Monocyte-Activation-Test

as a Pyrogen Test for

Medical Devices

Zwisler Lab – USER-Experience

Dr. Walter Zwisler, Zwisler Laboratorium GmbH, Konstanz

September 2018

Monocyte-Activation-Test

with human whole blood (cryoblood) and IL-1ß detection as a Pyrogen Test for Medical Devices

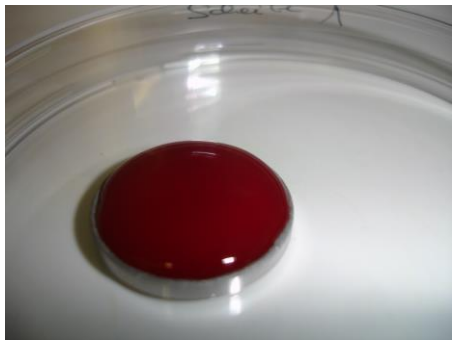
Zwisler Lab - USER-Experiences with the MAT 2003 – 2018

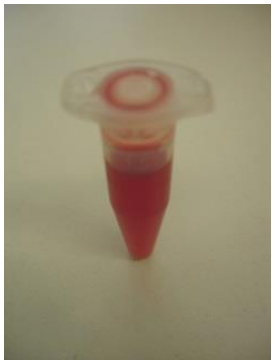
Dr. Walter Zwisler, Zwisler Laboratorium GmbH, Konstanz

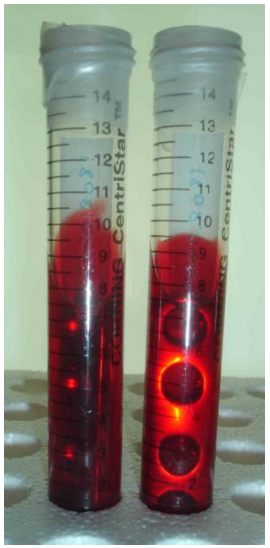
September 2018

Overview

- Introduction
- Pyrogens (Endotoxin an d Non-Endotoxin-Pyrogens)
- MAT for Medical Devices ⟷ Pharmaceutical Products
- MAT Application for Medical Devices
- Problems
- Questions / Future (?)







Introduction

Why am I standing here ?!
The Lab (Zwisler Laboratorium GmbH)

- Accredited Service Laboratory (ISO 17025)
- GLP, GMP compliance (EP 2.6.30)
- Medical Device-, Packaging- and Pharmaceutical Industry
- MAT-Customers:
  ⇨ **Med. Dev**: USA, Switzerland, Germany, Belgium, Austria

  ⇨ **Pharma:** Canada, France, Germany, Switzerland

Baden-Württemberg
REGIERUNGSPRÄSIDIUM TÜBINGEN
LEITSTELLE ARZNEIMITTELÜBERWACHUNG

Zertifikat-Nr./Certificate no.: Zwisler/Z001/2015
Aktenzeichen/Reference no.: 25-11/5483.0-1/Zwisler

BESTÄTIGUNG DER ÜBEREINSTIMMUNG MIT GMP

Teil 1

Ausgestellt nach einer Inspektion gemäß

- Art. 111 (5) der Richtlinie 2001/83/EG
- Art. 80 (5) der Richtlinie 2001/82/EG
- Art. 15 der Richtlinie 2001/20/EG

Die zuständige deutsche Überwachungsbehörde bestätigt:

Der Firma

Zwisler Laboratorium GmbH

Anschrift der Betriebsstätte

Blarerstr. 56

78462 Konstanz

Deutschland

• wurde im Rahmen der nationalen Arzneimittelüberwachung inspiziert in Verbindung mit der Tätigkeit gemäß § 14 Abs. 4 Nr. 3 Arzneimittelgesetz.

CERTIFICATE OF GMP COMPLIANCE

Part 1

Issued following an inspection in accordance with

- Art. 111 (5) of Directive 2001/83/EC
- Art. 80 (5) of Directive 2001/82/EC
- Art. 15 of Directive 2001/20/EC

The competent authority of GERMANY confirms the following:

The company

(see left)

Site address

(see left)

• has been inspected under the national inspection programme in connection with its activity according to Sect 14 para 4 no. 3 Arzneimittelgesetz (German Drug Law).

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Buslinie 2 · Haltestelle „Regierungspräsidium" oder „Landespolizeidirektion"

REGIERUNGSPRÄSIDIUM TÜBINGEN

Introduction

Why am I standing here ?!
The Lab and it´s IPT / MAT – know-how

We are involved into the MAT since 2003

The Lab was part of the international Validation
(our former Lab name: Qualis Lab, Konstanz)

ELSEVIER

Journal of Immunological Methods 316 (2006) 42 – 51

JIM Journal of Immunological Methods

www.elsevier.com/locate/jim

Research paper

International validation of pyrogen tests based on cryopreserved human primary blood cells

Stefanie Schindler [a], Ingo Spreitzer [b], Bettina Löschner [b], Sebastian Hoffmann [c], Hennes [d], Marlies Halder [c], Peter Brügger [e], Esther Frey [e], Thomas Hartung [c,*], Thomas Montag [b]

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[d] *Qualis Laboratories, Blarerstr. 56, D-78462 Konstanz, Germany*
[e] *Biological Analytics, Novartis Pharma AG, CH-4002 Basel, Switzerland*

Received 21 November 2005; received in revised form 22 June 2006; accepted 13 July 2006
Available online 7 September 2006

Introduction

Why am I standing here ?!
The Lab and it´s IPT / MAT – know-how

Over the years we used

- **Several liters of human fresh blood**
- **~ 40 different Lots of Human Cryoblood**
- 2004 – 2018: **~ 970 MAT – Kits**

Introduction

The test we use:

Monocyte **A**ctivation **T**est with **human whole blood (cryoblood)** – Interleukin-1beta (**IL1-ß**) as indicator

PyroDetect System – patented Procedure

IL-1β Detection

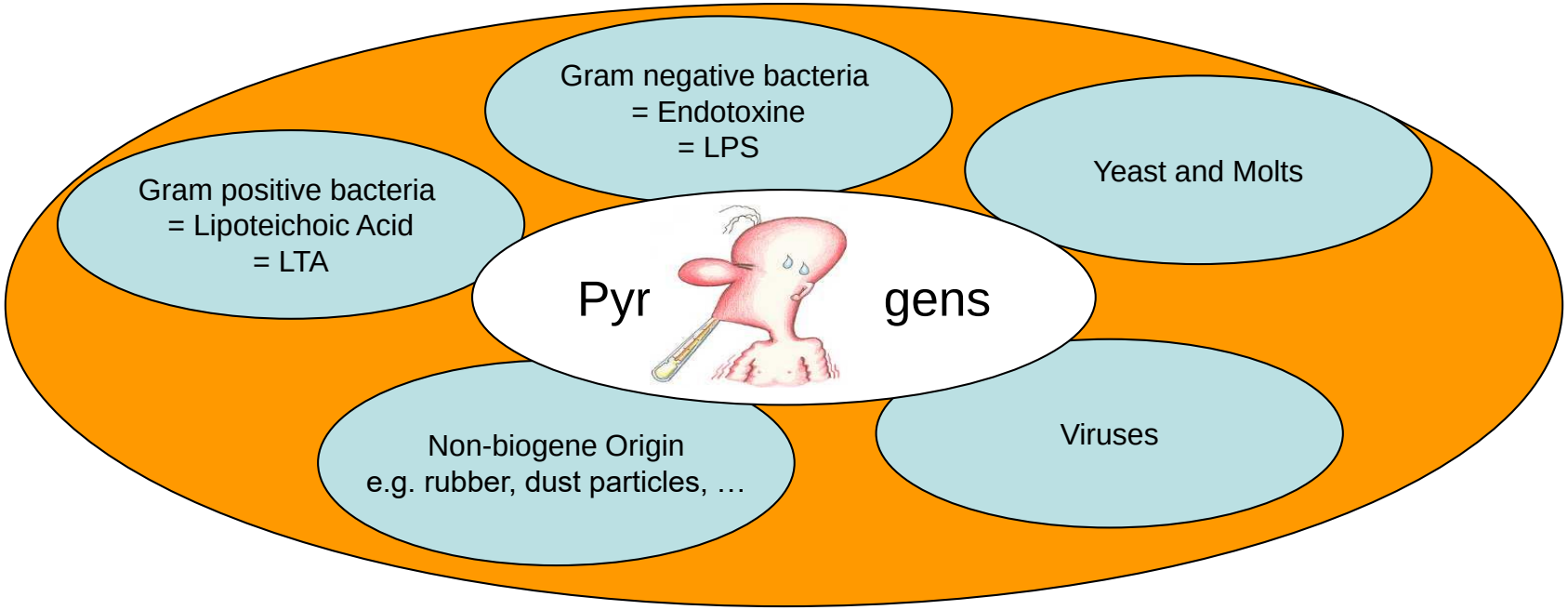
We also tried

IL-6
TNFa

With corresponding results

Pyrogens

… any substance that causes fever



Pyrogens

Pyrogens can be divided into **two classes**:

- **exogenous** pyrogens, such as endotoxin from Gram-negative bacteria that induce fever when applied intravenously; But of course also Non-Endotoxin Pyrogens (NEP)

and

- **endogenous** pyrogens that are induced inside the body as a reaction to the contact with exogenous pyrogens and which cause an elevation in body temperature (endogenous pyrogens have potent pyrogenic and inflammatory activities and include interleukin 1-a (IL-1a), interleukin-1b (IL-1b), tumor necrosis factor a (TNF-a) and interleukin-6 (IL-6))

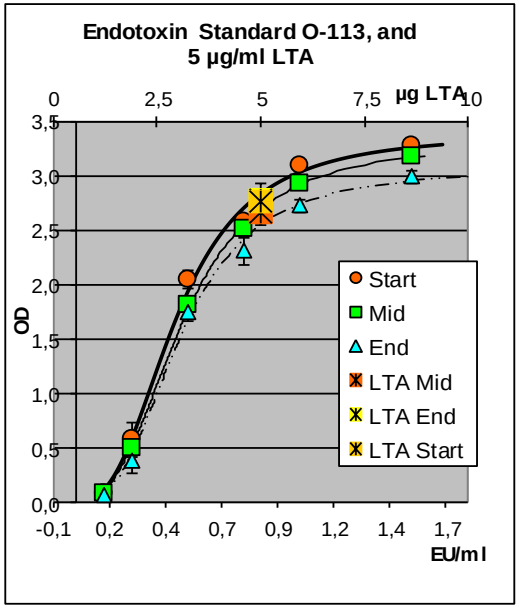
(Tim Sandle, 2015: Assessing Non-endotoxin Microbial Pyrogens in Relation in Pharmaceutical Processing)

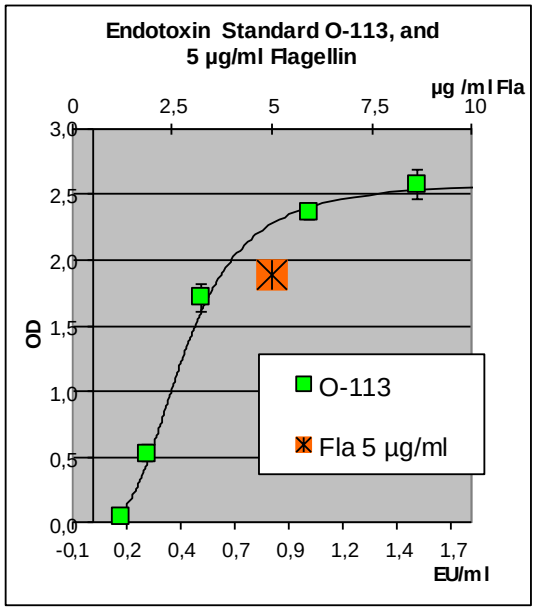
Pyrogens

Other Bacterial Pyrogens - Non-Endotoxin Pyrogens (NEP)

The actual EP 9.2, Chapter 2.6.30 wants to see Endotoxin and NEPs as pyrogenic substances, e.g. LTA and Flagellin to characterize the test system (e.g cryoblood)

Flagellin is the principal component of bacterial flagellum, and is present in large amounts on nearly all flagellated bacteria.





Pyrogens

Other Bacterial Pyrogens non-endotoxin pyrogens (NEP)

Bioburden and environmental monitoring assessments
can give information about the risk of significant levels of bacteria, fungi or other microbes, affecting products.

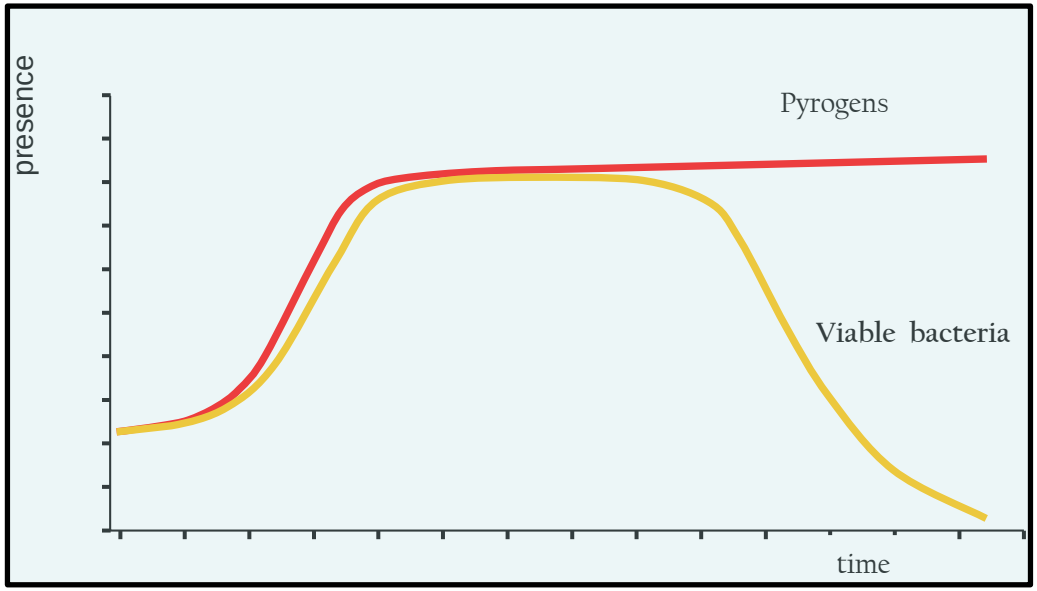
With focus on **Medical Device Productions**, the majority of detected microorganisms by an **microbiological monitoring** are **Gram Positive Bacteria** (Air, Surfaces, Humans/Skin),

… in contrast to **pharmaceutical** products, where **water** is a main source of pyrogens

Pyrogens

Other Bacterial Pyrogens non-endotoxin pyrogens (NEP)

Bioburden and environmental monitoring assessments
can give information about the risk of significant levels of bacteria, fungi or other microbes, affecting products, but:



Pyrogens

Major Pyrogens, Source, Relevance

Pyrogenic Substance	Primary Source	Relevance
Endotoxin	Water	Washing / Rinsing / WFI
Lipoteichoic acids Peptidoglycans	Skin, Air bacteria, Raw material, water	Humans / Production conditions e.g. clean room
Fungal components	Air	Production conditions
Peptidoglycans	Raw material, Skin, Air	Production conditions
Viruses	Plasma	Blood products
Material mediated pyrogens	Raw material, e.g. plastics	Material tested as `non-pyrogenic´ with LAL

Pyrogens – Medical Devices

ENDOTOXIN LIMITS For Parenteral Drug Products
By Mick Dawson , April, 2017 BET White Paper vol.1 no.2 :

Medical Devices

USP chapter <161> set generic endotoxin limits of **20 EU/device** for most devices labeled as non-pyrogenic and **2.15 EU/device** for devices that contact the cerebrospinal fluid (CSF).

For anterior segment solid devices, an endotoxin limit of **≤ 0.2 EU/device** is given.

(…why not pyrogen limits ???)

Pyrogens – Medical Devices

Material mediated Pyrogens …

exogenous pyrogens,

endogenous pyrogens

… two (– or three) classes:

Material mediated pyrogens – unknown origin, e.g particles or other molecules that can induce a fever reaction

Material mediated Pyrogens – so far tested with LAL (???) or rabbit test, but maybe not extractable ??!!

Pyrogen Tests

The Test Method and it`s application(s)

Pyrogen-Tests

- **Rabbit Test**
- **Limulus Amebocyte Lysate (LAL)**
- Monocyte-Activation Test

Rabbit Test
- Each rabbit reacts individually
- Stress can influence the result
- No positive control
- For Med Dev: indirect Test (**Elution**)
- **Animal Test** !
- Some substances can not be tested

LAL
- Only **endotoxin** detection
- For Med Dev: indirect Test (**Elution**)

- Sensitive, easy and low price
- Somehow a kind of Animal Test
- Some substances can not be tested

Pyrogens – Medical Devices

A bit provoking thesis

- Current (LAL-) tests do not adequately control pyrogen contamination of medical devices
- Testing directly on surface is a paradigm shift (no extraction)
- Human relevant testing is possible (test system is at least closer)

Pyrogen Tests

The Test Method and it`s application(s)

Pyrogen-Tests

- Rabbit Test
- Limulus Amebocyte Lysate (LAL)
- **Monocyte-Activation Test (since 2017 compendial test for pyrogens, EP)**

MAT (whole blood, IL1b)

- Detects pyrogenic and pro-inflammatory contaminants (**endotoxins and non-endotoxins**)
- Test system: Fresh or cryo-preserved human blood
- **Human in-vitro** system
- For Med. Devices: **direct Test** (!!)

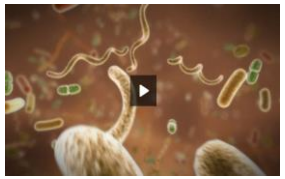
- Sensitivity (>0.125 EEU/ml)
- High price
- Some substances can not be tested (or only in high dilution)

MAT - IPT - Application for Medical Devices

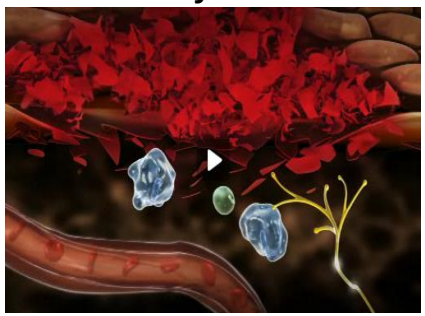
IPT (InVitro PyrogenTest, for Medical Devices, internal Lab Wording)

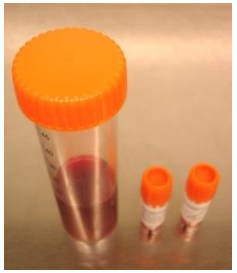
The general principle (very shortly , Dinarello et al. 1981)

Sample, Pyrogens e.g. LPS, LTA



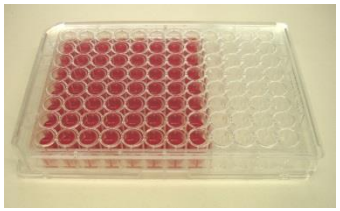
monocytes

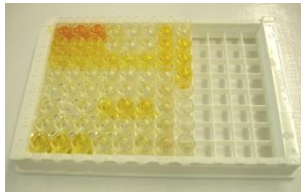




Cytokines,e.g.: IL-1β

http://www.cast-pharma.com





ELISA

OD

MAT - Application for Medical Devices vs. Pharma

IPT (InVitro PyrogenTest, for Medical Devices) **MAT (for liquid, pharmaceutical Samples)**

Main Differences – Regulatory Aspects:

Medical Devices	Pharmaceuticals
• ISO 10993-1/ -11 annex F (hint)	EP 2.6.30
• ISO/DTR 21582 (under develop. ?): Pyrogenicity … testing of medical devices	
• General: Limit of 20 EEU/device	Specified limits
2.15 EU/device (cerebrospinal fluid)	
0.2 EU/device (intraocular)	

MAT - Application for Medical Devices vs. Pharma

IPT (InVitro PyrogenTest, for Medical Devices) **MAT (for liquid, pharmaceutical Samples)**

Main Differences – Nature of pyrogens:

Medical Devices	Pharmaceuticals
Surface-attached pyrogens	Suspended pyrogens

MAT - Application for Medical Devices vs. Pharma

IPT (InVitro PyrogenTest, for Medical Devices) **MAT (for liquid, pharmaceutical Samples)**

Main Differences – Nature of sample:

Medical Devices	Pharmaceuticals
Solid sample	Solution
No dilution possible	Dilutions
No homogeneous sample-batch	Mainly homogeneous solutions
Spike on parallel sample	Spike in +/- same sample

MAT - Application for Medical Devices vs. Pharma

IPT (InVitro PyrogenTest, for Medical Devices) **MAT (for liquid, pharmaceutical Samples)**

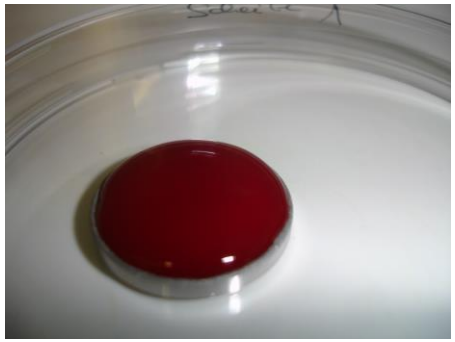
Main Differences – Test setup:

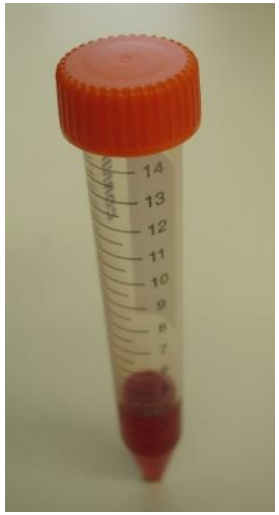
Medical Devices	Pharmaceuticals
• 1 blood-incubation – 3 (?)ELISA wells	4 blood incubations – 4 ELISA wells
• Semi-quantitative / quantitative	Quantitative Test (A); Limit Test (B) , Comparison Test (C)
At least two test items (NPC, PPC)	

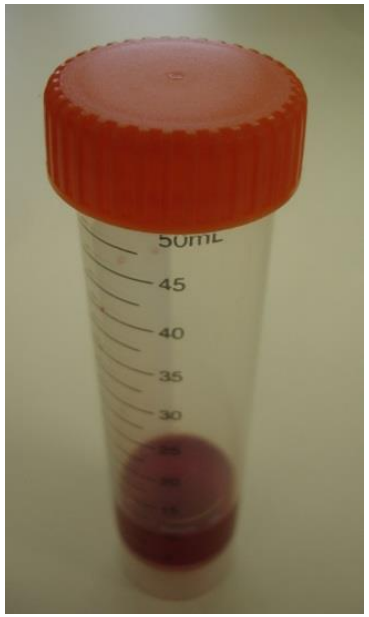
MAT - IPT - Application for Medical Devices

IPT (InVitro PyrogenTest, for Medical Devices)

1. Sample-Blood-Incubation in different Volumes / Containers
   Fresh human blood or Cryoblood







MAT - IPT - Application for Medical Devices

IPT (InVitro PyrogenTest, for Medical Devices)

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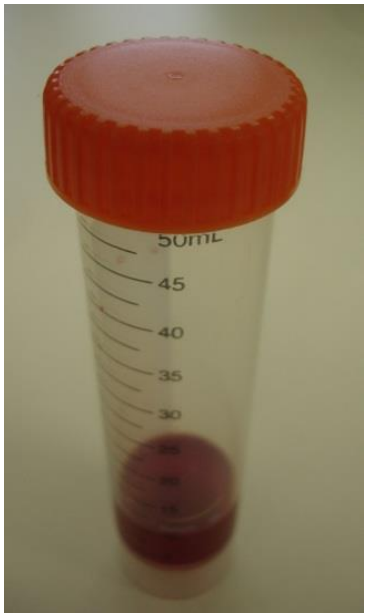
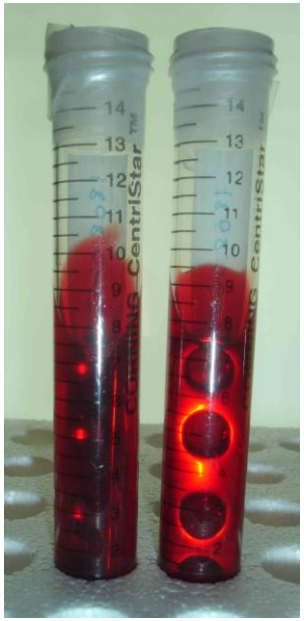


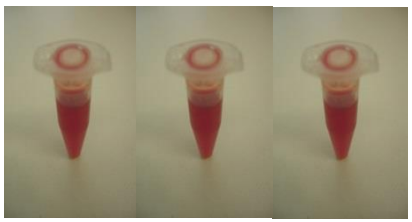


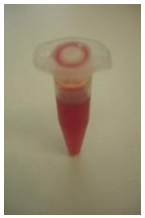
MAT - IPT - Application for Medical Devices

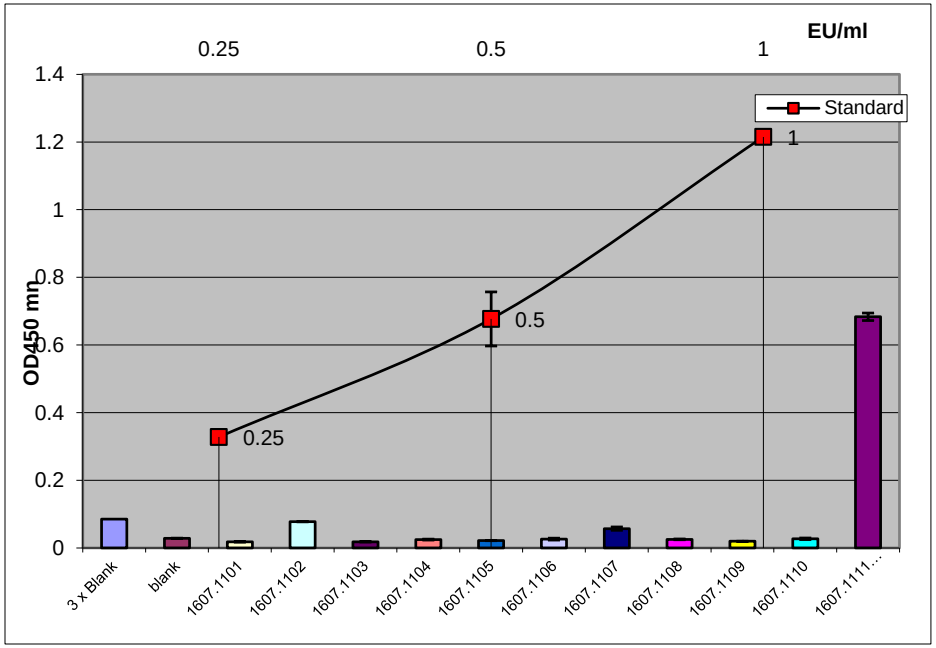
IPT (InVitro PyrogenTest, for Medical Devices)

Routine Analysis can consists of several NPC of a product and one spiked PPC (depending on batch size)

e.g. 3 + 1, 10 + 1







MAT - IPT - Application for Medical Devices

IPT (InVitro PyrogenTest, for Medical Devices)

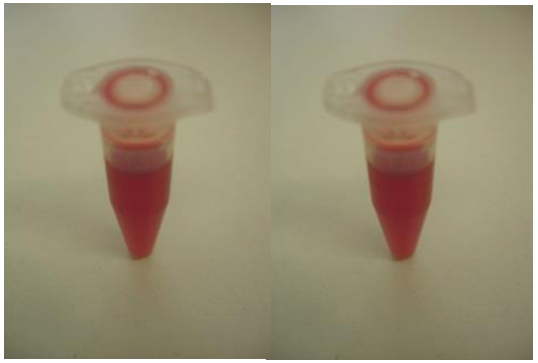
2. Normal ELISA (Triplicates)

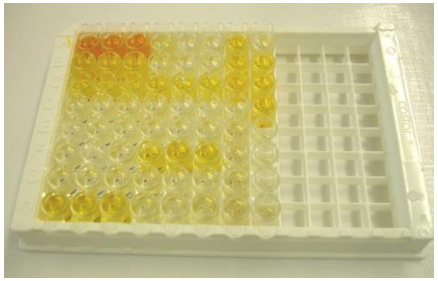
Standard, e.g.: Blank – 0.25 – 0.5 – 1.0 EU/ml

Approx. in the linear range of the sigmoid dose-response-curve

Samples: at least 2 Samples (NPC, PPC),

= test for interfering factors (50-200%)



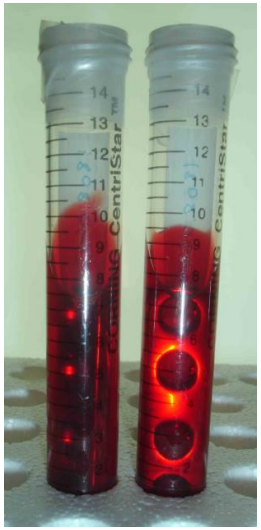


MAT - IPT - Application for Medical Devices

IPT (InVitro PyrogenTest, for Medical Devices)

3. Results:

optical densities from standard , NPC and PPC



Autobio Co.,LTD (2) 07.08.18

Messzeit: 2018-08-07 14:32 Mess.Einh.: OD Bediener: admin
Mess.Filter (nm): 450 Ref.Filter (nm): 620 Gerät: PHOMO

	1	2	3	4	5	6	7	8	9	10	11	12	
A	2,142	1,950	2,113	0,059	0,984								A
B	1,718	1,694	1,621	0,073									B
C	0,542	0,512	0,543	0,085									C
D	0,031	0,034	0,034	0,040									D
E	0,268	0,253	0,263	0,031									E
F	1,695	1,654	1,768	0,035									F
G	0,250	0,242	0,255	0,955									G
H	1,852	1,956	1,770	0,909									H
	1	2	3	4	5	6	7	8	9	10	11	12	

MAT - IPT - Application for Medical Devices

IPT (InVitro PyrogenTest, for Medical Devices)

3. Results:

Pyrogenic contaminants can be quantified with the use of a sigmoid standard curve (= **EEU/device**).

Autobio Co.,LTD ② 07.08.18

Messzeit: 2018-08-07 14:32 Mess.Einh.: OD Bediener: admin
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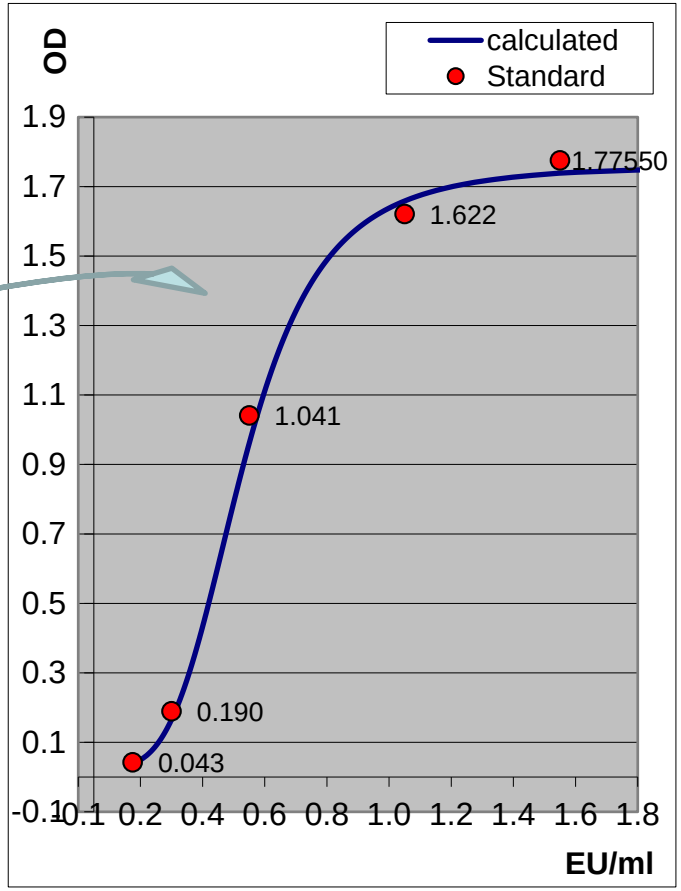
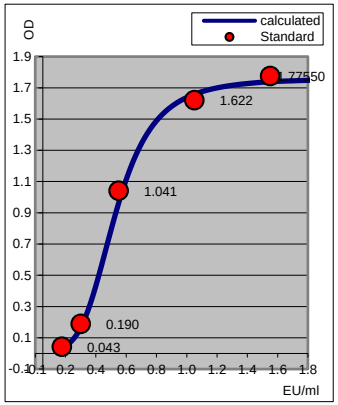


MAT - IPT - Application for Medical Devices



IPT (InVitro PyrogenTest, for Medical Devices)

3. Results:

Pyrogenic contaminants can be quantified with the use of a sigmoid standard curve (= **EEU/device**).

	Sample	Mean EEU/ml	Recovery (%)	EEU/device
A	1808.0602	0,195		0,117
B	1808.0602+	0,431	84,948	0,136
C	1808.0603	0,191		0,060
D	1808.0603+	0,484	95,232	0,153

or

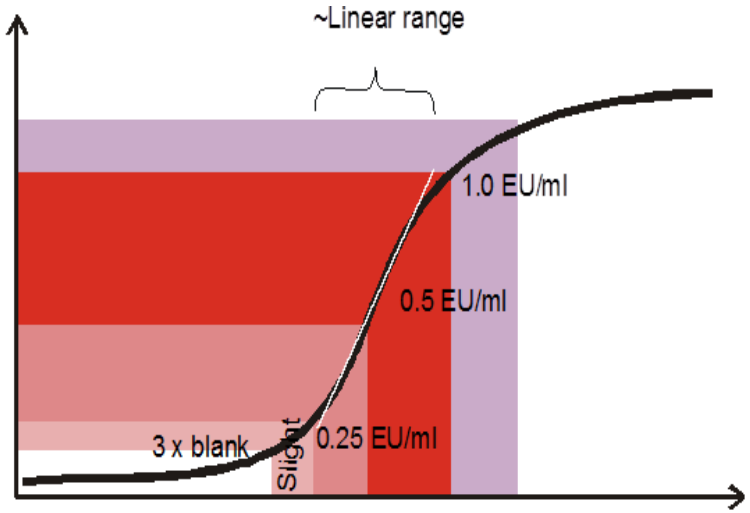
Pyrogenic contaminants can be analyzed by a semi-quantitative limit test

MAT - IPT - Application for Medical Devices

IPT (InVitro PyrogenTest, for Medical Devices)

3. Results:

Pyrogenic contaminants can be analyzed by a **semi-quantitative limit test**

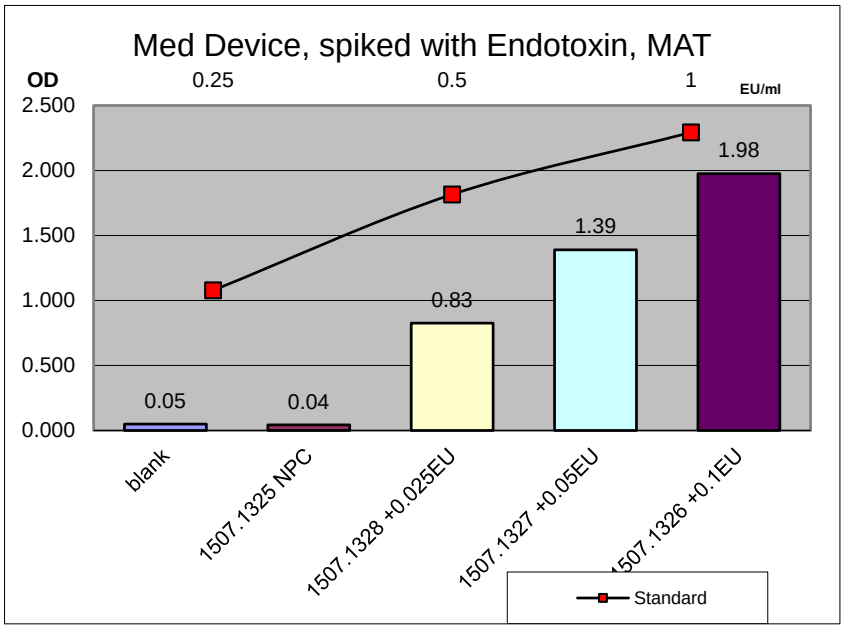


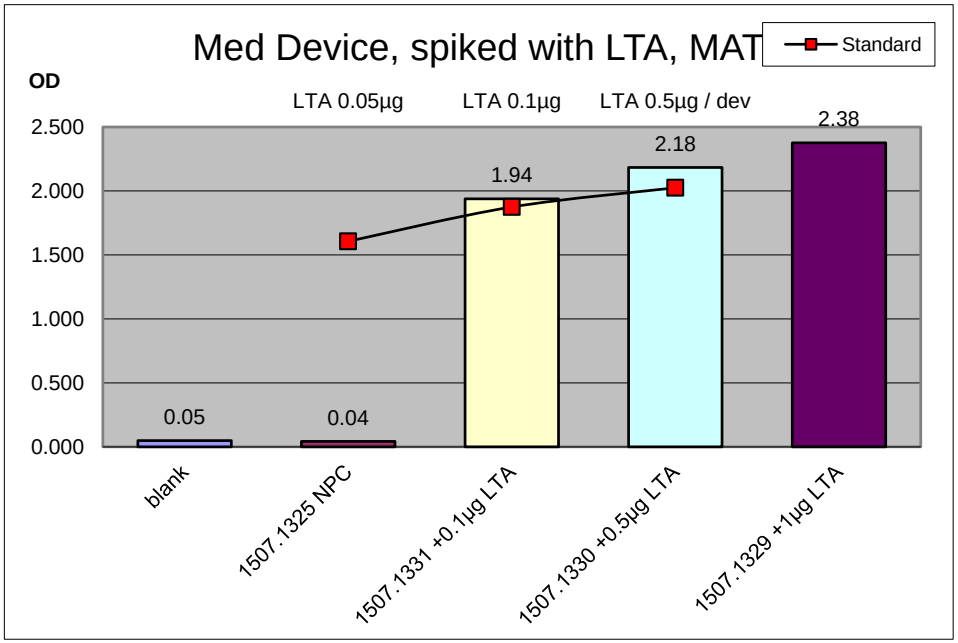
Internal LAB-comment	Internal Definition
No reactivity	<= 3 x blank signal
Slight reactivity	<= 0.25 EU/ml signal,
Mild reactivity	< 0.5 EU/ml signal
Moderate reactivity	>= 0.5 EU/ml signal, `pyrogen´
Severe reactivity	>= 1.0 EU/ml signal

MAT - IPT - Application for Medical Devices

IPT (InVitro PyrogenTest, for Medical Devices)

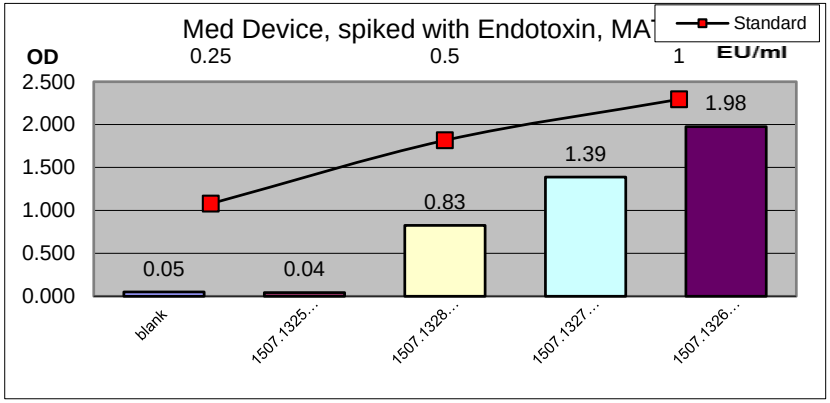
3. Results:

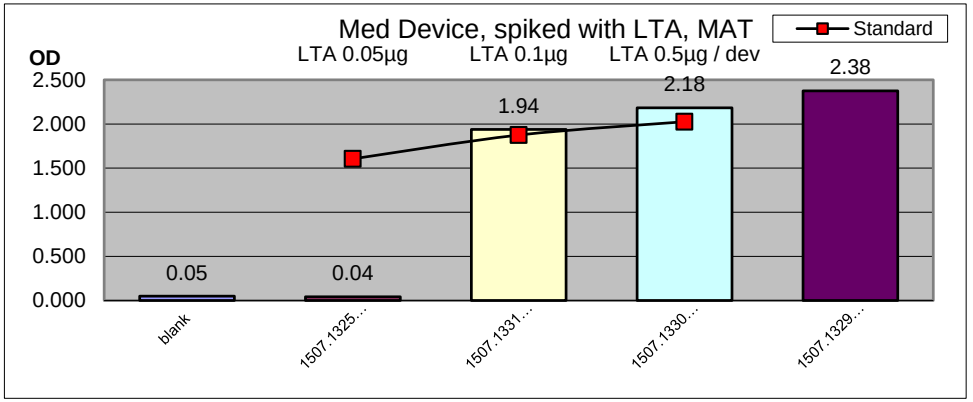


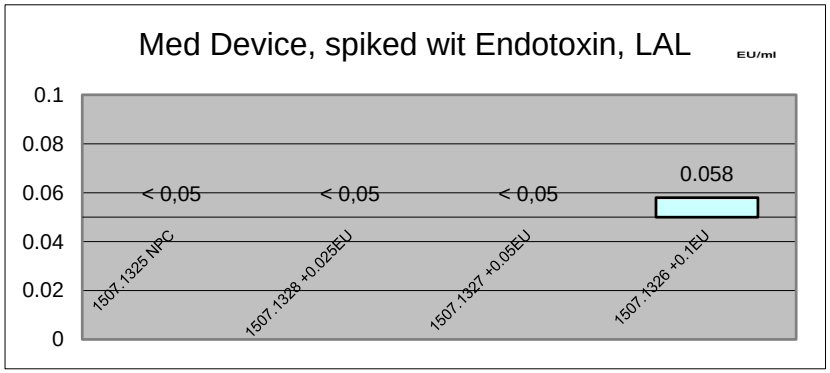


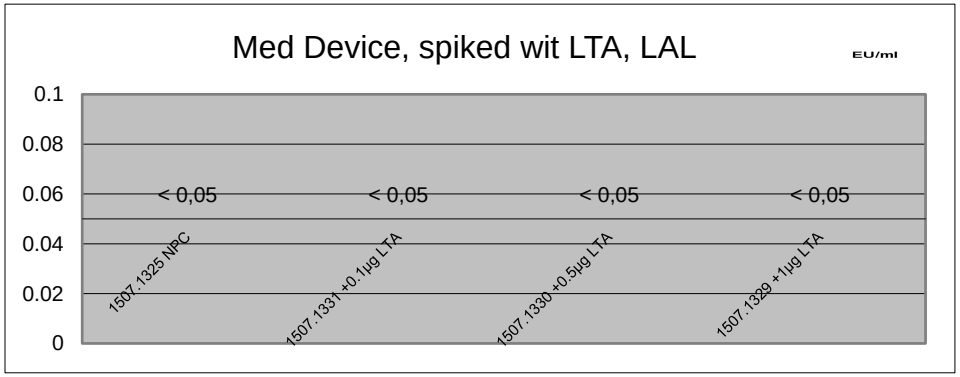
MAT - IPT - Application for Medical Devices

IPT (InVitro PyrogenTest, for Medical Devices) **MAT vs. LAL**
(with minimal extraction)







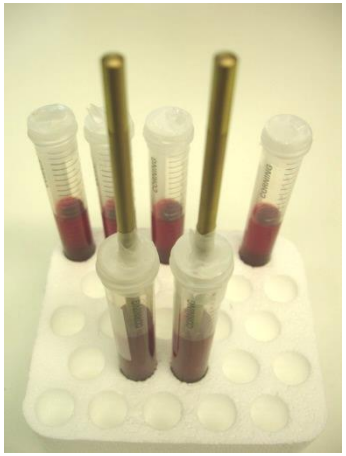


MAT - IPT - Application for Medical Devices

IPT (InVitro Pyrogen Test, for Medical Devices)

Samples:

- **Dental materials**
- **Material for Eye-surgery**
- **Implants**
- **Instruments**



MAT - IPT - Application for Medical Devices

IPT (InVitro PyrogenTest, for Medical Devices)

High Accuracy
Low Precision

Low Accuracy
High Precision
Wikipedia

ICH Q2 (R1) Validation of an Analytical Procedure

Testing for Impurities (quantitative)

•Accuracy	Spike-Recovery within **50-200%**
•Precision	Repeatability (same sample, same conditions)
	Intermediate Precision (same sample, different day, different conditions) ;**CV<20%**, but single events >40% (small values)
•Specificity	Detection of different Impurities: LPS**, LTA**, **Flagellin**, Bacteria, **Yeast** (Zymosan)
•Cut-off, IPT	Standard-curve (internal Lab Definition Limit Test: 3 * Blank)
•Cut-off, MAT	Standard-curve (Blank+ 3*sdv)
•LOD, MAT	First value above cut-off (Blank+ 3*sdv = **~ 0,1 EEU/ml**)
•LOD, IPT	First value above cut-off (= **Limit Test: 0.25 EEU/ml**)
•Linearity	Standard-curve (~ 0.5 EU/ml, p<0.01)
•Range	**Standard-curve (0,125 – 1.5 EU/ml)**

MAT - IPT - Application for Medical Devices

IPT (InVitro PyrogenTest, for Medical Devices)

Why the IPT:

- Direct Test – no Elution (Big Point!)
- Surface-attached Pyrogens detected
  = material-mediated Pyrogens?
- Detection of PYROGENS (Big Point!)
- Production-Monitoring: gram+ (Big Point!)
- Sensitivity is fine for Medical Devices
- Human Blood – close to reality (Big Point!)
- No Animal Test (Big Point!)

MAT - IPT - Application for Medical Devices - Problems

IPT (InVitro PyrogenTest, for Medical Devices)

In general the MAT is a straightforward method for Medical Devices,

with a (lower) detection limit of about 0.025 EEU / device up to 0.2 EEU/device (setups we use routinely)

depending of the blood/buffer volume of the assay – and this depends of the size of the Medical Device

MAT - IPT - Application for Medical Devices - Problems

IPT (InVitro PyrogenTest, for Medical Devices)

In general the MAT is a straightforward method for Medical Devices,

… but

MAT - IPT - Application for Medical Devices - Problems

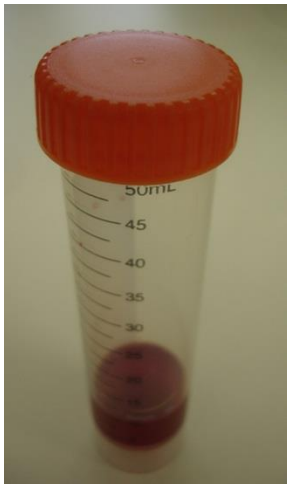
IPT (InVitro PyrogenTest, for Medical Devices)

In general the MAT is a straightforward method for Medical Devices,

… but

The size of a Med Dev. is critical

angled sample in a round hole



MAT - IPT - Application for Medical Devices - Problems

IPT (InVitro PyrogenTest, for Medical Devices)

The size of a Med Dev. is critical

Sometimes a dummy which went through the production process in parallel can be used to control the process

MAT - IPT - Application for Medical Devices - Problems

IPT (InVitro PyrogenTest, for Medical Devices)

Endotoxin Limits for Medical Devices

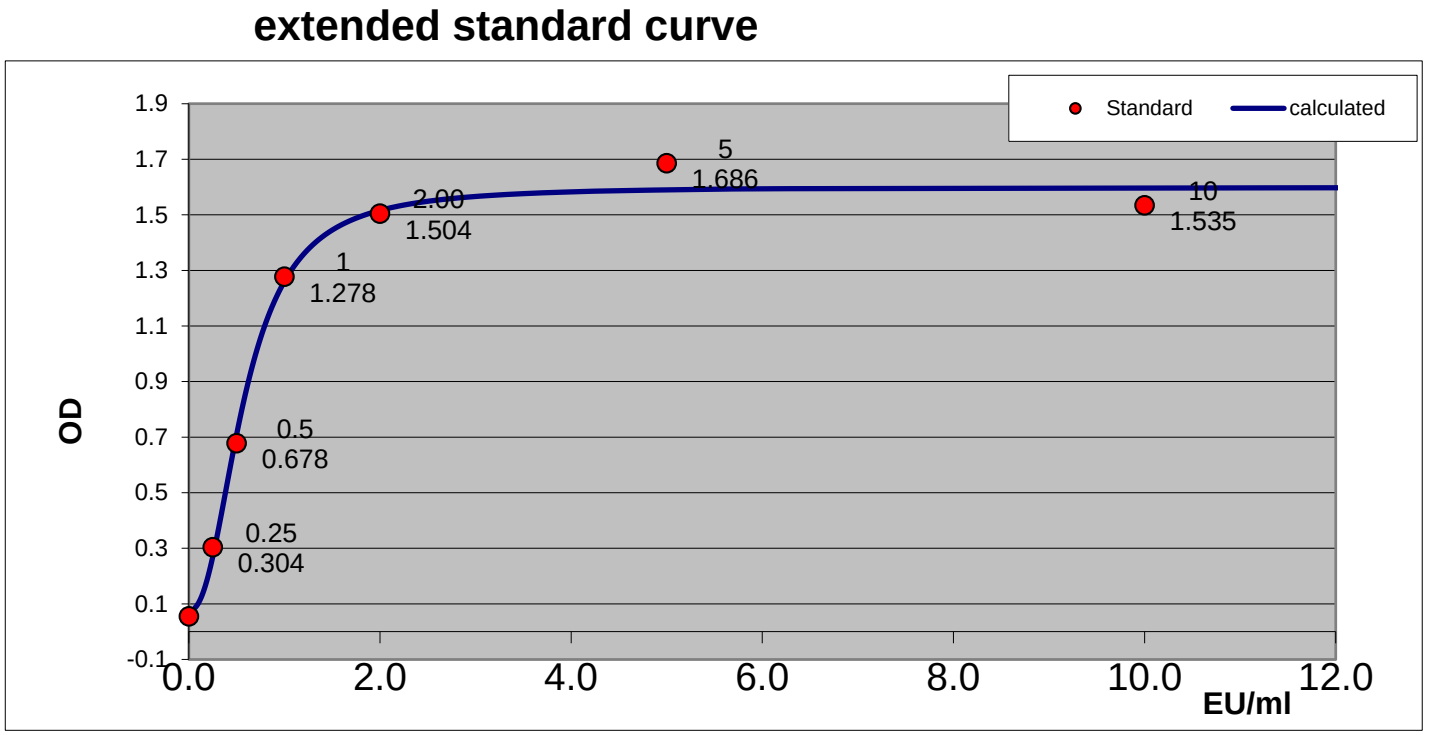
20 EU/device

2.15 EU/device

0.2 EU/device

MAT - IPT - Application for Medical Devices - Problems

IPT (InVitro PyrogenTest, for Medical Devices)



MAT - IPT - Application for Medical Devices - Problems

IPT (InVitro PyrogenTest, for Medical Devices)

Endotoxin Limits for Medical Devices

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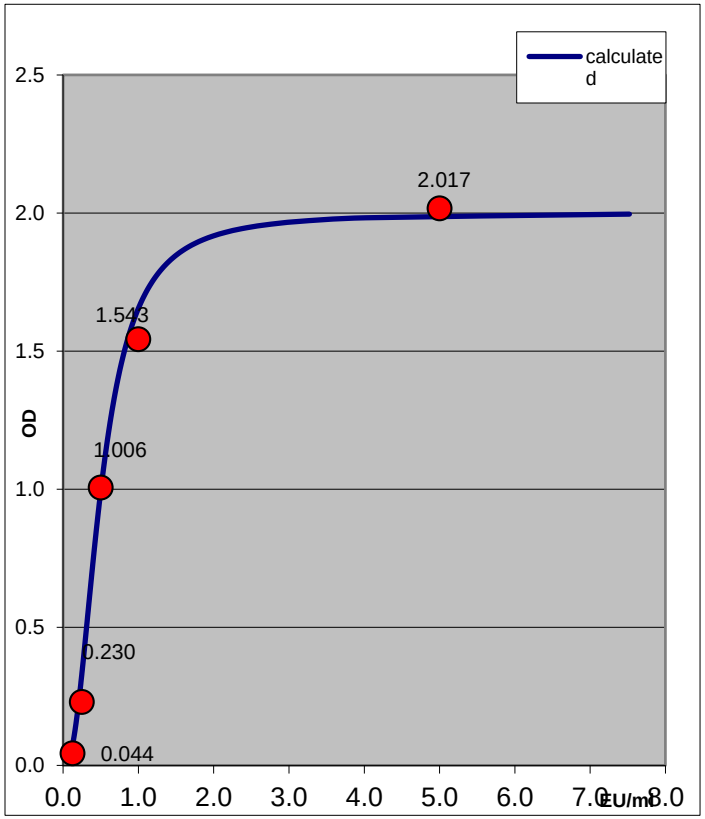

EU Standards > 2 EEU/ml

can not be calculate anymore

(sigmoid dose-response)

⟹ **Upper detection limit of 0.2 EEU/device to 1.6 EEU/device**

MAT - IPT - Application for Medical Devices - Problems

IPT (InVitro PyrogenTest, for Medical Devices)

Endotoxin Limits for Medical Devices

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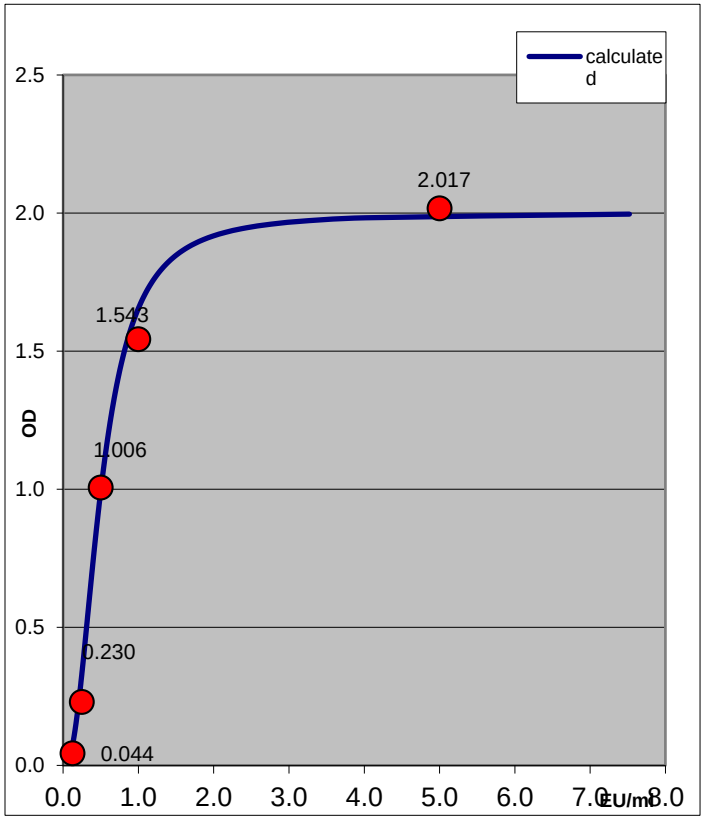
2.15 EU/device

0.2 EU/device

To verify a clean production / product the IPT is a very useful tool.

To quantify contaminated samples, the MAT / IPT is limited



MAT - IPT - Application for Medical Devices - Problems

IPT (InVitro PyrogenTest, for Medical Devices)

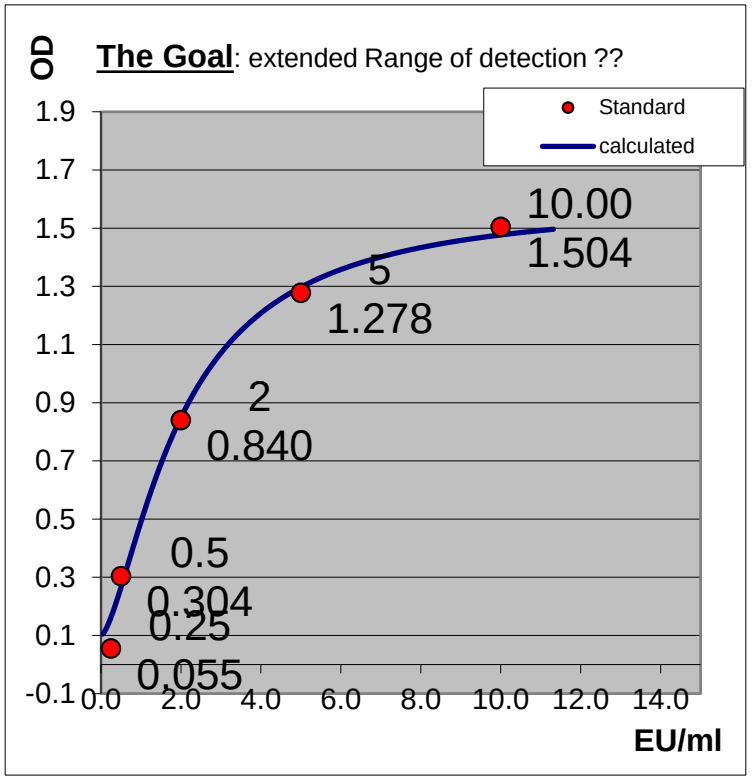
Endotoxin Limits for Medical Devices

20 EU/device

2.15 EU/device

0.2 EU/device

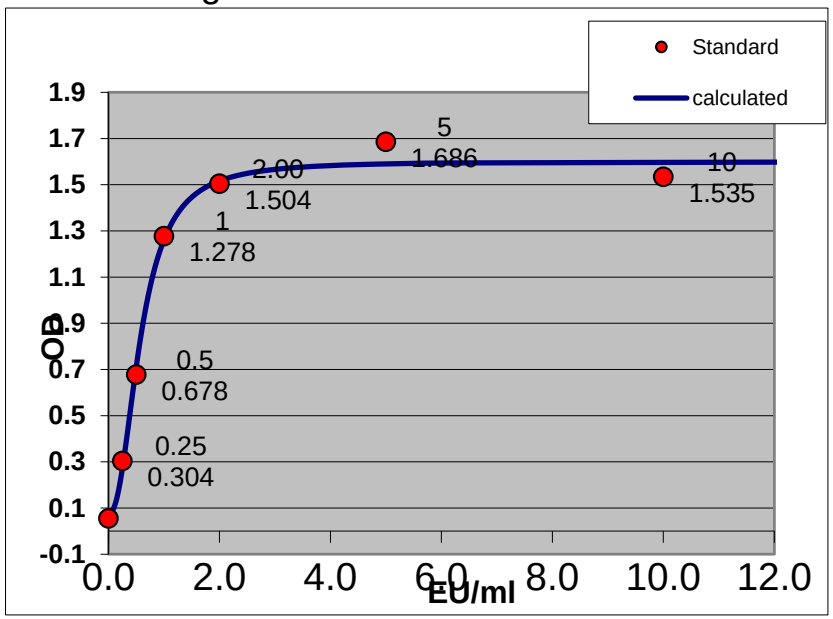
A wider range would be helpful for standard Medical Devices



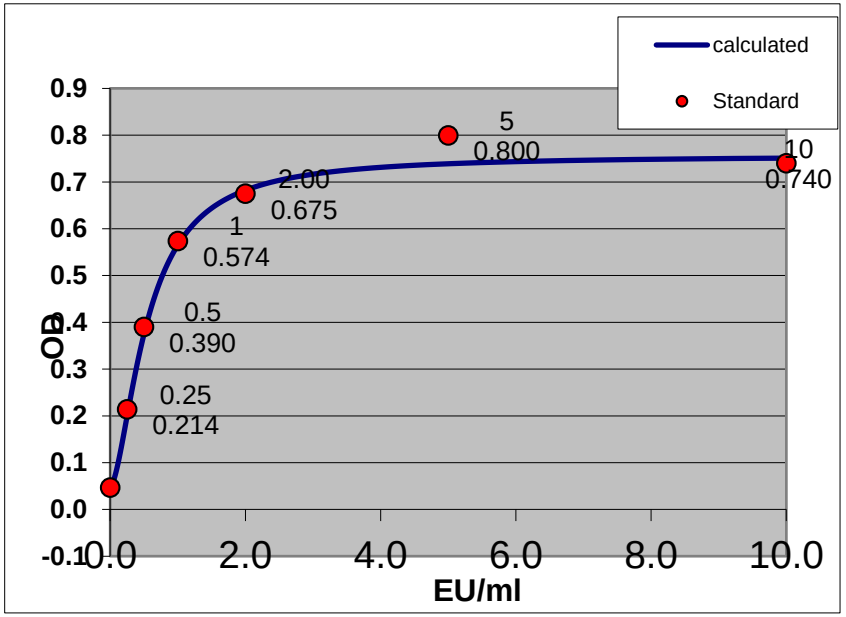
MAT - IPT - Application for Medical Devices - Problems

IPT (InVitro PyrogenTest, for Medical Devices)

regular amount of blood



half amount of blood



MAT - IPT - Application for Medical Devices - Problems

IPT (InVitro PyrogenTest, for Medical Devices)

Endotoxin Limits for Medical Devices

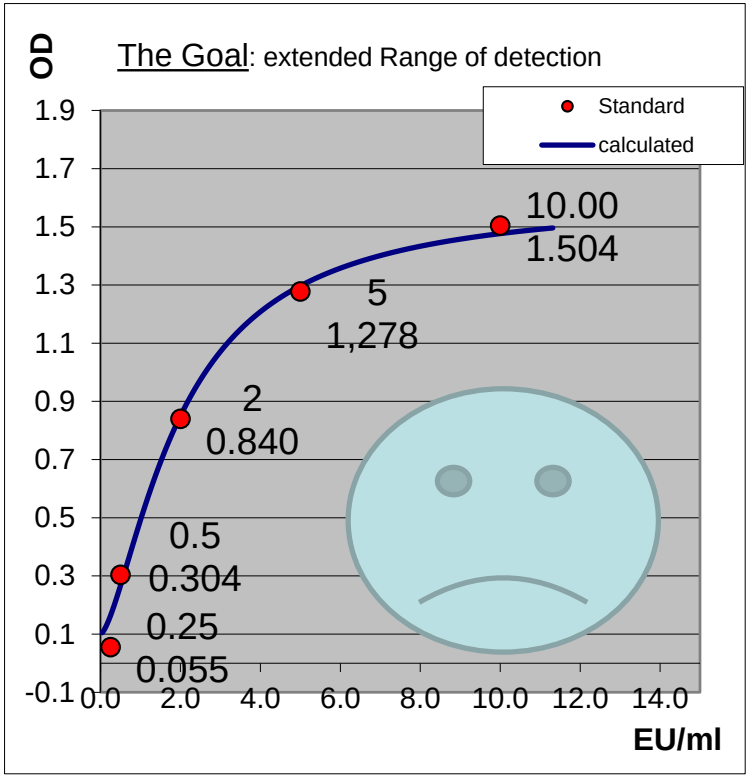
20 EU/device

2.15 EU/device

0.2 EU/device

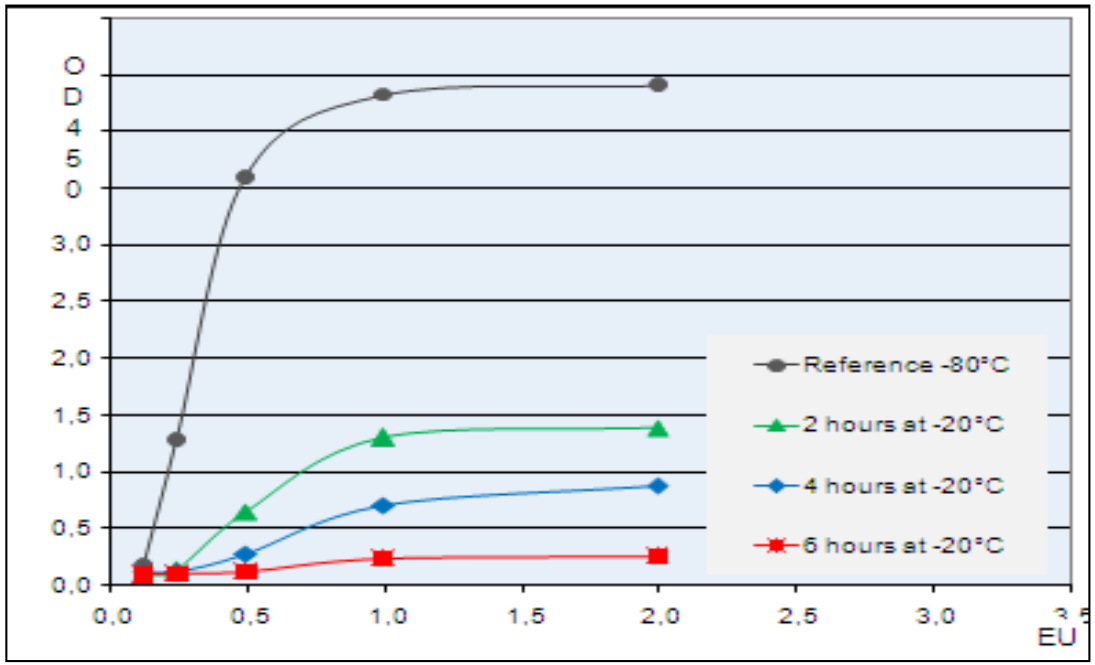
A wider range would be helpful for standard Medical Devices

… we are working on it



MAT - Cryoblood

Storage is important –80°C - and transport the challenge



Data: Biotest AG

MAT - Cryoblood

Storage is important –80°C - and transport the challenge

A new formulation of human Cryoblood seems to be more stable

Tests are in progress

MAT - IPT - Application for Medical Devices

IPT (InVitro PyrogenTest, for Medical Devices)

Why the IPT / MAT:

- Direct Test – no Elution (Big Point!)
- Surface-attached Pyrogens (Big Point!)
- Detection of PYROGENS (Big Point!)
- Production-Monitoring: gram+ (Big Point!)
- Sensitivity is fine for Medical Devices
- Even the range is ok because– we want clean MD
- Human Blood – close to reality (Big Point!)
- No Animal Test (Big Point!)
- Material mediated Pyrogens ?

MAT - IPT - Application for Medical Devices - Future ?

IPT (InVitro PyrogenTest, for Medical Devices) - Questions / Future

- Wider rage of detection ?
- LER (low endotoxin recovery) on Medical Devices ?
- Product/Material related pyrogenicity ?
- Nanomaterials ? (in progress)

Monocyte-Activation-Test used for Testing Medical Devices

Zwisler Lab – USER-Experience

Thank you

For your attention
To my Lab-Team
To Jeff Brown for the invitation

Dr. Walter Zwisler, Zwisler Laboratorium GmbH, Konstanz