Evaluating Novel Methods for Endotoxin Testing of Cellular Products – The PACT Experience

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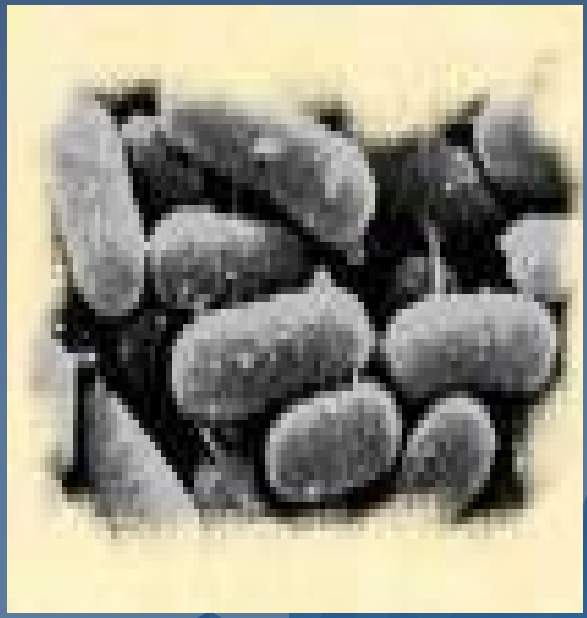
Objectives

- **Regulatory**
- **LAL Assay**
- **Multicenter study underway comparing 2 commercial endotoxin detection Assays.**
- **Preliminary data**
- **Points to Consider**

Endotoxin

- Found in water, food
- Lipopolysaccharide, integral part of cell wall in gram(-) bacteria.
- Fever, shock, DIC, leukopenia, leukocytosis, death



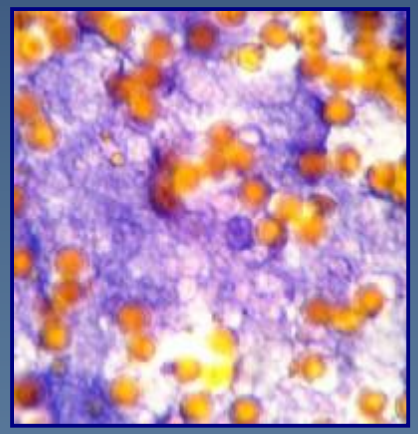
FDA Requirement

Instructions and Template for CMC Reviewers of Human SCT INDs:
CBER August 2003

Chemistry, Manufacturing and Control (CMC) section must provide sufficient information to assure proper identification, quality, *purity* and strength.

Purity

- "Products shall be free of extraneous material except that which is unavoidable in the manufacturing process described in the approved biologics license application." (21 CFR 610.13)
- Assays:
  - Pyrogenicity/Endotoxin
  - Contaminating cell phenotypes
  - Reagents/antibodies/serum

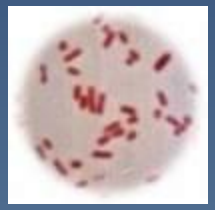


Purity - Endotoxin

- Limulus Amebocye Lysate (LAL) Test
  - Equivalency to Rabbit Pyrogenicity Testing [21 CFR 610.13(b)] for licensure
  - Upper limit per FDA 5 EU/kg body weight/dose (intra-thecal lower, 0.2 EU/kg
  - FDA Validation Guideline for LAL December 1987

LAL

- **Extract derived from circulating amebocytes of horse shoe crab *Limulus polyphemus***
- **Amebocyte + gram(-) bacteria = clot**
- **Assays: gel-clot, turbidimetric, chromogenic,**



+

Enzymatic Cascade
Serine Proteases

Ac-lle-Glu-Ala-Arg····· pNA

Clot /
Yellow color
405nm

Reaction time is inversely proportional to amount endo
Slower rxn time : â Endotoxin
Faster rxn time : á Endotoxin

Commercial LAL Detection Assays

Charles River Laboratories

Endosafe™–Portable Test System

Cambrex Bio Science

Gel clot, Chromogenic, Kinetic

Turbidimetric/Chromogenic

Endotoxin Comparison Study

Study Aim

- **Compare inhouse endotoxin method (Cambrex BioScience) to portable endotoxin detection system CRL Endosafe™**
- **Participants**
  - **NIH**
  - **PACT Institutions**
    - **Baylor College of Medicine**
    - **University of Pittsburgh**
    - **University of Minnesota**

PACT
Production Assistance for Cellular Therapies

Methods

- **CRL Endosafe**
  - Licensed endpoint kinetic chromogenic method
  - Device: incubating chamber, sample pump, 4 LEDs & microprocessor
  - 4 well cartridge
    - 1 sample/cartridge
    - Sample in duplicate + 2 spiked samples
  - Amount of Endotoxin is based upon archived standard curve

- **Cambrex Bio Science:**
  - Licensed for kinetic chromogenic/turbidimetric method
  - Device: incubating plate reader 96 well plate
    - Standards, blank, sample dilutions, spiked samples
  - Amount of Endotoxin is calculated based upon standard curve

Methods

- **Per FDA 1987 Validation guidance**
  - **Trained Operator**
  - **Qualify each new lot of endotoxin**
  - **Positive product control (PPC) be run with each sample at each dilution (50%-200% or w/in two-fold)**
- **Interfering substances**
  - **pH, albumin, EDTA**

PACT
Production Assistance for Cellular Therapies

The PACT Group provides education and production assistance to the cell therapy community through contract manufacturing of therapeutic cell products.

3 NHLBI-Funded Manufacturing Sites

Baylor College of Medicine's
Center for Cell and Gene Therapy

University of Minnesota's
Molecular and Cellular
Therapeutics

University of Pittsburgh's
Cancer Center

Endotoxin

- **Compare inhouse endotoxin method (Cambrex) to Endosafe Charles River Laboratories**
- **Study Design**
  - **Phase 1 –Test known lypholized endotoxin samples**
  - **Phase 2 - Each site test 12 cell and tissue samples (HCT/Ps) comparing Cambrex to Endosafe method**
  - **Phase 3 –Each site prepares blinded samples and ship frozen to each of the 3 participating institutions.**

Study Results Phase 1

Sample	Baylor	Baylor	UMN	UMN	Pitt #1	Pitt #1	Pitt #2	Pitt #2	NIH	NIH
Method	Kinet/ Chrom	Endo-Safe	Kinet/ Chrom	Endo-Safe	Gel Clot	Endo-Safe	Gel Clot	Endo-Safe	Kinet/ Turbid	Endo-Safe
Neg ctl	OK	OK	OK	OK	NA	OK	OK	OK	OK	OK
Pos #1	0.3207	0.221	0.266	0.243	0.16	0.143	0.45	0.36	0.231	0.237
Charles River		0.125-0.5		0.125-0.5		0.125-0.5		0.125-0.5		0.125-0.5
Pos #2	0.5582	0.393	0.617	0.628	0.41	0.481	0.67	0.476	0.465	0.491
Charles River		0.25-1.0		0.25-1.0		0.25-1.0		0.25-1.0		0.25-1.0

Phase 2
Endosafe Cell Products

- **Cultured T-cell subsets (TH2/TC2) with and w/o Rapamycin**
- **Cytotoxic T-lymphocytes**
- **EBV viral vectors**
- **Ferridex labeled CD34 cells**
- **Hematopoietic progenitor cells**
- **Mononuclear cells**
- **NK Cells**
- **Peripheral blood stem cells**
- **SKBR3 cells**
- **Vero cell line**

Endosafe Results

28 Total Samples

In-House	Endosafe Neg	Endosafe Pos
Neg	28 (100)	0 (0)
Pos	0 (0)	0 (0)

*** Positive result > 5EU/mL**

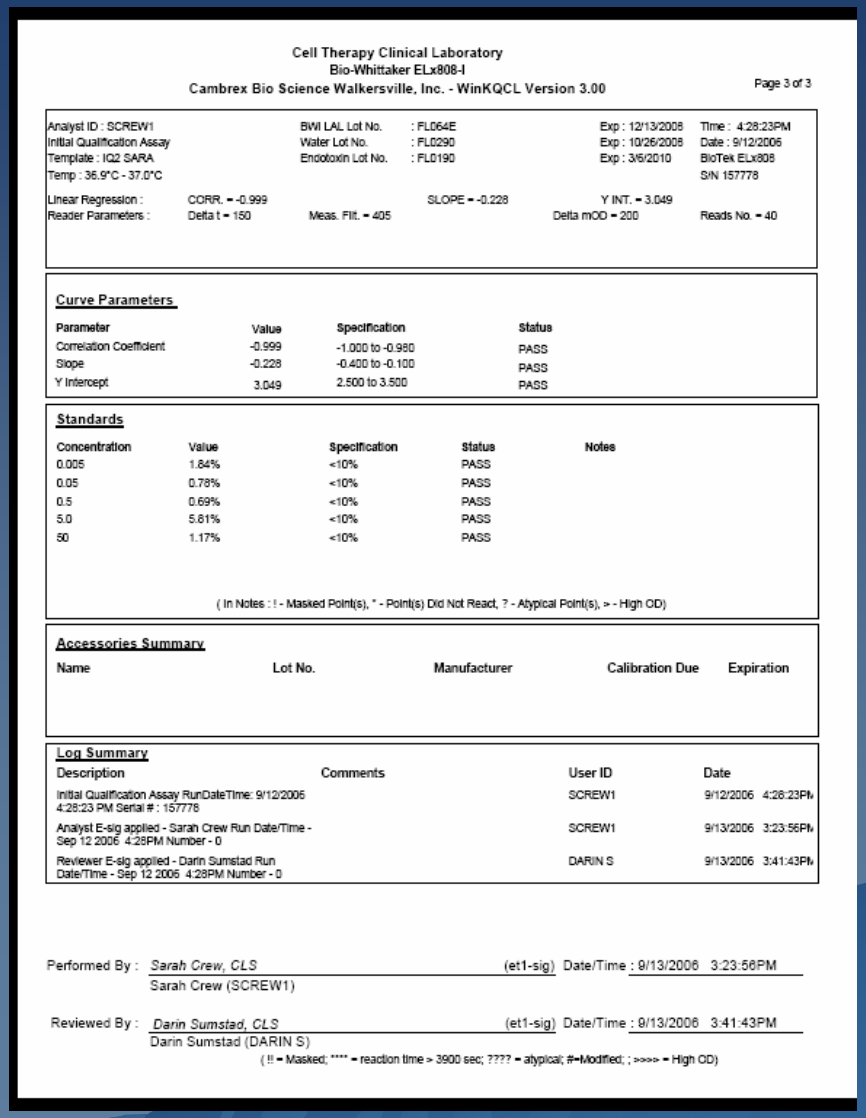
Points to Consider

Observations	Cambrex-Kinetic Chromo. OCL™	CRL Endosafe™
Ease of Use	Moderate – Std curve, multiple dilutions, +/- controls	Very Easy – Sample + spike sample Portable, training easy,
# of Samples/Run	Multiple sample dilutions	1 sample/cartridge
Time	1.5 hour	30 min (+15min for each addt'l dilution)
Software/Reports	21 CFR 11 compliant Data locked, audit, pass/fail reports	Text file
Costs	$16,500 – plate reader, computer, printer $553 rgt kt ~24 samples/kit $23/test	$4000 –plate reader + printer $330 cartridge pack 10/pack ~$33/test

ENDOSAFE

********* ENDOSAFE Test Record *********
V7.09R 01/10/06
DateTime: ..... 06/02/06 @ 15:44:08
Device: 0049
OperatorID: ..... SDA
Cartridge: ..... Endotoxin
Temperature: .. Start: 37.0C End: 37.0C
Method: KX-122
Cartridge Lot#: 5347144
Range: 5-0.05
Range Time: ..... Sec: 140-850
Onset Time (s): 504 250 660 298
Slope: -0.392 ..... Intercept: +2.420
Dilution: 20
Sample Lot: R060986/1
Sample ID: ..... TUFT
Sample Value: ..... 2.63 EU/ml
Sample CV: 19.0%
Spike Value: ..... 0.769 EU/ml
Spike CV: 12.4%
Spike Recovery: 104%

Cambrex



Cell Therapy Clinical Laboratory
Bio-Whittaker ELx808-I
Cambrex Bio Science Walkersville, Inc. - WinKQCL Version 3.00

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Analyst ID : SCREW1 | BWI LAL Lot No. : FL064E | Exp : 12/13/2006 | Time : 4:28:23PM
Initial Qualification Assay | Water Lot No. : FL0290 | Exp : 10/26/2006 | Date : 9/12/2006
Template : IQ2 SARA | Endotoxin Lot No. : FL0190 | Exp : 3/6/2010 | BioTek ELx808
Temp : 36.9°C - 37.0°C | | | S/N 157778

Linear Regression : CORR. = -0.999 SLOPE = -0.228 Y INT. = 3.049
Reader Parameters : Delta t = 150 Meas. Flt. = 405 Delta mOD = 200 Reads No. = 40

Curve Parameters

Parameter	Value	Specification	Status
Correlation Coefficient	-0.999	-1.000 to -0.980	PASS
Slope	-0.228	-0.400 to -0.100	PASS
Y Intercept	3.049	2.500 to 3.500	PASS

Standards

Concentration	Value	Specification	Status	Notes
0.005	1.84%	<10%	PASS	
0.05	0.78%	<10%	PASS	
0.5	0.69%	<10%	PASS	
5.0	5.61%	<10%	PASS	
50	1.17%	<10%	PASS	

(In Notes : ! - Masked Point(s), * - Point(s) Did Not React, ? - Atypical Point(s), > - High OD)

Accessories Summary

Name	Lot No.	Manufacturer	Calibration Due	Expiration

Log Summary

Description	Comments	User ID	Date
Initial Qualification Assay RunDateTime: 9/12/2006 4:28:23 PM Serial # : 157778		SCREW1	9/12/2006 4:28:23PM
Analyst E-sig applied - Sarah Crew Run Date/Time - Sep 12 2006 4:28PM Number - 0		SCREW1	9/13/2006 3:23:56PM
Reviewer E-sig applied - Darin Sumstad Run Date/Time - Sep 12 2006 4:28PM Number - 0		DARIN S	9/13/2006 3:41:43PM

Performed By : *Sarah Crew, CLS* (et1-sig) Date/Time : 9/13/2006 3:23:56PM
Sarah Crew (SCREW1)

Reviewed By : *Darin Sumstad, CLS* (et1-sig) Date/Time : 9/13/2006 3:41:43PM
Darin Sumstad (DARIN S)

(!! = Masked; **** = reaction time > 3900 sec; ???? = atypical; #=Modified; ; >>>> = High OD)