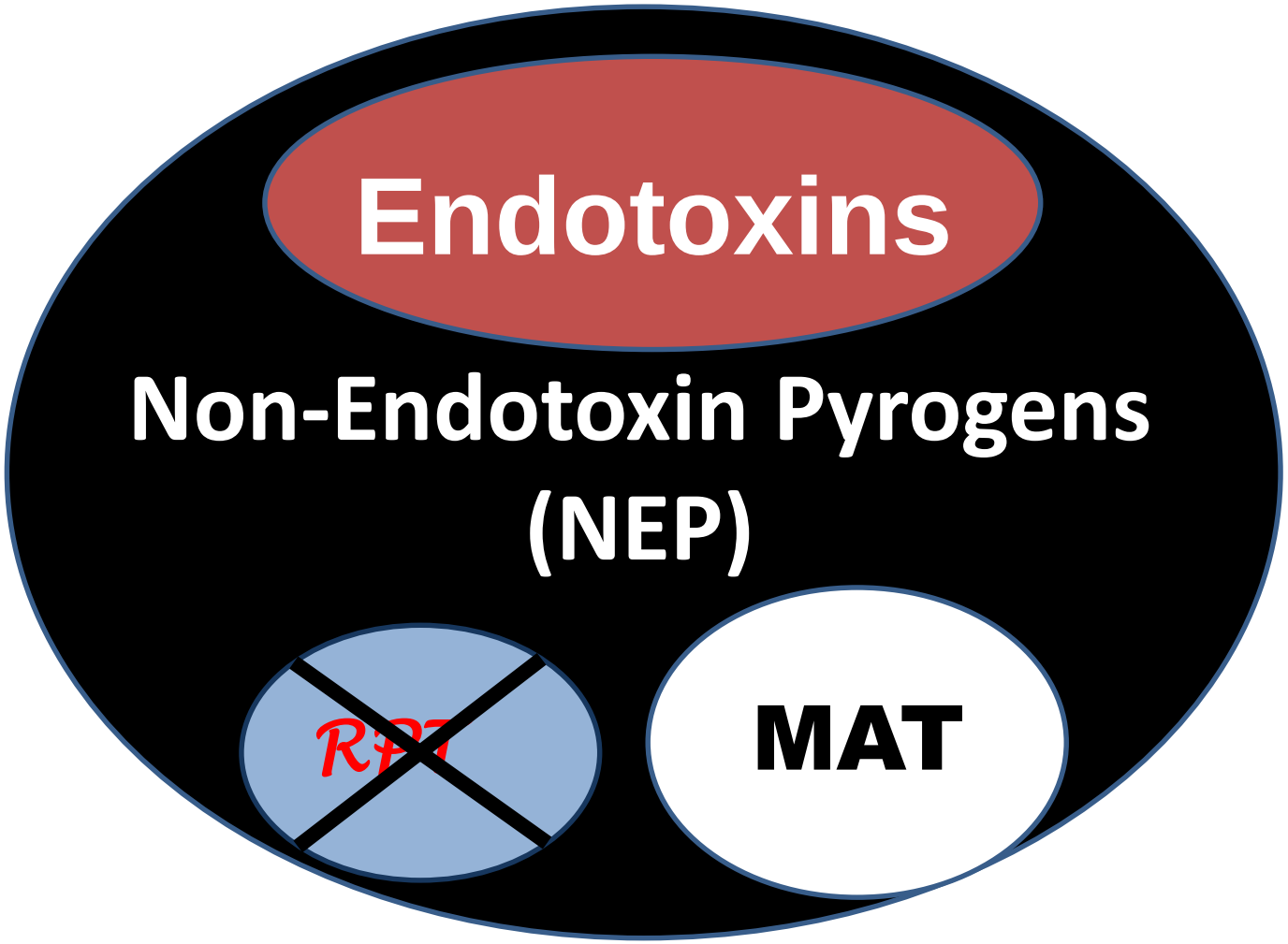
2 out of 7 cytokines	Treated/Reference Fold change
Severe CRS	**≥75**
No CRS	**<75**

Based on classification by Davila et (2014) and Lee et al (2014)

Stratification of Patients (Davila et al. 2014)

CRS Group	Clinical Characteristics
Severe CRS	• Persistent fever for 3 days • Selected cytokine elevations • Clinical evidence of toxicity • Closer attention Medical and pharmacologic intervention • Average hospitalization time: 56.7 days (range: 20 - 104 days)
No CRS	• Low-grade fever and mild cytokine elevations • No fever and absent CRS • Average hospitalization time: 15.1 days (4-61 days)

Global Perspectives on Pyrogens



Use MAT, Save more lives

LAL Issues: Alternative to animal use

- **RPT: Eliminated (Europe)**
- **MAT: Mandatory (EU and UK)**
- **rFC: optional**

Lead Organization:

- **European Pharmacopeia Agencies with practical MAT experience (Dr. I. Spreitzer, PEI):**
- **ANSM (France)**
- **FDA (United States)**
- **Health Canada**
- **INCQS (Brazil)**
- **NIBSC (United Kingdom)**
- **NIFDC (China)**
- **NIFDS (South Korea)**
- **NIID (Japan)**
- **NOMA**
- **PEI (Paul Ehrlich Institute)**
- **RIVM (The Netherlands)**

Summary

The MAT: a relevant alternative to RPT/BET

- Comprehensive
- Quantitative
- Versatile and scalable
- Relatively fast

The MAT currently available

- Commercial kits for in-house tests
- Testing service by specialized providers

MAT—Safer medical devices, better treatment, saving more lives