BIOMÉRIEUX

REF 423279

9316718- B - 2020-05

EN

BACT/ALERT® BPN

℞ only IVD

INTENDED USE

BACT/ALERT® BPN culture bottles are used with BACT/ALERT® Microbial Detection Systems (BACT/ALERT® 3D and BACT/ALERT® VIRTUO®) for quality control testing of leukocyte-reduced apheresis platelet (LRAP) units, and both single and pools of up to six (6) units of leukocyte-reduced whole blood platelet concentrates (LRWBPC).[1,2] BACT/ALERT® BPN culture bottles support the growth of anaerobic and facultative anaerobic microorganisms (bacteria).

BACT/ALERT® Microbial Detection Systems are used to detect bacteria in platelet components.

BACT/ALERT® Microbial Detection Systems are used as a safety measure, to extend dating beyond day 5 and up to day 7 for the following[A]:

- Large volume delayed sampling (LVDS) of platelets no sooner than 48 hours after collection; OR
- Secondary culture no sooner than day 4 after platelet collection.

BACT/ALERT® Microbial Detection Systems are used to extend dating to five days for the following [A]:

- Large volume, delayed sampling of platelets no sooner than 36 hours after collection; OR
- Secondary culture no sooner than day 3 after platelet collection.

SUMMARY AND EXPLANATION

BACT/ALERT® Microbial Detection Systems provide both a microbial detection system and a culture media with suitable nutritional and environmental conditions for organisms which might be present in the test sample. Inoculated bottles are placed into the instrument where they are incubated and continuously monitored for the presence of microorganisms that will grow in the BACT/ALERT® BPN bottles.

BACT/ALERT® Microbial Detection Systems may be used for quality control testing of platelets using the single-step large volume delayed sampling test strategy or for a two-step testing strategy where primary quality control testing of platelets along with a secondary or safety measure test are used to extend platelet outdating to five days or seven days per FDA guidelines. Bacterial tests are labeled as a safety measure when they show benefit for detection of bacterial contamination not revealed by previous bacterial testing. The laboratory should follow its own quality control procedures for these uses.

The performance of BACT/ALERT® Microbial Detection Systems for the detection of bacteria in non-leukocyte-reduced platelet products is not known since studies were conducted utilizing LRAP and leukocyte-reduced WBPC products.

Note: The information provided applies to all configurations of BACT/ALERT® Microbial Detection Systems, unless otherwise noted.

PRINCIPLE OF THE TEST

BACT/ALERT® Microbial Detection Systems utilize a colorimetric sensor and reflected light to monitor the presence and production of carbon dioxide (CO_2) that is dissolved in the culture medium. If microorganisms are present in the test sample, carbon dioxide is produced as the organisms metabolize the substrates in the culture medium. When growth of the microorganisms produces CO_2, the color of the gas-permeable sensor installed in the bottom of each culture bottle changes to yellow.[3]

REAGENTS

For *in vitro* diagnostic use only.

Caution: Handle specimens and inoculated culture bottles as though capable of transmitting infectious agents. All inoculated culture bottles and specimen collection needles should be decontaminated according to your institution's procedures.[4]

[1] Brecher ME, Hay SN, Rose AD, Rothenberg SJ. Evaluation of BacT/ALERT plastic culture bottles for use in testing of pooled whole blood derived leukocyte-reduced PRP platelets with a single contaminated unit. *Transfusion*, 2005; 45: 1512-1517.

[2] Brecher ME, Hay SN, Rothenberg SJ. Evaluation of a new generation of plastic culture bottle with an automated microbial detection system for nine common contaminating organisms found in platelet components. *Transfusion* 2004; 44: 359-363.

[A] Food and Drug Administration - Center for Biologics Evaluation and Research. Bacterial Risk Control Strategies for Blood Collection Establishments and Transfusion Services to Enhance the Safety and Availability of Platelets for Transfusion. Silver Spring, MD: FDA, 2019.

[3] Thorpe TC, Wilson ML, Turner JE, et al. BacT/ALERT: an Automated Colorimetric Microbial Detection System. *J Clin Micro* 1990; 28 (7), 1608-1612.

[4] Widmer AF, Frei R. Decontamination, Disinfection, and Sterilization, in Murray PR (ed.). *Manual of Clinical Microbiology*, ed. 7. Washington, D.C., American Society for Microbiology, 1999, pp 138-164.

BACT/ALERT® BPN (color-coded purple) – BACT/ALERT® BPN disposable culture bottles contain 40 mL of media and an internal sensor that detects carbon dioxide as an indicator of microbial growth. The media formulation consists of pancreatic digest of casein (1.36% w/v), papaic digest of soybean meal (0.24% w/v), sodium polyanetholesulfonate (SPS) (0.035% w/v), menadione (0.00005% w/v), hemin (0.0005% w/v), yeast extract (0.376% w/v), pyridoxine hydrochloride (0.0008% w/v), pyruvic acid (sodium salt, 0.08% w/v), reducing agents, and other complex amino acid and carbohydrate substrates in purified water. Bottles are prepared with an atmosphere of CO_2 in nitrogen under vacuum. The composition of the media may be adjusted to meet specific performance requirements.

Caution: The BACT/ALERT® plastic bottles contain polycarbonate. Since not all disinfectants are intended for use with polycarbonate surfaces, please refer to the product labeling of the disinfectant to verify compatibility.

Caution: Platelet specimens determined positive by BACT/ALERT® may contain organisms that are positive by smear that will not grow on routine subculturing media. These specimens should be subcultured on special media when such organisms are suspected. Also, BACT/ALERT® positive specimens may contain organisms that are not seen with routine smear methods and may require both specialized smears and subculturing media for detection and recovery.

Caution: On rare occasions, organisms may be encountered that grow in the BACT/ALERT® BPN culture bottle growth medium but do not produce sufficient carbon dioxide to be determined positive. Oxygen starvation in anaerobic culture bottle is an example that may cause this situation.

Caution: Prompt removal of positives as they are signaled by BACT/ALERT® is strongly recommended to avoid possible non-viable cultures due to autolysis or other reasons. Certain strains of *Streptococcus pneumoniae* may be particularly prone to autolysis if they are not removed promptly after being signaled positive.

Additional Materials Required

- BACT/ALERT® Microbial Detection Systems
- Sterile Airway Needle/Subculture Units
- Disposable gloves
- Appropriate biohazard waste containers for materials potentially contaminated with infectious agents
- Appropriate platelet coupling and sampling apparatus or platelet bag with integrally connected sample bag
- Alcohol pads or equivalent

Materials Available from bioMérieux

- BACT/ALERT® Microbial Detection Systems
- Sterile Airway Needle/Subculture Units

Storage Instructions

BACT/ALERT® BPN culture bottles are ready for use. Store in an upright position protected from direct light at room temperature (15-30°C). An expiration date is printed on each bottle label. Do not inoculate the culture bottles beyond the expiration date indicated. If the bottles are exposed to temperatures less than 15°C, precipitates may form that will disappear when the bottles are warmed to room temperature. Bottles must be at room temperature before use.

Chemical or Physical Indications of Instability

Prior to use, the BACT/ALERT® BPN culture bottles should be examined for evidence of damage or deterioration (discoloration). Bottles exhibiting evidence of damage, leakage, or deterioration should be discarded. The medium in undisturbed bottles should be clear, but there may be a slight opalescence or a trace of precipitate due to the anticoagulant SPS; do not confuse this with turbidity. Do not use a bottle which contains medium exhibiting turbidity, a yellow sensor, or excess gas pressure; these are signs of possible contamination.

INSTRUMENTS

Review the appropriate BACT/ALERT® Microbial Detection System User Manual before use.

SPECIMEN COLLECTION AND PREPARATION

The leukocyte-reduced platelet specimen must be collected using sterile procedures such that the collection set remains a closed system (e.g., use of an integrally connected sample bag or a sample bag connected with a sterile connection device, such as a tubing welder, per the device manufacturer's instructions). It is recommended to use disposable gloves when handling the sampling site and sampling bag to reduce the risk of contaminating the sampling site and sampling site coupler. Refer to Cumitech 1C[5] for the proper contamination avoidance procedure. When using the single-step LVDS strategy, the platelet specimen should be taken at ≥36 hours or ≥48 hours after collection and when using the two-step testing strategy where the primary quality control test and a secondary or safety measure test is performed, the primary QC platelet specimen should be taken at ≥24 hours or ≥36 hours after collection to allow for natural proliferation in the platelet product.[6] When testing

platelets as a secondary or safety measure test, the platelet specimen should be taken at no sooner than day 3 or day 4 from the time of collection. The laboratory should follow its own quality control procedures for specific days of testing to extend the outdating of platelets to five days or seven days per FDA guidelines.

General suggested guidelines for preparing and collecting the platelet specimen for testing are provided below.

1. Label the sample bag with the platelet product information.
2. The platelet specimen to be tested should be taken from the platelet bag(s) using an integrated sampling bag or sterile sampling device. If the platelet bag does not have an integrated sampling bag, a sterile connection device, such as a tubing welder, should be used to connect a sterile sampling bag or device in order to preserve the integrity of the platelet product, so that a closed system is maintained.
3. Strip the attached tubing between the platelet bag (LRAPs, single LRWBPC or a pool of up to 6 units of LRWBPC) and the sample bag toward the platelet bag, rotate contents of platelet bag to allow thorough mixing, and allow the tubing to refill from the platelet bag. Repeat an additional two times. Fill the sample bag with volume desired. Heat seal the tubing between the platelet bag and the sample bag. Aseptically remove the sample bag by cutting the tubing between two of the heat seal welds.

   Note: Per FDA guidelines, each culture bottle must be inoculated with a sample volume of 8-10 mL for quality control testing of platelets using either the single-step LVDS test strategy or the two-step test strategy where the primary quality control testing of platelets along with a secondary or safety measure test are used to extend platelet outdating. When testing more than 10 mL, the upper limit of sample volume recommended for one BACT/ALERT® BPN bottle, inoculate the sample over multiple bottles.
4. For single bag sampling of a unit of whole blood platelet concentrate or sample of a pool of up to 6 units of whole blood platelet concentrates, use an integrally connected sterile sample bag or a sample bag that has been attached using a sterile connection device, such as a tubing welder. Remove the desired test volume to the sample bag. Seal the sample bag off from the platelet bag, separate, and inoculate culture bottles from the sample bags.
5. Per U.S. FDA guidance, secondary testing can be used to extend dating of platelets provided the following conditions are met. Sampling should be done no sooner than Day 3 post collection with at least an aerobic bottle and with ≥8 mL of sample. Negative results from Day 3 secondary testing can be used to extend the dating of platelets to 5 days.
6. Per U.S. FDA guidance, safety measure testing can be used to extend dating of platelets provided the following conditions are met. Sampling should be done no sooner than Day 4 post collection with both aerobic and anaerobic bottles and with 8-10 mL sample per bottle. Negative results from Days 4-6 of safety measure testing can be used to extend the dating of platelets to 7 days. The platelets must be stored in FDA cleared or approved 7-day storage bags labeled with a requirement to test every product with a bacterial detection device cleared by FDA and labeled as a safety measure.
7. Per U.S. FDA guidance, large volume delayed sampling can be used to extend dating of platelets provided the following conditions are met. Sampling should be done at ≥36 or ≥48 hours post collection with both aerobic and anaerobic bottles and with 8-10 mL sample per bottle. Negative results from sampling ≥36 hours post collection of large volume delayed sampling can be used to extend the dating of platelets to 5 days unless a safety measure test is performed no sooner than day 4 to allow dating to 7 days. Negative results from sampling ≥48 hours post collection of large volume delayed sampling can be used to extend the dating of platelets to 7 days. The platelets must be stored in FDA cleared or approved 7-day storage bags.

BACT/ALERT® BPN CULTURE BOTTLE TEST PROCEDURE

Preliminary Comments and Precautions

1. Utilizing at least one aerobic and one anaerobic bottle, per FDA guidance, provides the best overall recovery for platelet contaminates. DO NOT VENT BACT/ALERT® BPN BOTTLES. Positive culture bottles should be transiently vented before subculturing, staining, or disposal to release any gas produced during microbial metabolism.
2. Use disposable gloves and handle inoculated bottles cautiously as though capable of transmitting infectious agents. Consult a physician immediately if contaminated materials are ingested or come in contact with open lacerations, lesions, or other breaks in skin.
3. When handling positive bottles that are bulging or leaking, wear appropriate personal protective equipment (PPE) to avoid coming in contact with microorganisms.
4. Immediately clean up any spillage of contaminated material using a 1:10 dilution of 5% sodium hypochlorite. Dispose of the cleaning material by an acceptable method.
5. All inoculated culture bottles and specimen collection needles should be decontaminated according to your institution's procedures.[7]
6. Culture bottles should be utilized by trained laboratory personnel.

   Caution: For US Only: US Federal Law restricts this device to sale by or on the order of a licensed practitioner.

[5] Baron EJ, Weinstein MP, Dunne WM Jr, Yagupsky P, Welch DF, Wilson DM. 2005. *Cumitech 1C, Blood Cultures IV*. Coordinating ed., Baron EJ. ASM Press, Washington, DC. Brecher ME, Holland PV, Pineda AA, Tegtmeier GE, Yomtovian

[6] Brecher ME, Holland PV, Pineda AA, Tegtmeier GE, Yomtovian R. Growth of bacteria in inoculated platelets: implications for bacteria detection and the extension of platelet storage. *Transfusion*. 2000; 40: 1308-1312.

[7] Widmer AF, Frei R. Decontamination, Disinfection, and Sterilization, in Murray PR (ed.). *Manual of Clinical Microbiology*, ed. 7. Washington, D.C., American Society for Microbiology, 1999, pp 138-164.

Procedural Notes and Precautions

1. Great care must be taken to prevent contamination of the platelet sample during inoculation into the culture bottles. Contamination could lead to a specimen being determined positive when a clinically relevant isolate is not actually present.

   Note: When sampling platelets, it should not be assumed that a sampling error leads to a positive culture of common skin contaminants (e.g., *Staphylococcus aureus*, *Staphylococcus epidermidis*).[8]
2. If inoculated culture bottles have been delayed in their receipt into the lab or have been incubated prior to entry into the BACT/ALERT® instrument, they should be visually inspected for indications of microbial growth. If microbial growth is evident, treat the bottles as positive and do not place in the BACT/ALERT® Microbial Detection System for monitoring.
3. Likely causes of contamination can occur from inadequate aseptic/sterile technique or operator error (e.g., operator lab coat, aerosol), sampling or inoculation in an inadequate environment, or a spore present on top of the BACT/ALERT® bottle septum when introducing the specimen which was not removed with the 70% alcohol wipe.

SPECIMEN TEST/INOCULATION PROCEDURE

Platelet Test Procedure

1. Label the culture bottles with the platelet product information. The bottle must be at room temperature.
2. Remove the plastic flip-top from each culture bottle and disinfect the septum with an alcohol pad or equivalent. Allow to air dry.
3. Disinfect the rubber septum on the surface of the platelet bag sampling site with an alcohol pad or equivalent, allow to air dry, and use a syringe and needle (using a needle gauge sufficiently large enough to allow easy drawback of platelet product into the syringe) to remove a sample from the sample bag. Alternatively, a sterile, integrally connected sampling device may be used to obtain a sample from the platelet bag.
4. **Note:** Each culture bottle must be inoculated with a sample volume of 8-10 mL. When testing more than 10 mL, the upper limit of sample volume recommended for one BACT/ALERT® BPN bottle, inoculate the sample over multiple bottles. Utilizing at least one aerobic and one anaerobic bottle, per FDA guidance, provides the best overall recovery for platelet contaminates.
   Insert the needle through the septum of the culture bottle and inject 4-10 mL of the platelet specimen into each bottle being inoculated. If using both an anaerobic and aerobic culture bottle, transfer to the anaerobic bottle first, so that any oxygen trapped in the syringe will not be transferred to this bottle. If a sterile, integrally connected sampling device is used, then the aerobic bottle must be inoculated first, followed by the anaerobic bottle, in order to minimize transfer of oxygen to the anaerobic bottle.

   Caution: Never force the syringe plunger down during inoculation, as splashing of sample may occur. Remove the syringe when the fill amount is reached, as the vacuum will automatically draw more than the recommended maximum. Puncture the bottle stopper vertically to avoid releasing the vacuum; a bottle without a vacuum should not be inoculated.
5. Ensure that the specimen is properly mixed with the reagents in the BACT/ALERT® BPN bottle.

Laboratory Procedure

1. DO NOT VENT BACT/ALERT® BPN BOTTLES.
2. Visually inspect bottles before testing. Bottles with a yellow sensor, turbidity, excess gas pressure, and/or evidence of growth should be treated as positive. Smear and subculture. Do not incubate unless smear is negative.
3. After collection, promptly transport the inoculated bottle to testing laboratory and test immediately.
4. After culture bottles have been loaded into the instrument, they should remain there for five to seven days or until designated positive, or until the platelet unit reaches its expiration date.
5. All bottles designated positive should be smeared and subcultured. If the smear is negative, indicating a possible false positive, the bottle should be reloaded into the instrument until growth of subculture or redesignation as positive. Cultures which were initially determined false positive and were redesignated positive should be smeared and subcultured.

[8] Sazama K. Bacteria in blood for transfusion. *Arch Pathol Lab Med*; April 1994; 118:359.

6. Negative cultures may be checked by smear and/or subculture at some point prior to discarding as negative.
7. Procedures for loading and unloading culture bottles into the appropriate BACT/ALERT® instrument are given in the User Manual.
8. **Do not reuse BACT/ALERT® culture bottles.** Dispose of inoculated BACT/ALERT® culture bottles according to your laboratory protocol. Autoclaving and/or incinerating inoculated BACT/ALERT® bottles is appropriate.[9]

   Note: A report of "negative" should not be interpreted as meaning that the original product is sterile. The negative status could be due to under-inoculation of the bottle, no organisms present in the inoculum, the number of organisms were too small for detection, or a culture bottle/medium that does not support the growth of the organism. Utilizing at least one aerobic and one anaerobic bottle, per FDA guidance, provides the best overall recovery for platelet contaminates.

   Note: When performing quality control testing of platelets using the single-step LVDS test strategy or the two-step testing strategy where primary quality control testing of platelets along with a secondary or safety measure test are used to extend platelet outdating, at a minimum, the culture bottle should be held through the expiration date of the product (The platelet unit with the shortest expiration date in the 6 unit pool will determine the final expiration of the pool.) or until designated positive. When performing the two step testing strategy, the primary platelet specimen should be taken at least 24 hours after collection to allow for natural proliferation in the platelet product.[10] When testing as a safety measure of platelets, it is recommended that the culture bottle should be held for seven days or until designated positive to allow for the growth and detection of slow growing organisms such as *Cutibacterium spp*. (formerly *Propionibacteria* spp.).

QUALITY CONTROL

A Certificate of Conformance is available for each lot of culture bottles. If desired, individual laboratories can perform quality control testing of BACT/ALERT® BPN culture bottles. Refer to the appropriate BACT/ALERT® User Manual and to CLSI® document M22-A3.[11]

Instrument

A BACT/ALERT® Reflectance Standards kit is provided with each instrument for the QC and Calibration procedures. All quality control should be part of normal system maintenance. Refer to the appropriate BACT/ALERT® User Manual for more information.

Caution: If your facility's LIS vendor sends bottle IDs and bottle type abbreviations to the BACT/ALERT® instrument, use the correct bottle type abbreviation to avoid possible false positive or false negative results. For more information, contact your local bioMérieux representative.

RESULTS

Positive or negative culture bottles are determined by decision-making software contained in the BACT/ALERT® Microbial Detection Systems. No action is required until the BACT/ALERT® instrument signals culture bottles positive or negative.

LIMITATIONS OF THE TEST

Many variables involved in platelet culture testing cannot be practically controlled to provide total confidence that results obtained are due solely to proper or improper performance of any culture medium or detection system.

1. A Gram-stained smear from a negative bottle may sometimes contain a small number of non-viable organisms that were derived from culture medium components, staining reagents, immersion oil, or glass slides, therefore, false-positive results are indicated.
2. False positive readings can occur due to noise on the powerline, placing the instrument in direct sunlight, or with dramatic temperature fluctuations.
3. Failure to achieve adequate leukocyte reduction may result in false positive readings.

[9] Brecher ME, Hay SN, Rothenberg SJ. Evaluation of a new generation of plastic culture bottle with an automated microbial detection system for nine common contaminating organisms found in platelet components. *Transfusion* 2004; 44: 359-363.

[10] Brecher ME, Holland PV, Pineda AA, Tegtmeier GE, Yomtovian R. Growth of bacteria in inoculated platelets: implications for bacteria detection and the extension of platelet storage. *Transfusion*. 2000; 40:1308-1312.

[11] CLSI®/NCCLS. *Quality Control for Commercially Prepared Microbiological Culture Media*; Approved Standard—Third Edition. CLSI®/NCCLS document M22-A3. Wayne, PA: NCCLS; 2004.

PERFORMANCE CHARACTERISTICS

BACT/ALERT® 3D Microbial Detection Systems

Detection of Organisms in Leukocyte-Reduced Apheresis Platelets

A study to determine the ability of the culture bottles to detect the presence of microorganisms in leukocyte-reduced apheresis platelets was performed at one clinical site. Bags were seeded at Day 2 with nine individual microorganisms to include:

- *Bacillus cereus* ATCC® 11778™
- *Escherichia coli* ATCC® 25922™
- *Enterobacter cloacae* clinical isolate
- *Klebsiella oxytoca* clinical isolate
- *Cutibacterium acnes* clinical isolate
- *Serratia marcescens* ATCC® 43862™
- *Staphylococcus aureus* ATCC® 27217™
- *Staphylococcus epidermidis* ATCC® 49134™
- *Streptococcus viridans* group clinical isolate

Three replicates of each bottle type were inoculated (4 mL) with each organism at each inoculum level. Seventy-two bottles at one site were inoculated with 4 mL of platelets (no seeded organisms) to serve as negative controls at the lower sample volume range, i.e., 4 mL, and 408 bottles at two sites were inoculated with 10 mL of platelets (no seeded organisms) to serve as negative controls of the higher sample volume range, i.e., 10 mL. There were no false positives from the negative controls inoculated at 4 mL and one false positive from the negative controls inoculated at 10 mL (1/408 or 0.25%). The initial concentration of organisms seeded varied for each organism and ranged from 1 CFU/mL to 300 CFU/mL. See the following table for results.

Table 1: Recovery of Organisms in Leukocyte-Reduced Apheresis Platelets

Microorganism	ATCC® Number	Actual Initial Inoculum (CFU/mL)*	Average Time to Detection (hours)			
			Plastic BACT/ALERT® BPA†	Glass BACT/ALERT® SA†	Plastic BACT/ALERT® BPN†	Glass BACT/ALERT® SN†
Bacillus cereus	11778™	5	8.7	8.9	9.7	10.4
			(8.7-8.8)	(8.8-9.0)	(9.5-9.8)	(9.7-10.7)
		2	9.3	9.7	10.9	10.8
			(9.1-9.5)	(9.3-10.1)	(10.7-11.1)	(10.2-11.1)
Escherichia coli	25922™	215	9.9	10.8	9.3	10.2
			(9.7-10.1)	(10.6-11.1)	(9.1-9.4)	(10.1-10.3)
		5	11.1	12.0	10.3	11.0
			(10.9-11.2)	(11.9-12.1)	(10.2-10.4)	(10.9-11.1)
Enterobacter cloacae	Clinical Isolate	300	9.9	10.7	9.6	10.5
			(9.8-10.0)	(10.7-10.8)	(9.5-9.7)	(10.4-10.5)
		21	11.0	11.8	10.3	11.5
			(10.9-11.2)	(11.7-11.8)	(10.2-10.4)	(11.4-11.5)
Klebsiella oxytoca	Clinical Isolate	32	10.1	10.8	10.4	11.2
			(9.9-10.3)	(10.8-10.8)	(10.3-10.5)	(11.1-11.3)
		5	10.9	11.5	11.1	12.1
			(10.7-11.0)	(11.5-11.5)	(11.0-11.2)	(12.0-12.2)

Microorganism	ATCC® Number	Actual Initial Inoculum (CFU/mL)*	Average Time to Detection (hours)			
			Plastic BACT/ALERT® BPA†	Glass BACT/ALERT® SA†	Plastic BACT/ALERT® BPN†	Glass BACT/ALERT® SN†
Cutibacterium acnes	Clinical Isolate	130	Negative‡	Negative‡	64.0	80.8
					(62.4-64.8)	(76.8-86.4)
		16	Negative‡	Negative‡	72.8	90.4
					(72.0-74.4)	(88.8-93.6)
Serratia marcescens	43862™	50	11.4	11.9	11.8	12.8
			(11.1-11.6)	(11.8-12.1)	(11.8-11.9)	(12.8-12.8)
		5	12.6	13.0	12.8	13.8
			(12.4-12.8)	(12.8-13.3)	(12.8-12.8)	(13.8-13.9)
Staphylococcus aureus	27217™	140	10.4	11.0	11.5	12.6
			(10.3-10.5)	(10.8-11.3)	(11.2-11.8)	(12.3-12.8)
		4	11.4	12.0	12.9	13.5
			(11.1-11.7)	(12.0-12.1)	(12.7-13.0)	(13.0-14.4)
Staphylococcus epidermidis	49134™	40	15.6	16.9	19.6	36.9
			(15.5-15.7)	(16.8-17.0)	(19.5-19.7)	(19.3-47.8)
		1	17.3	18.9	21.6	40.1
			(17.0-17.5)	(18.8-19.2)	(21.3-21.8)	(23.7-50.4)
Streptococcus viridans	Clinical Isolate	110	15.3	18.9	14.7	18.2
			(15.1-15.4)	(18.6-19.1)	(14.0-15.2)	(17.4-19.1)
		3	17.9	22.4	18.0	21.4
			(17.2-18.4)	(21.7-23.2)	(17.6-18.4)	(20.7-21.7)
Mean (n = 24 for each aerobic bottle type and n = 27 for each anaerobic bottle type at each inoculum level)		114	11.4§	12.5§	17.8	22.6
			(8.7-15.7)	(8.8-19.1)	(9.1-64.8)	(9.7-86.4)
		7	12.7§	13.9§	20.1	25.0
			(9.1-18.4)	(9.3-23.2)	(10.2-74.4)	(10.2-93.6)

* Prior to inoculation, two samples from each platelet bag were inoculated (4 mL per bottle) into plastic BACT/ALERT® BPA and glass BACT/ALERT® SA and plastic BACT/ALERT® BPN and glass BACT/ALERT® SN culture bottles (a total of 72 samples) to verify sterility of the apheresis bags (negative controls). These negative controls were negative, i.e., sterile. In addition, 204 bottles each of plastic BACT/ALERT® BPA and BACT/ALERT® BPN (408 bottles total) were inoculated with 10 mL of non-seeded, sterile, leukocyte-reduced apheresis platelets to serve as additional negative controls. There was one false positive result (0.25%).

† Three replicates of each bottle type were inoculated with each organism at each inoculum level. The average value is listed, with the range of values obtained listed in parenthesis below the average.

‡ High oxygen levels in this bottle prevent the growth of this organism. Recovery occurred in the BACT/ALERT® BPN and SN bottles. **Both BACT/ALERT® BPA and BACT/ALERT® BPN culture bottles should be inoculated from the platelet specimen for optimal recovery of contaminating microorganisms.**

§ Does not include data for *Cutibacterium acnes*.

Detection of Organisms in Leukocyte-Reduced Single Units of Whole Blood Platelet Concentrates

A study to determine the ability of the culture bottles to detect the presence of microorganisms in leukocyte-reduced single units of whole blood platelet concentrates was performed. Platelet bags were seeded at Day 2 with nine individual microorganisms to include:

- *Bacillus cereus* ATCC® 11778™
- *Escherichia coli* ATCC® 25922™

- *Enterobacter cloacae* clinical isolate
- *Klebsiella pneumoniae* clinical isolate
- *Cutibacterium acnes* clinical isolate
- *Serratia marcescens* ATCC® 43862™
- *Staphylococcus aureus* ATCC® 27217™
- *Staphylococcus epidermidis* ATCC® 49134™
- *Streptococcus viridans* group clinical isolate

Five replicates of each bottle type were inoculated (4 mL) with each organism at each inoculum level. The initial concentration of organisms seeded varied for each organism and ranged from <2 CFU/mL to 265 CFU/mL. An additional 180 bottles served as negative controls (WBPC with no seeded organisms added). No false positives or contaminated negative controls were detected. See the following table for results.

Table 2: Recovery of Organisms in Leukocyte-Reduced Single Units of Whole Blood Platelet Concentrates

Microorganism	ATCC® Number	Actual Initial Inoculum (CFU/mL)*	Number of Positive Cultures		
			BACT/ALERT® BPA†	BACT/ALERT® BPN†	Solid Media
Bacillus cereus	11778™	85	5	5	5
		5	5	5	5
Escherichia coli	25922™	110	5	5	5
		6	5	5	5
Enterobacter cloacae	Clinical Isolate	265	5	5	5
		17	5	5	5
Klebsiella pneumoniae	Clinical Isolate	20	5	5	5
		2	5	5	4
Cutibacterium acnes	Clinical Isolate	28	0‡	5	5
		< 2	0‡	5	5
Serratia marcescens	43862™	95	5	5	5
		2	5	5	5
Staphylococcus aureus	27217™	125	5	5	5
		4	5	5	5
Staphylococcus epidermidis	49134™	35	5	5	5
		3	5	5	5
Streptococcus viridans	Clinical Isolate	43	5	5	5
		3	5	5	5
Positive			80	90	89
Total % Recovery			88.9%	100%	98.9%
95% Confidence Interval			80.5-94.5	96.0-100.0	94.0-99.9
% Recovery of Facultative Organisms and Strict Aerobes			100%	-	-
95% Confidence Interval			95.5-100.0	-	-
% Recovery of Facultative Organisms and Strict Anaerobes			-	100%	-
95% Confidence Interval			-	96.0-100.0	-

* Prior to inoculation, 5 samples from each WBPC platelet bag were inoculated (10 mL into each bottle) into plastic BACT/ALERT® BPA and plastic BACT/ALERT® BPN culture bottles (a total of 180 samples) to verify sterility of the platelet bags (negative controls). All negative controls were negative (sterile), i.e., there were no false positives.

† Five replicates of each bottle type were inoculated with each organism at each inoculum level.

‡ High oxygen levels in this bottle prevent the growth of this organism. Recovery occurred in the BACT/ALERT® BPN bottles. **Both BACT/ALERT® BPA and BACT/ALERT® BPN culture bottles should be inoculated from the platelet specimen for optimal recovery of contaminating microorganisms.**

Detection of Organisms in Leukocyte-Reduced 6 Unit Pool of Whole Blood Platelet Concentrates (WBPC)
A study to determine the ability of the culture bottles to detect the presence of microorganisms in a pool of six (6) units of leukocyte-reduced pooled whole blood platelet concentrates was performed at two clinical sites. Platelet bags were seeded at Day 2 with 10 individual microorganisms to include:

- *Bacillus cereus* ATCC® 11778™
- *Escherichia coli* ATCC® 25922™
- *Enterobacter cloacae* clinical isolate
- *Klebsiella pneumoniae* clinical isolate
- *Cutibacterium acnes* ATCC® 11827™
- *Serratia marcescens* ATCC® 43862™
- *Staphylococcus aureus* ATCC® 27217™
- *Staphylococcus epidermidis* ATCC® 49134™
- *Streptococcus viridans* group clinical isolate
- *Clostridium perfringens* ATCC® 13124™

Ten replicates of each bottle type at each site were inoculated (4 mL) with each organism at each inoculum level. Each organism was seeded into one platelet unit at a target level of 10 and 100 CFU/mL, and then that unit was pooled with five sterile units. The concentration of organisms in the pool varied for each organism and ranged from <2 to 33 CFU/mL. An additional 207 BACT/ALERT® BPA and 207 BACT/ALERT® BPN bottles served as negative controls (LRWBPC with no seeded organisms added). One false positive was detected at Site B. See the following tables for results.

Table 3: Recovery of Organisms in a 6 Unit Pool of Leukocyte-Reduced Whole Blood Platelet Concentrates from Site A

Microorganism	ATCC® Number	Inoculum in Pooled Unit (CFU/mL)*	Number of Positive Cultures			
			BACT/ALERT® BPA†	BACT/ALERT® BPN†	BACT/ALERT® BPA + BACT/ALERT® BPN‡	Solid Media
Bacillus cereus	11778™	<2	10	10	10	6
		8	10	10	10	10
Escherichia coli	25922™	<2	10	10	10	6
		6	10	10	10	10
Enterobacter cloacae	Clinical Isolate	2	10	10	10	10
		24	10	10	10	10
Klebsiella pneumoniae	Clinical Isolate	<2	10	8	10	0
		2	10	10	10	9
Cutibacterium acnes	11827™	<2	0§	10	10	2
		2.5	0§	10	10	10
Serratia marcescens	43862™	<2	4	4	7	1
		<2	10	10	10	6
Staphylococcus aureus	27217™	2	10	10	10	4
		10	10	10	10	10
Staphylococcus epidermidis	49134™	2	10	10	10	6
		11	10	10	10	10
Streptococcus viridans	Clinical Isolate	<2	9	9	10	1
		<2	10	10	10	10

Microorganism	ATCC® Number	Inoculum in Pooled Unit (CFU/mL)*	Number of Positive Cultures			
			BACT/ALERT® BPA†	BACT/ALERT® BPN†	BACT/ALERT® BPA + BACT/ALERT® BPN‡	Solid Media
Clostridium perfringens	13124™	<2	0§	3	3	2
		<2	0§	10	10	1
Positive			153	184	190	124
Total % Recovery			76.5%	92.0%	95.0%	62.0%
95% Confidence Interval			70.0-82.2	87.3-95.4	91.0-97.6	54.9-68.8
% Recovery of Facultative Organisms and Strict Aerobes			95.6%	-	-	-
95% Confidence Interval			91.2-98.2	-	-	-
% Recovery of Facultative Organisms and Strict Anaerobes			-	92.0%	-	-
95% Confidence Interval			-	87.3-95.4	-	-

* One hundred and five bottles each of BACT/ALERT® BPA and BACT/ALERT® BPN were inoculated with 10 mL of non-seeded, sterile, leukocyte-reduced WBPC and incubated along with the seeded, inoculated bottles to serve as negative controls. These additional negative controls were also utilized to establish that leukocyte-reduced WBPC at a 10 mL sample volume do not cause a high rate of false positive results to occur. These negative controls (6 unit pool) also represent the upper sample volume range of leukocyte-reduced WBPC that can be inoculated into the BACT/ALERT® bottles. All negative controls were negative (sterile), i.e., there were no false positives at this site.

† Ten replicates of each bottle type were inoculated with each organism at each inoculum level.

‡ Number of positive results when one or both of the paired BACT/ALERT® bottles (BACT/ALERT® BPA and BACT/ALERT® BPN) were detected. For best overall recovery when culturing platelet specimens it is strongly recommended that more than one type of culture bottle be utilized (e.g., one aerobic and one anaerobic).

§ High oxygen levels in this bottle prevent the growth of these organisms. Recovery occurred in the BACT/ALERT® BPN bottles. **Both BACT/ALERT® BPA and BACT/ALERT® BPN culture bottles should be inoculated from the platelet specimen for optimal recovery of contaminating microorganisms.**

Table 4: Recovery of Organisms in a 6 Unit Pool of Leukocyte-Reduced Whole Blood Platelet Concentrates from Site B

Microorganism	ATCC® Number	Inoculum in Pooled Unit (CFU/mL)*	Number of Positive Cultures			
			BACT/ALERT® BPA†	BACT/ALERT® BPN†	BACT/ALERT® BPA + BACT/ALERT® BPN‡	Solid Media
Bacillus cereus	11778™	3	10	10	10	6
		11	10	10	10	10
Escherichia coli	25922™	3	10	10	10	6
		10	10	10	10	10
Enterobacter cloacae	Clinical Isolate	2	10	10	10	10
		14	10	10	10	10
Klebsiella pneumoniae	Clinical Isolate	<2	5	6	7	0
		3	10	10	10	3
Cutibacteriumacnes	11827™	<2	0§	0	0	0
		18	1	10	10	10
Serratia marcescens	43862™	4	10	10	10	8
		31	10	10	10	10

Microorganism	ATCC® Number	Inoculum in Pooled Unit (CFU/mL)*	Number of Positive Cultures			
			BACT/ALERT® BPA†	BACT/ALERT® BPN†	BACT/ALERT® BPA + BACT/ALERT® BPN‡	Solid Media
Staphylococcus aureus	27217™	<2	10	10	10	9
		17	10	10	10	10
Staphylococcus epidermidis	49134™	2	10	10	10	8
		19	10	10	10	10
Streptococcus viridans	Clinical Isolate	1	10	10	10	10
		33	10	10	10	10
Clostridium perfringens	13124™	<2	0§	2	2	8
		15	0§	10	10	10
Positive			156	178	179	162
Total % Recovery			78.0%	89.0%	89.5%	81.0%
95% Confidence Interval			71.6-83.5	83.8-93.0	84.4-93.4	74.9-86.2
% Recovery of Facultative Organisms and Strict Aerobes			96.9%	-	-	-
95% Confidence Interval			92.9-99.0	-	-	-
% Recovery of Facultative Organisms and Strict Anaerobes			-	89.0%	-	-
95% Confidence Interval			-	83.8-93.0	-	-

* One hundred and two bottles each of BACT/ALERT® BPA and BACT/ALERT® BPN were inoculated with 10 mL of non-seeded, sterile, leukocyte-reduced WBPC and incubated along with the seeded, inoculated bottles to serve as negative controls. These additional negative controls were also utilized to establish that leukocyte-reduced WBPC at a 10 mL sample volume do not cause a high rate of false positive results to occur. These negative controls (6 unit pool) also represent the upper sample volume range of leukocyte-reduced WBPC that can be inoculated into the BACT/ALERT® bottles. One negative control was positive and was determined to be a true false positive at this site.

† Ten replicates of each bottle type were inoculated with each organism at each inoculum level.

‡ Number of positive results when one or both of the paired BACT/ALERT® bottles (BACT/ALERT® BPA and BACT/ALERT® BPN) were detected. For best overall recovery when culturing platelet specimens it is strongly recommended that more than one type of culture bottle be utilized (e.g., one aerobic and one anaerobic).

§ High oxygen levels in this bottle prevent the growth of these organisms. Recovery occurred in the BACT/ALERT® BPN bottles. **Both BACT/ALERT® BPA and BACT/ALERT® BPN culture bottles should be inoculated from the platelet specimen for optimal recovery of contaminating microorganisms.**

Performance of the BACT/ALERT® 3D Systems for Use as a Secondary or Safety Measure Test to Extend the Shelf Life of Platelet Preparations

For instructions on platelet dating extension, refer to the PLATELET SPECIMEN COLLECTION AND PREPARATION section, item 5 and item 6.

A literature review was conducted and blood collection and transfusion services were queried to identify studies where the BACT/ALERT® 3D system was used for secondary testing and/or end-date QC surveillance of previously tested platelets. Evidence from the literature review indicates that the BACT/ALERT® 3D System is an effective safety measure for secondary testing of previously tested platelet products.

The reviewed studies used testing protocols consistent with the test parameters described above for quality control testing of platelets - i.e. 4 mL to 10 mL of sample per bottle, one aerobic BACT/ALERT® BPA bottle or a bottle pair (one aerobic BACT/ALERT® BPA bottle and one anaerobic BACT/ALERT® BPN bottle). The reviewed studies, in general, classified the test results based on AABB Bulletin 04-07 interpretation guideline, with some study specific modifications that included detailed,

subcategories of false positive results.[12] The overall Specificity and Sensitivity of the BACT/ALERT® 3D System were determined from external and internal studies.

A total of 128,124 LRAP units that were determined negative during quality control testing and released for transfusion were tested on Days 3, 4, and ≥6 days post collection. A total of 72 positive bottles (0.06%) were detected by the BACT/ALERT® 3D.

Table 5: Summary of Data from Secondary Testing of Apheresis Platelets

	Platelet Age			Total
	3 Days	4 Days	≥6 Days**	
Units Tested	19,404	76,578	32,142	128,124
% of Total Units Tested	15.0%	60.0%	25.0%	-
True Positives (TP)*	6	20	46	72
% of TP/Day Tested	0.03%	0.03%	0.14%	0.06%

* An instrument true positive is defined as a bottle signaled as positive by the instrument and growth is confirmed by subculture. All test results where a contaminant was isolated from the bottle, regardless of whether it was a true positive platelet contaminant or a procedural contaminant were considered in the BACT/ALERT® 3D System performance evaluation.

** Units tested expired on Days 5 or 7 and were tested on day 6 or later.

An analytical study was performed to determine if the age of platelets affected the time-to-detection (TTD) of organisms representing transfusion relevant contaminants. In this study, 4 mL aliquots of LRAP, 3 to 5 days post collection, were seeded with low levels (target 3 CFU/mL) of six organisms and tested in two lots each of BACT/ALERT® BPA and BACT/ALERT® BPN culture bottles. Additional bottles were inoculated with LRAP only, to serve as negative controls and to monitor for false positive detections. An analysis of variance based on a multi-sample median test (Brown-Mood test) was performed to determine whether the TTD was significantly different depending on the age of the platelets. A chi-square statistical analysis was used to determine whether platelet age has a significant effect on TTD. P-values greater than 0.05 indicate that there is no statistically significant evidence indicating that platelet age effects TTD. A separate median test was completed for each organisms / bottle combination. All p-values were greater than 0.05, providing confirmation that platelet age does not affect TTD. A total of 31 BACT/ALERT® BPA and 31 BACT/ALERT® BPN negative control bottles, each containing from 4 mL to 10 mL of LRAP, were tested. No false positive detections were observed (0/62).

Table 6: Analysis of BACT/ALERT® BPA and BACT/ALERT® BPN Bottle Time to Detection to Examine the Effects of Platelet Age – BACT/ALERT® 3D

Microorganism*	BACT/ALERT® Culture Bottle	Platelet Age (Days)	N	Mean TTD (Hours)	Median TTD (Hours)	Median Test χ^2 Exact P-value
Bacillus cereus NCTC 7464	BACT/ALERT® BPA	3	4	10.28	10.10	0.2191
		4	10	9.92	9.80	
		5	6	10.05	10.10	
	BACT/ALERT® BPN	3	4	14.70	14.40	0.6317
		4	10	15.06	15.10	
		5	6	14.12	14.40	
*Clostridium perfringens*** NCTC 8798	BACT/ALERT® BPN	3	3	13.67	10.30	0.5772
		4	10	19.83	10.70	
		5	6	11.78	10.30	

[12] Su L, Kamel H, Custer B, et al. Bacterial detection in apheresis platelets: Blood Systems experience with a two-bottle and one-bottle culture system. *Transfusion*. 2008,48:1842-1852.

Microorganism*	BACT/ALERT® Culture Bottle	Platelet Age (Days)	N	Mean TTD (Hours)	Median TTD (Hours)	Median Test χ^2 Exact P-value
Escherichia coli NCTC 12241	BACT/ALERT® BPA	3	4	12.98	13.10	0.4137
		4	10	12.86	12.70	
		5	6	12.68	12.60	
	BACT/ALERT® BPN	3	4	11.70	11.65	0.4622
		4	10	11.59	11.50	
		5	6	11.77	11.80	
*Pseudomonas aeruginosa*** NCTC 12924	BACT/ALERT® BPA	3	4	17.33	17.25	0.8363
		4	10	17.34	17.40	
		5	6	17.22	17.00	
Staphylococcus aureus NCTC 10788	BACT/ALERT® BPA	3	4	16.25	16.30	0.8687
		4	10	16.61	16.45	
		5	6	16.72	16.70	
	BACT/ALERT® BPN	3	4	17.50	17.40	0.9378
		4	10	17.68	17.60	
		5	6	17.50	17.40	
Streptococcus pyogenes NCTC 12696	BACT/ALERT® BPA	3	4	16.00	15.95	0.6006
		4	10	16.20	16.10	
		5	6	16.35	16.45	
	BACT/ALERT® BPN	3	4	12.80	12.85	0.7646
		4	10	12.99	14.00	
		5	6	12.97	13.00	

* All organisms tested were in the BIOBALL® SINGLESHOT 30 format.

** *C. perfringens* and *P. aeruginosa* were tested in the BACT/ALERT® BPN and BACT/ALERT® BPA bottles respectively per intended use.

The literature review reported the identifications of the organisms isolated from contaminated units observed during secondary testing, which for some studies also included the TTD of the isolates. The results are summarized in the following table.

Table 7: Organism(s) by Prevalence

	Microorganism(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	*Cutibacterium spp.* (includes 18 *C. 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

	Microorganism(s)	Number of Isolates	Range of Times to Detection in Days (Hours)
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)

Specificity & Sensitivity

The BACT/ALERT® 3D System overall specificity (false positive (FP) rate) and sensitivity (false negative (FN) rate) were determined from external and internal studies. In three of the studies where LRAP were tested, instrument false positive results were reported. These studies accounted for 36,943 units tested (19,404 on day 3 and 17,539 on day ≥6 (tested after expiry on days 5 or 7)). A total of 106 instrument false positive results were observed for an overall false positive rate of 0.3%. The individual studies reported 0%, 0.11% and 1.1% instrument FP.

Performance validation for the primary quality control testing of platelets is reported in Tables 1 through 4 above. During the performance validation testing for LRAP and LRWBPC (both single units and pools of 6 units) negative controls containing 4-10 mL of platelet concentrate were tested. Results for the negative controls are summarized in the following table.

Table 8: Instrument False Positive (FP) Results

Platelet Product (Table Number)	Quantity Tested per Bottle	#FP/Bottles Tested	%FP
LRAP (Table 1)	4 mL	0/72	0.00%
	10 mL	1/408	0.25%
LRWBDPC, single unit (Table 2)	10 mL	0/180	0.00%
LRWBDPC, pool of up to 6 units (Tables 3 and 4)	10 mL	Site A: 0/210	Site A: 0.00%
		Site B: 1/204	Site B: 0.49%
Overall	-	2/1074	0.19%
Range	-	-	0.00-0.49%

The overall BACT/ALERT® 3D System specificity, based on testing platelets on Day 2 after collection and in controlled studies, is 0.19% (range 0-0.49%). The overall FP rate reported in published studies from platelets tested on days 3 and ≥ 6 post collection was 0.3% (range 0-1.1%). Sensitivity for the secondary testing could not be determined from the study data since laboratories using the BACT/ALERT® 3D Systems would not typically subculture a bottle determined to be negative. During performance validation testing for LRAP and LRWBPC (both single units and pools of 6 units) and during testing to determine the effects of platelet age on TTD (Table 6), all negative control bottles and 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.

Equivalency of Platelet Preparation Methods for Performance Validation Testing of the BACT/ALERT Microbial Detection Systems

A retrospective review of time to detection (TTD) data collected during performance validation testing of the BACT/ALERT 3D and VIRTUO was conducted to determine if platelet preparation method had an impact on the TTD of microorganisms seeded into leukocyte reduced apheresis platelets (LRAP) or leukocyte reduced whole blood platelet concentrates (LRWBPC). Since recovery of seeded microorganisms is well established for the BACT/ALERT Microbial Detection Systems, TTD data were used to evaluate the platelet preparation methods. The goal of this data review was to establish the equivalency of platelet preparation methods for use in seeded studies to establish performance for the BACT/ALERT Microbial Detection Systems.

The data reviewed was collected from four independent studies conducted at two external sites where different instruments

different bottle lots and different volumes of seeded sample (8 mL or 4 mL) were tested. All studies used both aerobic (BPA) and anaerobic (BPN) culture bottles. Site 1 tested eight microorganisms resulting in 14 combinations of microorganism-bottle-platelet concentrate (LRAP or LRWBPC) on the BTA 3D and the VIRTUO. Site 2 tested nine microorganisms resulting in 17 combinations of microorganism-bottle-platelet concentrate (LRAP or LRWBPC) on the BTA 3D. Table 9 below is a summary of the comparison of the average of the mean TTD by platelet type and instrument.

Table 9: Comparison of the Average of the Mean TTD by Platelet Type on the BACT/ALERT Systems

Instrument | Platelet Type	LRAP TTD Hours	LRWBPC TTD Hours
VIRTUO (Site 1 – Study 1)	11.1	11.8[1]
BTA 3D (Site 1 – Study 2)	14.3	14.1[1]
BTA 3D (Site 2 – Studies 3 & 4)	16.5	17.0[2]

[1] 8 mL Sample Volume *[2] 4 mL Sample Volume*

The results show that the Average of the Mean TTD across platelet type tested on the BTA 3D or VIRTUO show there are no practical differences (LRAP or LRWBPC, <1 hour) in TTD between platelet type. The variability observed and reported in four independent studies for the average of the mean TTD across instruments and sites (LRAP ≤5.4 hours, LRWBPC ≤5.2 hours) supports that the platelet preparation method does not have a practical impact on TTD and that site-to-site and instrument-to-instrument differences have a greater impact on TTD.

Platelet preparation method does not impact the ability of the BACT/ALERT Microbial Detection Systems to detect microorganisms; therefore, data collected during performance validation testing of the VIRTUO using LRAP is representative of performance of the VIRTUO for testing LRWBPC.

BACT/ALERT® VIRTUO® Microbial Detection Systems

Note: The following tables with VIRTUO performance using LRAP can be applied to testing single and pools of up to six (6) units of LRWBPC.

Reproducibility Results from LRAP with BACT/ALERT® VIRTUO®

Data in the following table represent results from seeded leukocyte-reduced apheresis platelet (LRAP) units conducted with two lots of BACT/ALERT® BPA and BACT/ALERT® BPN culture bottles at three sites (two external and one internal), testing a

total of 270 replicates over 3 days, with two operators at each site. Reproducibility was evaluated on each of six microorganisms; five were inoculated into each bottle type (four facultative microorganisms and one obligate aerobic microorganism in BACT/ALERT® BPA and four facultative microorganisms and one obligate anaerobic microorganism in BACT/ALERT® BPN). All microorganisms were prepared from BIOBALL® products except for *P. aeruginosa* ATCC® 27853™ which was prepared as a serial dilution. The target inoculum was 5 CFU/mL of platelets with 4 mL of seeded platelets inoculated into each bottle. Percent recovery reflects a positive flag by the instrument and Gram-stain/subculture consistent with the seeded microorganism and from each bottle set identification was performed.

The following table shows the percent recovery for each of the microorganisms tested as well as the overall percent recovery by site and for all sites combined. The mean time to detection and corresponding range are shown for each microorganism for all sites, as well as the actual inoculum ranges.

Table 10: Reproducibility Detection Rates of Microorganisms in Leukocyte-Reduced Apheresis Platelets

Microorganism	% Recovery (95% Confidence Interval)				Time to Detection (hours)		Inoculum Ranges (CFU/mL)*
	Site 1	Site 2	Site 3	Overall	Mean	Range	
Staphylococcus aureus NCTC 10788	100% (18/18)	100% (18/18)	100% (18/18)	100% [93.4-100]% (54/54)	13.2	9.6-15.9	1-7
Streptococcus pyogenes NCTC 12696	100% (18/18)	100% (18/18)	100% (18/18)	100% [93.4-100]% (54/54)	11.7	9.8-16.9	1-7
Escherichia coli NCTC 12241	100% (18/18)	100% (18/18)	100% (18/18)	100% [93.4-100]% (54/54)	9.3	7.8-10.3	1-7
Bacillus cereus NCTC 7464	100% (18/18)	100% (18/18)	100% (18/18)	100% [93.4-100]% (54/54)	8.5	7.2-13.3	1-7
Pseudomonas aeruginosa† ATCC® 27853™	100% (9/9)	100% (9/9)	100% (9/9)	100% [87.2-100]% (27/27)	13.2	12.5-14.2	1-20
Clostridium perfringens‡ NCTC 8798	100% (9/9)	100% (9/9)	100% (9/9)	100% [87.2-100]% (27/27)	7.9	6.6-9.8	1-9
Overall	100%	100%	100%	100%	-	-	-
	[96.0-100]%	[96.0-100]%	[96.0-100]%	[98.6-100]%	-	-	-
	(90/90)	(90/90)	(90/90)	(270/270)	-	-	-

* 54 bottles (27 BACT/ALERT® BPA and 27 BACT/ALERT® BPN) were inoculated with 10 mL of unseeded LRAP to serve both as sterility checks for the platelets and as negative controls to ensure platelets inoculated into BACT/ALERT® BPA and BACT/ALERT® BPN bottles do not cause false positives to occur. Of the 27 BACT/ALERT® BPA bottles, 12 were from lot 1 and 15 were from lot 2. Of the 27 BACT/ALERT® BPN bottles, 15 were from lot 1 and 12 were from lot2.

† Strict Aerobe - not tested in anaerobic culture bottles (BACT/ALERT® BPN).

‡ Strict Anaerobe - not tested in aerobic culture bottles (BACT/ALERT® BPA).

Note: The percent recovery results, per microorganism, were 100% for all sites combined.

Note: The percent recovery result for BACT/ALERT® BPA lot 1 was 100% (60/60) with 95% CI: [94.0-100]% and for BACT/ALERT® BPA lot 2 was 100% (75/75) with 95% CI: [95.2-100]%.

Note: The percent recovery result for BACT/ALERT® BPN lot 1 was 100% (75/75) with 95% CI: [95.2-100]% and forBACT/ALERT® BPN lot 2 was 100% (60/60) with 95% CI: [94.0-100]%.

Note: Overall, the negative agreement rate for BACT/ALERT® VIRTUO® was 100% (54/54). The negative agreement rate for BACT/ALERT® BPA lot 1 was 100%, (12/12) of the negative controls were declared negative by BACT/ALERT® VIRTUO®. The negative agreement rate for BACT/ALERT® BPA lot 2 was 100%, (15/15) of the negative controls were declared negative by BACT/ALERT® VIRTUO®. The negative agreement rate for BACT/ALERT® BPN lot 1 was 100%, (15/15) of the negative controls were declared negative by BACT/ALERT® VIRTUO®. The negative agreement rate for BACT/ALERT® BPN lot 2 was 100%, (12/12) of the negative controls were declared negative by BACT/ALERT® VIRTUO®.

Clinical Study Results from LRAP with BACT/ALERT® VIRTUO®
A study to determine the ability of the BACT/ALERT® BPA and BACT/ALERT® BPN culture bottles to detect the presence of microorganisms in LRAP in plasma only was performed at two (2) external sites. The platelets were obtained from inventory of a platelet collection agency. Aliquots of the platelets were seeded with BIOBALL® microorganisms at a target inoculum of 5 CFU/mL or with microorganisms prepared from serial dilutions at a target inoculum of 10 CFU/mL. The aliquots from platelets were seeded with 11 individual microorganisms:

- *Bacillus cereus* NCTC 7464 Product 56023 BIOBALL® SINGLESHOT
- *Clostridium perfringens* NCTC 8798 Product 56028 BIOBALL® SINGLESHOT
- *Escherichia coli* NCTC 12241 Product 56035 BIOBALL® SINGLESHOT
- *Staphylococcus epidermidis* NCTC 6513 Product 56092 BIOBALL® SINGLESHOT
- *Staphylococcus aureus* NCTC 10788 Product 56045 BIOBALL® SINGLESHOT
- *Streptococcus pyogenes* NCTC 12696 Product 56046 BIOBALL® SINGLESHOT
- *Enterobacter cloacae* ATCC® 29005™
- *Klebsiella pneumoniae* ATCC® 8045™
- *Serratia marcescens* ATCC® 43862™
- *Streptococcus sanguinis* ATCC® 10556™
- *Pseudomonas aeruginosa* ATCC® 27853™

Four (4) mL of the seeded LRAP aliquots were inoculated into the appropriate BACT/ALERT® BPA and BACT/ALERT® BPN bottles and the bottles were tested in the BACT/ALERT® VIRTUO® and BACT/ALERT® 3D systems. A total of 10 repetitions/system were performed for each microorganism/bottle type/platelet preparation type (LRAP) and system (BACT/ALERT® VIRTUO®, BACT/ALERT® 3D). Recovery and time to detection for test microorganisms by bottle type, platelet type, and instrument are presented in the following table, as well as the actual inoculum ranges.

Table 11: Recovery of Microorganisms in Leukocyte-Reduced Apheresis Platelets with BACT/ALERT® BPA and BACT/ALERT® BPN – Sites Combined

Microorganism	BACT/ALERT® Culture Bottle*	Inoculum Ranges (CFU/mL)†	Number of Positive Cultures		Time to Detection (hours)			
			BACT/ALERT® 3D	BACT/ALERT® VIRTUO®	BACT/ALERT® 3D		BACT/ALERT® VIRTUO®	
					Mean‡	Range	Mean‡	Range
Bacillus cereus NCTC 7464	BACT/ALERT® BPA	1-7	20	19	10.3	10.1-10.7	7.7§	7.3-8.2
	BACT/ALERT® BPN	1-8	20	20	11.8	9.8-16.7	9.4	7.8-12.0
Clostridium perfringens¶ NCTC 8798	BACT/ALERT® BPA	-	-	-	-	-	-	-
	BACT/ALERT® BPN	1-7	20	20	11.1	10.7-12.2	8.0	7.1-9.1
Enterobacter cloacae ATCC® 29005™	BACT/ALERT® BPA	3-16	20	20	12.7	12.5-13.2	10.0	9.8-10.8
	BACT/ALERT® BPN	4-20	20	20	11.6	11.3-12.0	9.0	8.8-9.3

Microorganism	BACT/ALERT® Culture Bottle*	Inoculum Ranges (CFU/mL)†	Number of Positive Cultures		Time to Detection (hours)			
			BACT/ALERT® 3D	BACT/ALERT® VIRTUO®	BACT/ALERT® 3D		BACT/ALERT® VIRTUO®	
					Mean‡	Range	Mean‡	Range
Escherichia coli NCTC 12241	BACT/ALERT® BPA	1-8	20	20	12.6	12.0-12.9	9.6	9.1-10.2
	BACT/ALERT® BPN	1-7	20	20	11.9	11.5-12.5	9.2	8.6-9.8
Klebsiella pneumoniae ATCC® 8045™	BACT/ALERT® BPA	2-17	20	20	14.5	13.9-15.4	11.7	11.0-12.6
	BACT/ALERT® BPN	3-19	20	20	13.8	13.0-14.4	11.2	10.6-12.1
*Pseudomonas aeruginosa*** ATCC® 27853™	BACT/ALERT® BPA	2-20	20	20	16.7	16.3-17.0	13.3	12.7-13.9
	BACT/ALERT® BPN	-	-	-	-	-	-	-
Serratia marcescens ATCC® 43862™	BACT/ALERT® BPA	2-14	20	20	12.4	11.7-13.9	10.2	9.7-10.9
	BACT/ALERT® BPN	1-12	20	20	12.5	11.8-13.0	10.2	9.3-10.8
Staphylococcus aureus NCTC 10788	BACT/ALERT® BPA	1-10	20	20	17.1	16.6-17.3	14.5	13.7-15.9
	BACT/ALERT® BPN	2-6	20	20	15.7	14.6-17.1	12.5	11.8-13.3
Staphylococcus epidermidis NCTC 6513	BACT/ALERT® BPA	1-7	20	20	20.4	19.6-21.4	18.1	17.2-18.9
	BACT/ALERT® BPN	1-8	20	20	17.7	16.3-20.8	14.6	13.2-15.9
Streptococcus pyogenes NCTC 12696	BACT/ALERT® BPA	1-7	20	20	15.0	14.2-15.8	12.2	11.4-13.4
	BACT/ALERT® BPN	2-9	20	20	13.9	13.4-14.4	11.2	10.5-12.0
Streptococcus sanguinis ATCC® 10556™	BACT/ALERT® BPA	1-16	20	20	21.8	19.2-26.4	17.4	13.8-22.0
	BACT/ALERT® BPN	1-17	20	19	27.0	19.2-39.6	20.7§	13.2-34.3
Positive	BACT/ALERT® BPA	-	200	199	-	-	-	-
	BACT/ALERT® BPN	-	200	199	-	-	-	-
Total % Recovery	BACT/ALERT® BPA	-	100%	99.5%	-	-	-	-
	BACT/ALERT® BPN	-	100%	99.5%	-	-	-	-
95% Confidence Interval	BACT/ALERT® BPA	-	98.2%-100%	97.2%-99.9%	-	-	-	-
	BACT/ALERT® BPN	-	98.2%-100%	97.2%-99.9%	-	-	-	-

* Ten replicates each of BACT/ALERT® BPA and BACT/ALERT® BPN bottles were inoculated with each microorganism at the inoculum level indicated.

† One BACT/ALERT® BPA and one BACT/ALERT® BPN bottle for each system was inoculated with 10 mL of “unseeded” apheresis platelets to serve as negative controls each time seeded testing was performed. These negative controls served as a sterility test of the platelet units and to establish that LRAP at a 10 mL inoculum volume do not cause false positive results to occur. The number of negative controls tested was supplemented from leftover platelets from the seeded testing such that 208 repetitions of negative controls (104 BACT/ALERT® BPA and 104 BACT/ALERT® BPN) were performed on BACT/ALERT® 3D and 211 repetitions of negative controls (106 BACT/ALERT® BPA and 105 BACT/ALERT® BPN) were performed on BACT/ALERT® VIRTUO®. For BACT/ALERT® 3D, 207 of 208 negative controls were negative (sterile), i.e., there was 1 false positive. For BACT/ALERT® VIRTUO®, all (211) negative controls were negative (sterile), i.e. there were no false positives.

‡ Mean of 20 replicates

§ Mean of 19 replicates

¶ Strict Anaerobe - not tested in aerobic culture bottles (BACT/ALERT® BPA).

**Strict Aerobe - not tested in anaerobic culture bottles (BACT/ALERT® BPN).

The seeded bottle data were analyzed using a non-inferiority approach with a -5% non-inferiority margin based on the overall detection rate.

For BACT/ALERT® BPA, BACT/ALERT® VIRTUO® detected 199/200 (99.5%, CI 97.2% - 99.9%) and BACT/ALERT® 3D detected 200/200 (100%, CI 98.2% - 100%) of the seeded bottles. For the one BACT/ALERT® VIRTUO® status negative bottle, there was no microorganism seen on Gram stain and no colonies on subculture. Repeat testing duplicates were status positive and were considered in agreement with the expected result.

The difference of the two detection rates (BACT/ALERT® VIRTUO® – BACT/ALERT® 3D) is -0.5% with a 95% confidence interval for the difference of the two detection rates of [(-3.2%) - 1.9%], using the Newcombe hybrid score method with continuity correction. Since the lower bound of the confidence interval is > -5%, the BACT/ALERT® VIRTUO® detection rate is considered acceptable.

For BACT/ALERT® BPN, BACT/ALERT® VIRTUO® detected 199/200 (99.5%, CI 97.2% - 99.9%) and BACT/ALERT® 3D detected 200/200 (100%, CI 98.2% - 100%) of the seeded bottles. For the one BACT/ALERT® VIRTUO® status negative bottle, there was no microorganism seen on Gram stain and no colonies on subculture. Repeat testing duplicates were status positive and were considered in agreement with the expected result.

The difference of the two detection rates (BACT/ALERT® VIRTUO® – BACT/ALERT® 3D) is -0.5% with a 95% confidence interval for the difference of the two detection rates of [(-3.2%) - 1.9%], using the Newcombe hybrid score method with continuity correction. Since the lower bound of the confidence interval is > -5%, the BACT/ALERT® VIRTUO® detection rate is considered acceptable.

BACT/ALERT® 3D and BACT/ALERT® VIRTUO® Systems Comparative Data

An internal study was conducted for analytical sensitivity during instrument validation to establish substantial equivalence between the BACT/ALERT® VIRTUO® and the BACT/ALERT® 3D Microbial Detection Systems. The LRAP in plasma units were obtained from the inventory of a platelet collection agency. Aliquots of the platelets were seeded with BIOBALL® microorganisms, and from stock cultures, at a target inoculum of 5 CFU/mL or with microorganisms prepared from serial dilutions at a target inoculum of 10 CFU/mL. The aliquots from platelets were seeded with 13 individual microorganisms:

- *Bacillus cereus* NCTC 7464 Product 56023 BIOBALL® SINGLESHOT
- *Clostridium perfringens* NCTC 8798 Product 56028 BIOBALL® SINGLESHOT
- *Enterobacter cloacae* ATCC® 35549™
- *Escherichia coli* NCTC 12241 Product 56035 BIOBALL® SINGLESHOT
- *Pseudomonas aeruginosa* NCTC 12924 Product 56040 BIOBALL® SINGLESHOT
- *Klebsiella pneumoniae* ATCC® 35657™
- *Proteus mirabilis* ATCC® 7002™
- *Salmonella enterica* subsp. *enterica* serovar Pomona ATCC® 10729™
- *Salmonella enterica* subsp. *enterica* serotype Typhimurium NCTC 12023 Product 56044 BIOBALL® SINGLESHOT
- *Serratia marcescens* ATCC® 43862™
- *Staphylococcus aureus* NCTC 10788 Product 56045 BIOBALL® SINGLESHOT
- *Staphylococcus epidermidis* NCTC 6513 Product 56092 BIOBALL® SINGLESHOT
- *Streptococcus sanguinis* ATCC® 10556™

Four (4) mL of the seeded LRAP aliquots were inoculated into the appropriate BACT/ALERT® BPA and BPN bottles and the bottles were tested on 3 BACT/ALERT® VIRTUO® and 1 BACT/ALERT® 3D. A total of 2 repetitions/system were performed for each microorganism/bottle type with LRAP and system (3 BACT/ALERT® VIRTUO® and 1 BACT/ALERT® 3D). Recovery and time to detection for test microorganisms by bottle type and instrument are presented in the following table, as well as the actual inoculum ranges.

Table 12: Analytical Sensitivity: Growth Performance in BACT/ALERT® BPA and BACT/ALERT® BPN Culture Bottles

Microorganism	BACT/ALERT® Culture Bottle	BACT/ALERT® 3D				BACT/ALERT® VIRTUO®			
		% Recovery (n=2)	Mean Inoculum (CFU/mL)*	Time to Detection (hours)		% Recovery (n=6)	Mean Inoculum (CFU/mL)*	Time to Detection (hours)	
				Mean	Range			Mean	Range
Bacillus cereus NCTC 7464	BACT/ALERT® BPA	100.0	5	10.4	10.3-10.6	100.0	5	7.5	7.2-7.9
	BACT/ALERT® BPN	100.0	5	13.8	13.7-13.9	100.0	5	11.5	9.5-12.7
Clostridium perfringens† NCTC 8798	BACT/ALERT® BPA	-	-	-	-	-	-	-	-
	BACT/ALERT® BPN	100.0	1	10.2	10.1-10.3	100.0	1	7.5	7.0-7.8
Enterobacter cloacae ATCC® 35549™	BACT/ALERT® BPA	100.0	1	22.7	22.1-23.3	100.0	1	22.5	18.3-26.6
	BACT/ALERT® BPN	100.0	1	13.2	13.2‡	100.0	1	10.0	9.8-10.1
Escherichia coli NCTC 12241	BACT/ALERT® BPA	100.0	1	13.0	12.7-13.2	100.0	1	9.8	9.7-10.0
	BACT/ALERT® BPN	100.0	1	11.6	11.5-11.8	100.0	1	9.0	8.9-9.2
Klebsiella pneumoniae ATCC® 35657™	BACT/ALERT® BPA	100.0	1	12.0	12.0‡	100.0	1	9.4	9.1-9.7
	BACT/ALERT® BPN	100.0	1	12.1	12.0-12.2	100.0	1	9.6	9.4-9.7
Proteus mirabilis ATCC® 7002™	BACT/ALERT® BPA	100.0	3	13.1	13.0-13.2	100.0	3	10.8	10.3-11.4
	BACT/ALERT® BPN	100.0	3	11.6	11.5-11.8	100.0	3	9.2	8.9-9.6
Pseudomonas aeruginosa§ NCTC 12924	BACT/ALERT® BPA	100.0	1	17.0	16.6-17.5	100.0	1	13.8	13.2 - 14.6
	BACT/ALERT® BPN	-	-	-	-	-	-	-	-
Salmonella enterica subsp. *enterica* serovar Pomona ATCC® 10729™	BACT/ALERT® BPA	100.0	4	12.8	12.7-13.0	100.0	4	10.1	9.9-10.3
	BACT/ALERT® BPN	100.0	4	11.6	11.5-11.8	100.0	4	9.3	9.1-9.4
Salmonella enterica subsp. *enterica* serotype Typhimurium NCTC 12023	BACT/ALERT® BPA	100.0	1	13.9	13.9‡	100.0	1	10.8	10.5-11.3
	BACT/ALERT® BPN	100.0	1	12.1	12.0-12.2	100.0	1	9.6	9.0-10.1

Microorganism	BACT/ALERT® Culture Bottle	BACT/ALERT® 3D				BACT/ALERT® VIRTUO®			
		% Recovery (n=2)	Mean Inoculum (CFU/mL)*	Time to Detection (hours)		% Recovery (n=6)	Mean Inoculum (CFU/mL)*	Time to Detection (hours)	
				Mean	Range			Mean	Range
Serratia marcescens ATCC® 43862™	BACT/ALERT® BPA	100.0	3	12.1	12.0-12.2	100.0	3	9.7	9.6-9.9
	BACT/ALERT® BPN	100.0	3	12.5	12.5‡	100.0	3	10.1	9.8-10.3
Staphylococcus aureus NCTC 10788	BACT/ALERT® BPA	100.0	1	16.8	16.6-17.0	100.0	1	14.1	13.3-15.1
	BACT/ALERT® BPN	100.0	1	18.1	17.8-18.5	100.0	1	14.3	13.9-14.9
Staphylococcus epidermidis NCTC 6513	BACT/ALERT® BPA	100.0	1	18.7	18.5-19.0	100.0	1	14.9	14.2-15.3
	BACT/ALERT® BPN	100.0	1	22.2	21.8-22.6	100.0	1	18.0	17.4-18.7
Streptococcus sanguinis ATCC® 10556™	BACT/ALERT® BPA	100.0	7	19.3	19.2-19.4	100.0	7	15.6	15.3-16.3
	BACT/ALERT® BPN	100.0	7	21.8	21.6 - 22.1	100.0	7	19.0	16.9-21.6

* Four negative controls were tested at a volume of 10 mL of unseeded LRAP per bottle type per bottle lot randomly across the four instruments (3 BACT/ALERT® VIRTUO® and 1 BACT/ALERT® 3D) to serve as negative controls each time seeded testing was performed. These negative controls also served as a sterility test of the platelet units. In addition, 4 bottles of each bottle type per each lot were loaded to establish that LRAP at a 10 mL inoculum volume do not cause false positive results to occur. A total of 10 BACT/ALERT® BPA and 10 BACT/ALERT® BPN bottles were tested on the BACT/ALERT® VIRTUO® and 2 BACT/ALERT® BPA and 2 BACT/ALERT® BPN bottles were tested on the BACT/ALERT® 3D. All negative controls were declared negative by both the BACT/ALERT® VIRTUO® and BACT/ALERT® 3D and were negative upon subculture, i.e. there were no false positives.

† Strict Anaerobe - not tested in aerobic culture bottles (BACT/ALERT® BPA).

‡ Both replicates have the same time-to-detection value.

§ Strict Aerobe - not tested in anaerobic culture bottles (BACT/ALERT® BPN).

Within-Laboratory Precision (Repeatability) of BACT/ALERT® BPA and BACT/ALERT® BPN Culture Bottles
An internal study was conducted during instrument validation to establish evidence of repeatability of growth performance of the BACT/ALERT® VIRTUO® when tested with 4 mL of LRAP over 10 days with BACT/ALERT® BPA and BACT/ALERT® BPN culture bottles. Bottles were also tested on the BACT/ALERT® 3D, for reference. The platelets were obtained from the inventory of a platelet collection agency. Aliquots of the platelets were seeded with BIOBALL® microorganisms at a target inoculum of 5 CFU/mL. The aliquots from platelets were seeded with 6 individual microorganisms:

- *Bacillus cereus* NCTC 7464 Product 56023 BIOBALL® SINGLESHOT
- *Escherichia coli* NCTC 12241 Product 56035 BIOBALL® SINGLESHOT
- *Pseudomonas aeruginosa* NCTC 12924 Product 56040 BIOBALL® SINGLESHOT
- *Clostridium perfringens* NCTC 8798 Product 56028 BIOBALL® SINGLESHOT
- *Staphylococcus aureus* NCTC 10788 Product 56045 BIOBALL® SINGLESHOT
- *Streptococcus pyogenes* NCTC 12696 Product 56046 BIOBALL® SINGLESHOT

Table 13: Within-Laboratory Precision (Repeatability) of BACT/ALERT® BPA and BACT/ALERT® BPN Culture Bottles

Microorganism	BACT/ALERT® Culture Bottle	BACT/ALERT® 3D				BACT/ALERT® VIRTUO®			
		% Recovery (n=20)*	Mean Inoculum (CFU/mL)†	Time to Detection (hours)		% Recovery (n=60)*	Mean Inoculum (CFU/mL)†	Time to Detection (hours)	
				Mean	Range			Mean	Range
Bacillus cereus NCTC 7464	BACT/ALERT® BPA	100.0	3	10.0	9.6-10.8	100.0	3	7.5	7.0-8.2
	BACT/ALERT® BPN	100.0	3	14.7	11.3-16.3	100.0	3	11.1	8.1-13.4
Clostridium perfringens‡ NCTC 8798	BACT/ALERT® BPA	-	-	-	-	-	-	-	-
	BACT/ALERT® BPN	95.0	3	16.3	9.6-102.2§	100.0	3	11.4	6.8-62.4§
Escherichia coli NCTC 12241	BACT/ALERT® BPA	100.0	3	12.8	12.2-13.7	100.0	3	9.8	9.2-11.2
	BACT/ALERT® BPN	100.0	3	11.7	11.3-12.2	100.0	3	9.1	8.6-9.7
Pseudomonas aeruginosa¶ NCTC 12924	BACT/ALERT® BPA	100.0	< 1	17.3	16.8-18.2	98.3	< 1	13.9	13.1-15.4
	BACT/ALERT® BPN	-	-	-	-	-	-	-	-
Staphylococcus aureus NCTC 10788	BACT/ALERT® BPA	100.0	3	16.5	15.6-18.0	100.0	3	13.8	12.4-15.1
	BACT/ALERT® BPN	100.0	3	17.6	16.8-18.2	100.0	3	14.2	13.1-15.4
Streptococcus pyogenes NCTC 12696	BACT/ALERT® BPA	100.0	3	16.3	15.6-17.0	100.0	3	13.6	12.2-15.5
	BACT/ALERT® BPN	100.0	3	12.9	12.5-13.7	100.0	3	10.3	9.6-11.0

* The number of replicates for *P. aeruginosa* and C. *perfringens* were 20 for the BACT/ALERT® 3D and 60 for the BACT/ALERT® VIRTUO®.

† A total of 250 negative control bottles were tested with volumes from 4-10 mL of unseeded LRAP (188 on BACT/ALERT® VIRTUO® (96 BACT/ALERT® BPA and 92 BACT/ALERT® BPN) and 62 on BACT/ALERT® 3D (31 BACT/ALERT® BPA and 31 BACT/ALERT® BPN)) to assess the risk of false positives. All negative controls were declared negative by the instruments after 7 days and upon subculture. No false positive bottles were observed.

‡ Strict Anaerobe - not tested in aerobic culture bottles (BACT/ALERT® BPA).

§ C. *perfringens* demonstrated sporadic, prolonged time-to-detection in some bottles on each detection system. Bottles were subcultured and identified as C. *perfringens*.

¶ Strict Aerobe - not tested in anaerobic culture bottles (BACT/ALERT® BPN).

Performance of the BACT/ALERT® VIRTUO® System for Use as a Secondary or Safety Measure Test to Extend the Shelf Life of Platelet Preparations

For instructions on platelet dating extension, refer to the PLATELET SPECIMEN COLLECTION AND PREPARATION section, item 5 and item 6.

Study data summarized in Tables 10 through 13 demonstrate the BACT/ALERT® VIRTUO® System is equivalent to the BACT/ALERT® 3D for detecting bacterial contamination in LRAP. To establish the equivalency of the BACT/ALERT® VIRTUO® to the BACT/ALERT® 3D as a safety measure for secondary testing of previously tested platelet products, an analytical study was performed to show that the age of platelets does not affect the time-to-detection (TTD) of organisms representing transfusion

relevant contaminants. This study shows that the BACT/ALERT® VIRTUO® is an effective secondary or safety measure for retesting of previously tested platelet products for the purpose of extending the outdating.

In this study, 4 mL aliquots of LRAP, 3 to 5 days post collection, were seeded with low levels (target 3 CFU/mL) of six organisms and tested in two lots each of BACT/ALERT® BPA and BACT/ALERT® BPN culture bottles. Additional bottles were inoculated with LRAP only, to serve as negative controls and to monitor for false positive detections. An analysis of variance based on a multi-sample median test (Brown-Mood test) was performed to determine whether the TTD was significantly different depending on the age of the platelets. A chi-square statistical analysis was used to determine whether platelet age has a significant effect on TTD. P-values greater than 0.05 indicate that there is no statistically significant evidence indicating that platelet age effects TTD. A separate median test was completed for each organism / bottle combination. All p-values for both the BACT/ALERT® 3D (Table 6) and BACT/ALERT® VIRTUO® (Table 14) were greater than 0.05, providing confirmation that platelet age does not affect TTD. A total of 96 BACT/ALERT® BPA and 92 BACT/ALERT® BPN negative control bottles were tested on the BACT/ALERT® VIRTUO®, each containing from 4 mL to 10 mL of LRAP. No false positive detections were observed (0/188).

Table 14: Analysis of BACT/ALERT® BPA and BACT/ALERT® BPN Bottle Time to Detection to Examine the Effects of Platelet Age – BACT/ALERT® VIRTUO®

Microorganism*	BACT/ALERT® Culture Bottle	Platelet Age (Days)	N	Mean TTD (Hours)	Median TTD (Hours)	Median Test χ^2 Exact P-value
Bacillus cereus NCTC 7464	BACT/ALERT® BPA	3	12	7.50	7.50	0.4592
		4	30	7.59	7.55	
		5	18	7.50	7.50	
	BACT/ALERT® BPN	3	12	11.54	11.55	0.0928
		4	30	11.30	11.50	
		5	18	10.56	10.95	
*Clostridium perfringens*** NCTC 8798	BACT/ALERT® BPN	3	12	13.42	8.10	0.6948
		4	30	11.11	8.05	
		5	18	10.58	7.95	
Escherichia coli NCTC 12241	BACT/ALERT® BPA	3	12	9.81	9.80	0.7230
		4	30	9.84	9.85	
		5	18	9.79	9.75	
	BACT/ALERT® BPN	3	12	9.21	9.10	0.2486
		4	30	9.19	9.15	
		5	18	9.04	9.00	
*Pseudomonas aeruginosa*** NCTC 12924	BACT/ALERT® BPA	3	12	13.97	14.05	0.2076
		4	29	13.98	13.80	
		5	18	13.67	13.70	
Staphylococcus aureus NCTC 10788	BACT/ALERT® BPA	3	12	13.61	13.50	0.3623
		4	30	13.85	13.90	
		5	18	13.79	13.95	
	BACT/ALERT® BPN	3	12	14.22	14.20	0.2226
		4	30	14.31	14.30	
		5	18	14.04	14.00	

Microorganism*	BACT/ALERT® Culture Bottle	Platelet Age (Days)	N	Mean TTD (Hours)	Median TTD (Hours)	Median Test χ^2 Exact P-value
Streptococcus pyogenes NCTC 12696	BACT/ALERT® BPA	3	12	13.48	13.40	0.8416
		4	30	13.70	13.80	
		5	18	13.66	13.65	
	BACT/ALERT® BPN	3	12	10.38	10.45	0.4334
		4	30	10.30	10.35	
		5	18	10.17	10.10	

* All organisms tested were in the BIOBALL® SINGLESHOT 30 format.

** *C. perfringens* and *P. aeruginosa* were tested in the BACT/ALERT® BPN and BACT/ALERT® BPA bottles respectively per intended use.

Specificity & Sensitivity

The BACT/ALERT® VIRTUO® System overall specificity (false positive (FP) rate) and sensitivity (false negative (FN) rate) were determined from external and internal studies conducted during performance validation utilizing LRAP and reported in Tables 10 through 13. During the performance validation testing for LRAP, negative controls containing 4-10 mL of platelet concentrate were tested. No instrument false positives were observed during the performance validation. Results for the negative controls are summarized in the following table.

Table 15: BACT/ALERT® VIRTUO® Instrument False Positive (FP) Results Observed When Testing LRAP

Study Data Source (Table Number)	Quantity Tested per Bottle	#FP/Bottles Tested	%FP
Reproducibility (Table 10)	10 mL	0/54	0.00%
Recovery (Table 11)	10 mL	0/211	0.00%
Analytical sensitivity (Table 12)	10 mL	0/20	0.00%
Within-laboratory precision (Table 13)	4-10 mL	0/188	0.00%
Overall	-	0/463	0.00%

Sensitivity of the BACT/ALERT® VIRTUO® instrument was determined during performance validation testing for LRAP. All negative control bottles and any seeded bottles that were determined negative by the instrument were confirmed to be true negatives through subculture to plated media. There were no false negative bottles observed.

Performance of the BACT/ALERT® Detection Systems for Use with Large Volume Delayed Sampling of Platelet Preparations

For instructions on platelet dating extension, refer to the PLATELET SPECIMEN COLLECTION AND PREPARATION section, item 7.

A data review was conducted and blood collection and transfusion services were queried to identify sources of data where the BACT/ALERT 3D (BTA 3D) was used to perform large volume delayed sampling (LVDS) for quality control testing of leukocyte-reduced apheresis (LRAP) and whole blood platelet concentrates (LRWBPC).

The reviewed data used testing protocols consistent with the test parameters described in the FDA guidance for using LVDS for quality control testing of platelets - i.e. 8 mL to 10 mL of sample per bottle, a bottle pair (one aerobic BACT/ ALERT® BPA bottle and one anaerobic BACT/ALERT® BPN bottle) tested at either greater than or equal to (≥) 36 hours or (≥) 48 hours. The reviewed studies, in general, classified the test results based on AABB Bulletin 04-07 interpretation guideline, with some study specific modifications that included detailed, subcategories of false positive results. The overall Specificity of the BTA 3D when used to perform the LVDS test was determined from external studies. The overall Sensitivity of the BACT/ALERT Microbial Systems has been previously reported in Tables 8 and 15 above.

The VIRTUO has been established as substantially equivalent to the BTA 3D through the 510(k) clearance process and as reported in Table 9 above, platelet preparation method has no impact on the ability of the BACT/ALERT Microbial Detection Systems to detect microorganisms, therefore, the performance data presented for the LVDS test apply to the BTA 3D and VIRTUO.

Using LVDS performed at either ≥36 hours or ≥48 hours, a combined total of 1,499,268 LRAP and LRWBPC tests were

performed with 1333 positive bottles (0.09%) detected by the BTA 3D. A combined total of 10,428 outdated LRAP and LRWBPC units were also tested resulting in 2 positive bottles (0.019%).The data reviewed indicate that the BACT/ALERT® Detection Systems using the large volume delayed sampling strategy is an effective test method for detecting contamination of platelets.

Table 16: Summary of Data from Large Volume Delayed Sampling of Leukocyte-Reduced Apheresis (LRAP) and Whole Blood Platelet Concentrates (LRWBPC)

	Post collection test time (h)		Outdate Testing of Platelets	
	≥ 36 h	≥48 h	LVDS initial testing ≥ 36 h	LVDS initial testing ≥ 48 h
LRAP Units Tested	986,234	85,294	-	-
# LRAP True Positives* (%)	600 (0.06%)	74 (0.09%)	-	-
LRWBPC Units Tested	411,764	15,976	-	-
# LRWBPC True Positives* (%)	634 (0.15%)	25 (0.16%)	-	-
Total Units (LRAP + LRWBPC)	1,397,998	101,270	6842	3586
Total # True Positives (%)	1,234 (0.09%)	99 (0.10%)	2 (0.029%)	0 (0.000%)

*An instrument true positive is defined as a bottle signaled as positive by the instrument and growth is confirmed by subcultures. All test results where a contaminant was isolated from the bottles, regardless of whether it was a true positive platelet contaminant or a procedural contaminant were considered in the BACT/ALERT System performance evaluation.

Table 17 below is a summary of the identifications and TTD of the microorganisms associated with the positive LVDS test results summarized in Table 16 above.

Table 17: Organism(s) by Prevalence

	Microorganisms	Number of Isolates	Range of Times to Detection in Days (Hours)
1	*Cutibacterium spp.* (including 160 C. *acnes*)	177	2.1-6.0 (50-143)
2	Coagulase negative *Staphylococcus* (including 28 *S. saccharolyticus and 3 S. epidermidis*)	81	0.2-3.4 (4.8-81)
3	*Viridans Streptococcus* (including 12 *S. pneumoniae*)	56	0.3-2.3 (6.2-54.0)
4	*Streptococcus beta-hemolytic group* (including 6 *S. agalactiae and 10 S. dysgalaticae*	25	0.2-0.8 (4.0-19.0)
5	*Gram negative bacilli (including Bacteriodes vulgatus, Campylobacter lari, Enterobacter cloacae, Escherichia coli, Haemophilus aphrophilus, Klebsiella oxytoca, Klebsiella pneumoniae, Pseudomonas aeruginosa, Serratia marcescens)*	19	0.1-1.7 (1.9-41.0)
6	*Gram positive cocci (Enterococcus faecalis, Gemella haemolysans, Gemella morbillorum, Granulicatella adiacens)*	12	0.3-2.8 (8.0-67.2)
7	*Staphylococcus aureus*	11	0.1-0.7 (2.0-17.0)
8	*Streptococcus spp.*(including *9 S. bovis*)	10	0.3-1.4 (7.2-34.0)
9	*Gram positive bacilli (Eggerthella lenta, Lactobacillus casei, Lactobacillus lactis, Listeria monocytogenes)*	9	0.6-1.7 (14.0-40.0)
10	*Corynebacterium spp. (including diphtheroids)*	4	1.0-4.9 (24.0-117.1)
11	*Bacillus spp.*	3	0.1-0.2 (2.0-4.1)
12	*Anaerobic Streptococcus*	1	2.1-2.3 (50.0-56.0)

Specificity & Sensitivity

The overall specificity (false positive (FP) rate) observed when performing LVDS was determined from external studies.

Table 18: Instrument False Positive (FP) Results Reported in LVDS Data

Platelet Product	#FP / Bottles Tested	%FP
LRAP	3621 / 1,071,528	0.34%
LRWBPC	737 / 427,740	0.17%
Overall	4358 / 1,499,268	0.29%
Range	-	0.17% - 0.34%

The overall instrument FP rate observed during LVDS testing was 0.29% (4358/1,499,268) with a range of 0.17% - 0.34% across platelet preparation. The overall instrument false positive rate for LVDS fell within the FP rate observed in controlled studies and reported in Table 8 above.

LIMITED WARRANTY

bioMérieux warrants the performance of the product for its stated intended use provided that all procedures for usage, storage and handling, shelf life (when applicable), and precautions are strictly followed as detailed in the instructions for use (IFU).

Except as expressly set forth above, bioMérieux hereby disclaims all warranties, including any implied warranties of merchantability and fitness for a particular purpose or use, and disclaims all liability, whether direct, indirect or consequential, for any use of the reagent, software, instrument and disposables (the "System") other than as set forth in the IFU.

AVAILABILITY

bioMérieux BACT/ALERT® BPN 100/case	REF 423279

For technical assistance in the USA, contact bioMérieux Customer Service at 1-800-634-7656. Outside the USA, contact your local bioMérieux Representative.

INDEX OF SYMBOLS

Symbol	Meaning
	Catalogue number
	Manufacturer
	Date of manufacture
	Temperature limit
	Use by date
	Batch code
	Consult Instructions for Use
	Contains sufficient for <n> tests
	This way up
	*In Vitro* Diagnostic Medical Device
	Do not reuse
	Latex-free
	For US Only: Caution: US Federal Law restricts this device to sale by or on the order of a licensed practitioner

Instructions for use provided in the kit or downloadable from www.biomerieux.com/techlib

REVISION HISTORY

Change type categories

N/A	Not applicable (First publication)
Correction	Correction of documentation anomalies
Technical change	Addition, revision and/or removal of information related to the product
Administrative	Implementation of non-technical changes noticeable to the user

Note: *Minor typographical, grammar, and formatting changes are not included in the revision history.*

Release Date	Part Number	Change Type	Change Summary
2020-05	9316718-B	Correction	**Performance Characteristics** - • Corrected microorganism's ATCC number: *Enterobacter cloacae* (Table 11) • Corrected microorganism's name: *Salmonella enterica* subsp. *enterica* serovar Pomona (Table 11)
2018-12	9315170-A	N/A	Document creation

bioMérieux, Inc.
100 Rodolphe Street
Durham, North Carolina 27712 USA
www.biomerieux.com