German NCL MAT experience with blood derived products

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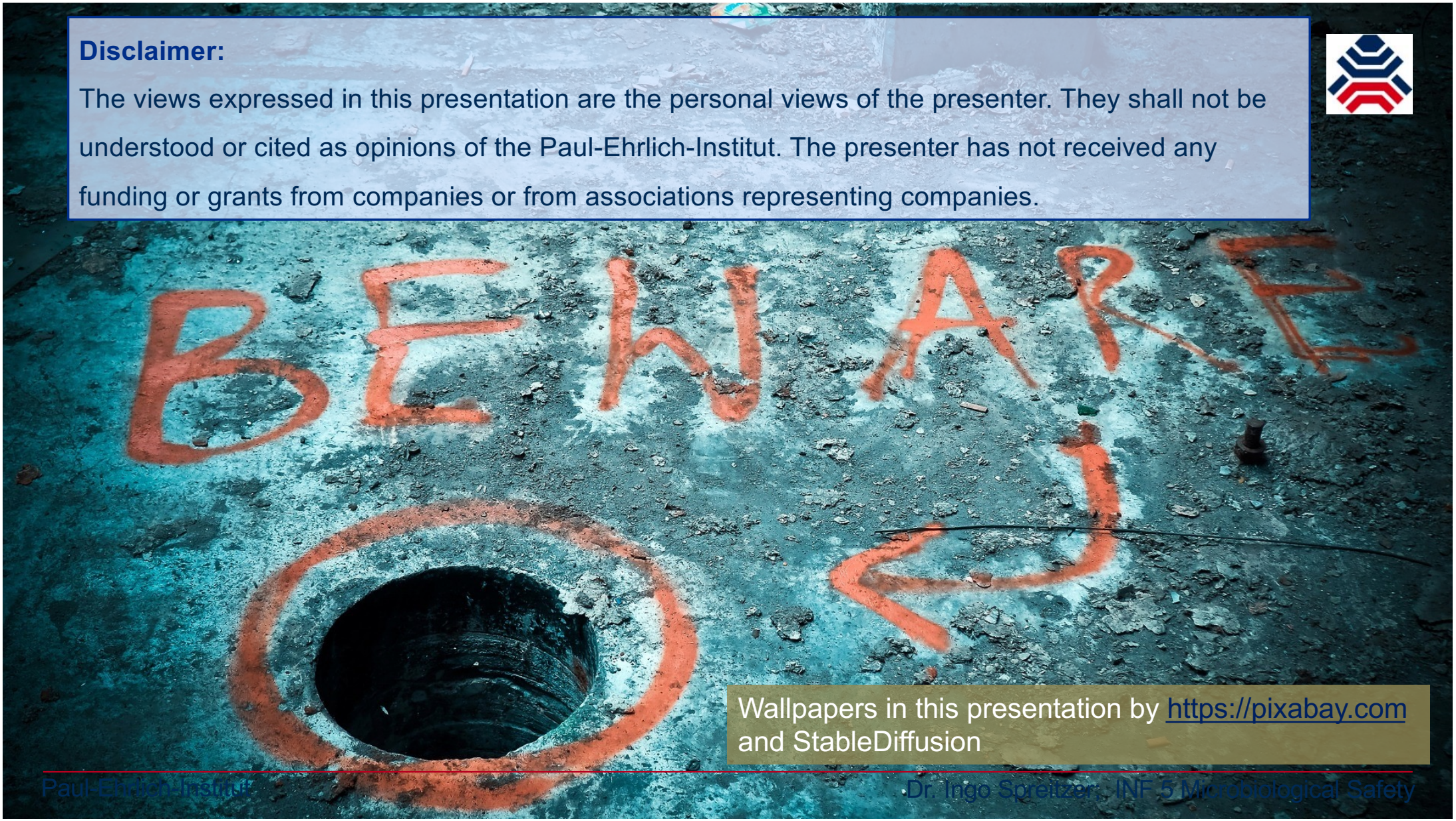

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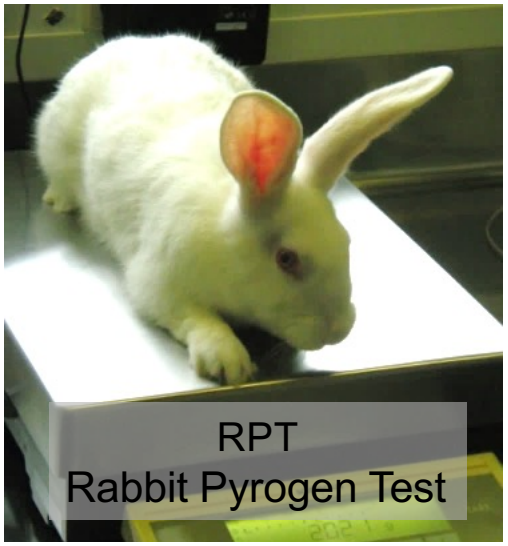
Outlook

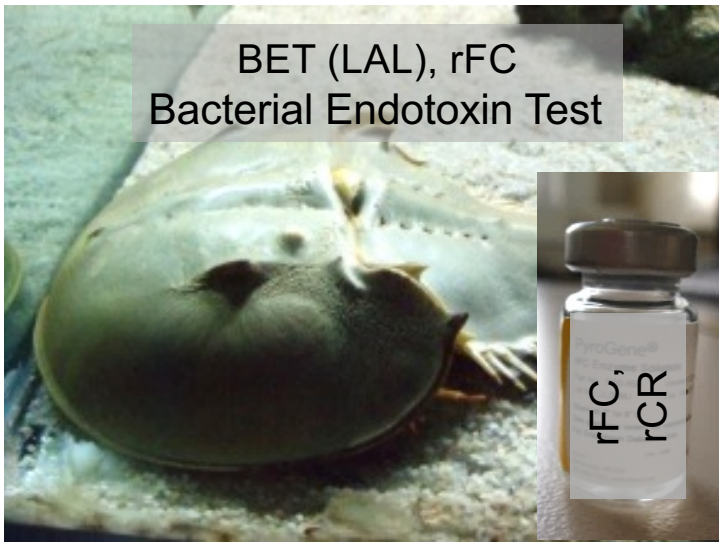
- current Pyrogen / Endotoxin tests
- blood products and „pyrogenicity“
- how EU-Directive 2010/63 affected Pyrogen/Endotoxin testing
- the pyrogenicity strategy in Europe and its consequences for drug im- and export
- The global future of Pyrogen / Endotoxin testing

- Animal experiment
- no controls



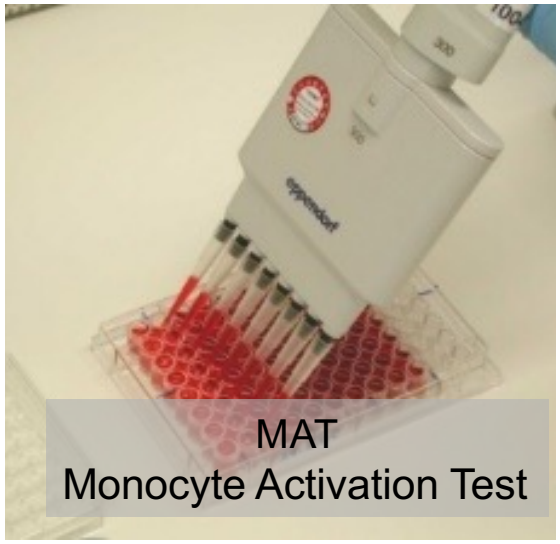
- **Sensitivity relatively unknown**, depends on Injection volume
- **no positive control**
- no standard curve
- no Spiking/Recovery

- 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.

Blood products, pyrogenicity (in RPT and humans)

- Several publications on pyrogenicity of Albumin and other products before the main development of MAT

  Transfusion
  1978 Jan-Feb;18(1):102-7. doi: 10.1046/j.1537-2995.1978.18178118551.x.
  Pyrogen reactions associated with the infusion of normal serum albumin (human)
  A C Steere, M K Rifaat, E B Seligmann Jr, H D Hochstein, G Friedland, P Dasse, K O Wustrack, K J Axnick, L F Barker

  J Clin Apher
  1995;10(2):81-4. doi: 10.1002/jca.2920100205.
  Pyrogen reactions to human serum albumin during plasma exchange
  M Pool 1, B C McLeod

Early MAT-versions (whole blood, PBMC) wellsuited for blood products; classical pyrogenicity

J Immunoassay
1999 Feb-May;20(1-2):79-89. doi: 10.1080/01971529909349315.
Differentiation between endotoxin and non-endotoxin pyrogens in human albumin solutions using an ex vivo whole blood culture assay
E J Pool 1, G Johaar, S James, I Petersen, P Bouic

ALTEX
2002:19 Suppl 1:73-5.
Comparative study of rabbit pyrogen test and human whole blood assay on human serum albumin
Ingo Spreitzer 1, Matthias Fischer, Katja Hartzsch, Ursel Lüderitz-Püchel, Thomas Montag

ALTEX
2011;28(3):227-35. doi: 10.14573/altex.2011.3.227.
Monocyte activation test (MAT) reliably detects pyrogens in parenteral formulations of human serum albumin
Rolando Perdomo-Morales 1, Zenia Pardo-Ruiz, Ingo Spreitzer, Alicia Lagarto, Thomas Montag

MAT: classical pyrogenicity **+**
rare immuniological issues rabbit / human resolved by MAT

Biologicals
2019 May:59:12-19. doi: 10.1016/j.biologicals.2019.04.001. Epub 2019 Apr 22.
Immunoglobulin G from single plasma donor in immune globulin intravenous causes false positive pyrogen test
Clark Zervos et al**: false-positive RPT due to 1 out of 4500 Donors; revealed and released by MAT**

Similar case reported by Prof. Peter Tureczek (Takeda) in Brussels 2023 (EDQM-EPAA Pyrogenicity Event The future of pyrogenicity testing: phasing out the rabbit pyrogen test)

The **„ Eau Claire Incident";** statement Prof. Tureczek: "…very similar to case described by Zervos et al., …

Fever threshold of rabbits; 2.6.8.; RSE-data PEI

Journal of Endotoxin Research; Vol. 11; No. 1; 25-31; 2005

RPT not harmonised

Table 1. Experimental designs and criteria for product assessment of the pyrogen tests of the EP, JP and USP

Pyrogen test	Cumulative number of rabbits	Product passes/negative test result	Product fails/positive test result
EP	3	If summed response ≤ 1.15°C	If summed response > 2.65°C
	6	If summed response ≤ 2.80°C	If summed response > 4.30°C
	9	If summed response ≤ 4.45°C	If summed response > 5.95°C
	12	If summed response ≤ 6.60°C	If summed response > 6.60°C
JP	3	If all individual responses < 0.60°C and if summed response ≤ 1.40°C	If 2 or 3 individual responses ≥ 0.60°C
	5[a]	If 4 or 5 individual responses < 0.60°C	If 2 or more individual responses ≥ 0.60°C
USP[b]	3	If all individual responses < 0.50°C	– (USP-2: if summed response > 3.30°C)
	8	If summed response ≤ 3.30°C **and** if not more than 3 individual responses ≥ 0.50°C	If summed response > 3.30°C **or** if 4 or mor individual responses ≥ 0.50°C

[a]Five additional animals are tested and the test result is determined only considering these.
[b]Although the USP lacks a criterion for failure in the first step, it can be anticipated (USP-2) that if already the three rabbits in this step show a summed response larger than 3.30°C, which is a criterion for failure in the second step, the test will be terminated.

28 *Hoffmann, Lüderitz-Püchel, Montag, Hartung*

Fever threshold USP, EP, JP

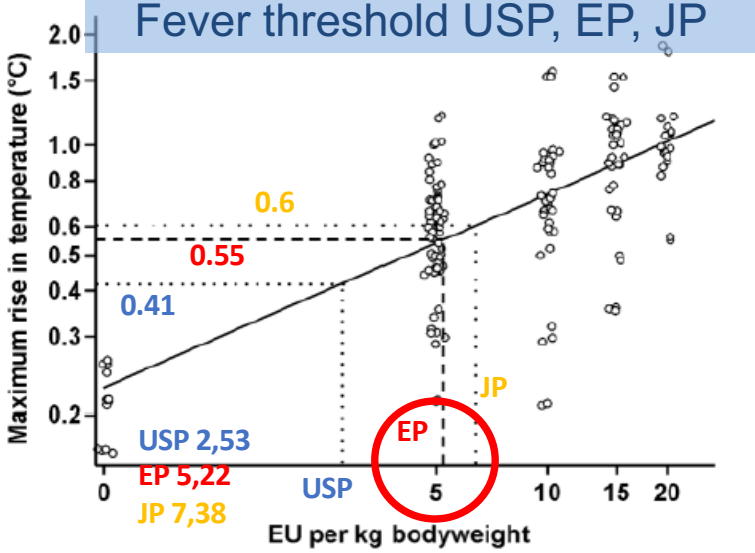
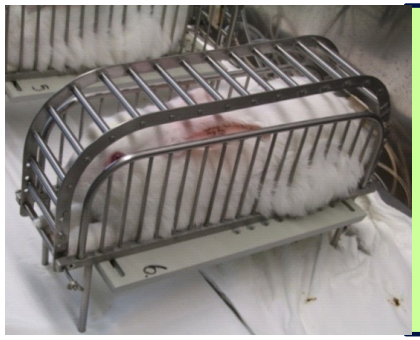


Fig. 1. Biometrical model of rabbit fever describing the temperature rise induced by standard endotoxin per kg body weight based on the data of 171 rabbits (circles) with the regression line (straight line) and the pharmacopoeial rabbit fever thresholds (USP: dotted line; EP: dashed line; JP: dashed/dotted line). A jitter-effect was used to present the data to make identical data distinguishable.

The EP EU/kg bw for the fever threshold is nearly identical to the BET i.v.-Limit!

RPT



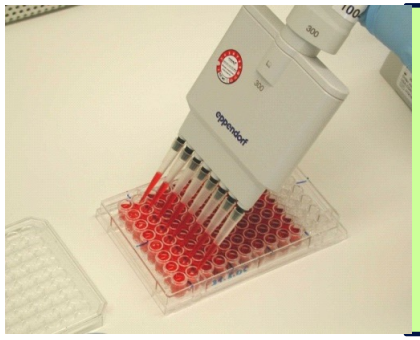
Sensitivity 500pg LPS (RSE) /kg body weight
Injection-Volume 10 ml /kg bw Sensitivity = 50pg LPS /ml
1 ml /kg bw Sensitivity = 500pg LPS /ml

Injection-Volume EP 0.5 – 10 ml/kg acc. to monograph

Journal of Endotoxin Research; Vol. 11; No. 1; 25-31; 2005

1000-50 pg/ml

MAT

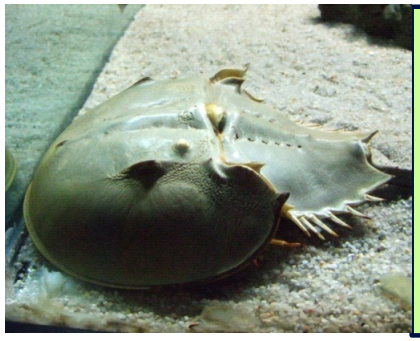


Known Sensitivities 50 pg LPS (RSE)/ ml
down to 3 pg LPS (RSE)/ ml.

Every known MAT is more sensitive than the RPT, the question remains if this is always necessary
The sensitivity of the MAT is defined as for the BET (low or lowest LPS-value of the standard curve)

Increasing sensitivity

BET



Sensitivities (λ) range from 6 pg RSE/ml down to 0.001 pg/ml

These sensitivities are mainly not needed because of the Applied Drug volume, but to overcome sample interference by diluting (approx. 70% of samples show interference in the BET)

0.001 pg/ml

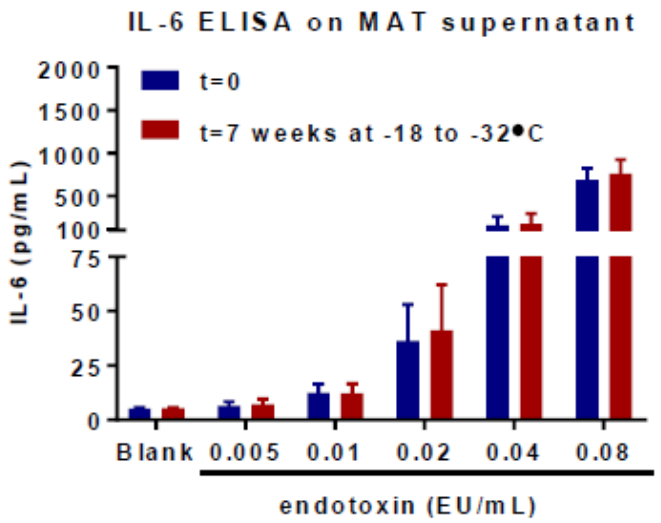
RPT/MAT comparison PEI 10/2005-11/2010; RPT skipped at end of 2014, (Method B (Limit Test) Ph. Eur. 2.6.30) inhouse MAT (cryopreserved human whole blood) setup relative insensitive on purpose (has to detect at least 50 pg RSE/ml)

Product class	Number of lots	RPT pass	MAT pass
Antithrombin III	8	✔	✔
FVIII-Inhibitor	7	✔	✔
Coagulation Factors	82	✔	✔
Human Plasma virus inactivated	26	✔	✔
Human Albumin	40	✔	✔
Immunglobulins	75	✔	✔
Vaccines	43	✔	✔
Protein C	1	✔	✔
Proteinase inhibitors	3	✔	✔
Research / adverse events	7	fail	fail
Total	292 lots	>= 876 rabbits	

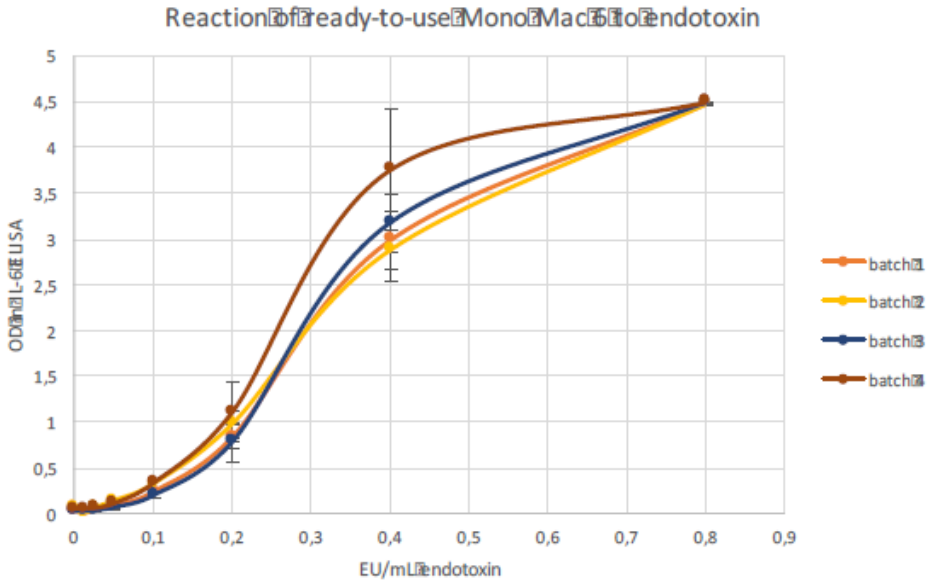
„good batches“

The published MAT-sensitivities for LPS

(concentration in the sample, not in the reaction mix) 0.03 to 0.5 EU/ml (sample); mainly affected by sample amount in the final reaction vessel („dosage“); **very small dynamic range** compared to kinetic BET



Sanquin, E. Gitz
Pharmalab 2019



Merck Millipore; A. Fritsch
Pharmalab 2019

ALTEX
2016;33(1):47-53. doi: 10.14573/altex.1509291. Epub 2015 Dec 2.
Limitations of the rabbit pyrogen test for assessing meningococcal OMV based vaccines
Caroline Vipond 1, Lucy Findlay 2, Ian Feavers 1, Rory Care 1

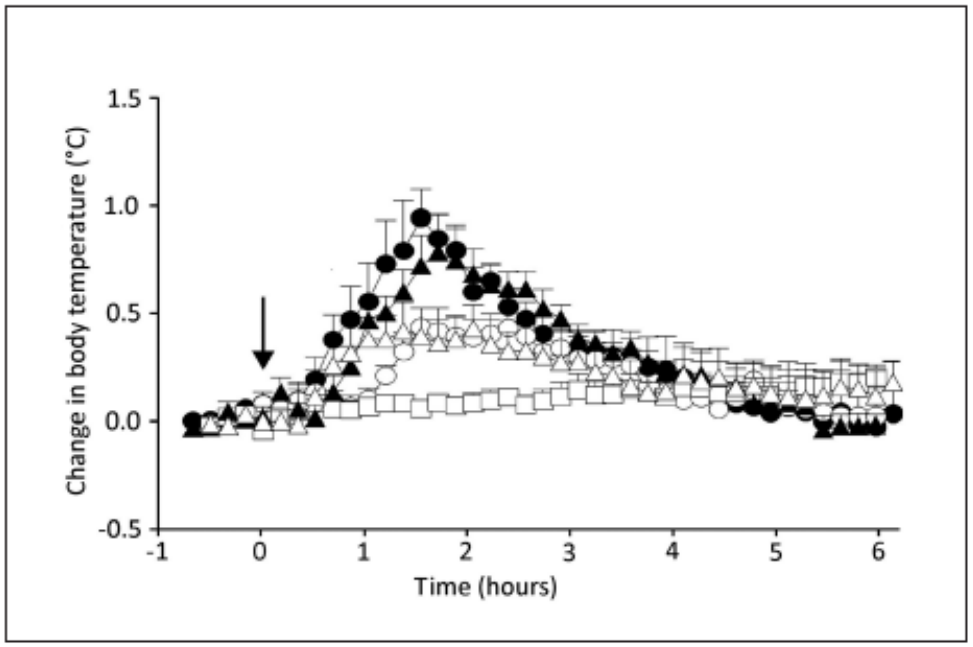


Fig. 1: Temperature change in rabbits following administration of saline or endotoxin

□ saline, ○ 20 IU/kg, △ 30 IU/kg, ▲ 40 IU/kg and ● 80 IU/kg of endotoxin. The temperatures were taken rectally according to EP and USP pyrogen test methods.

ALTEX
. 2023;40(1):117-124. doi: 10.14573/altex.2202021. Epub 2022 Jun 24.
Comparison of pyrogen assays by testing products exhibiting low endotoxin recovery
Tammy L Thurman 1, Carol J Lahti 2, Jeanne M Mateffy 3, Ren-Yo Forng 4, Friedrich von Wintzingerode 5, Lindsey M Silva 5, Sven M Deutschmann 6, Ned Mozier 1

the same time. Results show high sensitivity to endotoxin of both the BET and MAT and much lower sensitivity in the RPT, indicating that much higher levels of reference standard endotoxin are required to induce pyrogenicity in the RPT than the 5 endotoxin units (EU) per kg common threshold. The results of the BET and MAT correlated well for

Interestingly, 5 of 8 rabbits receiving the LRW T0 sample and 3 of the rabbits receiving the LRW T3 sample did not respond with a notable temperature increase. These findings suggest that 35 EU/kg is inadequate to reliably produce fever in these rabbits, even in the water control where no sample effects are present. Another observation is that none of the samples

The estimation that 50% of the rabbits will react pyrogenic to 5EU/kg might depend on the rabbit species, animal staple and chosen Endotoxin. RPT-Users typically don´t know the sensitivity of their own assay. BET and MAT are much more sensitive and sensitivity is known and confirmed

Do I know of issues in testing blood products in MAT?

- Sanquin, the Dutch national blood service (and MAT-supplier) releases its blood products via MAT, Differences on use of either FCS or HSA as supplement have been published; 2 Kit versions available

ALTEX
.2021;38(2):307-315. doi: 10.14573/altex.2008261. Epub 2020 Oct 28.
Performance of monocyte activation test supplemented with human serum compared to fetal bovine serum
Marijke W A Molenaar-de Backer et al.1, https://www.altex.org/index.php/altex/article/view/2025/version/2085

- Some users report MAT-issues swith spike-recovery (mostly below 50%, but > 200% reported too)

- In one case these issues arose after successful validation of Method 1: Problem of drug batch consistency or MAT-cell batch consistency?

How to overcome issues in testing blood products in MAT?

- If during product-specific validation spike-recovery issues (spiking only in Method 1) occur you have those choices:

  - optimize your setup for Method 1 **or**
  - try to validate for **Method 2** (no spiking but batch-to batch comparison)

recovery below 50%:

- use a spike a little above the middle of the standard curve
- use a less sensitive MAT-setup (MVD changes!): less sample = less interference, resulting shift of the dose-response to the right allows higher spike value; **even with a sensitivity of 50 pg LPS/ml you outperform any RPT**
- sample pretreatment

recovery above 200%:

- use a spike a little below the middle of the standard curve

How to overcome issues in testing blood products in MAT?

Spike recovery above 200%:

- If this happens during product-specific validation you have revealed an unknown characteristic of your drug: enhancing LPS-induced pro-inflammatory response.
  Whatever MAT you choose you should rethink the LPS-Limits for your product

- If this happens with a single batch it is suspicious

In general PPC-issues in the MAT are not solved by sticking with the RPT (no spiking)

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

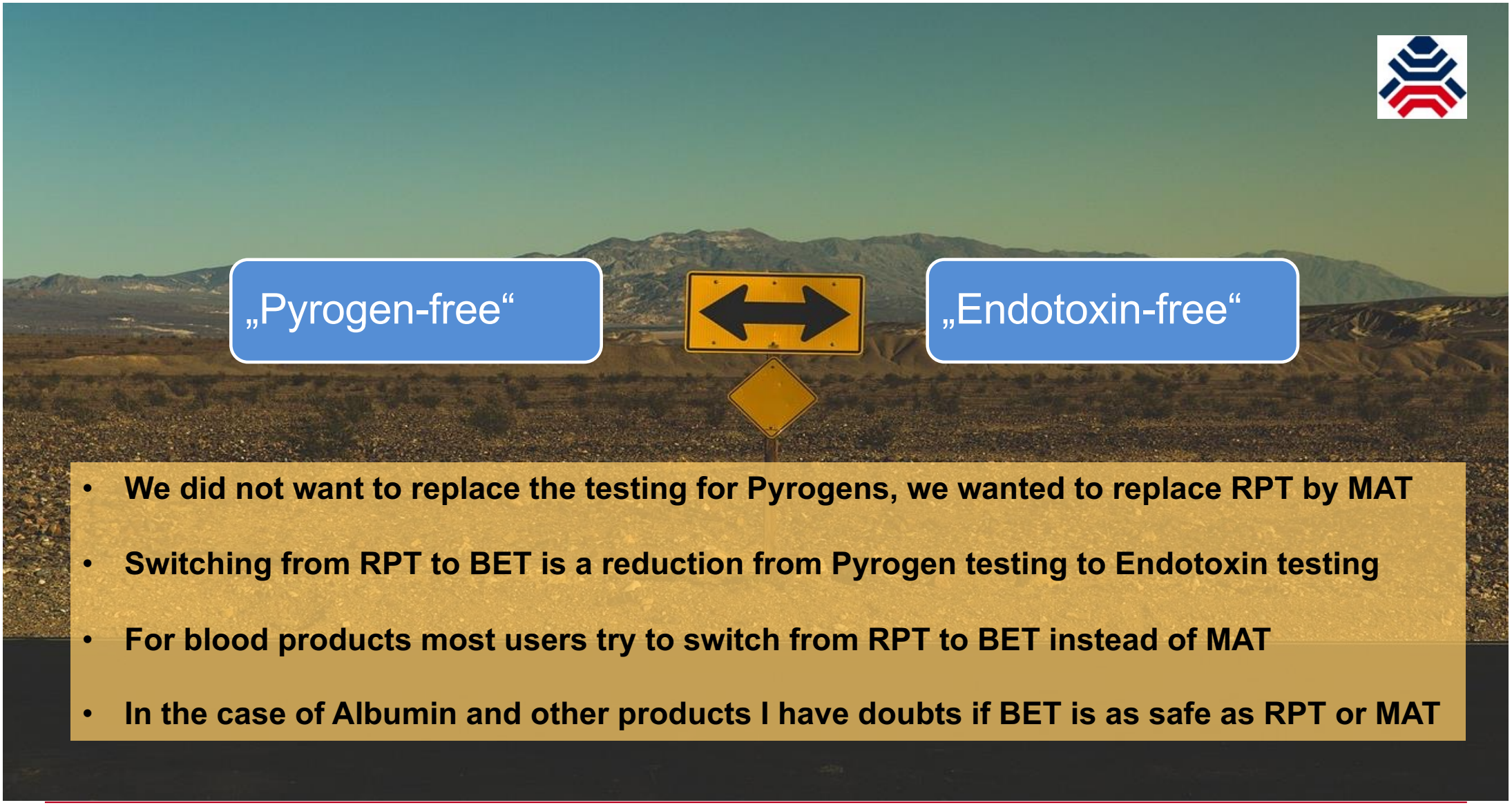
MAT and rFC now are OFFICIAL AND COMPENDIAL in Europe

Monocyte-Activation Test as an Alternative Method for Pyrogen Test ⟨*G4-13-190*⟩
(単球活性化試験による発熱性物質試験法の代替法⟨*G4-13-190*⟩)

September 2024 Japan: https://www.pmda.go.jp/english/rs-sb-std/standards-development/jp/pub-comments/jp/0044.html

Drug produced and tested in EU	Import to EU = reduced retest in EU; Export from EU	Drug produced and tested in Non-EU
BET official and compendial*		BET official and compendial*
rFC official and compendial*	Full method validation	rFC alternative
rCR alternative	Full method validation / Full method validation	rCR alternative
RPT ends 2026; replaced mainly by MAT	No RPT-data from EU	RPT official and compendial*
MAT official and compendial*	Full method validation	MAT alternative

*** if referenced**



- **We did not want to replace the testing for Pyrogens, we wanted to replace RPT by MAT**
- **Switching from RPT to BET is a reduction from Pyrogen testing to Endotoxin testing**
- **For blood products most users try to switch from RPT to BET instead of MAT**
- **In the case of Albumin and other products I have doubts if BET is as safe as RPT or MAT**

Albumin …

Original Article

Innate Immunity

Innate Immunity
0(0) 1–8
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sagepub.co.uk/journalsPermissions.nav
DOI: 10.1177/1753425915583365
ini.sagepub.com
SAGE

An improved monocyte activation test using cryopreserved pooled human mononuclear cells

Shabnam Solati[1], Lucien Aarden[1], Sacha Zeerleder[1,2] and Diana Wouters[1]

Table 4. Testing the pyrogenicity of different batches of human albumin.

	BET	RPT	MAT
Batch 1	–	–	–
Batch 2	–	+	+
Batch 3	–	+	+
Batch 4	–	+	+

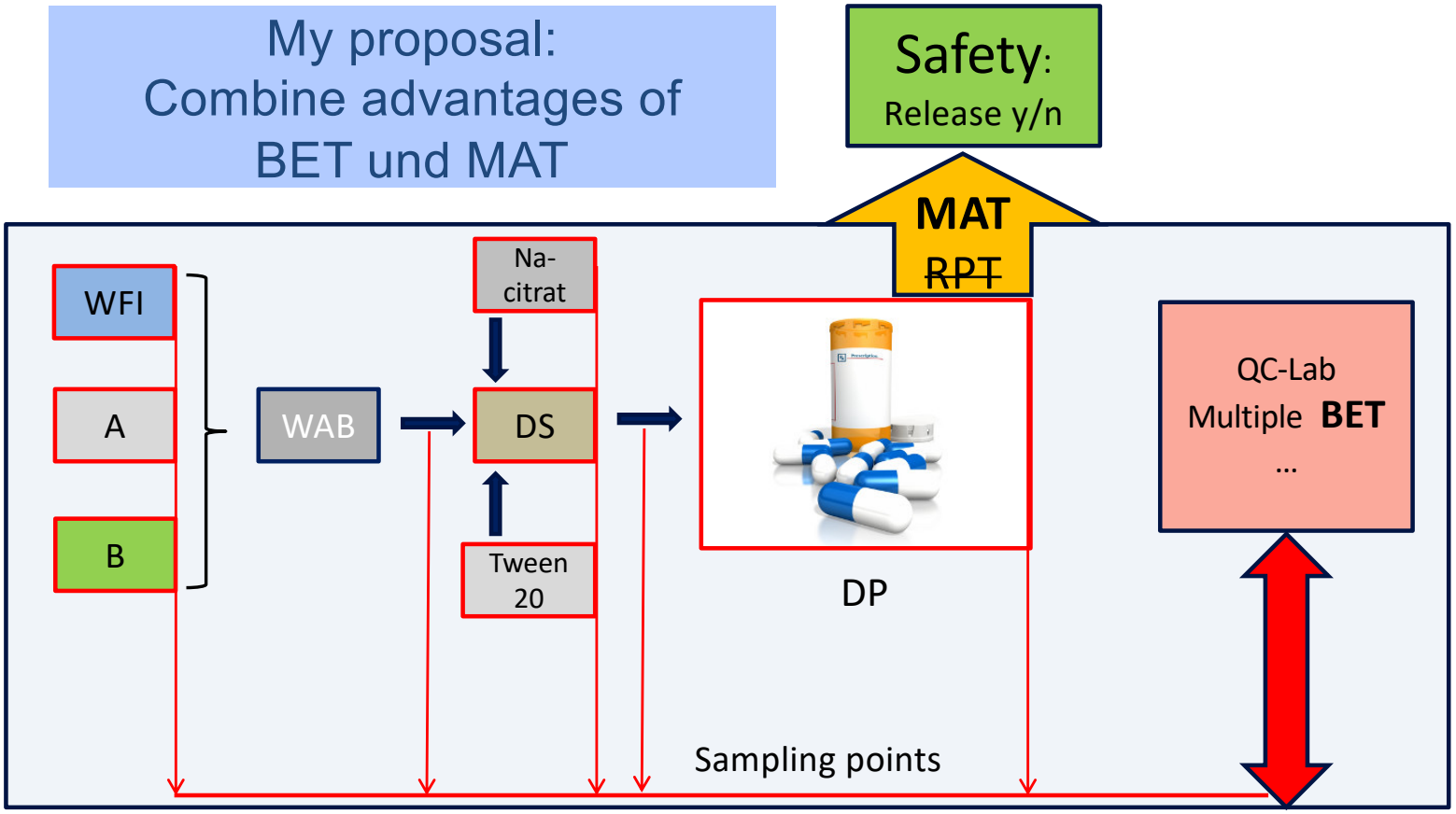
NEP?

Four albumin batches were tested, of which some showed different outcomes in the RPT and the BET. As seen in this table, the MAT and the RPT gave identical results. However, batches #2, #3 and #4, which were pyrogenic and induced IL-6 in the MAT, passed the BET.

From our production plant we selected four batches of albumin that behaved anomalously in the RPT and the BET. It is clear that the RPT and the MAT correlate well, whereas the BET deviates from the two, which is in line with the fact that the BET does not detect non-endotoxin pyrogens.

Data from the Dutch national blood service (Sanquin)

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

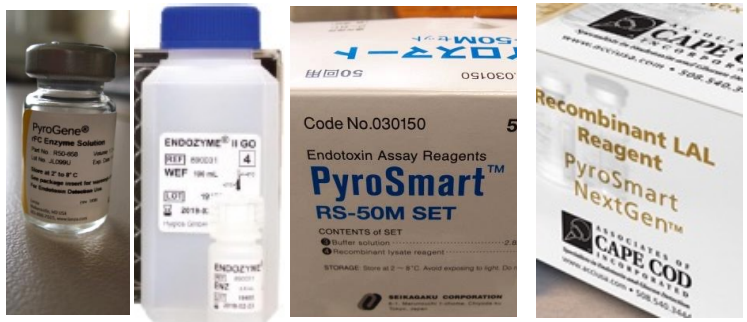
BET

1980

New BET-setups like PTS or Eclipse

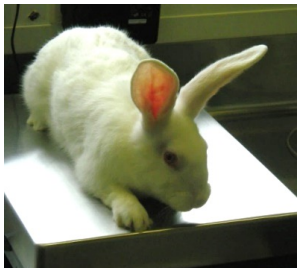
rFC, (rCR)

2021



Sensor on a chip?; direct analytical detection techniques?

Time

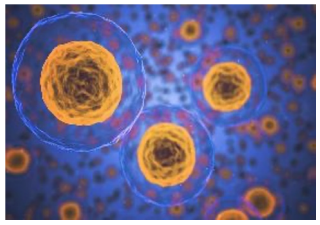


RPT;
Prediction model for humans

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

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