Use of the BACT/ALERT® BPA and BPN Culture Bottles for Secondary Safety Measure Testing of Platelets

Mary Beth Anheuser
Staff, Regulatory Affairs Specialist
bioMérieux, Inc.

PIONEERING DIAGNOSTICS

Background

Background

- BACT/ALERT® 3D Systems (BTA) have been widely used for in-process QC testing of platelets since 2004, when the AABB Standard 5.1.5.1. was made effective. This Standard required 100% testing of all platelet products for bacteria before releasing for transfusion.

Aerobic

- In 2005, the BTA BPA / BPN culture bottles received FDA Clearance – the BPA culture bottle was cleared under BK050037, and the BPN culture bottle was cleared under BK050043.

Anaerobic

- The BTA BPA / BPN culture bottles were cleared as an in-process QC method for testing Leukocyte Reduced Apheresis Platelets (LRAP), and for both Leukocyte Reduced Single Units and pools of up to six units of Leukocyte Reduced Whole Blood Platelet Concentrates (LRWBPC).

Background (continued)

- **In March of 2016, FDA issued Version 2 of the Draft Guidance (DG) "Bacterial Risk Control Strategies for Blood Collection Establishments and Transfusion Services to Enhance the Safety and Availability of Platelets for Transfusion." The Draft Guidance stated the following.**
  - **DG**: "Surveillance data on platelets stored for up to 5 days have shown that 95 percent of platelet transfusion-related septic reactions and 100 percent of associated fatalities have occurred with transfusion of day 4 and day 5 stored platelets, with almost even distribution between these two days."
  - **DG**: Recommendations for extending platelet dating beyond Day 3, and up to Day 7.
    - Secondary testing of previously cultured, but not pathogen-reduced platelets, using an FDA-cleared rapid bacterial detection device; or, using a device cleared by FDA and labeled as a "safety measure", one of the following:
      - Repeat culture on day 4 (release 12 hours after sampling, transfuse through day 5);
      - Repeat culture on day 4 (release 24 hours after sampling, transfuse through day 6);
      - Repeat culture on day 5 (release 24 hours after sampling, transfuse through day 7).
  - **DG**: "Currently, culture-based detection devices labeled as a "safety measure" for the extension of dating beyond day 5 are not available. The recommendations to extend platelet dating beyond day 5….using a culture-based device may not be implemented until the availability of such devices."
  - **NOTE**: Although the DG discussed the use of larger sample volumes (LSV) and delayed testing (DT), these options alone, or in combination, were not offered as alternatives to secondary testing. Based on the literature, it is the opinion of the bioMérieux Platelet Scientific Advisory Board that LSV / DT is an option for producing platelets that are safe for transfusion through day 7.

Development of the BTA BPA and BPN Bottles as a Safety Measure Test

Development of the BTA BPA and BPN Bottles as a Safety Measure Test

Objective:

- Provide evidence that the BTA 3D Systems, including the BPA & BPN bottles, are an effective safety measure for secondary testing of platelet products (apheresis and whole blood derived) to extend platelet dating beyond day 3 and up to day 7;

Method

- A literature review was conducted to identify clinical studies where the BTA 3D system was used as the primary in-process QC test and also for secondary testing, at any point post collection, to extend platelet dating or for end-date QC testing and surveillance.

- In addition, blood collection establishments and transfusion services were queried for unpublished data where the BTA 3D System was being used for secondary testing and/or end-date QC testing of previously tested platelets.

- Nine publications and two* unpublished studies were identified that met these criteria.
  - Four of the studies were based in the U.S., where testing was performed using apheresis platelets.
  - Seven studies were ex-U.S. and testing was conducted on apheresis platelets and whole blood derived buffy coat platelets.
  - * **NOTE**: Of the two unpublished studies reviewed for the safety measure claim, one of the studies has since been published.

Development of the BTA BPA and BPN Bottles as a Safety Measure Test (continued)

Success Criteria

- The data review needed to demonstrate the following:
  - 1) A benefit for the use of the BTA 3D Systems for detecting contamination which was not revealed by previous bacterial testing;
  - 2) Clinical specificity.

Data Analysis

- In general, the studies classified test results based on AABB Bulletin 04-07 definitions and subcategories of false positive results, with some study specific modifications.
- Based on the data reviewed, the general assumptions listed below were made:
  - Data are summarized as total number of positive tests, observed by the total number of tests performed on each day post collection.
  - Since the data of interest are secondary and QC surveillance test results, it was assumed that one test was performed per platelet unit;
  - All units eligible for secondary and QC surveillance testing were negative by the primary test.
  - The objective of the data review was to show that the BTA 3D System detects contamination missed in previous testing as determined by a positive secondary or QC surveillance test result; therefore, the differences in testing protocols (i.e. volume tested, or the bottle(s) used (aerobic only, aerobic/anaerobic pair), were not considered.

Development of the BTA BPA and BPN Bottles as a Safety Measure Test (continued)

- For development of the BTA BPA / BPN Safety Measure Test, the AABB classified result definitions had to be reclassified to evaluate / demonstrate the performance of the BTA 3D System for detecting organisms:

  - Instrument True Positive – the bottle is signaled as positive by the instrument and growth is confirmed by subculture of the bottle.

    Includes platelet results designated:

    - true positive (positive on both initial / confirmatory test),
    - false positive (positive on initial test, negative on confirmatory test),
    - discordant negative (growth of organism from bottle, no growth from platelet component),
    - discordant positive (growth of different organisms from bottle and platelet component) and indeterminate (positive on initial test, no confirmatory test performed or confirmatory test cannot be interpreted, aka unconfirmed positive).

Development of the BTA BPA and BPN Bottles as a Safety Measure Test (continued)

- BTA 3D Instrument False Positive (FP) - the bottle is signaled as positive by the instrument and no organism is recovered from the subculture.

  Includes platelet results designated false positive due to instrumentation error.

- BTA 3D True Negative – The bottle result is signaled negative by the instrument at the end of the designated test duration and confirmed through subculture of the bottle.

- BTA 3D Instrument False Negative (FN) – bottles inoculated with microorganisms for a seeded study that are signaled negative by the instrument and show the expected organism on Gram stain/smear, subculture and identification.

  - **NOTE**: True negative and false negative categories are typically used to evaluate performance of the BTA 3D Systems during a seeded study. The data, as defined here, are not available from the studies evaluated. Study sites performing primary and secondary testing of platelets typically do not culture bottles determined negative by the instrument. As such, study data collected by bioMérieux were used to determine the true negative and false negative rates for the BTA 3D Systems.

Results & Discussion

Results & Discussion

- **Slide 7 outlined the methods by which data from the 11 studies was analyzed. Table 1 below summarizes the study data where Secondary and QC Surveillance Testing of Platelets* was performed.**

Table 1: Summary of Data from Secondary and QC Surveillance Testing of Platelets*

	Platelet Age			Total
	3 Days	4 Days	≥6 days**	
Units Tested	19,404	95,993	102,535	217,932
% of Total Units Tested	8.9%	44.0%	47.0%	100%
True Positives (TP)***	6	40	128	174
% of TP by Day Tested	0.03%	0.04%	0.12%	0.08%

- *Apheresis platelets (AP) (128,124), Buffy Coat (BC) platelets (32,621), AP+BC (57,187)
- **Units tested expired on Days 5 or 7 and were tested on Day 6 or later
- ***BTA 3D instrument true positive (as defined on Slide 8).

Results & Discussion (continued)

- **Table 2:** Analysis of Bottle Time to Detection (TTD) to Examine the Effects of Platelet Age

The results of the study data shows that the age of platelets does not impact the ability of the BTA 3D System to recover and detect organisms. This gives additional support to the data summarized in **Table 1**, which shows the BTA 3D System can be used as a safety measure for secondary testing of platelets to extend out dating.

Organism (BioBall® SingleShot 30)	Bottle	Platelet Age (Days)	N	Mean TTD (Hours)	Median TTD (Hours)	Median Test χ^2 Exact P-value
Bacillus cereus NCTC7464	BPA	3	4	10.28	10.10	0.2191
		4	10	9.92	9.80	
		5	6	10.05	10.10	
	BPN	3	4	14.70	14.40	0.6317
		4	10	15.06	15.10	
		5	6	14.12	14.40	
Clostridium perfringens NCTC8789	BPN	3	3	13.67	10.30	0.5772
		4	10	19.83	10.70	
		5	6	11.78	10.30	
Escherichia coli NCTC12241	BPA	3	4	12.98	13.10	0.4137
		4	10	12.86	12.70	
		5	6	12.68	12.60	
	BPN	3	4	11.70	11.65	0.4622
		4	10	11.59	11.50	
		5	6	11.77	11.80	
Pseudomonas aeruginosa NCTC12924	BPA	3	4	17.33	17.25	0.8363
		4	10	17.34	17.40	
		5	6	17.22	17.00	
Staphylococcus aureus NCTC10788	BPA	3	4	16.25	16.30	0.8687
		4	10	16.61	16.45	
		5	6	16.72	16.70	
	BPN	3	4	17.50	17.40	0.9378
		4	10	17.68	17.60	
		5	6	17.50	17.40	
Streptococcus pyogenes NCTC12696	BPA	3	4	16.00	15.95	0.6006
		4	10	16.20	16.10	
		5	6	16.35	16.45	
	BPN	3	4	12.80	12.85	0.7646
		4	10	12.99	14.00	
		5	6	12.97	13.00	

Results & Discussion (continued)

- **Table 3:** Organism(s) by Prevalence

The study data show that the BTA 3D System used as a safety measure detects the most prevalent and clinically significant transfusion related microorganisms identified in secondary testing in ≤ 27 hours allowing for interdiction of the units prior to transfusion.

	Organism(s)	Number of Isolates	Range of Times to Detection in Days (Hours)
1	Coagulase Negative Staphylococcus (includes S. epidermidis and S. saccharolyticus)	33	0.13 - 1.11 (3.1 - 26.6)
2	Propionibacterium spp. (includes 18 P. acnes)	21	3.57 - 6.6 (85.7 - 158.4)
3	Staphylococcus spp. (coagulase activity not determined)	9	Not Reported
4	Corynebacterium spp. (includes diphtheroids)	3	0.4 - 4.2 (9.6 - 100.8)
5	Staphylococcus aureus	2	0.15 - 0.3 (3.6 - 7.2)
6	Viridans streptococcus spp.	2	Not Reported
7	Bacillus spp.	2	0.13 (3.1)
8	Gram positive bacilli (includes Leuconostoc spp., Brevibacterium spp.)	2	0.25 – 4.8 (6-115.2)
9	Gram negative bacilli (includes Acinetobacter baumannii, Leclercia adecarboxylata)	2	0.24 - ≤0.33 (5.8 - 7.9)
10	Anaerobic Streptococcus spp.	1	2.1 (50.4)
11	Mixed Culture: gram negative coccobacilli, Acinetobacter spp., Microbacterium spp.	1	0.21 (5.0)
12	Micrococcus luteus	1	2.32 (55.7)

Results & Discussion (continued)

- **Instrument Specificity**
  - Five of 11 studies included data on false positive (FP) results.
    - BTA 3D had an overall FP rate of 0.27% (with a range of 0 – 1.1%).
    - FP by platelet type:
      - LRAP - @ Day 3: 0/19,404 (0%); @ Days 6-7: 96/8,498 (1.1%); and @ Days 7-8: 10/9,041 (0.11%)
      - WBDBC - @ Days 6-7: 53/8535 (0.62%)
      - AP + WBDBC - @ Days 6-7: 0/6438 (0%); and @ Days 7-8: 21/15560 (0.13%)

- **Table 4:** Study Associated BTA 3D False Positive Results

	Platelet* Age			Total
	3 Days	4 Days	≥6 days**	
Units Tested	19404	-	48072	67,476
% of Total Units Tested	29%	-	71%	-
False Positives (FP)	0	-	180	180
% of TP by Day Tested	0	-	0.37%	0.27%

- *Apheresis platelets (AP) (36,943), Buffy Coat (BC) platelets (8,535), AP+BC (21,998)
- **Units tested expired on Days 5 or 7 and were tested on Day 6 or later

Results & Discussion (continued)

- **Instrument Sensitivity**

  - Sensitivity of the BTA 3D System when used for secondary testing could not be determined from the study data since laboratories do not typically subculture a bottle determined to be negative.

  - However, during the previous performance validation testing for LRAP and LRWBPC (for both single units and pools of up to 6 units), and during testing to determine the effects of platelet age on TTD, all negative control bottles (1,136), as well as any seeded bottles that were determined negative by the instrument, were confirmed to be true negatives through subculture to plated media.

    - **No false negative bottles were observed.**

Summary

Summary

- Data from the 11 studies reviewed were collected using a variety of platelet collection protocols. Regardless of the sample volume tested (4 to ≥ 10 mL), the number / types of bottles used (a BPA aerobic bottle, or a BPA aerobic / BPN anaerobic bottle pair), or the age of the platelets (3 to 8 days post collection), the contaminants missed during primary quality control testing were detected by the BACT/ALERT 3D (BTA 3D) System when secondary testing was initiated on Days 3 through 8, post collection.

- *Staphylococcus spp*. were the most prevalent contaminants reported during secondary testing. The study data show the BTA 3D will detect all of the *Staphylococcus spp*. reported in ≤ 27 hours after incubation with the majority detected in ≤ 24 hours (with a range 3.1 to 26.6 hours).

- *Propionibacteria spp.* were the second most prevalent contaminants identified during secondary testing. The data show the BTA 3D will detect these slower growing organisms in 3 to 7 days. Although this would not allow for the unit to be interdicted, performing the safety measure test could provide valuable information to initiate patient follow-up that could otherwise be delayed.
  - 18 strains of 21 reported were *P. acnes*

- The current BPA / BPN bottle IFUs report an overall false positive (FP) rate of 0.19% (with a range 0 to 0.49%) for the BTA 3D system that was based on testing platelets on Day 2 after collection. The overall FP rate reported in the studies as related to secondary safety measure test indicate a rate of 0.27% (with a range 0 to 1.1%).

- No false negative results were observed in previous performance validation studies.

Conclusion

Conclusion

- The BACT/ALERT 3D (BTA 3D) System is an effective safety measure for secondary testing of platelet products, Leukocyte Reduced Apheresis Platelets (LRAP), and both single and pools of up to six units of Leukocyte Reduced Whole Blood Derived Platelet Concentrates (LRWBDPC), to extend platelet dating beyond Day 3 and up to Day 7 when testing is conducted using the test parameters described in the BPA and BPN bottle IFUs and according to the FDA Draft Guidance “Bacterial Risk Control Strategies for Blood Collection Establishments and Transfusion Services to Enhance the Safety and Availability of Platelets for Transfusion”.

- The BACT/ALERT® BPA Culture Bottle and the BPN Culture Bottle were cleared February, 1, 2018, under 510(k) BK170142, as Safety Measure Test.

PIONEERING DIAGNOSTICS