BACTERIAL DETECTION
Experience In the Blood Center

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FLORIDA BLOOD SERVICES
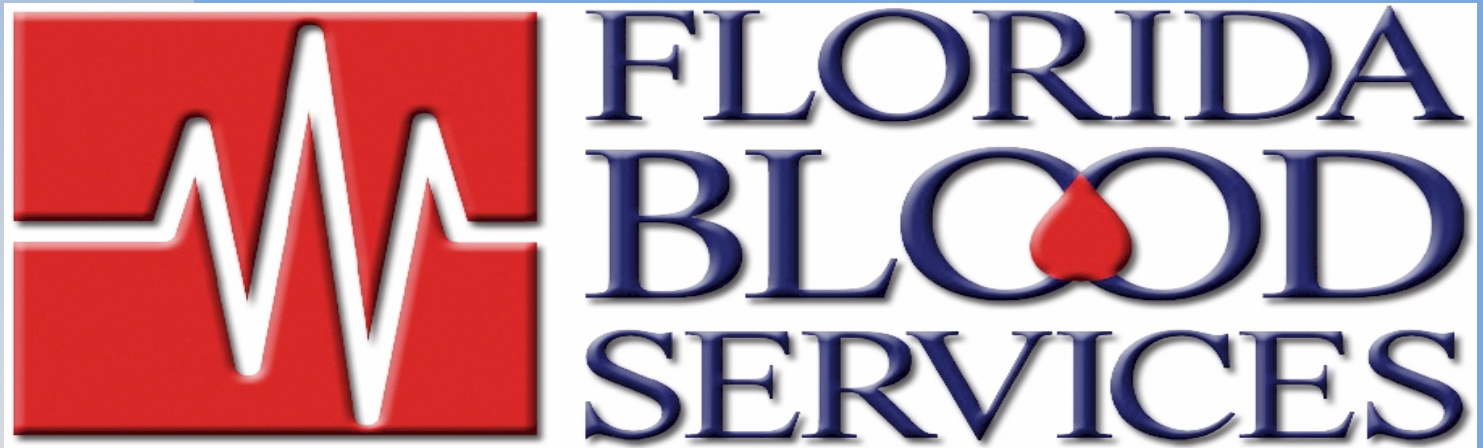
Accreditation Requirements

- **AABB Standards**

"5.1.5.1 The Blood Bank or Transfusion Service shall have methods to limit and detect bacterial contamination in all platelet components…

5.1.5.1.1 Standard 5.1.5.1 shall be implemented by March 1, 2004"

- **CAP Inspection TM-Checklist**

"TRM.44955 – Phase I

Does the laboratory have a system to detect the presence of bacteria in Platelet components?"

Florida Blood Services

- 170,000 Whole Blood Collections
- 11,000 Platelets Pheresis Donations Yielding 17,000 Components
- 70,000 Whole Blood Derived Platelets Distributed
- Total of 500,000 Blood Components Manufactured Annually

Bacterial Contamination

- Most recognized residual TTD risk
- Bacteria in Platelets as Defined in the Literature:
  - Detected: 1 in 1,000
  - Causes reactions: 1 in 10,000
  - Sepsis: 1 in 100,000
  - Death: 1 in 200,000 (??)

Avoidance Strategies

- Limiting opportunities for contamination

- Detection of contamination

- Pathogen inactivation

Limiting Contamination

- Good aseptic technique in phlebotomy
- Effective scrubbing solutions:
  - Tincture of iodine
  - Chlorhexidine
- Diversion of initial blood flow

Bacterial Detection
Culture Methods

- Detection by
  - Oxygen consumption (Pall BDS)
  - CO_2 generation (BacT/Alert)
- Highest sensitivity ($<10^2$ CFU/mL)
- Require lag phase
- Costly

Bacterial Detection
other methods

- Staining: sensitivity 10^6 CFU/mL
  - ◆ Gram's stain
  - ◆ Wright's stain
  - ◆ Acridine orange
- Dry chemistry (Dipstick) 10^7 CFU/mL
  - ◆ Glucose
  - ◆ pH
- Swirling 10^7 CFU/mL

Validation Strategy
Performance Qualification

- Detection
  - ◆ Seeding Known Organisms
    - ★ Negative Control
    - ★ Positive Control CFU/unit
      - Dilution by plasma volume of component
      - 10-100 CFU per unit
    - ★ Lag Time Variables from Seeding to Inoculation
    - ★ Volume of Inoculate
    - ★ Repeatability
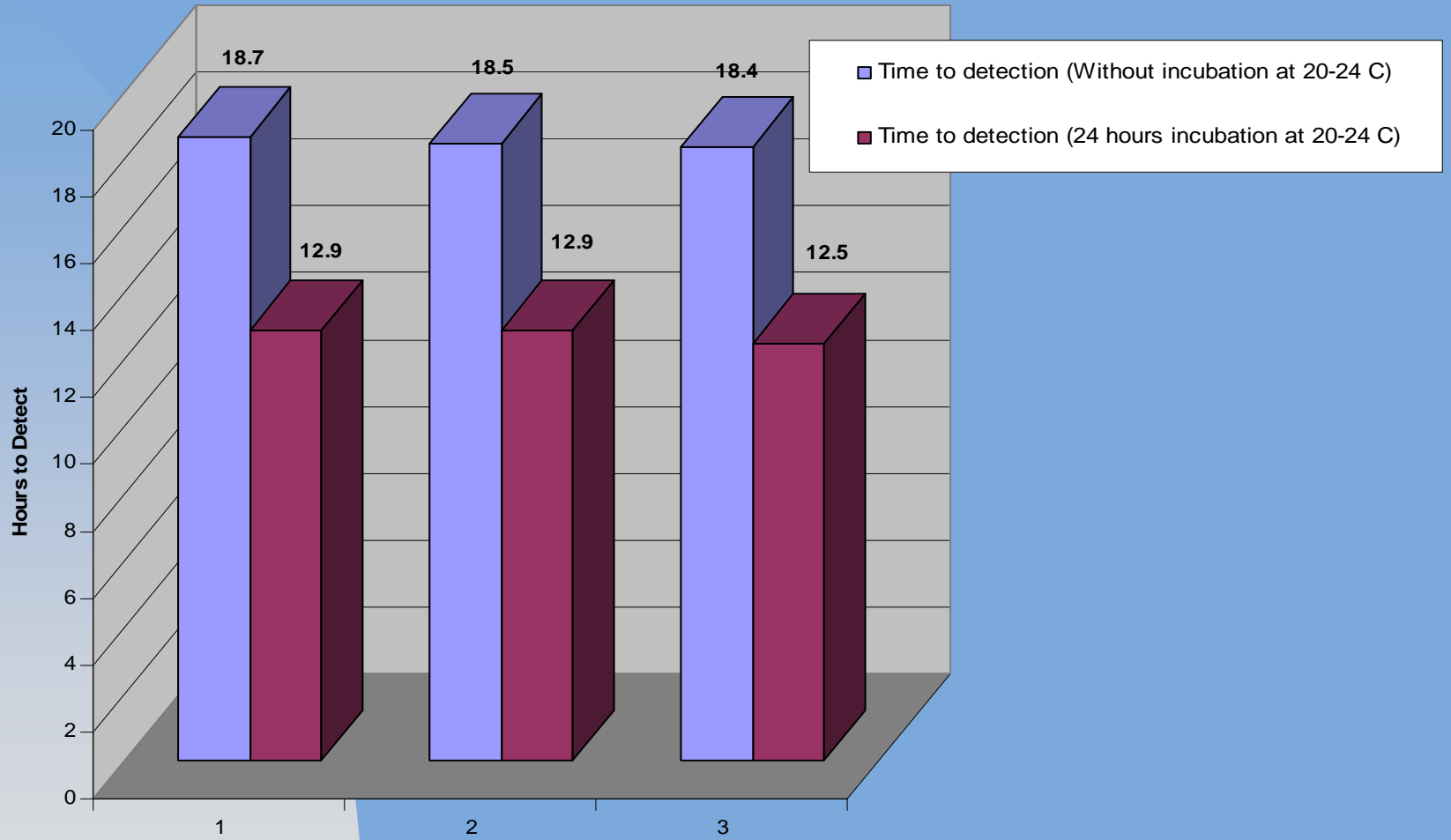- Personnel
  - ◆ Training and Competency

# Validation Strategy
Operational Qualification

- Computer Platform
  - ★ Positive ID – sample integrity from storage bag to culture medium
- Elapsed Time
- Temperature of Incubator

- Messages
  - ★ Error Codes
  - ★ Print Functions and Status
  - ★ Problem log
- Operation System Entry
  - ★ Label Control

| Platelet pheresis BUN	Volume	24 Hr	5 Day	Time to Detection No Lag	Time to detection (after 24 hours incubation at 20-24 C)	Time to Detection No Lag	Time to detection (after 24 hours incubation at 20-24 C)	Time to detection	Time to detection (after 24 hours incubation at 20-24 C)	
O819162	412 mL	neg-to-date	neg	18.7 hours	12.9 hours					
				18.5 hours	12.9 hours					
				18.4 hours	12.5 hours					
O821285	197 mL	neg-to-date	neg			27.4 hours	16.3 hours			
						27.3 hours	15.2 hours			
						20.1 hours	15.0 hours			
O819999	298 mL	neg-to-date	neg					not detected	not detected	
								not detected	not detected	
								not detected	not detected	
Ecoli pellets inoculated directly into the bottle								11.8 hours		
								Seeded with E.coli ~22 CFU's		
								Time to detection (after 24 hours incubation at 20-24 C)		
O820734	267 mL	neg-to-date	neg					17.8 hours		
								not detected		
								not detected		

S. aureus 10-20 CFU/mL
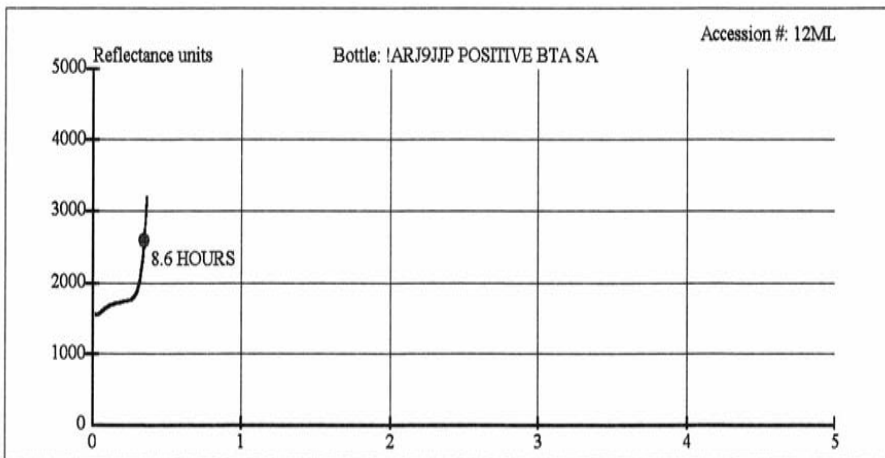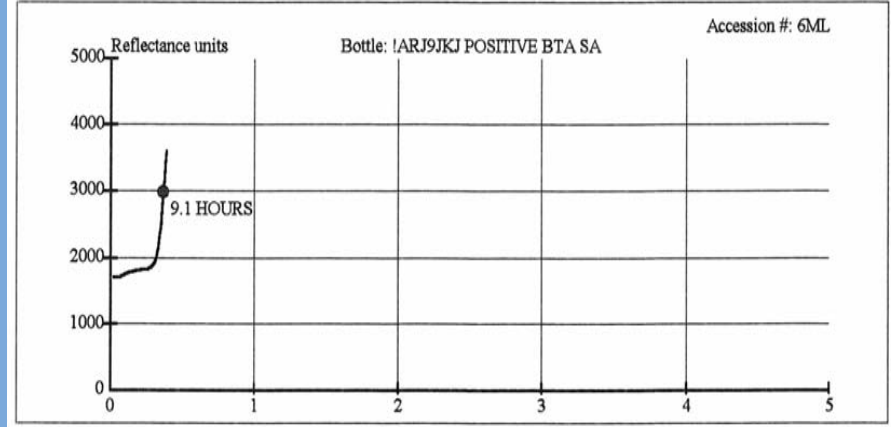
Time to Detection (S. aureus)
Varied Volume of Inoculate
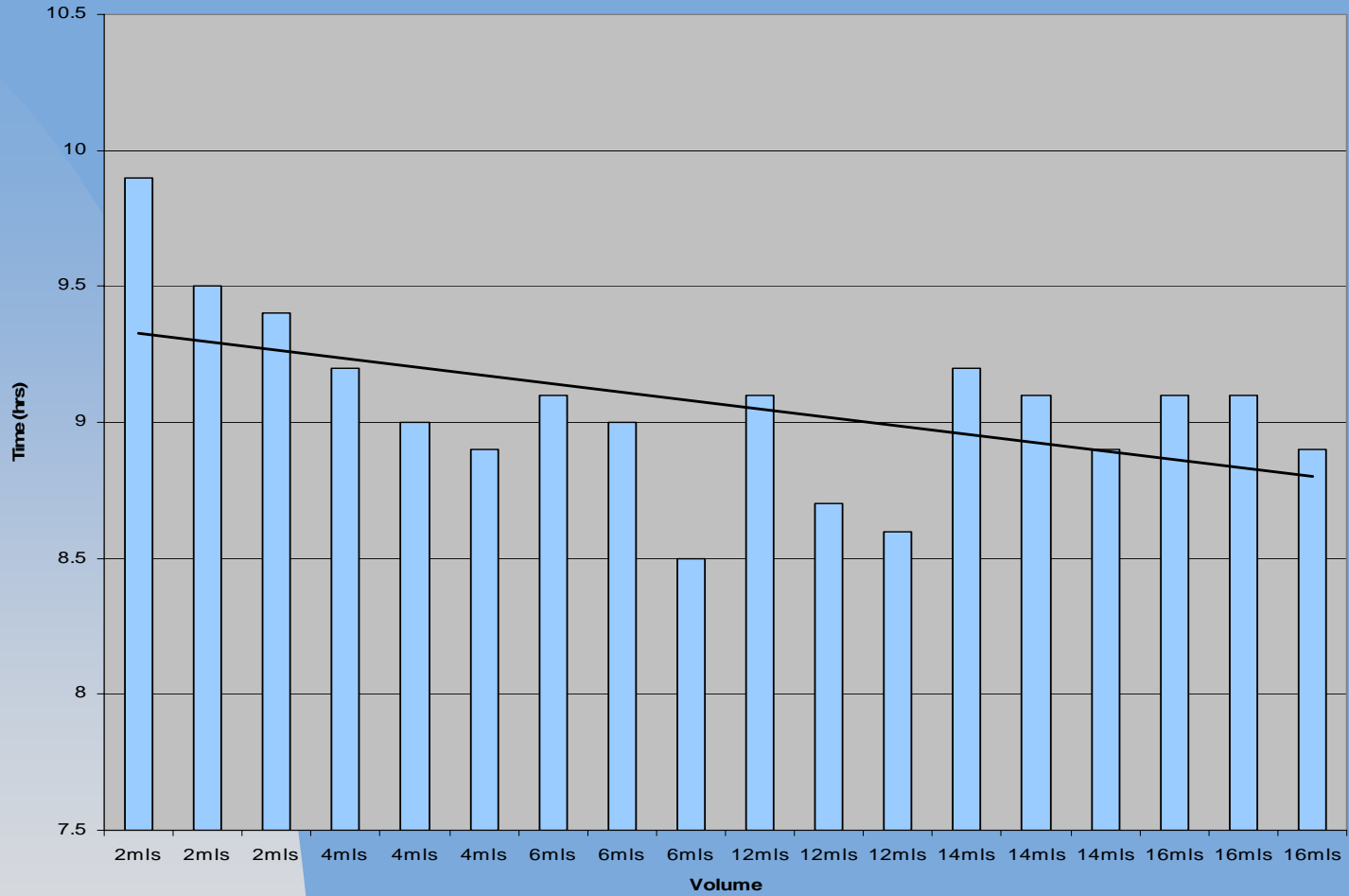
Variable Volumes
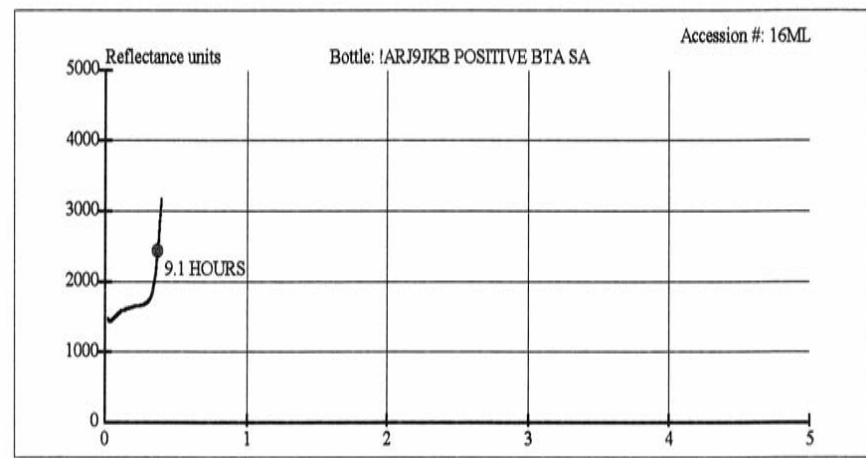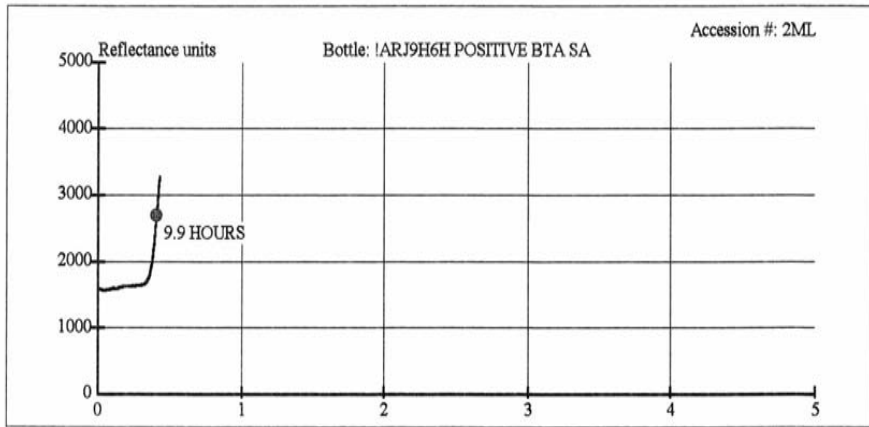
Operations

- ➢ Isolate and Sample for Daily QC Cell Counts
  - • **Platelet Count**
  - • **WBC Count (Flow)**
- ➢ Incubate 24 hrs lag phase at 22-24^0 C in Three (3) Daily Batches
- ➢ Sterile Connect Sample Pouch
- ➢ Fill Sample Pouch to 8 -10 mL
- ➢ Seal to Remove Pouch and Isolate Platelets Pheresis

Operations

➢ Inoculate Blood Culture Medium

➢ Incubate Blood Culture 12 hours

➢ Obtain 12 hr "Neg-to-Date" Report

➢ Enter Preliminary Result (BD1) into Operation's Computer System to Allow for Labeling and Release

➢ Monitor Culture through 5th Day and Enter Final Result (BD5) into Operation's Computer System
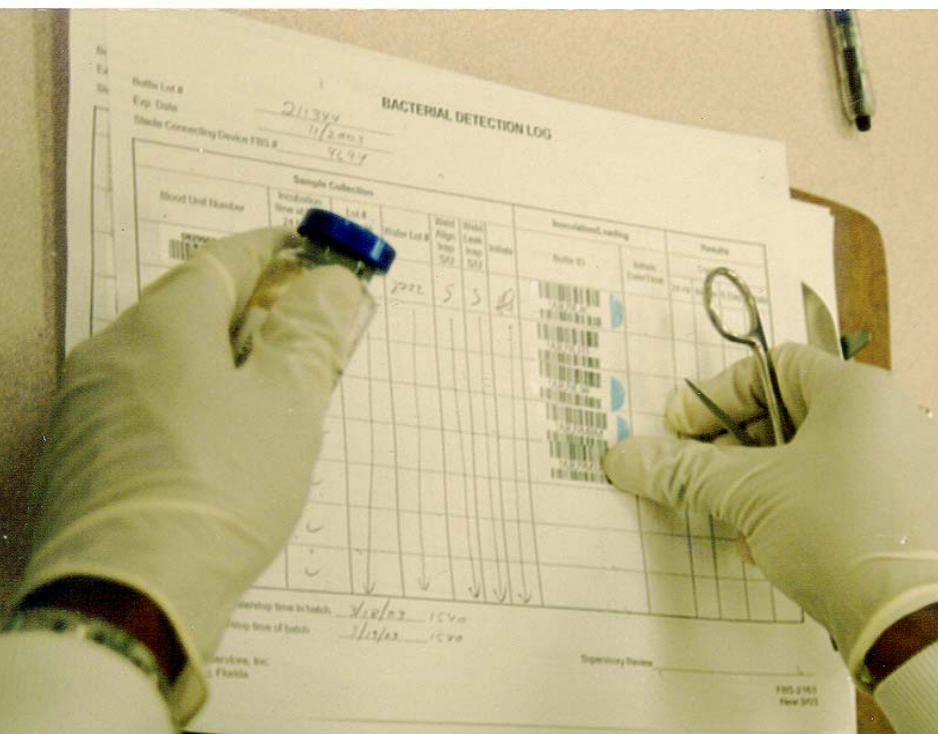
Culture Methods
Implementation issues

- Handling of positive results
  - ◆ Notification of physician if unit was released
  - ◆ False vs. True positive
  - ◆ Donor notification, deferral, flagging (2x deferred)
  - ◆ Organism ID and sensitivity
  - ◆ Computer interface/Data recording

Positive Results on Released Units

- Contract Transfusion Service at hospital:

  - Notify patient's physician/nurse (manage as "panic value')

- At Hospital consignee:

  - Notify Lab

- Out of Service Area:

  - Notify Blood Center

Root Cause Analysis

- Evaluate:
  - ◆ Phlebotomy staff
  - ◆ Donor
- Perform RCA on both, true positives and false positives determined by replicate growth study

Root Cause Analysis

- Phlebotomy staff:
  - Review records
  - Observe technique
- Donor:
  - Obtain detailed medical history
  - Physical exam
  - Cultures: skin, urine, and blood

Bacterial Detection Stats

BACTERIAL DETECTION Of PLATELETS PHERESIS		
March 10, 2003 - March 10, 2004		
Number Tested	10,737	
Number Positive	11	
% Positive	0.10%	
Catagorization of Positives		
-Contaminates	5	(4) Bacillus sp. - positive at 94hr; 31.7hr; 25.4hr; 22.9hr
		(1) Klebsiella pneumo 13.9 hr
% Contamination	0.047%	
-True Positives	6	(1) S. aureus - positive at 10.2hr
		(1) E. coli - 6.6hr
		(4) Staph Epi - 24.9 hr, 24.3hr, 13.2hr, 21.6hr
% Positive	0.056%	

CHALLENGES REMAIN

- Inventory Control of 3-day shelf life
  - ◆ 7-day Expiration Pending Bacti Data
- Hospital Inventories – to credit or not to credit returns
- Whole Blood Derived Platelets
- Work all the "bugs" out – Pun intended!

Status of Bacterial Detection

- Currently Exists A Dichotomy of Safety
  - Two Different Safety Profiles For Platelet Doses
    - Issue 70% as Platelets Pheresis
      - Tested by Blood Culture
    - Issue 30% as Whole Blood Derived Platelets
      - Tested by surrogate markers for bacteria (pH/Glucose)

Platelets Sampling Device

4" Tube
with RF weld

3-Port Y Connector
BR3.9000

1.5" Tube

Y-Connector
BR3.9001

Female Luer Lock
BR3.3007

Pinch Clamp
BR9.1004

20 mL BD Syringe
84-020-14

12" Tube
81-000-18

Swivel Lock Connect
80-008-79

BD Eclipse Needle Assembly
84-751-04
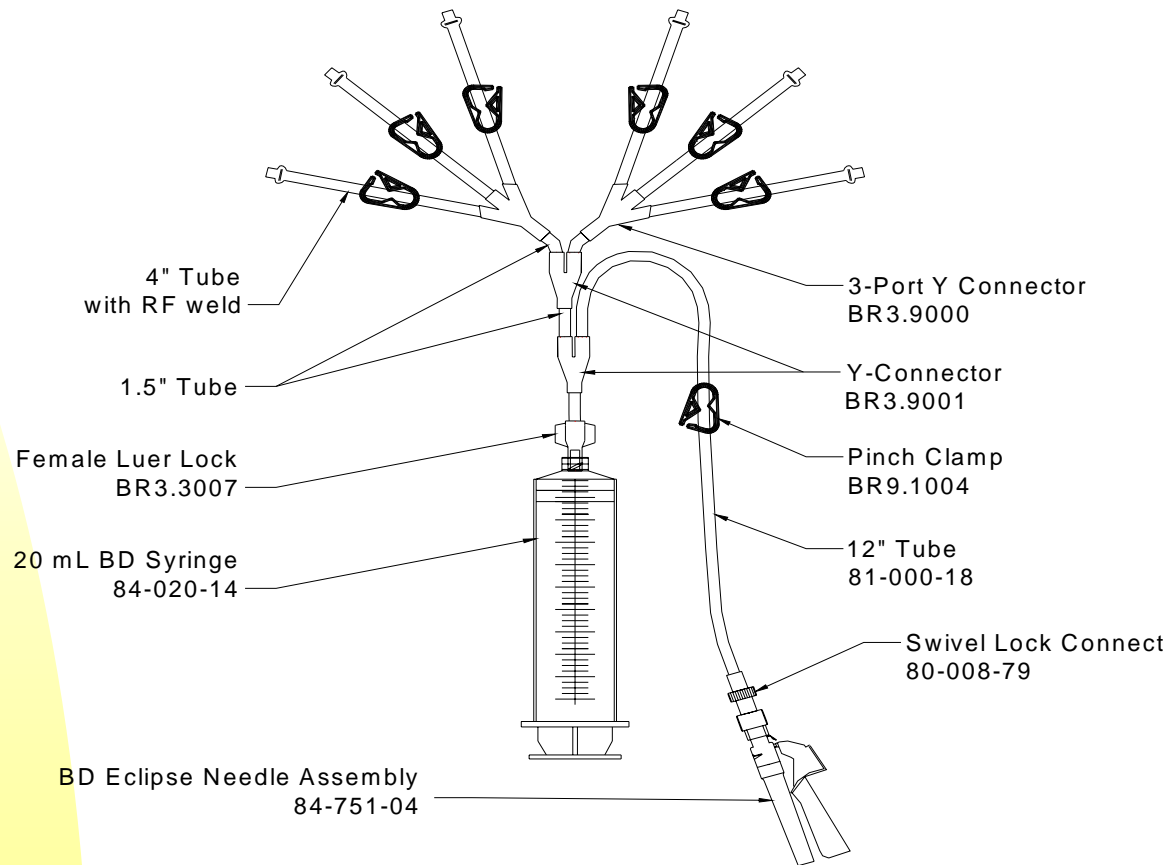
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Platelets Segments

Stripping Tubing Segments

Lag Phase at 37⁰ C

Sterile Connection of Segments
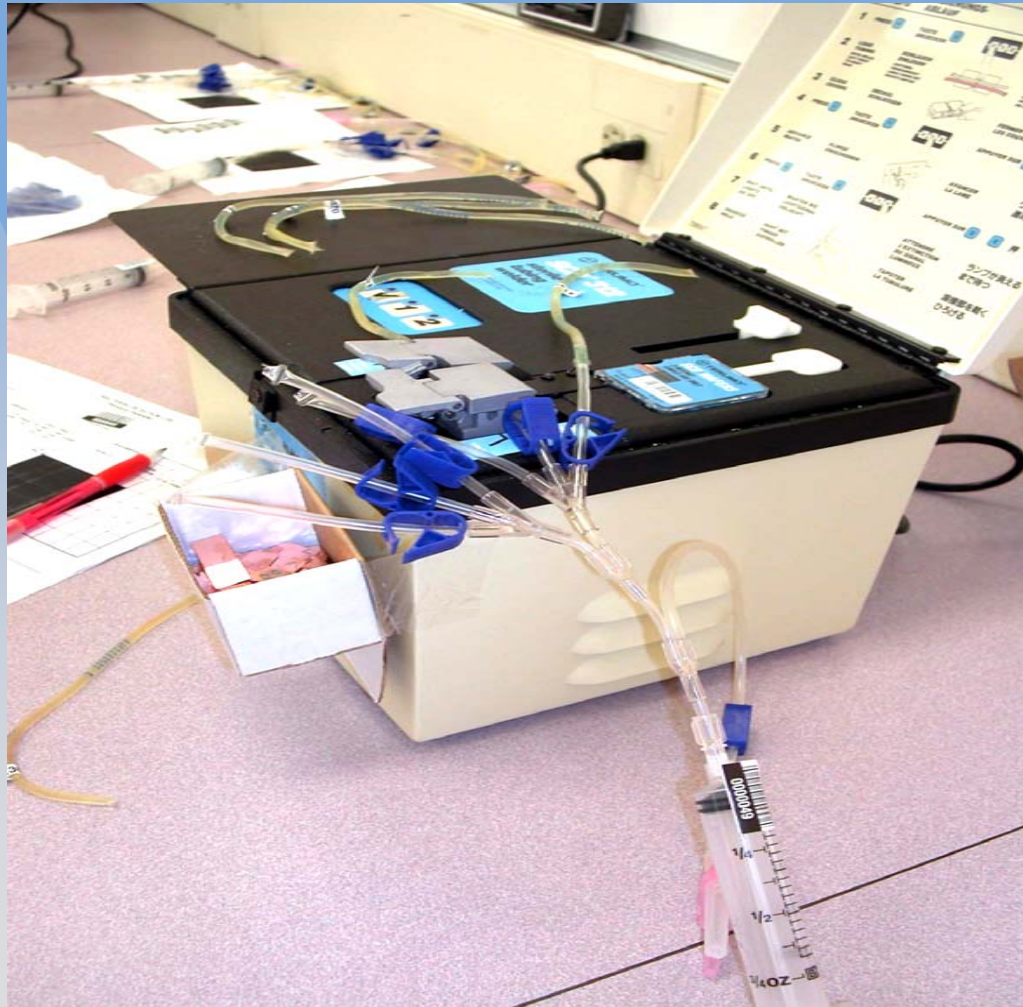
Platelets Sampling Process

A METHOD OF BACTERIAL DETECTION OF WHOLE BLOOD DERIVED PLATELETS

Variables: CFU/bag – 75-100 CFU
Temp of lag phase – 22-24 ^{0}C vs 37 ^{0}C
Time of lag phase – 12hr vs 24 hr
Time to detection of positive blood culture

RESULTS:

Organism	Lag Phase	Temp of Lag	Time to Pos
S Epidermidis	12 hrs	37 ^{0}C	18.3 hrs
S Epidermidis	12 hrs	22-24 ^{0}C	18.8 hrs
E Coli	24 hrs	37 ^{0}C	4.1 hrs
E Coli	24 hrs	22-24 ^{0}C	7.8 hrs
Pool of six (6), 1/6 seeded with S Epidermidis	24 hrs	37 ^{0}C	7.4 hrs

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BLOOD
SERVICES

Platelets Bacterial Detection Validation

POOL#	BUN	POOL RESULTS	SINGLET RESULTS	INTERP	PH	GLUCOSE	INTERP
0000003	4100093	no growth	no growth	S	7.0	250	S
	4100095		no growth	S	7.5	100	U
	4100112		no growth	S	7.0	250	S
	4100102		no growth	S	7.5	250	S
	4117816		no growth	S	7.5	250	S
	4100124		no growth	S	7.5	100	U
0000004	4118842	no growth	no growth	S	7.5	250	S
	4118843		no growth	S	7.5	250	S
	4118847		no growth	S	7.0	100	U
	4113058		no growth	S	6.0	neg	U
	4113060		no growth	S	7.0	250	S
	4118825		no growth	S	7.5	250	S

Platelets Bacterial Detection Validation Summary

POOL#	Total Number	POOL RESULTS	SINGLET RESULTS	INTERP	PH < 7.0	Glucose < 250 mg/dl	Both pH < 7; glucose < 250 mg/dl
1-100	594	no growth	no growth	S	36	151	33
		0%	0%		6.1%	25.4%	5.6%

Platelets Bacterial Detection Validation (Seeded)

S. aureus					
	CFU's	Temp	Detection (hours)		
Pool of 6	~15	37 C	5.3		
Pool of 6	~15	24 C	12.7		
Singlet	~15	24 C	12.7	pH=6.0	Glucose=neg
S. epidermidis					
	CFU's	Temp	Detection (hours)		
Pool of 6	~15	37 C	8.7		
Pool of 6	~15	24 C	17.3		
Singlet	~15	24 C	15.2	pH=6.0	Glucose=neg
E.coli					
	CFU's	Temp	Detection(hours)		
Pool of 6	~15	37 C	9.8		
Pool of 6	~15	24 C	10.3		
Singlet	~15	24 C	9.1	pH=7.5	Glucose=250

Conclusions

- pH and glucose levels, as surrogate markers are not consistently maintained in platelet storage day-5

- Correlation of Surrogate Markers to actual Bacterial Contamination is poor

- Time to detection is reduced by half in a 37C lag phase

Blood Component Costs

- Platelets Pheresis and Red Blood Cells
  - Cost Load (1:1 Whole Blood Collected)
    - Recruitment
    - Collections
    - Processing and Testing
    - Inventory and Distribution
- Platelets/Plasma/Cryo
  - By-Product Cost (Variable Ratio to WB Collected)
    - Incremental Bag Cost
    - Quality Control
    - Production Labor
    - Inventory and Distribution

TESTING COST

- Equipment
  - ★ > $175K in hardware
  - ★ 80,000 tests/yr/3yr depr = $0.75 / unit
- Labor (platelets pheresis – platelets (6))
  - ★ $2.99 - $1.05 / unit
- Consumables (platelets pheresis – platelets (6))
  - ★ $10.16 - $4.90 / unit
- Total Direct Costs (platelets pheresis – platelets (6))
  - ★ $13.90 - $6.70

TESTING COST

- Real Cost Includes Increased Expiration (platelets pheresis - out date 3-day post detection)

  - ★ Mar – Sep 2002:   5.52%
  - ★ Mar – Sep 2003:   12.75%
  - ★ Platelets - ? +15% = 30%?

- Need For A Variance to Allow For 7-day Storage

Emerging Technologies

- Immunoassay in dry media
- Spectrophotometric analysis
  - ◆ Swirl and Shimmer
- Concentration and mass spectrometry
- Molecular probes
- Pathogen inactivation=Holy Grail