FDA's Current Thinking on Bacterial Detection in Platelets

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Current status of bacterial detection in platelet products

- Two culture–based automatic bacterial detection systems are cleared by the FDA for quality control testing

- AABB standard 5.1.5.1 requires bacterial detection on every platelet unit (100% QC)
  - Culture-based automatic bacterial detection is limited to apheresis products
  - Whole blood platelets are being tested for pH and glucose levels by dipstick

FDA concerns with bacterial detection as currently applied

- Test performance characteristics unknown
- Use of non-validated tests (glucose and pH by dipstick, swirling)
- Non-standardized methodology even with culture-based devices
- Potential for excessive false positives
- Less reliable methods are used on whole blood derived platelets creating a two tiered safety system for apheresis and whole blood derived platelets

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Desired improvements to the current state of bacterial detection and storage

- Standardized methodology for automatic culture systems (timing of sample, volume collected, duration of culture)
- Application of automatic culture systems to whole blood derived platelets
  - Eliminate use of non-validated methods
  - Pre-storage pooling of platelets or bacterial testing sample pooling
- Validation of automatic culture systems for a release test claim

Extension of platelet dating from 5 to 7 days

- Platelet storage was reduced from 7 to 5 days in 1986 by FDA on advice of Blood Products Advisory Committee (BPAC) over concerns of increased bacterial contamination at day 7
- Return to 7 day storage will require that
  - 1) platelet storage containers are validated to preserve platelet efficacy out to 7 days based on current standards (2 bags already cleared)
  - **2) bacterial detection method is validated as a release test for 7 day platelets**

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Criteria required for a release test

- **Sensitivity**- accuracy in detection of contaminated units $Se = TP/(TP + FN)$
- **Specificity**-accuracy in detection of non-contaminated units $Sp = TN/(TN + FP)$
- **Positive predictive value**- how many units with a positive test result are contaminated with bacteria $PPV = TP/(TP + FP)$
- **Negative predictive value**- how many units with a negative test result are free of bacteria $NPV = TN/(TN + FN)$

Evaluation of bacterial detection devices

- Analytical testing
- Field studies

Analytical testing of bacterial detection devices

- Analytical testing: sensitivity in detecting certain level of contamination in intentionally contaminated units (1 CFU/mL, 10 CFU,mL)

- Define optimal volume for detection

- Define optimal medium conditions (leukoreduced vs non-leukoreduced)
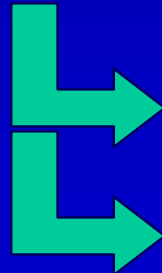
Analytical testing of bacterial culture devices

"SPIKE" IN BACTERIA, 1-10 CFU/ML

DAYS IN STORAGE

D1	D2	D3	D4	D5

SAMPLE

CULTURE RESULT 24-48 HOURS LATER

DETERMINE CFU/ML AT TIME OF SAMPLING

Issues to consider in designing field studies

- Level of contamination at time of collection unknown

- Level of contamination changes as bacteria proliferate (very low at donation to very high within 3-4 days,

- Rate of growth can differ for some slow growing organisms (Staph epidermidis)

- Timing of sampling can significantly effect success in detecting contaminated units
  - sample too early and there may not be enough bacteria to detect)

- Volume of sampling will effect sensitivity
  - Relevant in sample pooling strategies

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Estimation of bacterial risk in extending the shelf life of PLT concentrates from 5 to 7 days. C.K. Lee et al. *Transfusion* 43:1047-1052, 2003

6020 platelet units sampled on day 2* of storage
1.5 mL x 5 (pooled samples) tested with BacT/Alert

3010 units sampled on **day 5**
7 mL tested with BacT/Alert

3010 units sampled on **day 7**
7 mL tested with BacT/Alert

4 units contaminated
3 P. acnes
1 coagulase neg. Staph

4 units contaminated
2 P. acnes
2 coagulase neg. Staph

Total residual risk   4/3010   4/3010
Risk from Staph   1/3010   2/3010

(*Day 2 cultures detected a 1/2800 contamination rate)

Lessons from the study by **C.K. Lee et al.** *Transfusion* **43:1047-1052, 2003**

- Demonstrates how a field study identifies "weak" spots in bacterial testing process
- Demonstrates that a second culture, either at day 5 or 7, is necessary to confirm the early culture
- Sample pooling can decrease sensitivity of the early culture
- Relatively small study will detect only a few contaminated units

Original field study design-BPAC Dec. 2002

- Compare bacterial contamination rates on day 1 vs day 5 to validate the day 1 test

- Additional culture on day 7 to support extension of dating

- Set criteria for an acceptable difference between day 5 and 7 contamination rates to permit extension of dating

- Study size depended on goal to establish a minimum 80% sensitivity of the day 1 culture

FIELD TRIAL OF AUTOMATIC CULTURE DEVICES FOR 7 DAY STORED PLATELETS- Using outdated units

DAYS IN STORAGE   **OUTDATE**

| D1 | D2 | D3 | D4 | D5 | | D7 |

1ST SAMPLE   2ND SAMPLE   3RD SAMPLE

CULTURE   CULTURE   CULTURE

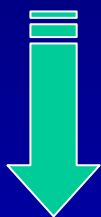**CONFIRMATION OF RESULTS**

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Modified study design

- <u>New endpoint:</u> estimate residual bacterial risk for a 7 day old platelet unit tested for bacteria on day 1

- Approve 7 day platelet storage if the bacterial risk at day 7 is lower than the current bacterial risk of untested platelet products

- Current contamination risk 1/2,000-1/4,000

- Goal is to demonstrate a point estimate of risk at day 7 to be 1/10,000 with a 95% upper confidence limit that the risk is <1/5,000

- Study size ~ 50,000 platelet units

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FIELD TRIAL OF AUTOMATIC CULTURE DEVICES FOR 7 DAY STORED PLATELETS - Using "off- line" units
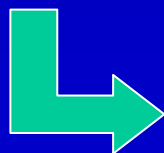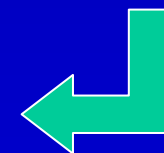
DAYS IN STORAGE

D1	D2	D3	D4	D5	D6	D7

1ST SAMPLE

2ND SAMPLE

CULTURE

CULTURE

CONFIRMATION OF RESULTS

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Cost saving advantages of the new study design

- Eliminates day 5 cultures
- Day 7 samples could be pooled
  - Bacterial load by day 7 is high so dilution would not decrease sensitivity
  - 10 samples into 1 bottle could reduce the total number of bottles to ~5,000
- Only whole blood derived units tested
  - Units will be produced for the study as a byproduct of whole blood collection
  - extrapolation of data to apheresis platelets
- Pre-storage pooling can be validated separately

Application of bacterial testing to whole blood-derived platelets

- Final transfusion product is a "pool" of 4-6 units collected from individual donors
- The pooling step is made up to 4 hours prior to transfusion
- Pooling at beginning of storage for 5 days has not been cleared due to concerns over bacterial proliferation in the pool to higher levels than in individual units
- There are no FDA cleared storage containers for storing platelet pools out to 5 days or longer

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Post-Storage vs Pre-Storage Pooling of Whole Blood-Derived Platelets

transfusion

pooling

Storage up to 5 days

**transfusion**

pooling

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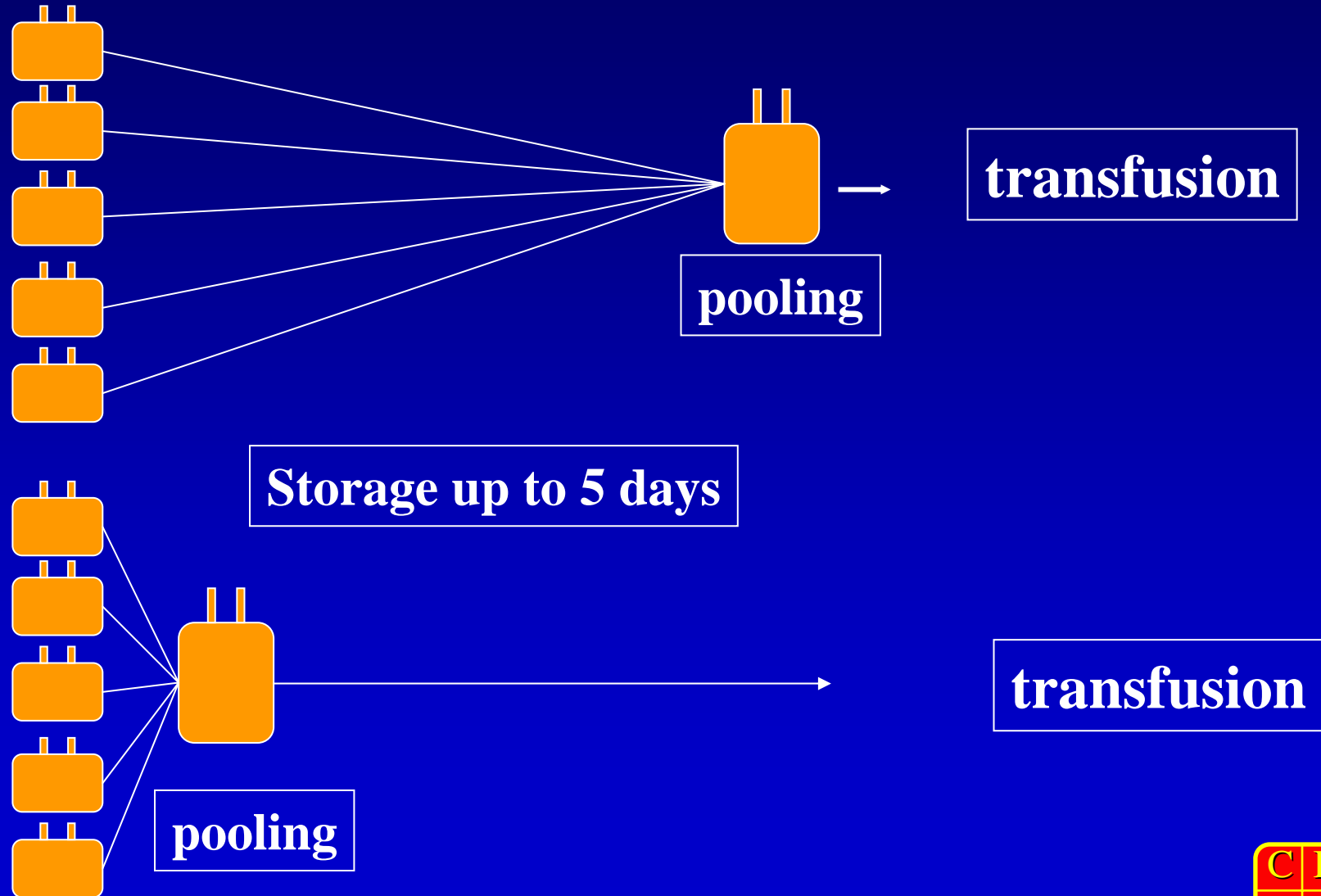
Pre-storage pooling vs sample pooling

- The pool of whole blood derived platelets could be most economically tested by
- 1) a single test of the pool early in storage
- 2) a single test on a pooled sample prepared from the individual un-pooled units

In vitro spiking protocol for pooled platelets

SPIKE IN BACTERIA AT 1-10 CFU/ML

Pooled samples

Pooled transfusion product

Test for bacteria

Single units

Test for bacteria
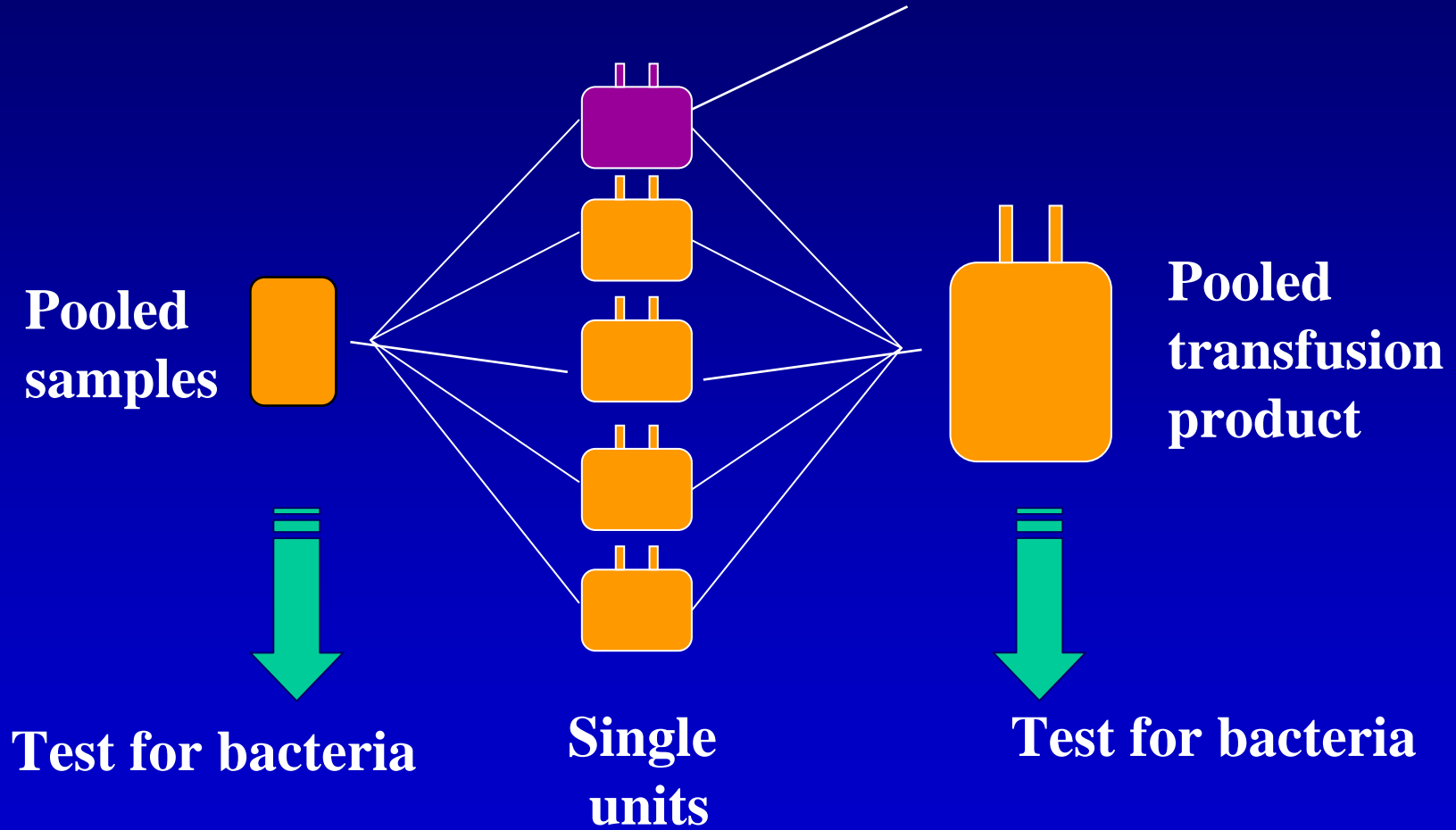
Pre-storage pooling

- Bacterial detection devices applied to pools will need to be validated by analytical testing to demonstrate sufficient sensitivity to account for the dilution of the bacterial inoculum by the pooling process
  - Testing of pre-storage pools
  - Testing of pooled samples
- FDA had taken the position that pre-storage pooling systems should not be cleared unless linked to the use of a validated bacterial detection release test.

- FDA's current thinking is that such systems can be cleared if culture monitoring QC is performed by tests with analytical sensitivity similar to that cleared for single units.

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Pre-storage pooling cont.

- Approval of pre-storage pooling will require validation of platelet storage containers to preserve platelet efficacy in a pool for 5 days or longer

- Validation approach discussed in March 2003 BPAC
  - Testing of platelet "efficacy" by following corrected count increments in thrombocytopenic patients
  - Compare pre-storage pooled platelets to post-storage pooled platelets
  - Approximately 50 patients per arm

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Summary of progress towards having a validated bacterial detection release test

- FDA and AABB joint protocol development for validation of bacterial detection devices as a release test

- Modified field study design to reduce costs

- Clarification of bacterial testing requirements for pre-storage pools (analytical testing)

- Outline of studies necessary for clearance of pre-storage pooling containers