Connecting People, Science and Regulation

Monocyte Activation Test using PyroDetect System, human(e) alternative to rabbit in pyrogen testing

Pre-conference Workshop on Bacterial Endotoxin Testing, February 17th 2014, Berlin,Germany

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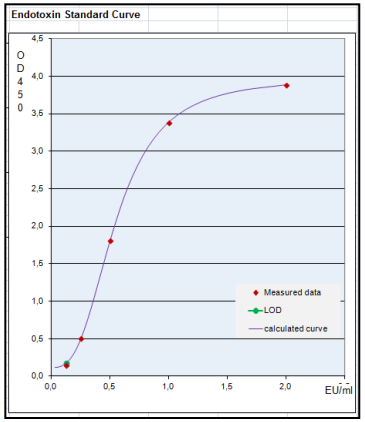
Merck Millipore, France

Objective of short presentation

- Overview of the procedure of the PyroDetect System including quality criteria and fulfillment of EP requirements
- Focus on Data Analysis tool software



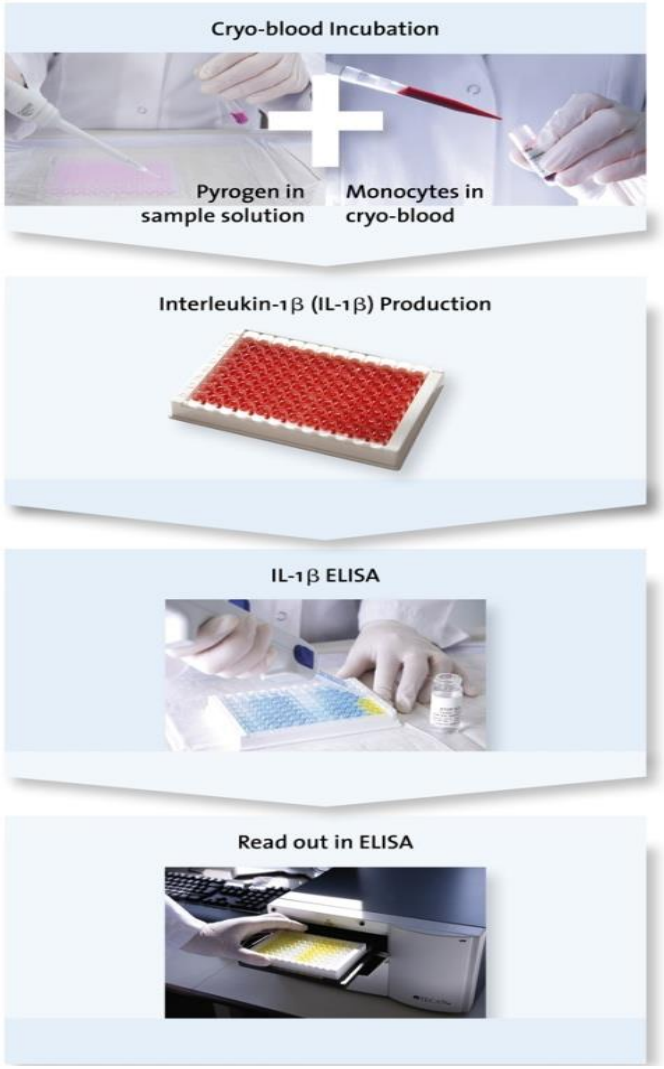
Data Interpretation PyroDetect System - Method B

sample 1							
Tests of validity	Minimal sensitivity:	EC 1xLOD>LOD			0,613	Passed	Failed
	Recovery of spike [%]:	Sample conc.			Sample 1:2		Sample 1:4
		124,25			95,19		100,58
Limits				CLC [EU/ml]:	0,5		
				MVD (CLC x conc. of test solution/LOD):	4,4		
				Sample pre-dilution 1:	4		
Results		Final dilution	OD of dilution	EU ≥ LOD?	Dil. ≥ MVD	Data interpretation	
	Sample conc.	4	0,182	Yes	No	Not conclusive	
	Sample 1:2	8	0,167	Yes	Yes	Pyrogenic [EU]>CLC	
	Sample 1:4	16	0,154	Yes	Yes	Pyrogenic [EU]>CLC	

What is the PyroDetect System?

- Test Kit to perform the Monocyte-Activation Test (MAT) according to EP chapter 2.6.30
- MAT using pooled cryo-preserved human whole blood and interleukin-1β ELISA
- PyroDetect Data Analysis Tool to support EP required calculations
  - P-values (regression, non-linearity)- curve validity criteria
  - 4-parameter endotoxin standard curve
  - Pyrogenicity based on the Contaminant Limit Concentration (CLC)
  - Calculation of Limit of Detection (LOD) and Maximum Valid Dilution (MVD)
- Standards: endotoxin standard (RSE) and Interleukin-1β standard

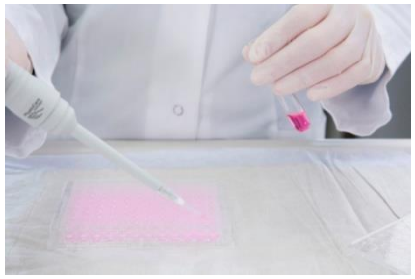
Procedure of the PyroDetect System



1. Cryoblood incubation
2. Interleukin-1β ELISA
3. Read out and data analysis

Protocol of the PyroDetect System
1. Cryoblood Incubation

RPMI Medium



Endotoxin dilutions

Sample dilutions

Cryo-preserved human whole blood

Pipetting of RPMI-cryo-blood mixture

10-24 hours
37° Incubation

Protocol of the PyroDetect System
2. Interleukin-1β ELISA

Transfer of cryo-blood
mix into IL-1β ELISA plate

Washing steps

Addition of conjugate

Washing steps
Addition of substrate

Addition of stop solution

Read out and analysis

Focus on Data Analysis tool

Focus on Data Analysis tool

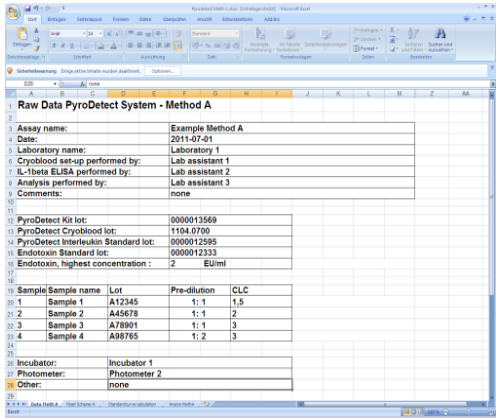
Back up slides

PyroDetect Data Analysis tool : Overview (1/2)

- Software tool including 4 Excel-files for the different methods and an integrated statistic software (Statconn Version 3.12 and R Version 2.12.2):
  - Quantitative method (Method A)
  - Semi-quantitative method (Method B)
  - Reference-lot-comparison (Method C)
  - Test of Interfering Factors (TIF)
- All Excel-files comprise the sheets: Raw Data, Pipetting Scheme, Standard curve Calculation & Data Analysis
- OD values of the ELISA Reader are copied to the appropriate Excel-file for further analysis

PyroDetect Data Analysis tool : Overview (2/2)

1. Raw data sheet



Raw Data PyroDetect System - Method A

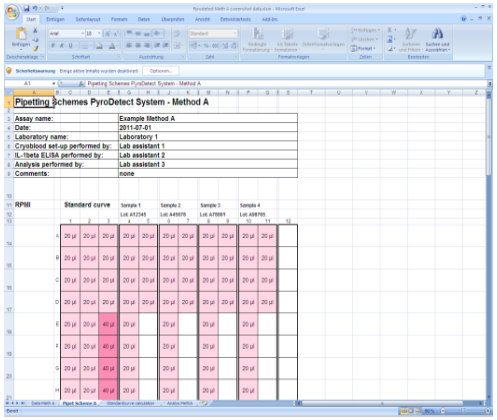
Assay name:	Example Method A
Date:	2011-07-01
Laboratory name:	Laboratory 1
Cryoblood set-up performed by:	Lab assistant 1
IL-1beta ELISA performed by:	Lab assistant 2
Analysis performed by:	Lab assistant 3
Comments:	none

PyroDetect Kit lot:	0000013569
PyroDetect Cryoblood lot:	1104.0700
PyroDetect Interleukin Standard lot:	0000012595
Endotoxin Standard lot:	0000012333
Endotoxin, highest concentration :	2 EU/ml

Sample	Sample name	Lot	Pre-dilution	CLC
1	Sample 1	A12345	1: 1	1,5
2	Sample 2	A45678	1: 1	2
3	Sample 3	A78901	1: 1	3
4	Sample 4	A98765	1: 2	3

Incubator:	Incubator 1
Photometer:	Photometer 2
Other:	none

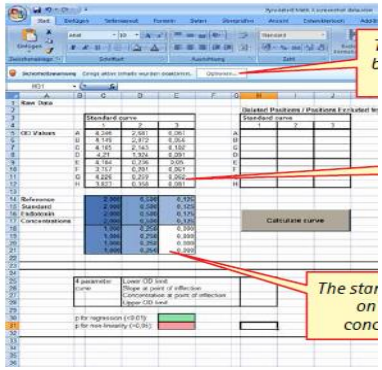
2. Pipetting scheme



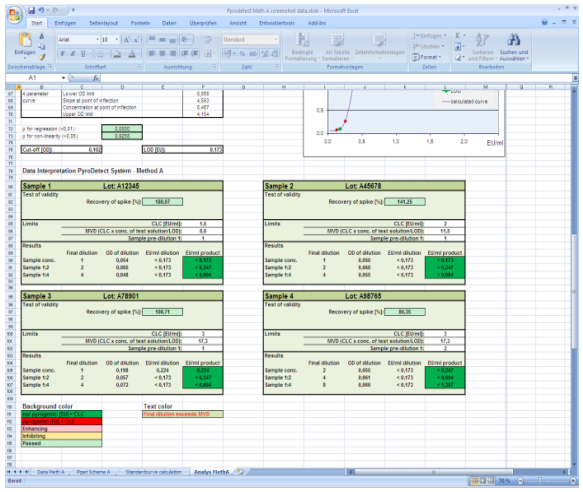
Pipetting Schemes PyroDetect System - Method A

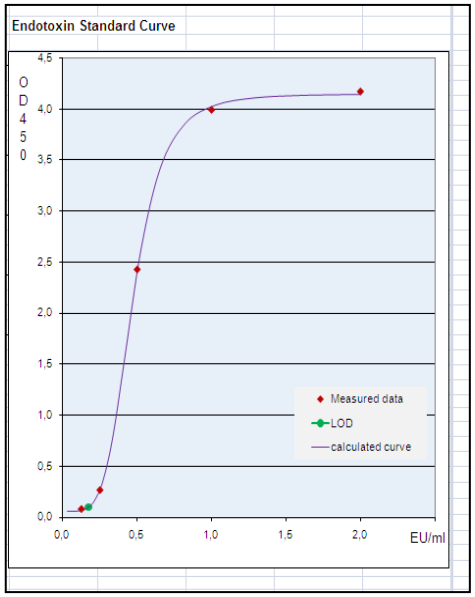
Assay name:	Example Method A
Date:	2011-07-01
Laboratory name:	Laboratory 1
Cryoblood set-up performed by:	Lab assistant 1
IL-1beta ELISA performed by:	Lab assistant 2
Analysis performed by:	Lab assistant 3
Comments:	none

3. Standard curve calculation

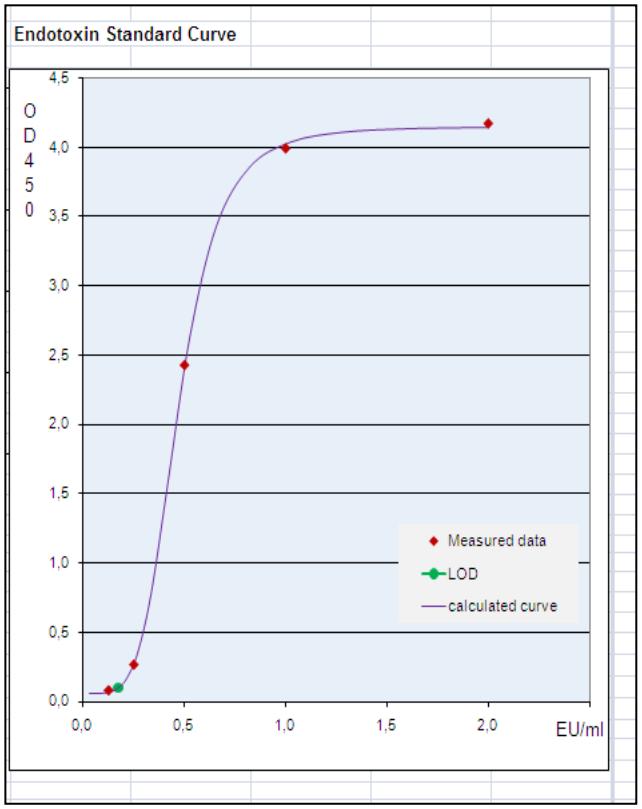


4. Data Analysis





PyroDetect Data Analysis tool, Method A

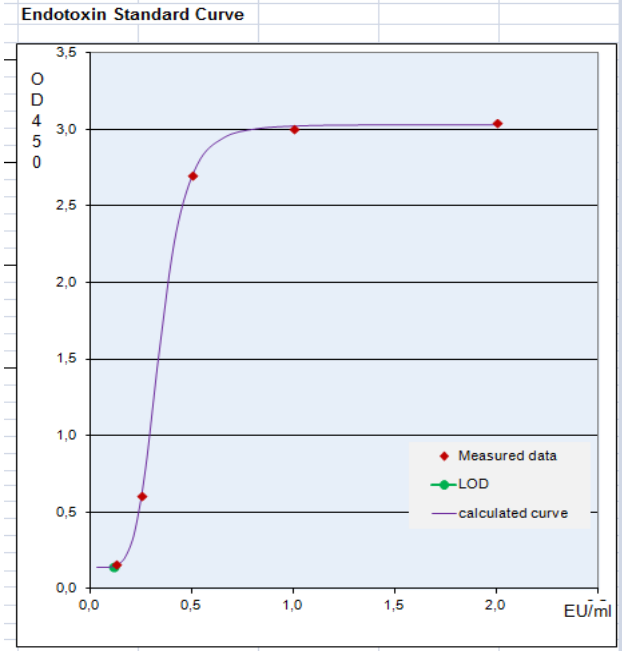


p for regression (<0,01):	0,0000		
p for non-linearity (>0,05):	0,9258		
Cut-off [OD]:	0,102	LOD [EU]:	0,173

Sample 2	Lot: A45678			
Test of validity				
	Recovery of spike [%]:	85,88		
Limits			CLC [EU/ml]:	1,5
			MVD (CLC x conc. of test solution/LOD):	8,6
			Sample pre-dilution 1:	2
Results				
	Final dilution	OD of dilution	EU/ml dilution	EU/ml product
Sample conc.	2	3,750	0,758	1,517
Sample 1:2	4	1,325	0,392	1,566
Sample 1:4	8	0,313	0,258	2,061

Sample 4	Lot: A98765			
Test of validity				
	Recovery of spike [%]:	80,35		
Limits			CLC [EU/ml]:	3
			MVD (CLC x conc. of test solution/LOD):	17,3
			Sample pre-dilution 1:	2
Results				
	Final dilution	OD of dilution	EU/ml dilution	EU/ml product
Sample conc.	2	0,055	< 0,173	< 0,347
Sample 1:2	4	0,061	< 0,173	< 0,694
Sample 1:4	8	0,066	< 0,173	< 1,387

PyroDetect Data Analysis tool, Method B



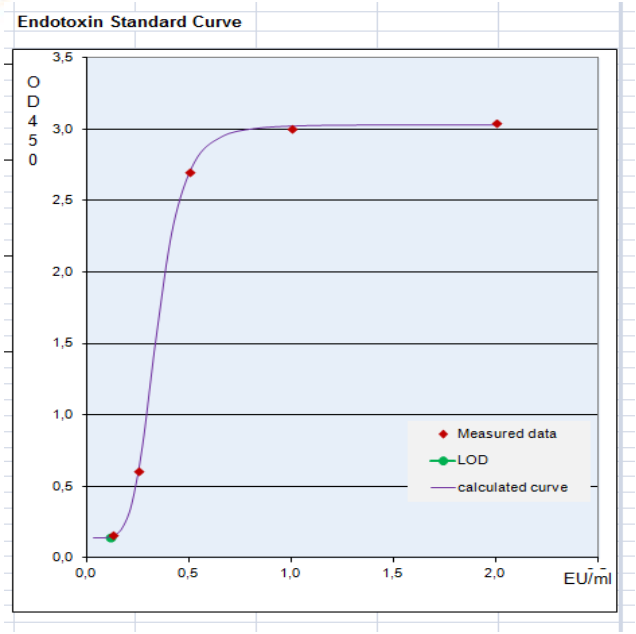
73					
74	p for regression (<0,01):		0,0000		
75	p for non-linearity (>0,05):		0,9620		
76					
77	Cut-off [OD]:	0,147		LOD [EU]:	0,114
78					
79					

Data Interpretation PyroDetect System - Method B

sample 1

Tests of validity	Minimal sensitivity:		EC 1xLOD>LOD		0,613	Passed	Failed
	Recovery of spike [%]:		Sample conc.		Sample 1:2		Sample 1:4
			134,69		102,52		103,86
Limits				CLC [EU/ml]:	10		
				MVD (CLC x conc. of test solution/LOD):	87,4		
				Sample pre-dilution 1:	1		
Results		Final dilution	OD of dilution	EU ≥ LOD?	Dil. ≥ MVD	Data interpretation	
	Sample conc.	1	0,123	No	No	Not pyrogenic [EU]<CLC	
	Sample 1:2	2	0,126	No	No	Not pyrogenic [EU]<CLC	
	Sample 1:4	4	0,121	No	No	Not pyrogenic [EU]<CLC	

PyroDetect Data Analysis tool, Method B



74	p for regression (<0,01):		0,0000		
75	p for non-linearity (>0,05):		0,9620		
76					
77	Cut-off [OD]:	0,147		LOD [EU]:	0,114
78					

Data Interpretation PyroDetect System - Method B

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Limits			CLC [EU/ml]:	0,5			
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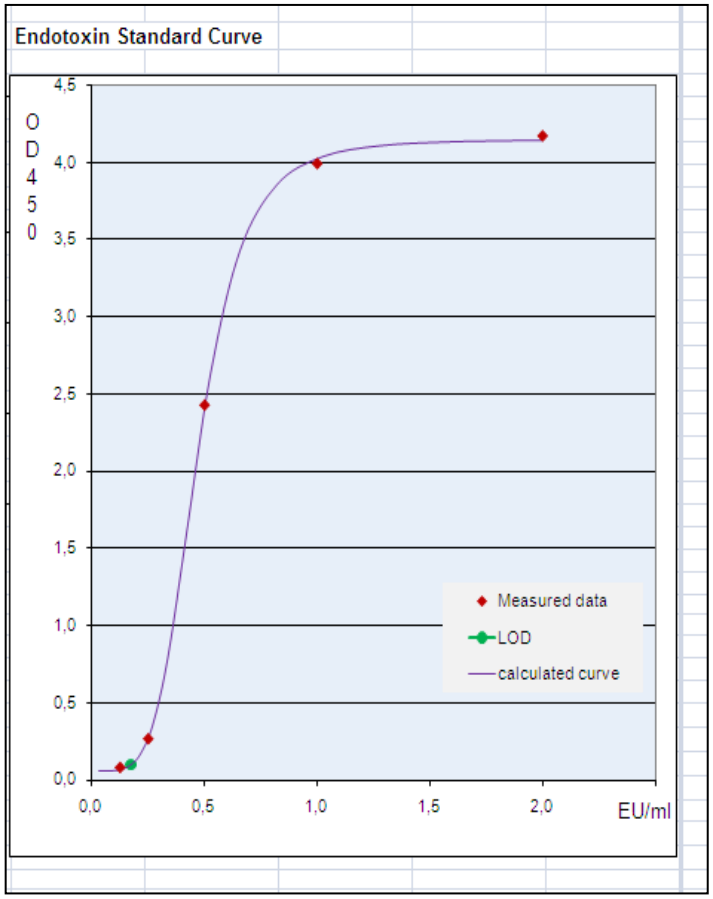
	Sample conc.	4	0,182	Yes	No	Not conclusive	
	Sample 1:2	8	0,167	Yes	Yes	Pyrogenic [EU]>CLC	
	Sample 1:4	16	0,154	Yes	Yes	Pyrogenic [EU]>CLC	

PyroDetect Data Analysis tool, TIF



p for regression (<0,01):	0,0000
p for non-linearity (>0,05):	0,9624

Cut-off [OD]:	**0,170**	**LOD [EU]:**	**0,123**

Data Interpretation PyroDetect System - Test for Interfering Factors

Antibiotic

Tests of validity

Recovery of spike [%]:	Sample conc.	109,34
	Sample 1:2	100,46
	Sample 1:4	111,98
	Sample 1:8	109,34

Tests of validity

Recovery of spike [%]:	Sample conc.	
	Sample 1:2	
	Sample 1:4	
	Sample 1:8	

Background color

Enhancing
Inhibiting
Passed