Medical device testing in vitro - pyrogenicity testing and beyond -

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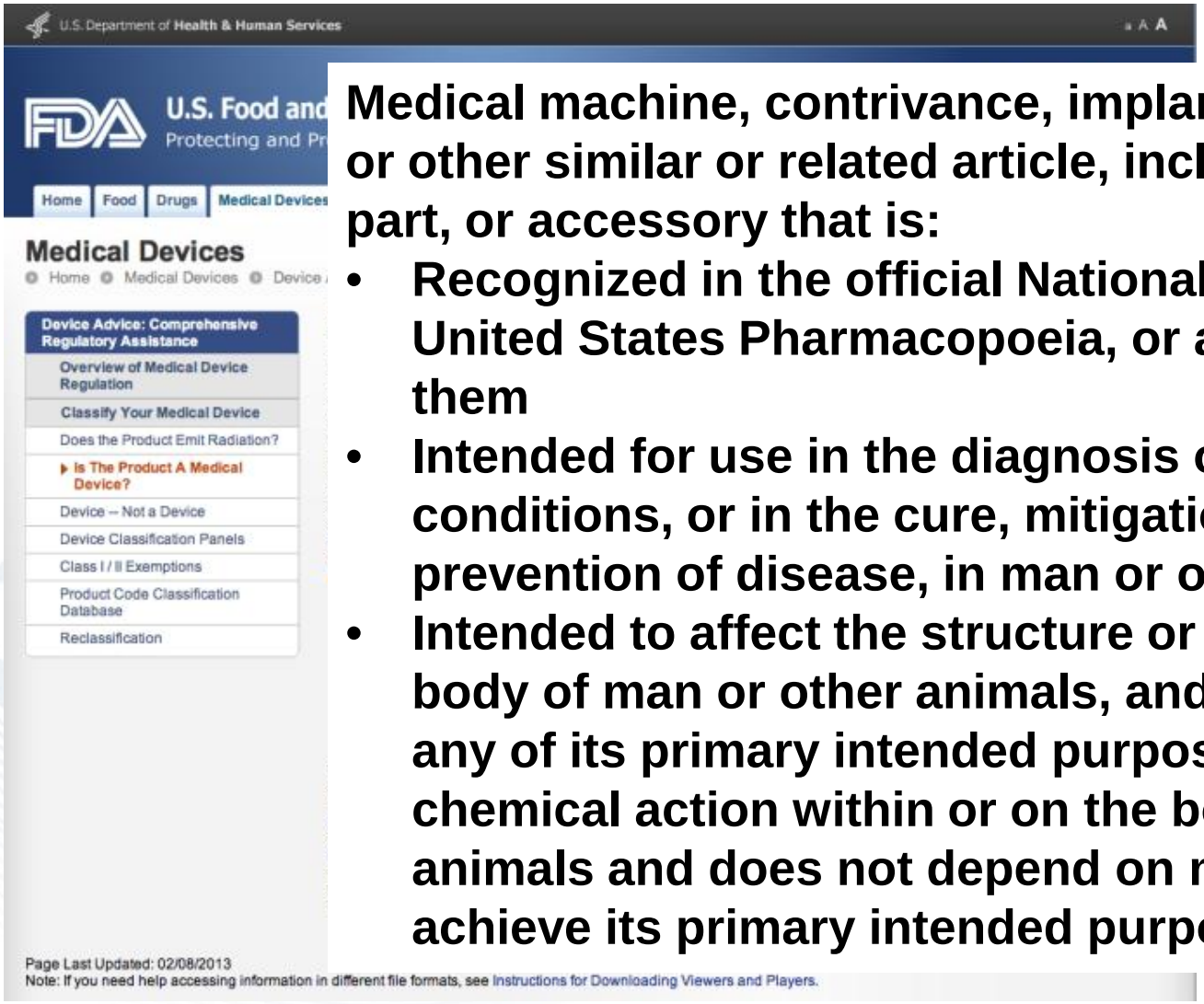
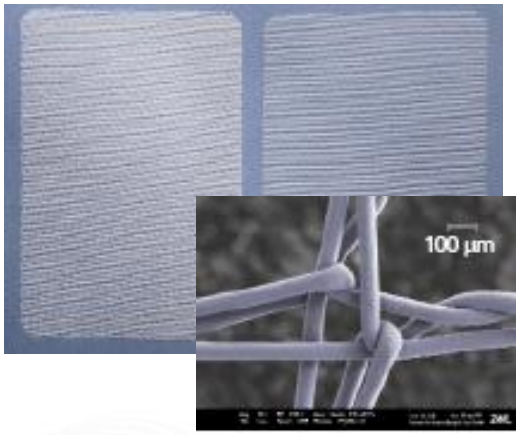
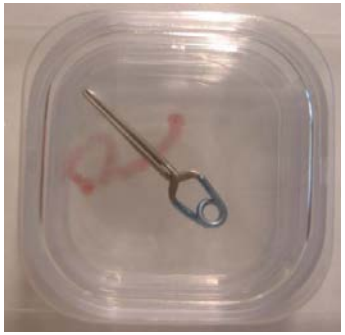


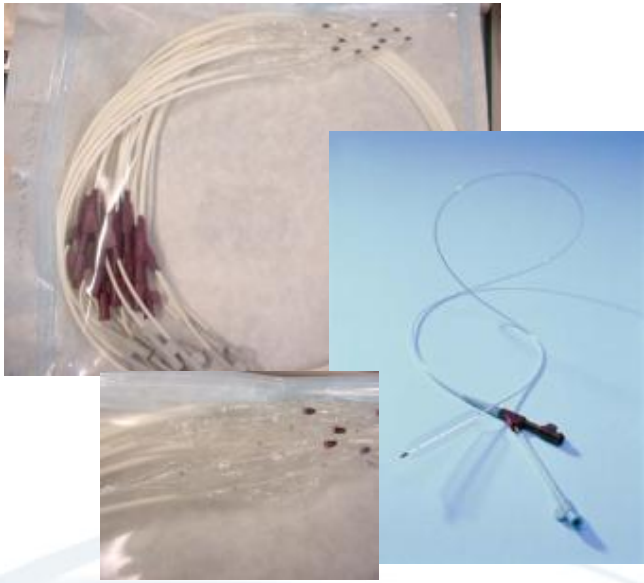
Medical machine, contrivance, implant, in vitro reagent, or other similar or related article, including a component part, or accessory that is:

- **Recognized in the official National Formulary, or the United States Pharmacopoeia, or any supplement to them**
- **Intended for use in the diagnosis of disease or other conditions, or in the cure, mitigation, treatment or prevention of disease, in man or other animals**
- **Intended to affect the structure or any function of the body of man or other animals, and does not achieve any of its primary intended purposes through chemical action within or on the body of man or other animals and does not depend on metabolic action to achieve its primary intended purposes.**

Examples for implants



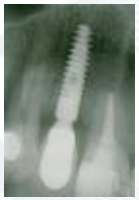




Implants

- **Classification according to duration of implantation:**

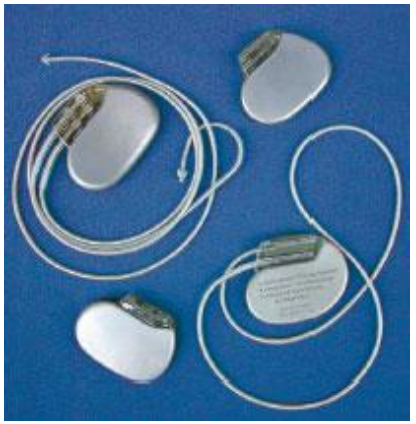
  - ***Ultra-short-time-implant:*** **minutes to hours**
    e.g. surgical instructions for operations



  - ***Short-time-implant:*** **days to weeks**
    e.g. orthopaedic nails, screws and plates

  - ***Long-time-implant:*** **years**
    e.g. hip prosthesis

Implants



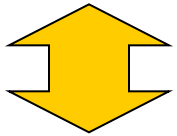
- **Classification according to function:**

  - ***active*: takes over of a function which is dysfunctional or reduced**
    e.g. cardiac pacemaker

  - ***passive*: replacement of tissue (may support/ enable maintenance of function)**
    e.g. mammary implant

Biocompatibility

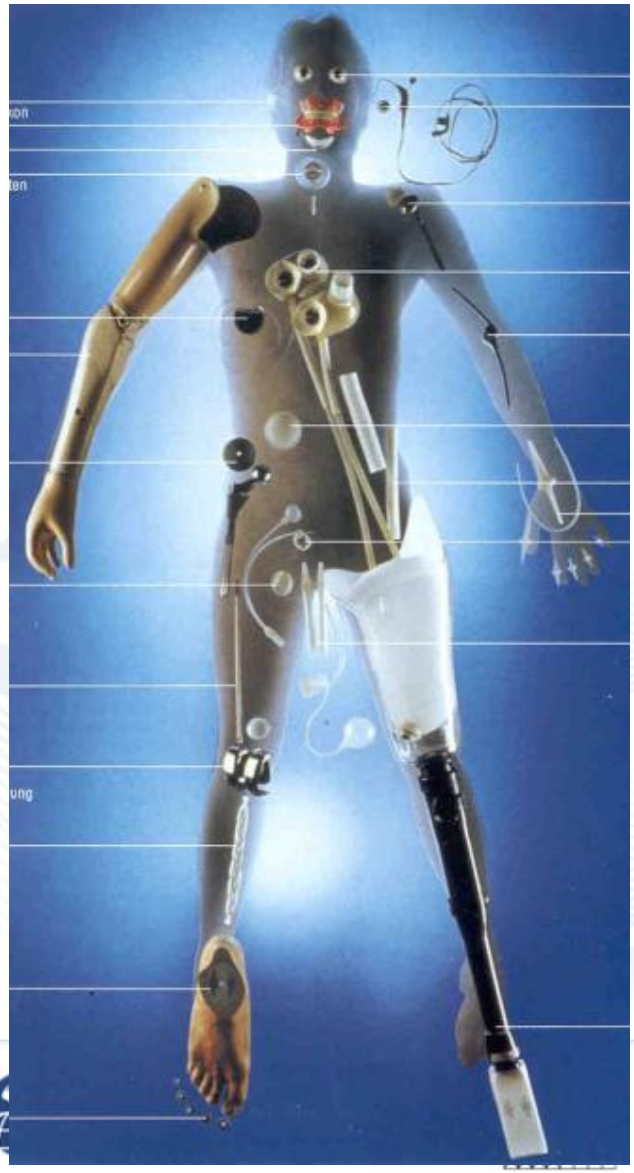
- **Incompatible: Release of substances in toxic concentrations or of antigens**



- **Biotolerant: Release of substances in non-toxic concentrations**
- **Bioinert: No release of toxic substances**
- **Bioactive: positive interaction leading to reaction of tissue**

Effect of Contaminants?

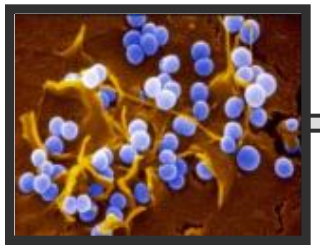
Pyrogen?



Medical device testing

- Material v contaminant
- Surgery vs. material
- Surface effects
- Material effects
- Long-term effects
- Immune reactions
- Sh nt
- Batch-dependent

Pyrogen contamination

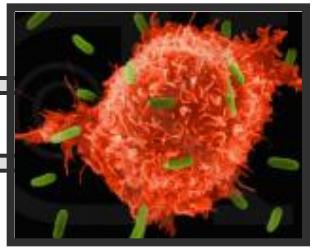


PAMPs

S.aureus

TLRs

NODs



cell associated immune receptors

killing of bacteria

release of inflammatory mediators

10^{13} own cells versus 10^{14} bacteria per person

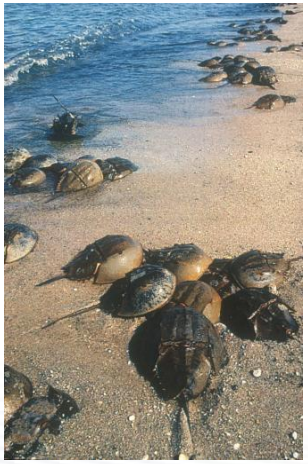
the gut of one person contains 1-2kg of bacteria

= 50g of Gram-negative endotoxin

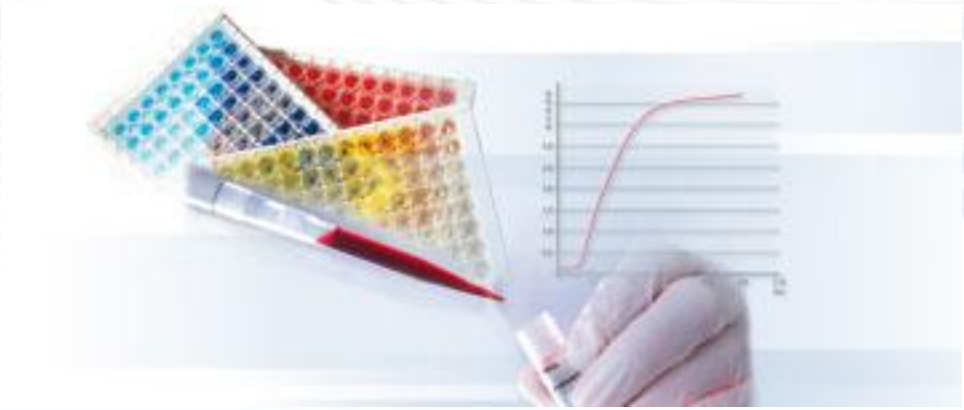
= enough to kill one million people by injection

= enough to induce fever in one billion people

Pyrogen (bacterial and fungal contamination) testing still consumes more than 300.000 rabbits per year

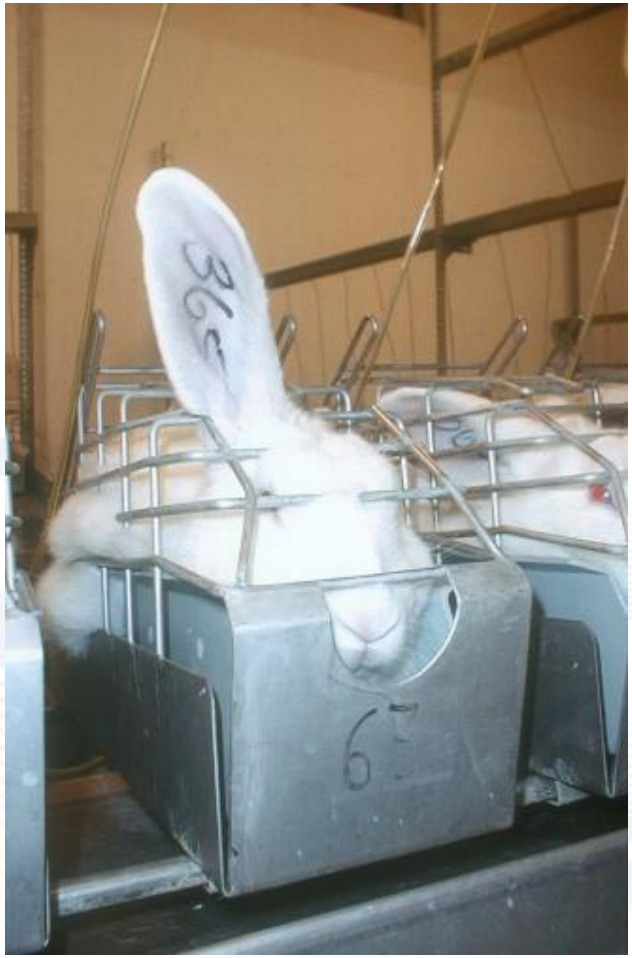


Limulus assay is restricted to Gram-negative endotoxin, disturbed by many substances and does not reflect human pyrogen potency



1995 Whole blood pyrogen test
2003 and 2004 Validated
2006 Validity statement
2009 Accepted Eur. Pharmacopoeia and with limitations by ICCVAM / FDA
PyroDetect (Biotest, now Merck/Millipore)

Limitations of the Rabbit Pyrogen Test

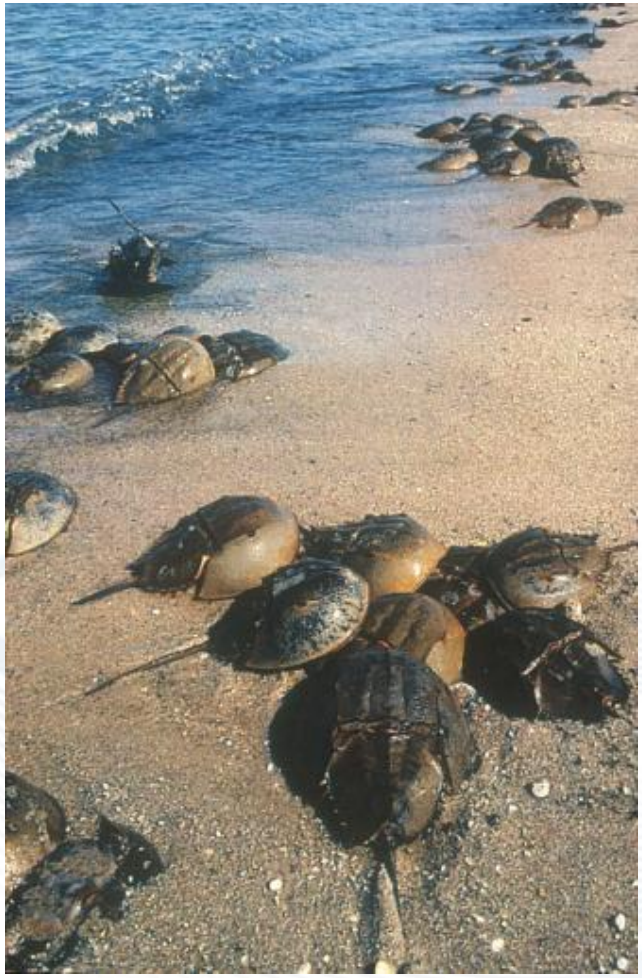


Animal consumption
Costly and laborious
Species variations, strain variations
Examined only for endotoxins
Stress affects body temperature
Replicate experiments
No positive controls

Test is not applicable for certain drugs

Radiopharmaceuticals (short half-life)
Chemotherapy (toxic)
Sedatives/analgetics (lower body temp.)
Cytokines (increase body temperature)
Antibiotics/plasma proteins/antigens (allergies)

Limitations - Limulus Amebocyte Lysate Assay

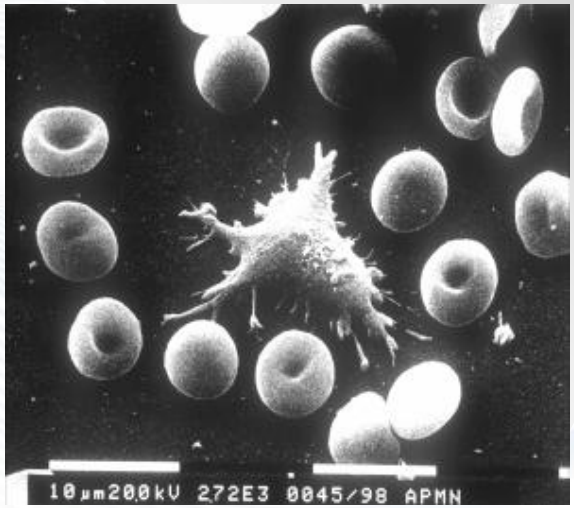


- Restricted to endotoxins
- Disturbed by endotoxin-binding components (spike duration !!!) e.g.
  - blood components
  - lipids
  - solid materials
  - cell therapies
- Potencies of pyrogens and synergies not reflected; difficult to correlate with rabbit test
- False-positive for glucans, cellulose and many herbal preparations
- Not a full *in vitro* substitute; US landings down from 3 to 0.7 million (1998– 2005)

Novel Pyrogen tests based on the human fever reaction

Exogenous Pyrogen

1. Whole Blood
2. Leukocytes
3. Cell lines
4. Cryopreserved blood cells



Endogenous Pyrogen

Fever

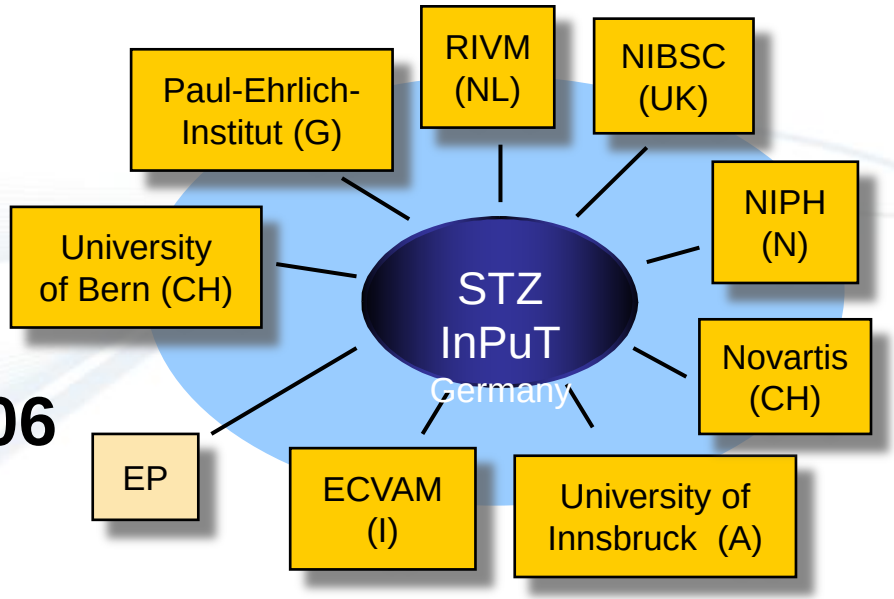
ELISA

Hoffmann et al. (2005). International validation of novel pyrogen tests based on human monocytoid cells.

***J Immunol Methods*, 298: 161-73**

Collaborative study

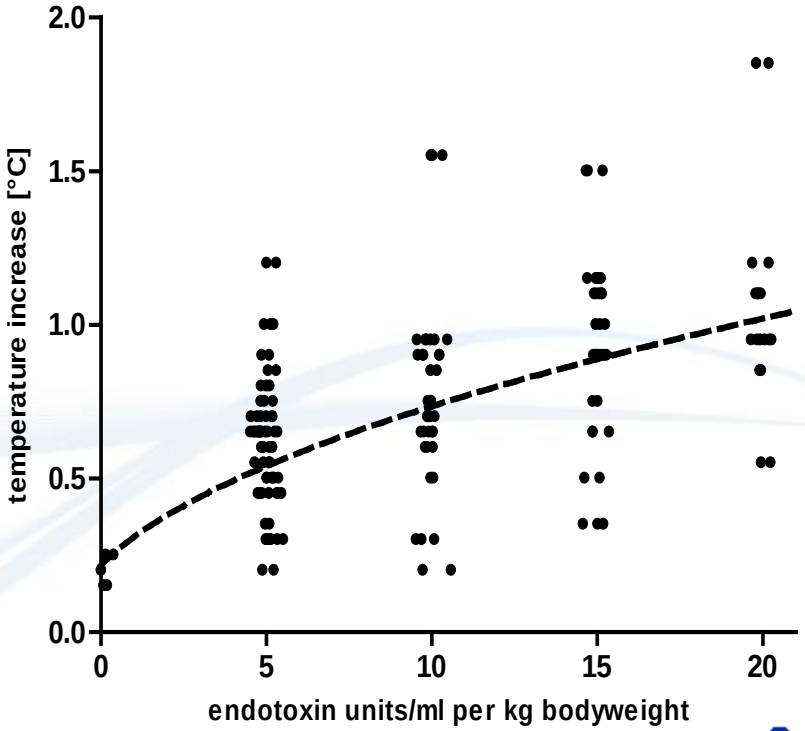
- **EU FP5 project**
- **2000-2003**
- **10 partners**
- **6 in vitro tests**
- **Validated by ESAC 2006**



The rabbit test as reference

The temperature reaction of a sensitive strain (n = 171)

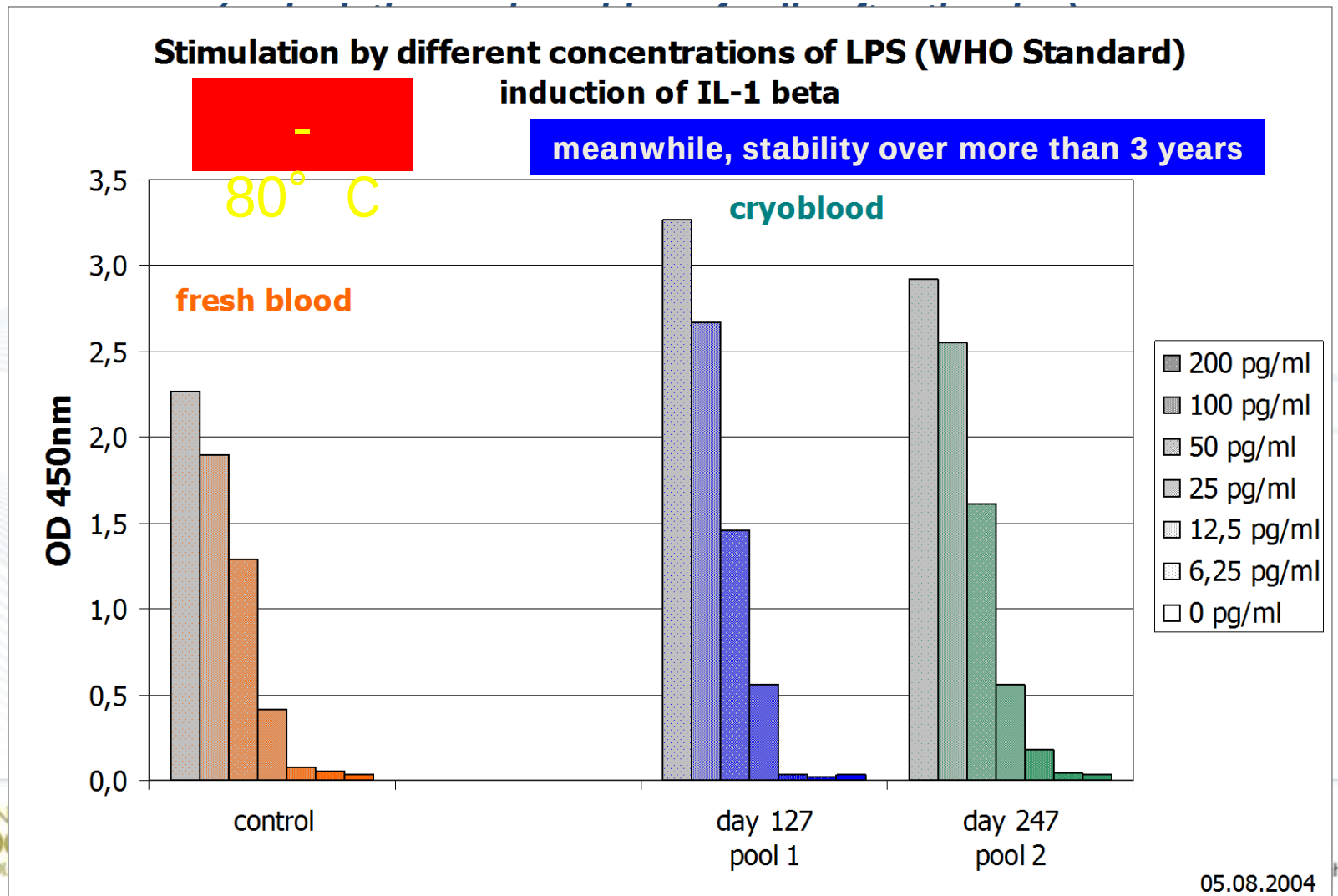
- limit temperature increase in Europe: 0.55° C
  - ➡ 5 EU/10 ml = 0.5 EU/ml defined as threshold
- 13 drugs with five contaminations each (0, 0.25, 0.5, 1 EU/ml)



challenging original study design

➡ (58% specific, 88% sensitive for rabbit)

Viability of cryopreserved in whole blood at – 80° C



Results – Predictive Capacity

Prediction Model 'PM0'

- non-pyrogenic: 0 EU/ml
- pyrogenic: 0.25, 0.5, 1 EU/ml

➡ 30 non pyrogenic and 120 pyrogenic samples

Original Prediction Model

- non-pyrogenic: 0, 0.25 EU/ml
- pyrogenic: 0.5, 1 EU/ml

➡ 60 non pyrogenic and 90 pyrogenic samples

Test	Specificity	Sensitivity
PBMC	96.7%	100%
WBT A	92.6%	96.2%
WBT B	85.2%	99.0%
WBT fresh	85.7%	99.1%

Test	Specificity	Sensitivity
PBMC	93.1%	77.6%
WBT A	98.8%	83.6%
WBT B	82.4%	89.1%
WBT fresh	97.4%	81.4%

Conclusion

The validated methods using cryopreserved cells

- are an important standardisation

  ➡ widely applicable by overcoming limitations

  - availability of primary cells
  - exclusion of abnormal donors
  - test for infectious agents

- are reproducible and robust
- perform well in terms of predictive capacity
- should augment regulatory acceptance

Tab. 14: Result of LAL-testing of the 10 spiked drugs used in the MAT validation study

NIPH		"Truth" –	"Truth" +	Σ
PM	+	**12**	**4**	16
	–	**0**	**8**	8
Σ		12	12	24
NIBSC		**"Truth" –**	**"Truth" +**	**Σ**
PM	+	4	2	6
	–	**8**	**10**	18
Σ		12	12	34

sens: 67%
spec: 100%

sens: 83%
spec: 33%

Specific characteristics whole blood assays

- **Blood donor dependent, primary cells, no isolation, cell suspension**
- **Saline, thermoblock incubation possible**
- **Extensive supportive data (90 different LPS, 40 LTA, 50 fungal spores, 5 exotoxins etc.), incriminated samples and >80 products, direct comparisons to rabbit tesing; not disturbed by adjuvants**
- **Variants: commercial kit available, medical devices/solid materials, air-born-pyrogens, adsorb/wash-extraction/enrichment, cell therapies, one-plate assay**

Specific characteristics cryo-blood assays

- **Standardised and pretested primary cell pool, no isolation after thawing, cell suspension**
- **Culture medium, thermoblock incubation possible**
- **Supportive data for various non-LPS-pyrogens and routine product testing**
- **Variants: see whole blood assays, storage at -80 degree vs. liquid nitrogen**

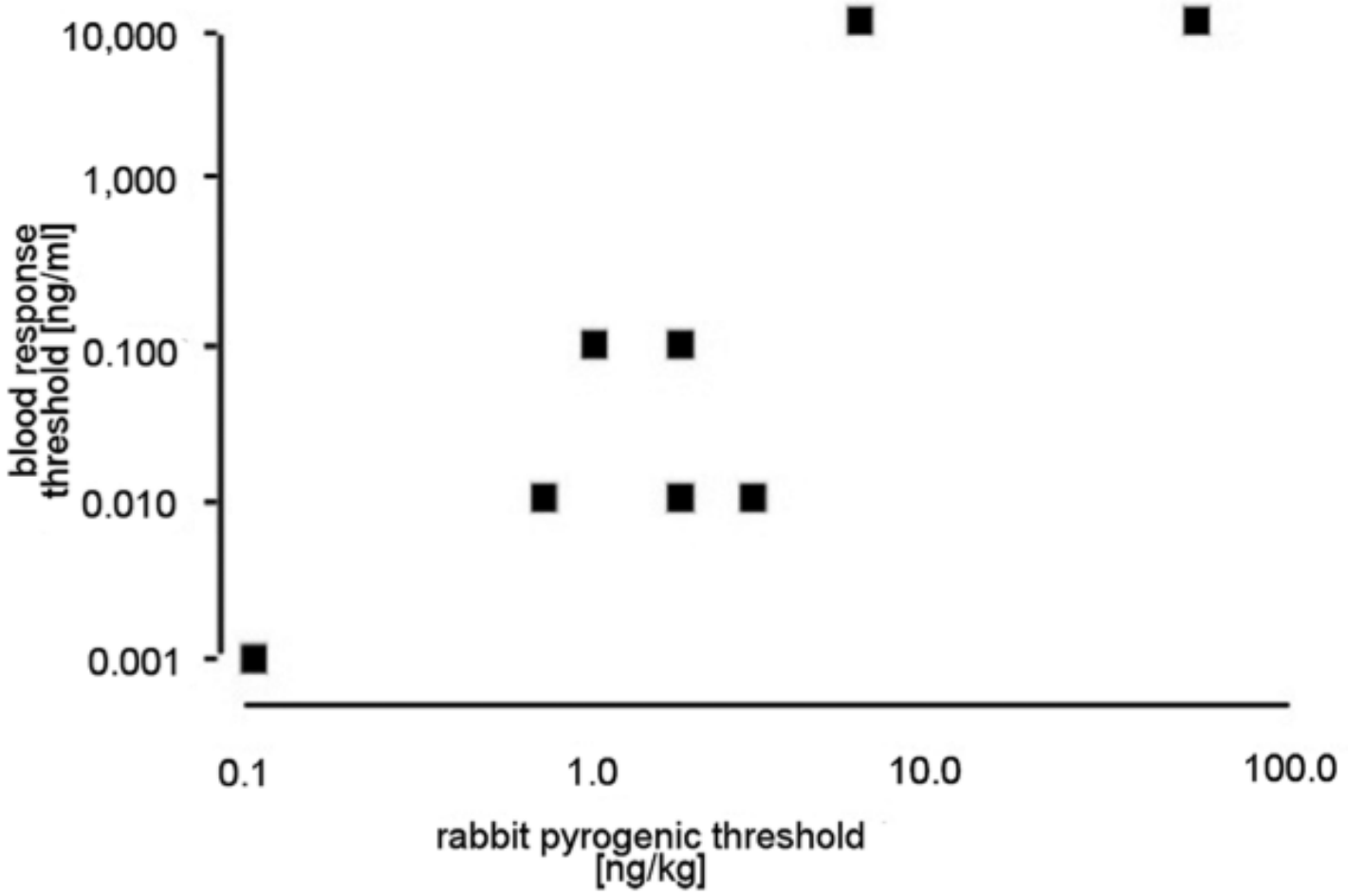
Comparison rabbit test and MAT for human serum albumin

Spreitzer et al., ALTEX 2002

	Rabbit pyrogen test (n = 261)			WB-IL-1	
Spike	**Positive [>2,5° C]**	**Repeti-tion [> 1,15° C]**	**Negative [< 1,15° C]**	**Positive**	**Negative**
None	-	-	29	-	29
5 EU	5	23	1	29	-
10 EU	10	21	8	29	-

9 different batches of albumin were tested pure and spiked with 5 or 10 IU/ml

MAT in contrast to BET / LAL reflects in vivo potency of different LPS



New challenges

Patient safety: non-endotoxin pyrogens

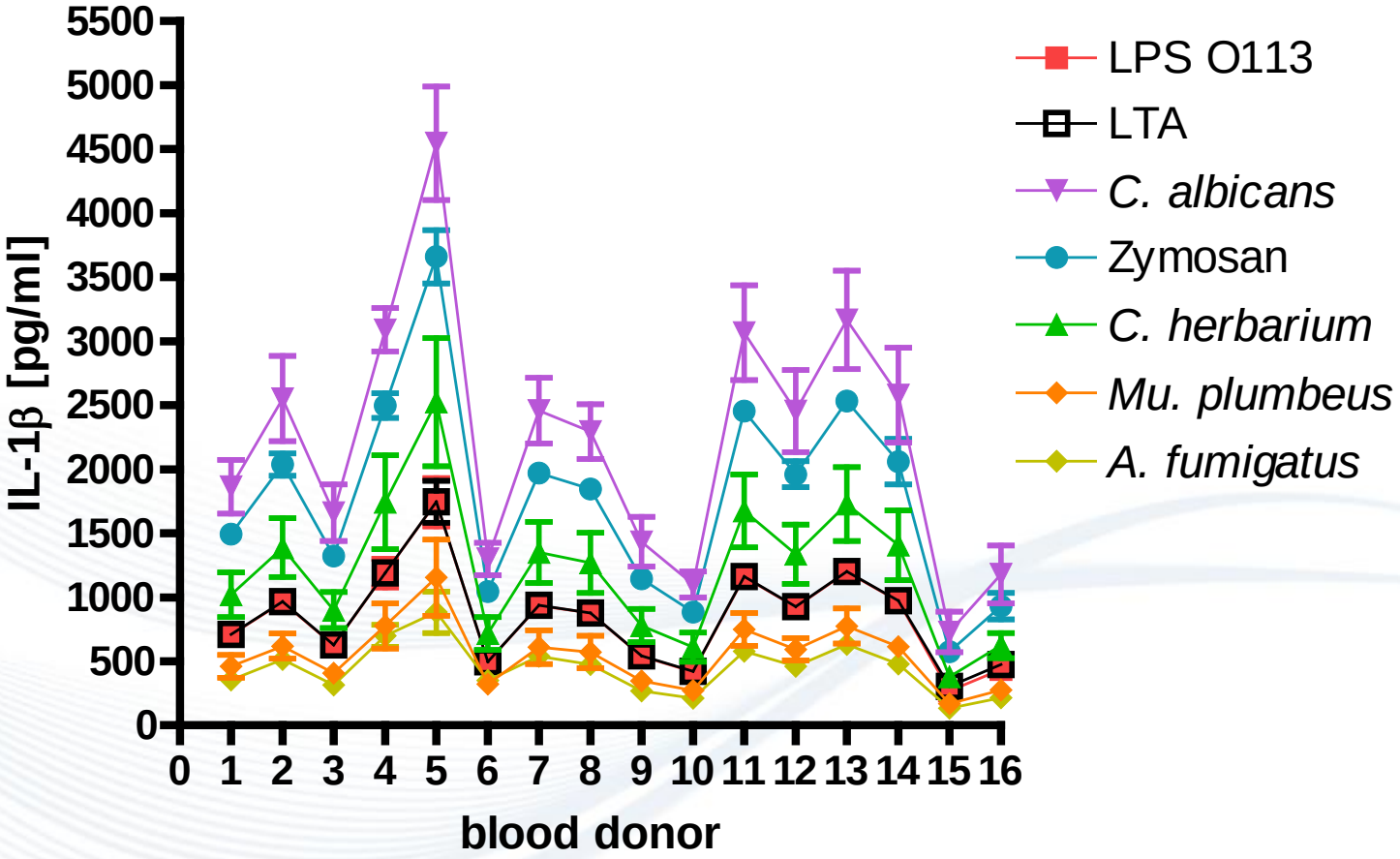
New expression systems in gene technology
(Gram-positive bacteria, insect and fungal cells)

Medical devices

Cellular therapies

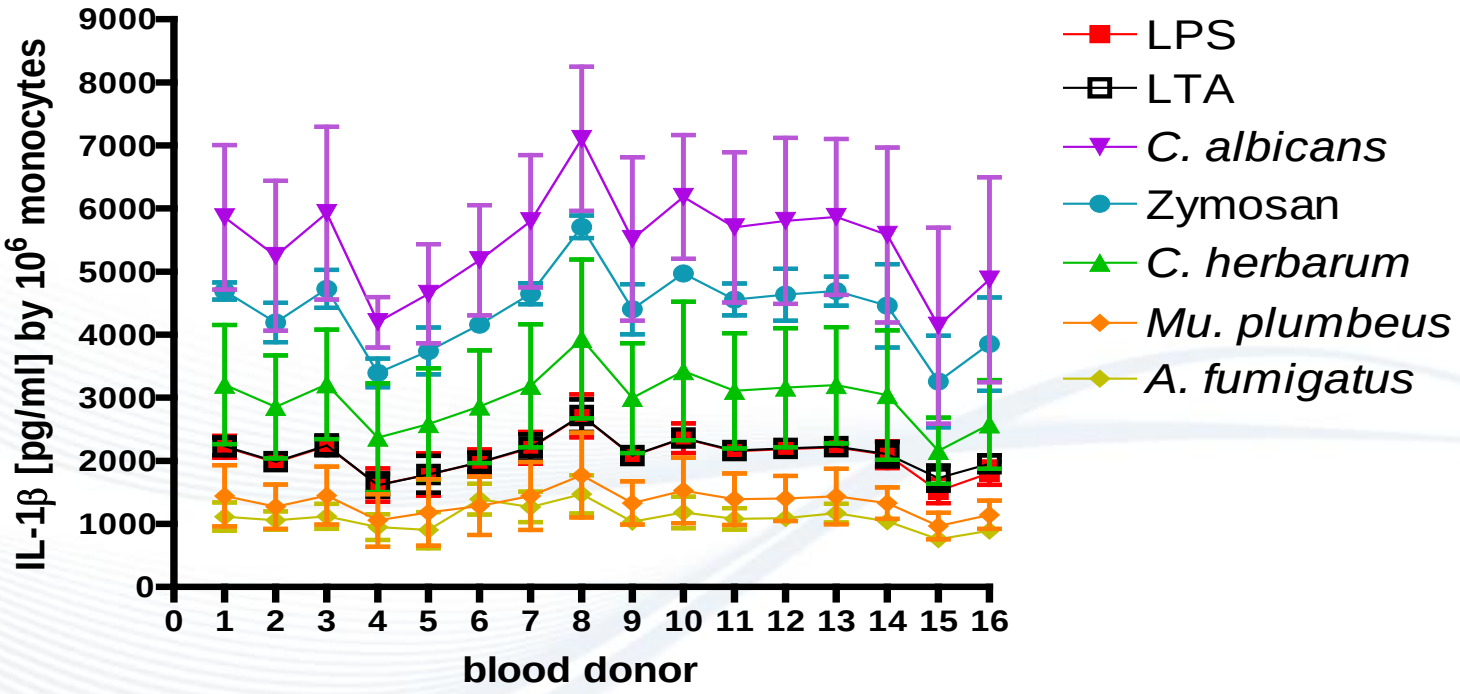
Environmental pyrogens

Comparison of different donors



Different absolute but similar relative response of donors

Relative response of donors



Differences caused mainly by different monocyte counts

transatlantic think tank for toxicology

ALTEX 2013, 30:169-208

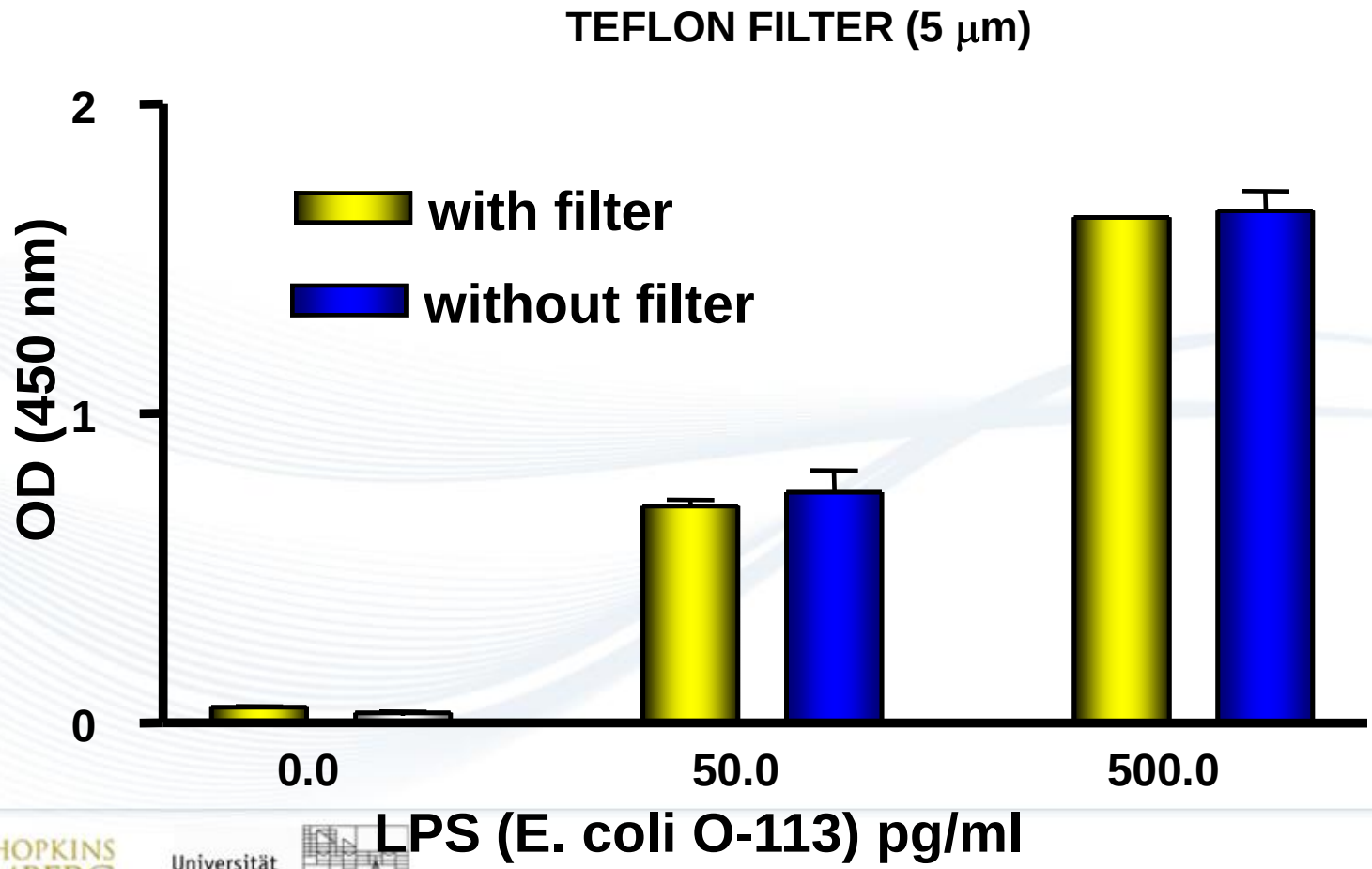
t4 Report*

Evidence for the Detection of Non-Endotoxin Pyrogens by the Whole Blood Monocyte Activation Test[1]

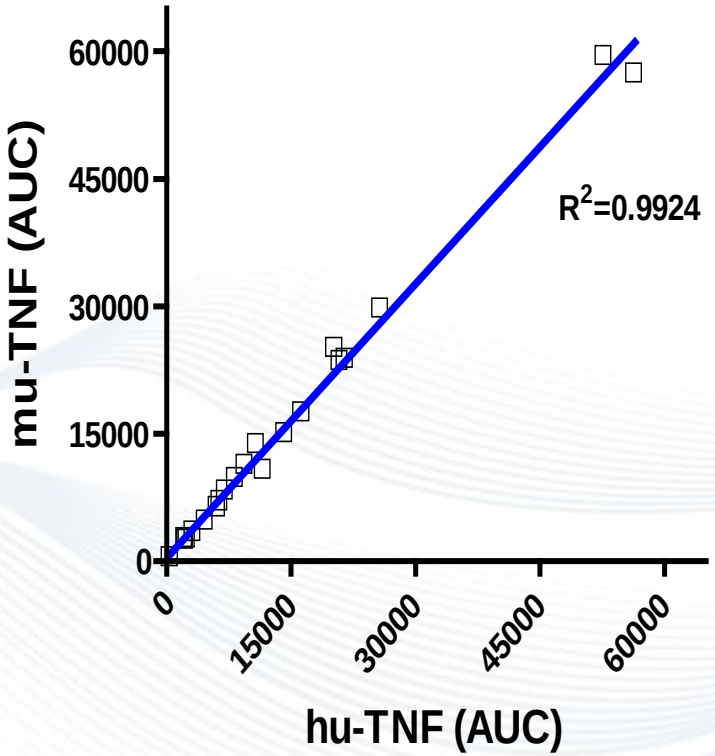
Nina Hasiwa [1,2], Mardas Daneshian [1], Peter Bruegger [4], Stefan Fennrich [5], Astrid Hochadel [2], Sebastian Hoffmann [6], Felix E. Rivera-Mariani [3], Christoph Rockel [7], Stefanie Schindler [8], Ingo Spreitzer [9], Sandra Stoppelkamp [5], Kranthi Vysyaraju [3], and Thomas Hartung [1,3]

Submitted to FDA / ICCVAM

Endotoxin determination on PTFE filters



Air-born pyrogens as risk factor for asthma and COPD



Whole human blood and murine alveolar macrophages respond similar to fungal spores

- **Human relevant test system**
- **Easy field sampling**
- **Measurement on filter**
- **Patent**
- **Ongoing studies childhood asthma, COPD *(with F. Mariani-Rivera, K. Vysyaraju, P. Breysse)***

PALL-monitor with PTFE (teflon) membrane

Lessons learned

- alternative methods can outperform animal tests
- importance of commercialization
- 15 years from development to acceptance
- Importance International harmonization
- lack of enforcement of implementation
- enabling technology
- importance of public-private partnership

Risk of Pyrogenic Contamination

contact with tissue / blood
consequences

short-term	long-term
pyrogenic reactions • inflammatory reactions	**example hemodialysis** • immunodeficiency • amyloidosis • erythropoietin response ↓ • atherosclerosis

Impairment of the patient
reduced biocompatibility

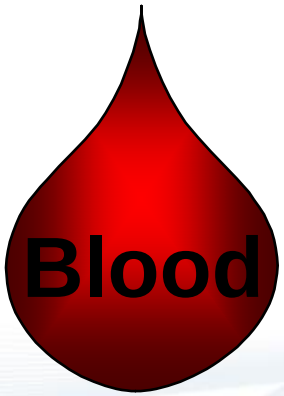
Pyrogen detection on biocompatible materials

Limulus-Assay

Rinsing solution

(contaminated)

Biocompatible Material



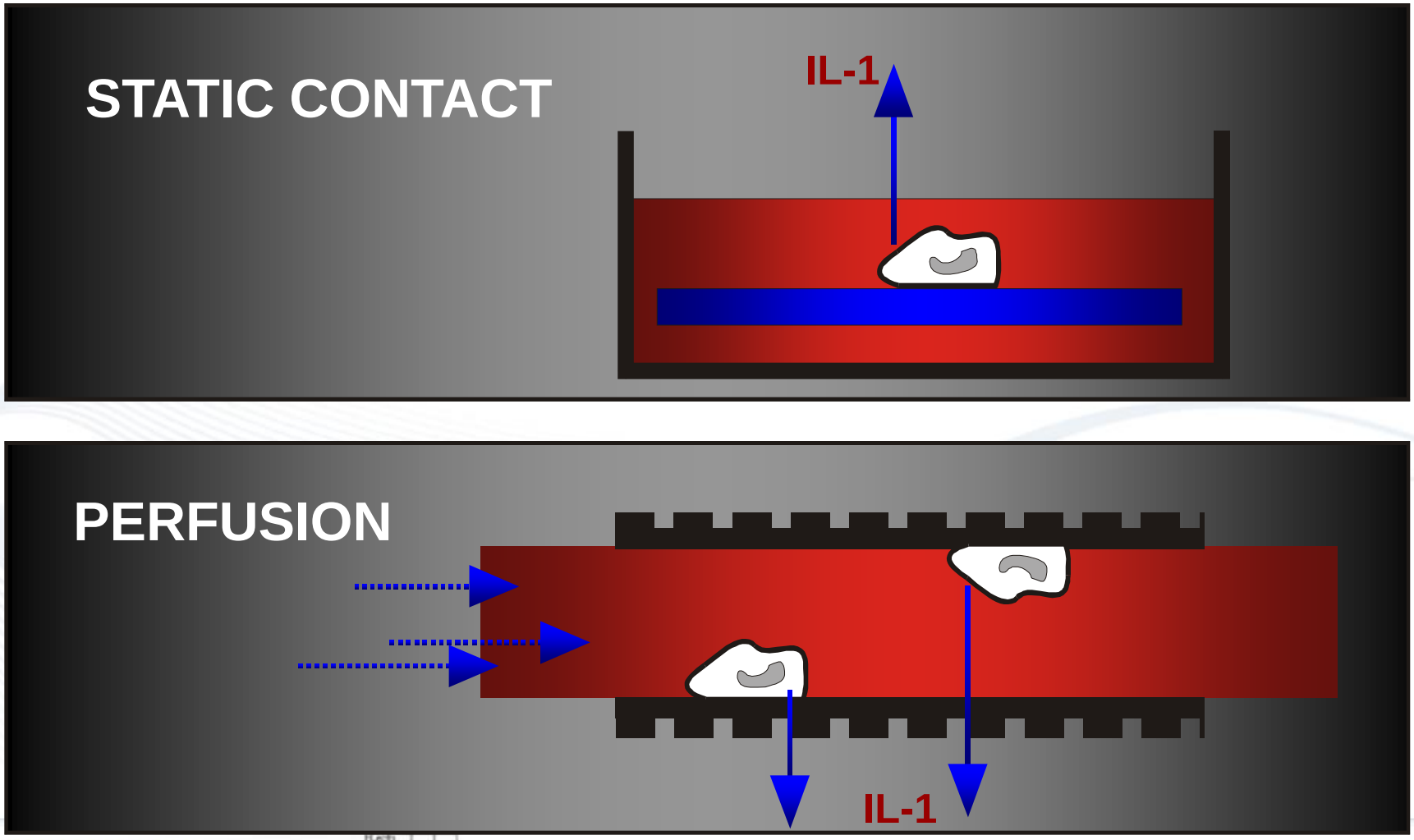
Cytokine,
e.g. IL-1β

Rinsing solution/
Implant

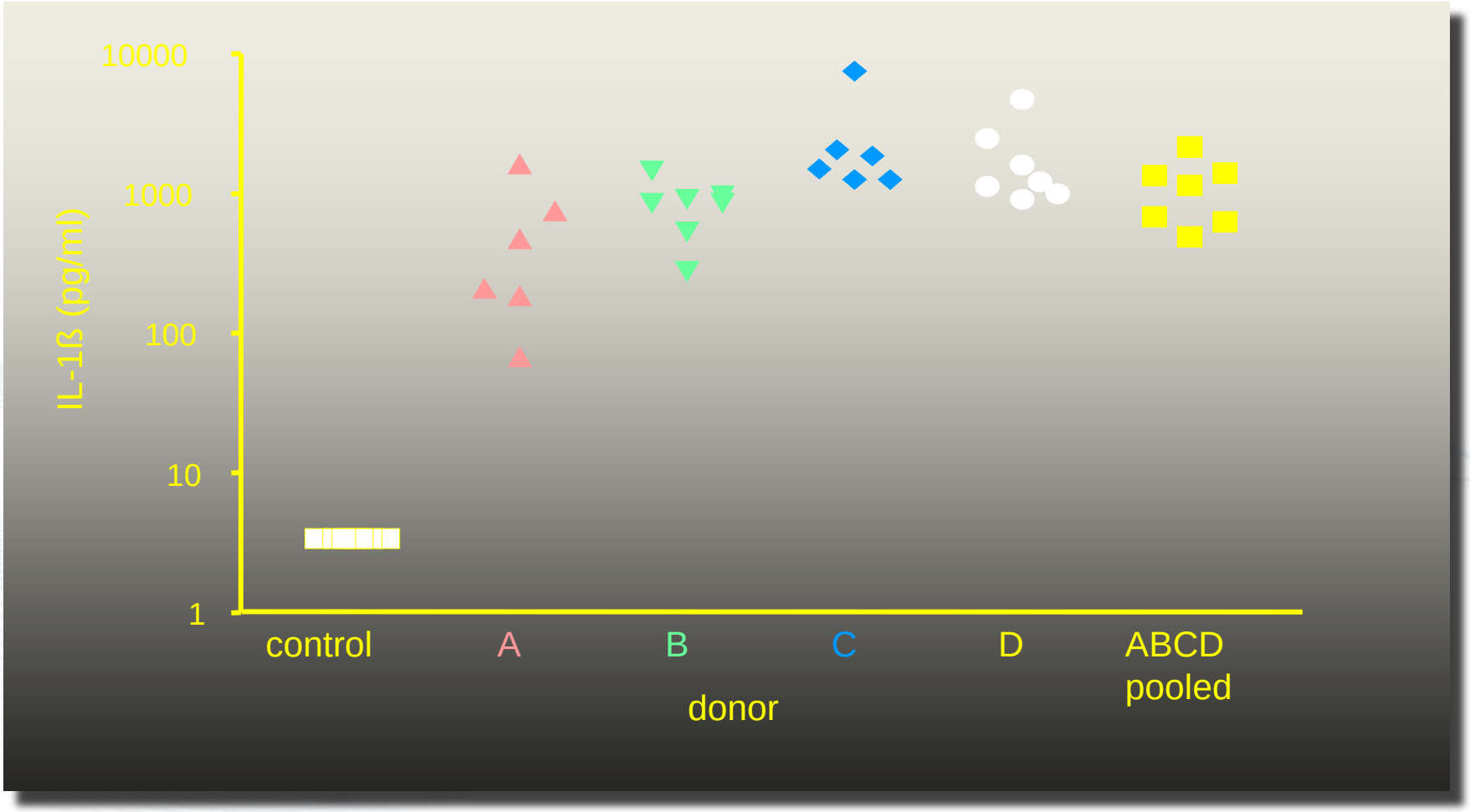
Rabbit Assay

ELISA (MAT)

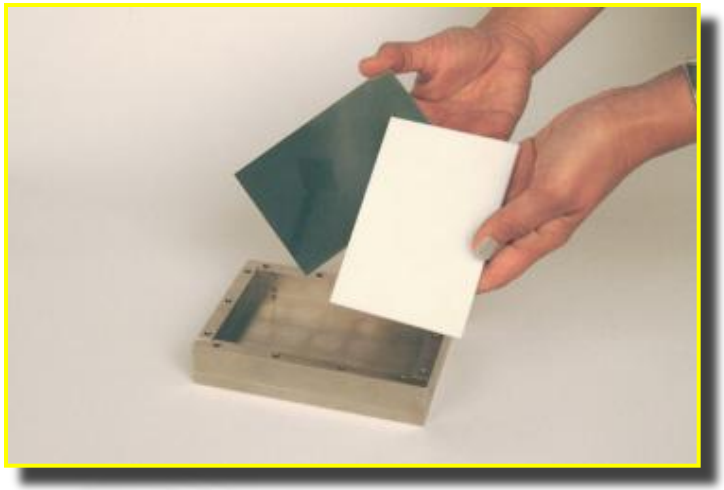
Performance of the MAT for Devices

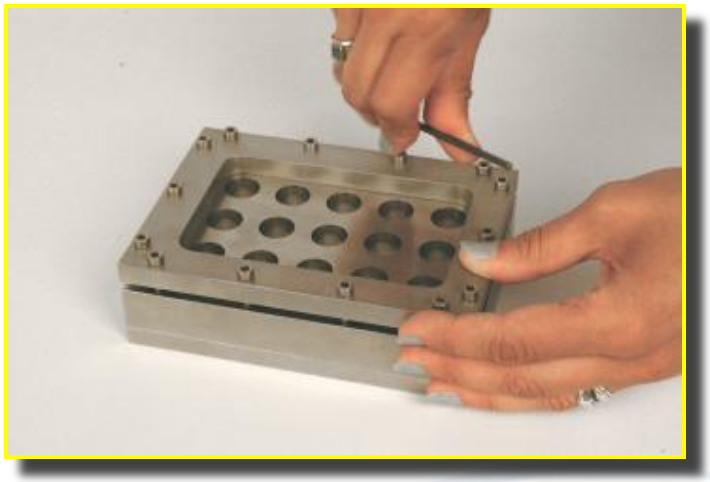


Contamination of steel by manual handling

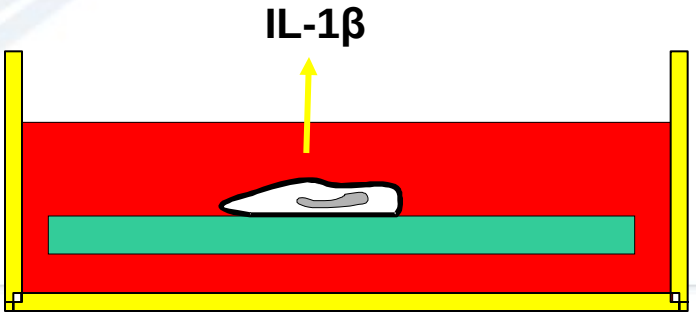


15-well steel block

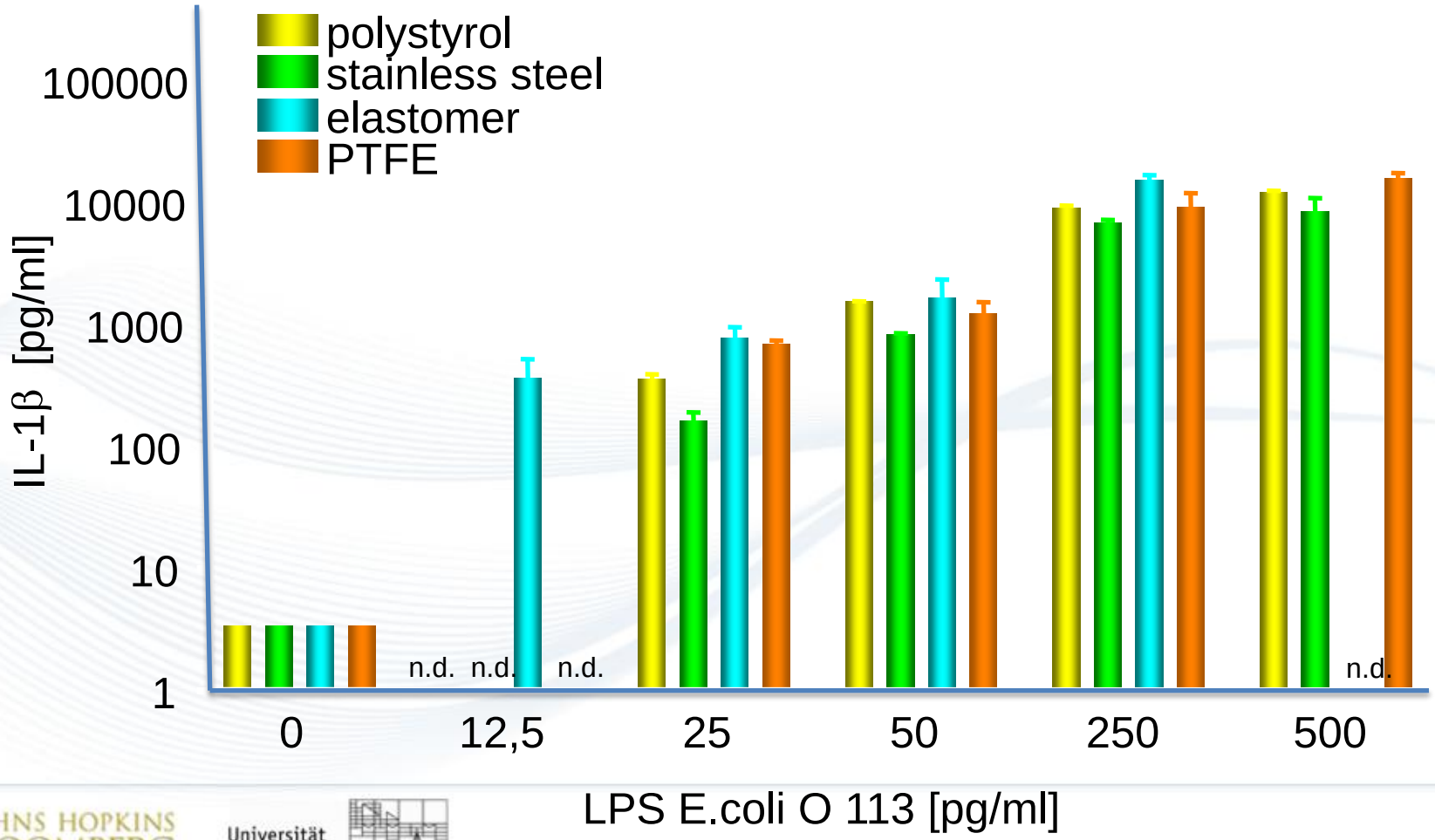




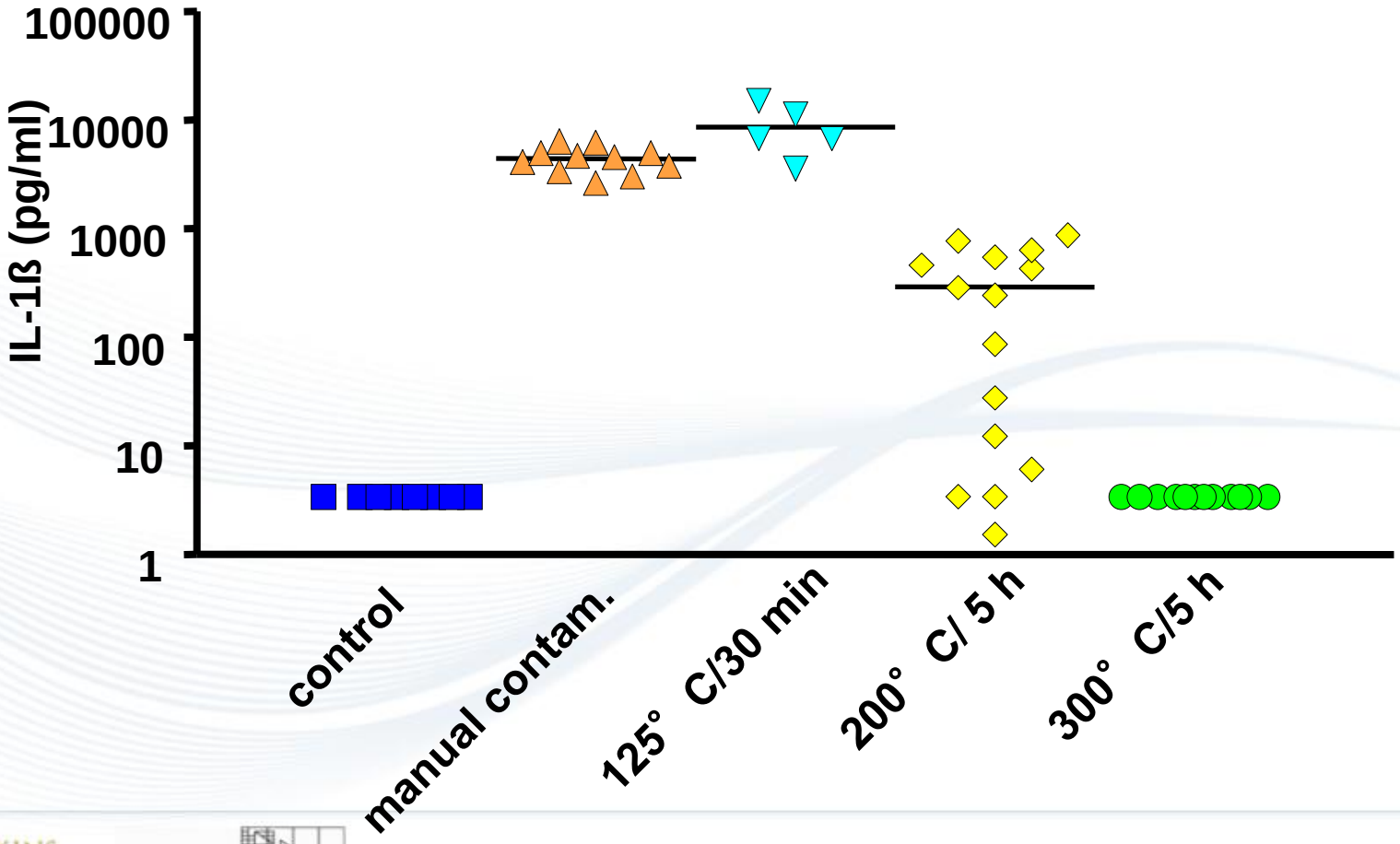
Static Contact



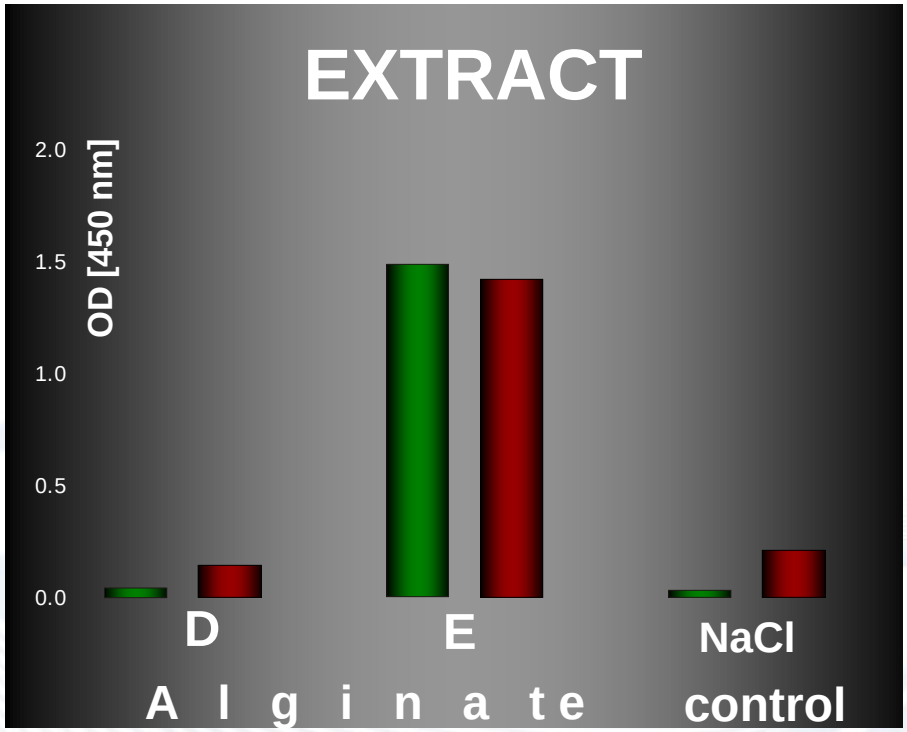
Endotoxin response on different materials tested in the 15well steel block

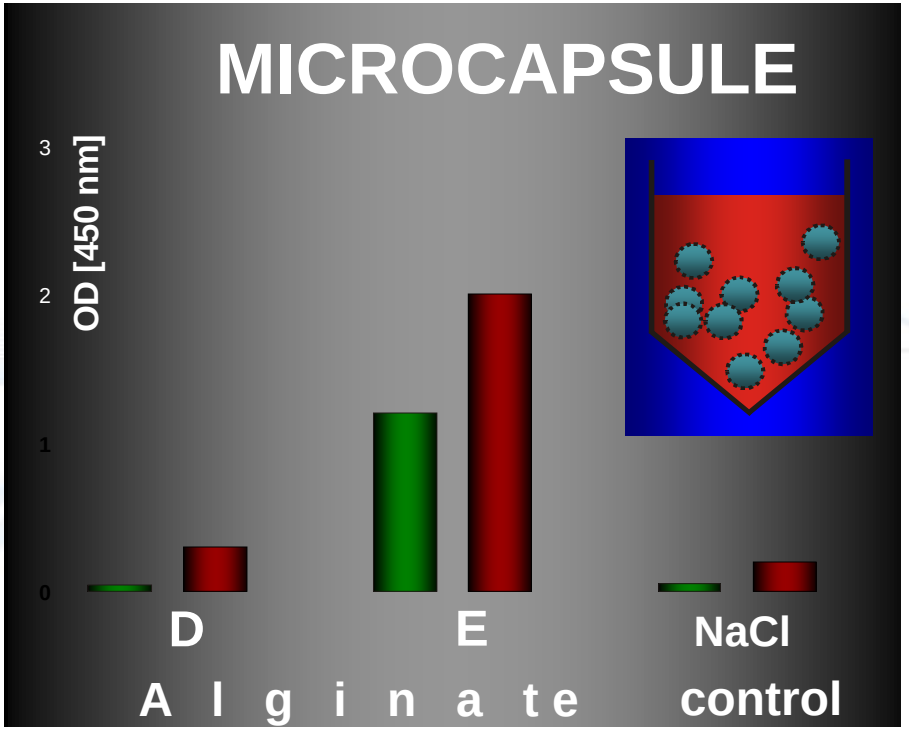


Elimination of pyrogenic residuals on titanium plates contaminated by manual handling



Test of alginate microcapsules in the whole blood pyrogen test





Spider Silk

The development of novel spider protein derived implants and devices for orthopedic surgery

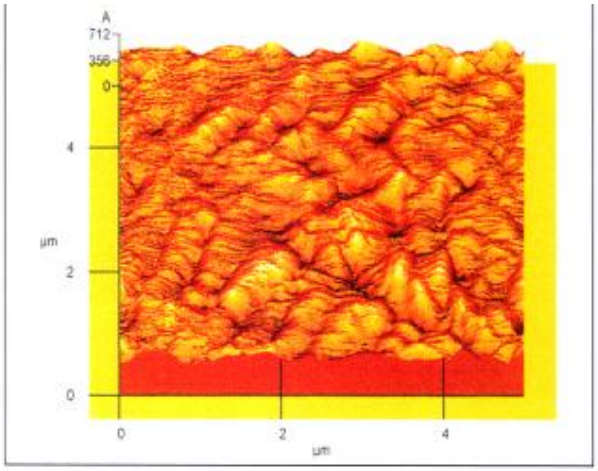
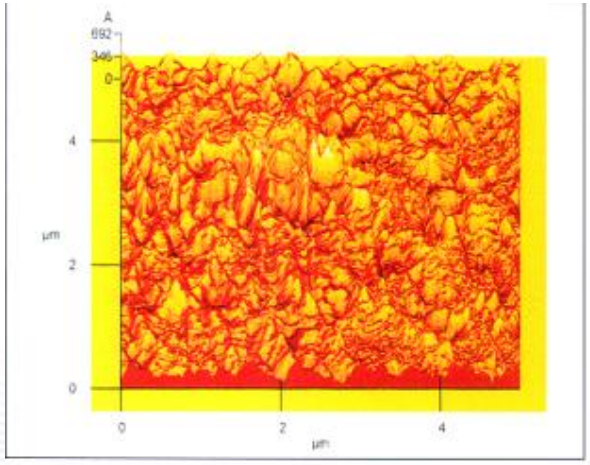
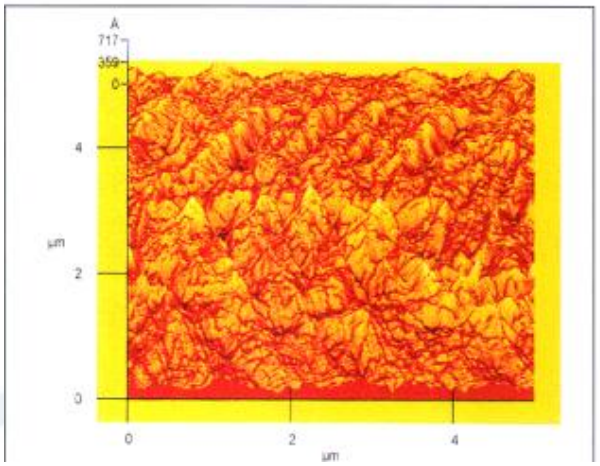
Acronym: SPIDERMAN
Start: 6/1/2002
End: 5/31/2007

JOHNS HOPKINS BLOOMBERG SCHOOL of PUBLIC HEALTH

Universität Konstanz

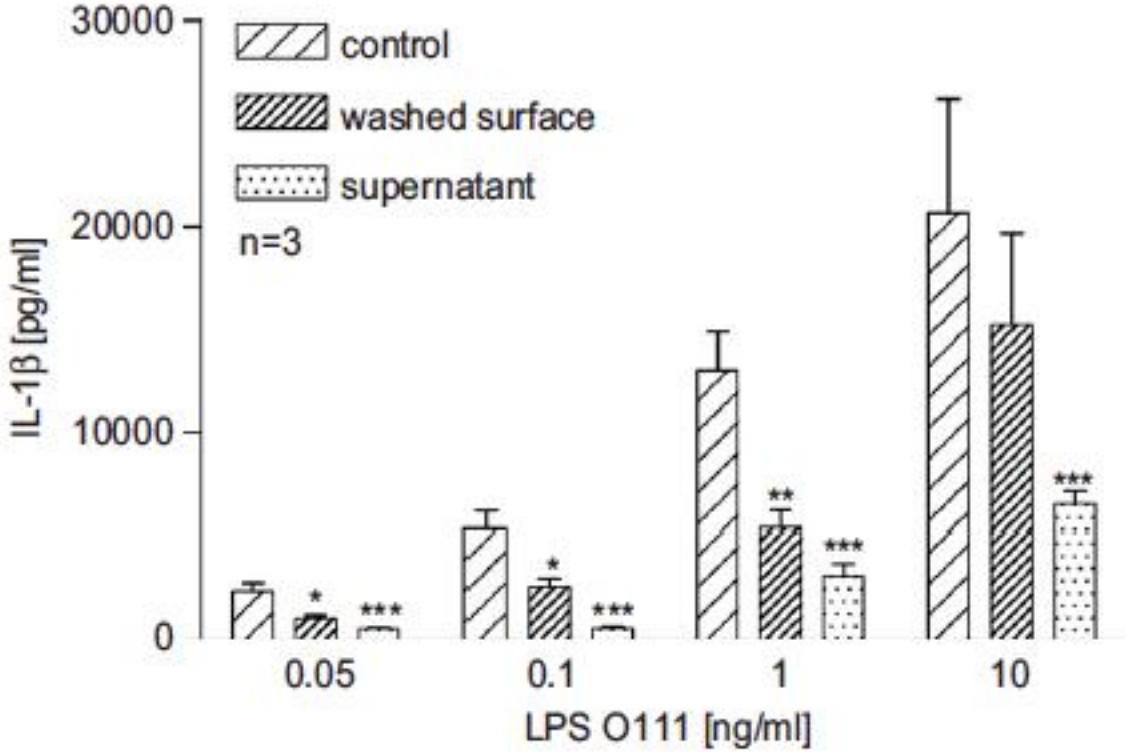
Atomic Force Microscopy of Polypropylen surface

LPS Spike (500 ng/ml) and washing

Spike	washed	No Spike
		
20,6 nm	32,4 nm	43,4 nm

Average peak height

LPS is only partially removed



Biomaterials

www.elsevier.com/locate/biomaterials

une-stimulating

von Aulock[b],

a,b,*

Fig. 3. LPS on surfaces. LPS residuals on polystyrene surfaces after washing for 6 h with sterile PBS in comparison to the resulting supernatant and a contaminated not washed plate verified by the human whole blood incubation and following IL-1β in ELISA in pg/ml $\pm$ SEM. All the samples were done in triplicates with blood from three different donors. *$p<0.05$, **$p<0.01$, ***$p<0.001$ versus the control (two-way ANOVA with Bonferroni post-test on the logarithmized data).

In vitro pyrogen test medical devices

Francesca Mazzotti,[1] Julia Beuttler,[1] Albrecht Wendel,[1] Thomas Hartung

Neurosurgical aneurysm clips

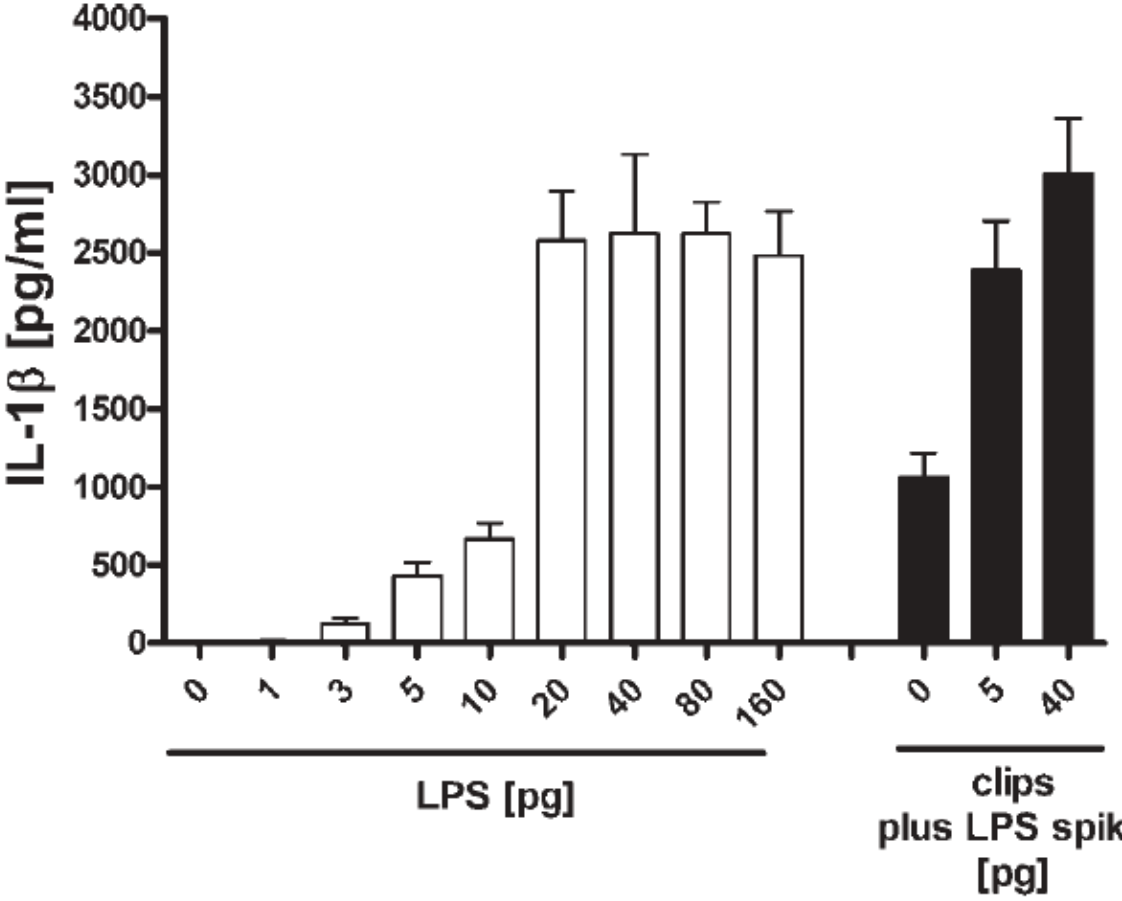


Figure 6. Low contamination of titanium clips during production. Titanium clips packaged for sale were employed directly in the whole blood assay in the presence or absence of LPS. IL-1β release was measured by ELISA. Data are means ± SEM of blood from four donors.

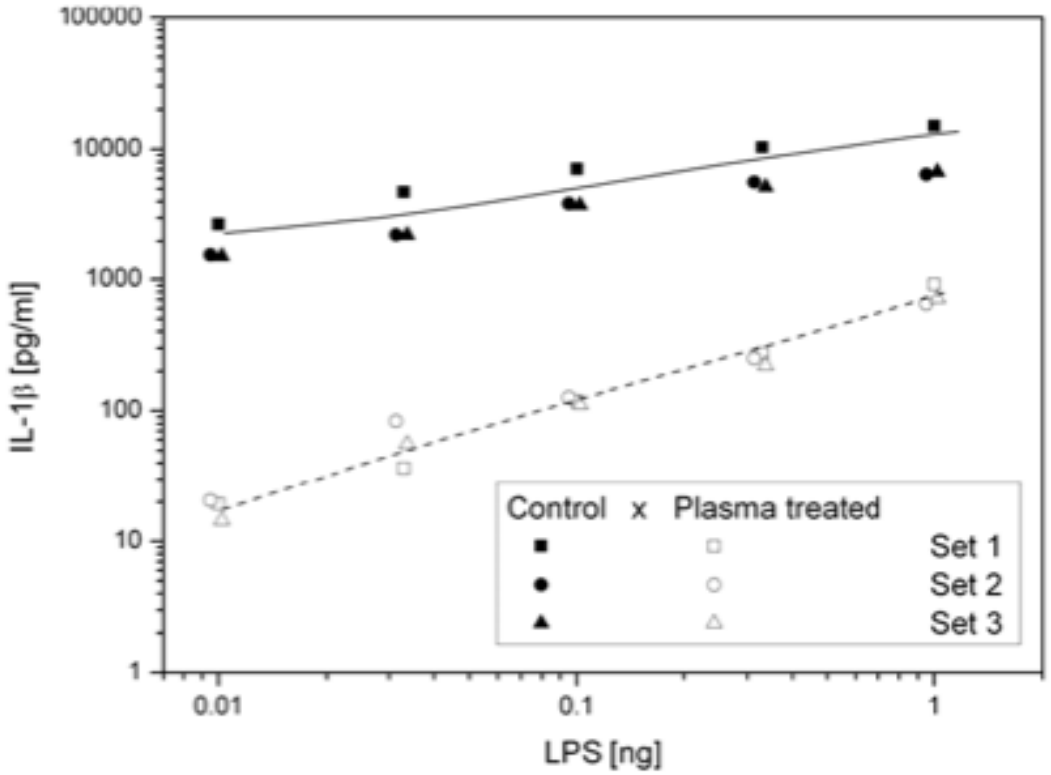


Fig. 3. LPS at different concentrations, plasma-treated 60 s to verify the reproducibility of the sample preparation and the plasma treatment (human whole blood incubation with following IL-1β ELISA, Applied power, 1000 W; pressure, 13.3 Pa; gas flow, 100 sccm; gas mixture, O_2/H_2 (50/50)).

Innate Immunity
14(2) (2008) 00–00

Los Angeles, London,
New Delhi and Singapore
ISSN 1753-4259 (print)
10.1177/1753425907088243

Conclusions pyrogen testing

- **Current tests do not adequately control pyrogen contamination of medical devices**
- **Testing on surface is a paradigm shift**
- **Human relevant testing is possible**

Perspectives for other alternative methods

- *Skin assays*
- *Genotoxicity*
- *Sensitization*
- *Cytotoxicity*
- *Cell Transformation*
- **Immunotoxicity**

In Vitro Medical Device Testing Symposium

Mt. Washington Conference Center, Baltimore MD

December 10-11, 2013

website
http://altweb.jhsph.edu/news/2012/med_device_symp.html

In Vitro Pyrogen Test

The Konstanz‘ group

Slides available at: http://www.toxicology.org/isot/ss/mdss/events.asp

Patents

Method for assaying flowing media for microbial toxins
EP 02 729 818.9 submitted 25.03.2002, under evaluation
US 10/474 694 submitted 10.10.2003, under evaluation
JP 2002-581 998 submitted 04.12.2003, granted 10.04.2009

Test procedure with biological system
EP 0 741 294 B1 submitted 24.04.1996, granted 05.11.2003 (countries: AT, BE, CH/LI, DE, ES, FR, GB, IT, NL, SE)
USP 5 891 728 submitted 02.05.1996, granted 06.04.1999
JPP 3 667 439 submitted 07.05.1996, granted 15.04.2005

Test for determining pyrogenic effect of a material
EP 0 851 231 B1 granted 15.12.1997 (countries: AT, BE, CH/LI, DE, ES, FR, GB, IT, NL, SE)
US CIP 10/761,237 submitted 23.12.1997 (under evaluation)
JP 9-354572 submitted 24.12.1997, granted 04.04.2008