Evolution of pyrogen and endotoxin testing in Plasma-Derived Medicinal Products (PDMPs) and Vaccines

- Aim of the session:
  - to share knowledge to establish viable alternatives to Limulus Amebocyte Lysate (LAL) test

- Pyrogen and endotoxin testing of PDMP and vaccines (**Swissmedic perspective)**
  - Present: 2026
    - LAL is the standard pyrogen test despite available recombinant alternatives (rFC, rCR) or MAT
  - Past: Until 2025
    - Focus on replacement of rabbit pyrogen test (RPT) with Limulus Amebocyte Lysate (LAL) or monocyte activation test (MAT)
  - Future: Replacement of LAL with recombinant alternatives or MAT
    - Manufacturers? what we know and expect?
    - Swissmedic? what an agency can do?

Replacement of rabbit pyrogen testing with an alternative test for Plasma-Derived Medicinal Products (PDMPs) and Vaccines

- Disclaimer
  - Presented views and opinions are my own
- Disclosure of Conflict of Interest
  - I have no financial conflicts to disclose

European regulation for Plasma-Derived Medicinal Products (PDMP) and Vaccines

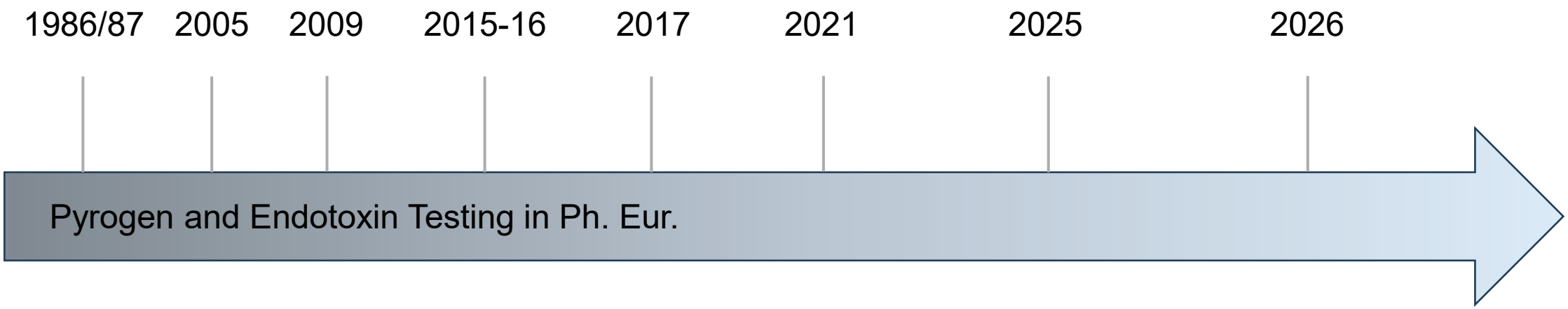
- Swissmedic (Swiss Agency for Therapeutic Products)
  - is not member of EMA (European Medicines Agency)
    - ➢ Swissmedic follows the same regulations for PDMPs and vaccines as the EMA
- Swissmedic is full member of EDQM (European Directorate for the Quality of Medicines)
  - ➢ European Pharmacopoeia (Ph. Eur.) requirements are binding on Swissmedic
- Swissmedic is part of the OCABR network (Official Control Authority Batch Release)
  - ➢ EMA and Swissmedic mutually recognise OMCL batch release
- Swissmedic is part of the PIC/S (Pharmaceutical Inspection Co-operation Scheme)
  - ➢ EMA and Swissmedic are collaborating on inspections of manufacturers of medicinal products

Why the focus on pyrogen testing of Plasma-Derived Medicinal Products (PDMPs) and Vaccines

- Most of PDMPs and vaccines are parenteral preparations and must be controlled for pyrogenic contaminations
  - Ph. Eur. general monograph: Parenteral preparations (0520)
  - (The requirements of this monograph do not necessarily apply to products derived from human blood, to immunological preparations)

- Product group specific considerations
  - PDMPs are fractionated from human plasma and plasma donations have a high risk for viral and bacterial contaminations
  - Vaccines can contain substances that are inherently pyrogenic (e.g. adjuvants, membrane proteins) or live attenuated viral vaccines can interfere with pyrogen tests

Evolution of pyrogen testing in PDMPs and Vaccines between 2009-2025



RPT 2.6.8
BET 2.6.14

MAT 2.6.30

how using BET 5.1.10

Rev. 5.1.10 Risk Assessment

MAT 2.6.40

rFC 2.6.32

Strategy to fully replace RPT with BET

Pyrogenicity 5.1.13

Removal of RPT
rFC as methode G of LAL 2.6.14 (acceptance as equivalent)

Why the focus on pyrogen testing of Plasma-Derived Medicinal Products (PDMPs) and Vaccines

- Regulatory impact on pyrogen testing
  - Ph. Eur. General Monograph: Parenteral preparations (0520)
    - Bacterial endotoxins - pyrogens. Parenteral preparations for human use comply with the test for bacterial endotoxins (2.6.14) or, where justified and authorised, with the test for pyrogens (2.6.8).
  - Monographs for PDMPs (e.g. Albumin, Immunoglobulin, Factor VIII)
    - Pyrogens (2.6.8) or Bacterial endotoxins (2.6.14). It complies with the test for pyrogens or, preferably and where justified and authorised, with a validated *in vitro* test such as the bacterial endotoxin test.
  - Ph. Eur. 0153 general monograph for Vaccines for human use
    - Bacterial endotoxins (2.6.14)

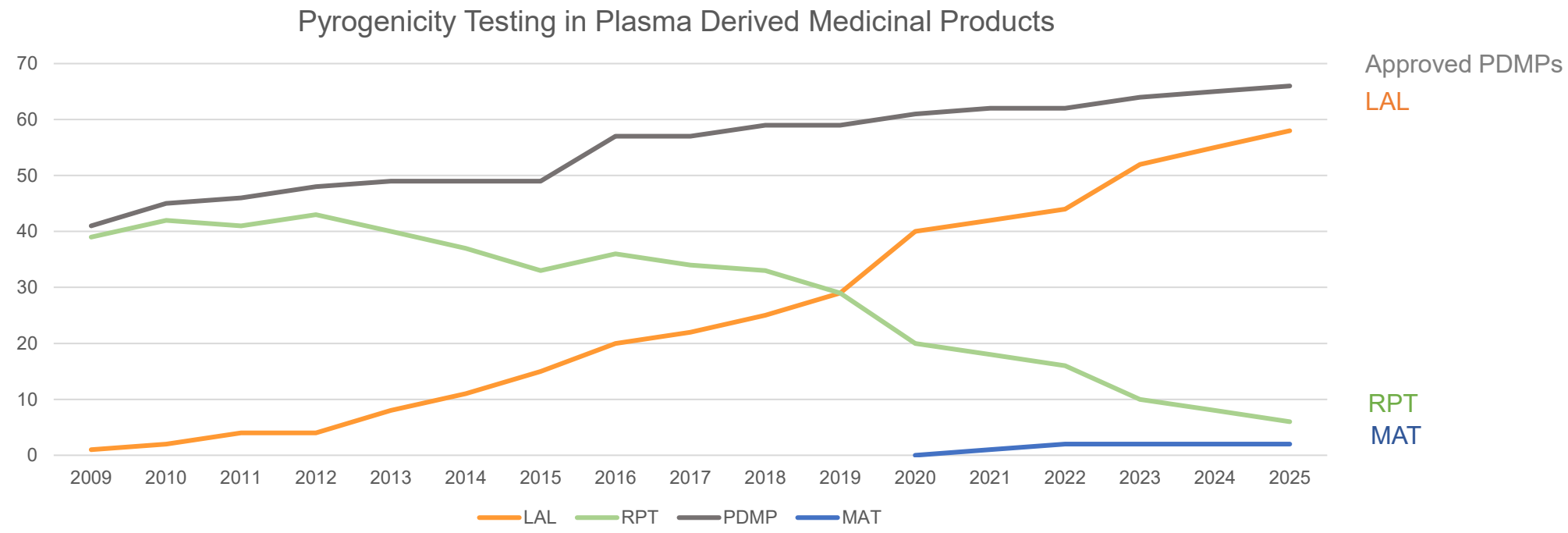
➢ Until 2025 Rabbit pyrogen test Ph. Eur. (2.6.8) was mandatory for PDMPs and Vaccines (during development) and could only be replace by alternative tests, if comparability was demonstrated

Evolution of pyrogen testing in PDMPs and Vaccines between 2009-2025 in Switzerland

- 2009 EMEA Guideline on the replacement of rabbit pyrogen testing by an alternative test for plasma derived medicinal products

Evolution of pyrogen testing of PDMPs 2009-2025 in Switzerland

	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024	2025
Approvals with **RPT**	40	3		2	1			3									
Approvals with **LAL**	1	1	1					5		2		2	1		2	1	1
Replacements of RPT by LAL			1		4	3	4		2	1	4	9	2	2	6	2	2
Addition of **LAL** or **MAT**												1	1	1			



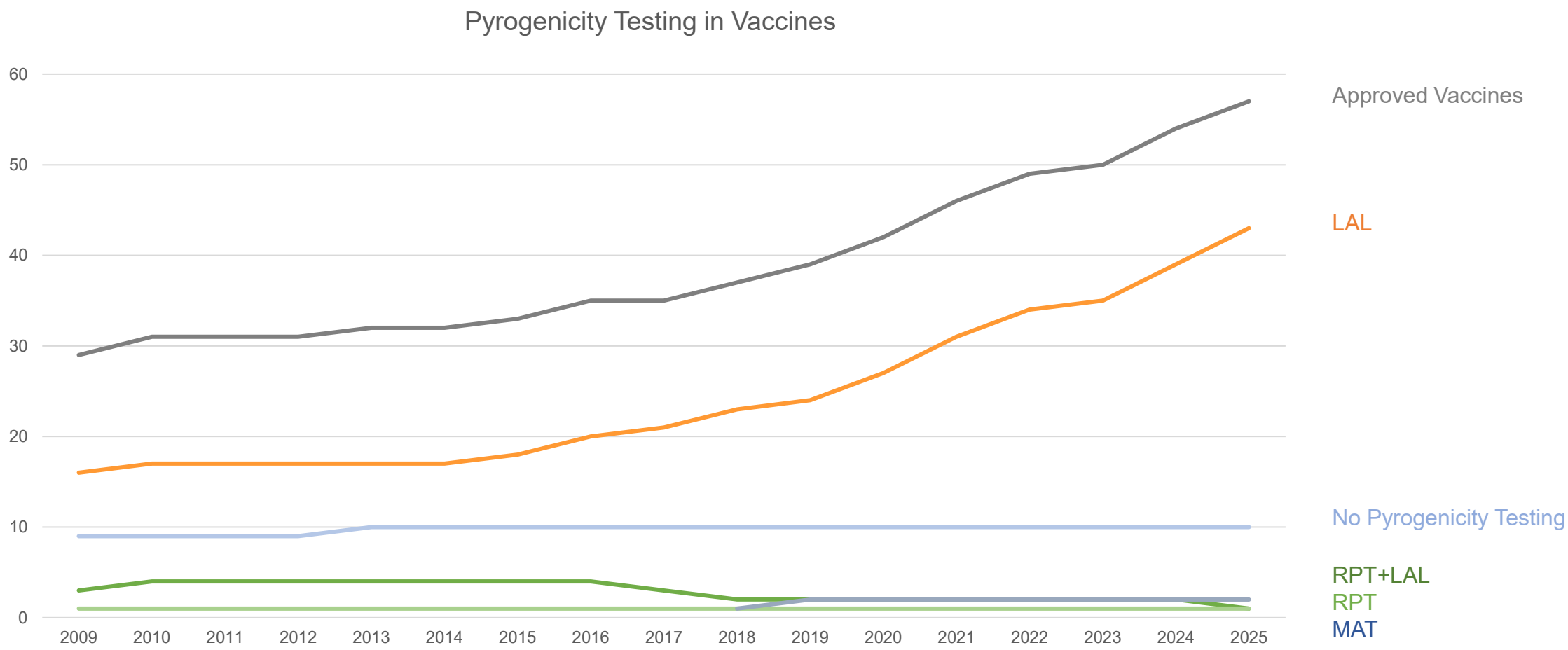
Evolution of pyrogen testing of PDMPs between 2009-2025 in Switzerland

➔ Successful replacement of the RPT with LAL for the release of PDMPs
- 41 replacements of RPT with LAL
- 17 approvals of new PDMPs directly with LAL

➔ The LAL has become the standard release test for PDMPs

➔ No manufacturer uses an animal-free alternative (rFC or rCR) of the LAL

Evolution of pyrogen testing of Vaccines 2009-2025 in Switzerland



Evolution of pyrogen testing of Vaccines between 2009-2025 in Switzerland

➔ The LAL has become the standard release test for Vaccines

➔ No manufacturer uses an animal-free alternative (rFC or rCR) of the LAL

Pyrogen testing of PDMPs and Vaccines in 2026

- Successful replacement of the rabbit pyrogen test with the Limulus Amebocyte Lysate (LAL) test
- The LAL test has become the standard endotoxin release test for PDMPs and vaccines
- Manufacturers of PDMPs and vaccines do not yet use an animal-free (rFC, rCR) version of the LAL test

Pyrogen/endotoxin testing in PDMPs and Vaccines after 2026?

	2026	> 2026
▪ During development	LAL /MAT	Risk Assessment or MAT
▪ Raw materials	LAL/MAT	rFC/rCR/MAT?
▪ Buffers, WFI	LAL	rFC/rCR?
▪ Resins, Filters	LAL	rFC/rCR?
▪ IPC	LAL	rFC/rCR?
▪ Intermediates	LAL	rFC/rCR?
▪ Drug substance	LAL	rFC/rCR?
▪ Drug product	LAL	rFC/rCR?
▪ Excipients	LAL	rFC/rCR?
▪ Adjuvants	LAL	rFC/rCR?

Pyrogen testing of PDMPs and Vaccines in 2030

- Do manufacturers have to replace the LAL with a recombinant alternative (rFC, rCR)?
- Regulatory requirements
  - Ph. Eur. 0520 general monograph for parenteral preparations
    - Parenteral preparations for human use comply with a suitable test for pyrogenicity (5.1.13)
    - LAL, rFC (2.6.14) or MAT (2.6.30)
  - Ph. Eur. 0153 general monograph for Vaccines for human use
    - Pyrogenicity characterisation during development studies (5.1.13)
  - 3R Directive 2010/63/EU on the protection of animals used for scientific purposes does apply to non-human vertebrate animals and cephalopods, **but does not apply to Limulus Amebocytes**
- Other aspects
  - ✓ Supply security: animal source, dependence on horseshoe crab blood from U.S. Atlantic Coast
  - ✓ rFC, rCR: Improved lot-to-lot consistency
  - ✓ rFC, rCR: no β-glucan false positives
  - ✓ rFC and rCR are encouraged to replace LAL

What do manufacturers intend to change for PDMPs and Vaccines?

- Established vs new drug products?
  - Established products
    - Remaining RPT will be replaced soon by MAT or LAL
    - No information whether LAL will be replaced by rFC soon
  - New parenteral drug products will be developed with MAT and rFC

Pyrogen testing of PDMPs and Vaccines, what Swissmedic is doing?

- Recommendations to Swissmedic
  - Swissmedic uses the LAL test for official control authority batch release (OCABR) and market surveillance
    - Swissmedic OMCL introduces MAT and an animal-free LAL alternative (rFC or rCR)
  - Swissmedic contributes to international harmonisation of guidelines and pharmacopeia for animal-free pyrogen testing (MAT, LAL or rFC is requested in Europe, whereas RPT is still requested in many other parts of the world)
  - Swissmedic is strengthening its expertise for assessments and to provide sound scientific advices
  - ➢ Thereby supporting and encouraging manufacturers to establish viable alternatives to LAL

Conclusions?

- Successful replacement of the rabbit pyrogen test with the LAL test
- The LAL test has become the standard pyrogen test
- Significant potential for replacing the LAL test with an animal-free alternative test
- No worldwide harmonisation of pyrogenicity testing
- Manufacturers have the intention to establish recombinant alternatives for new products
- Swissmedic is actively developing expertise in recombinant alternatives to the LAL test
- Swissmedic aims to support manufacturers in their efforts to replace the LAL test with alternative methods

Thank you for your attention