BacT/ALERT® BPA

℞only IVD

INTENDED USE

BacT/ALERT® BPA culture bottles are used with the BacT/ALERT® Microbial Detection Systems for quality control testing of platelets. BacT/ALERT BPA culture bottles support the growth of aerobic microorganisms (bacteria and fungi).

- For the BacT/ALERT® 3D Systems, the following platelet preparation methods have been validated: 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

SUMMARY AND EXPLANATION

BacT/ALERT Microbial Detection Systems and culture bottles 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 BPA bottles.

BacT/ALERT Microbial Detection Systems and culture bottles may be used for quality control testing of platelets and as a secondary safety measure test. Bacterial tests are labeled as 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.

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]

BacT/ALERT® BPA (color-coded blue) – BacT/ALERT BPA disposable culture bottles contain 40 mL of media and an internal sensor that detects carbon dioxide as an indicator of microbial growth. The BacT/ALERT BPA culture bottle does not require venting. The media formulation consists of pancreatic digest of casein (1.7% w/v), papaic digest of soybean meal (0.3% w/v), sodium polyanethol sulfonate (SPS) (0.035% w/v), pyridoxine HCl (0.001% w/v), and other complex amino acid and carbohydrate substrates in purified water. Bottles are prepared with an atmosphere of CO_2 in oxygen 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 BPA culture bottle growth media but do not produce sufficient carbon dioxide to be determined positive.

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 Needles/Subculture Units
- Disposable gloves
- Appropriate biohazard waste containers for materials potentially contaminated with infectious agents
- Appropriate platelet coupling and sampling device or platelet bag with integrally connected sample bag

Materials available from bioMérieux

- BacT/ALERT® Microbial Detection Systems
- Sterile Airway Needles/Subculture Units

Storage instructions

BacT/ALERT BPA culture bottles are ready for use. Store protected from direct sunlight 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, visually inspect all BacT/ALERT BPA bottles for evidence of damage or contamination. A bottle should not be used if any evidence of leakage is noted. The media should be clear, but there may be a slight opalescence or a trace of precipitate due to the anticoagulant SPS. Do not confuse opalescence with turbidity. Do not use a bottle which contains media exhibiting turbidity, excess gas pressure, or a yellow sensor; these are signs of possible contamination.

Instruments

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

PLATELET SPECIMEN COLLECTION AND PREPARATION (LRAP, SINGLE AND A POOL OF UP TO 6 UNITS OF LRWBPC)

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. For quality

control testing, the platelet specimen should be taken at least 24 hours after collection to allow for natural proliferation in the platelet product.[6] When testing platelets as a secondary safety measure, the laboratory should follow its own quality control procedures for specific days of testing beyond Day 2.

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. (Note: A sample volume of 4-10 mL is required for each culture bottle to be inoculated for quality control testing and secondary safety measure testing. When testing more than 10 mL, the upper limit of sample volume recommended for one BPA bottle, inoculate the sample over multiple bottles.) 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.
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. 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. If you are a U.S. customer 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.

BacT/ALERT BPA CULTURE BOTTLE TEST PROCEDURE

Preliminary comments and precautions

1. 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).
2. DO NOT VENT BACT/ALERT BPA BOTTLES. Positive culture bottles should be transiently vented before subculturing, staining, or disposal to release any gas produced during microbial metabolism.
3. 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.
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.[4]
6. Culture bottles should be utilized by trained laboratory personnel.
7. For US Only: Caution: US Federal Law restricts this device to sale by or on the order of a licensed practitioner.

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*).[7]

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. (Note: A sample volume of 4-10 mL is required for each culture bottle to be inoculated for quality control testing and secondary safety measure testing. When testing more than 10 mL, the upper limit of sample volume recommended for one BPA bottle, inoculate the sample over multiple bottles. 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.) Alternatively, a sterile, integrally connected sampling device may be used to obtain a sample from the platelet bag.
4. 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 automatically will 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.

Laboratory procedure

1. DO NOT VENT BACT/ALERT BPA 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.
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. [2]

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. 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).

NOTE: For quality control testing of platelets, 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. The platelet specimen should be taken at least 24 hours after collection to allow for natural proliferation in the platelet product.[6] 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 a *Propionibacteria* spp.

QUALITY CONTROL

A Certificate of Conformance is provided with each lot of culture bottles. If desired, individual laboratories can perform quality control testing of BacT/ALERT BPA Culture Bottles. Refer to the appropriate BacT/ALERT User Manual and to CLSI® document M22-A3.[8]

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 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.

PERFORMANCE CHARACTERISTICS OF THE TEST

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
Propionibacterium 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 Table 1 for results.

Table 1. Recovery of Organisms in Leukocyte Reduced Apheresis Platelets

			Average Time to Detection (hours)			
Microorganism	**ATCC® #**	**Actual Initial Inoculum (CFU/mL)***	**Plastic BacT/ALERT BPA†**	**Glass BacT/ALERT SA†**	**Plastic BacT/ALERT BPN†**	**Glass BacT/ALERT SN†**
Bacillus cereus	11778™	5 2	8.7 (8.7 - 8.8) 9.3 (9.1 - 9.5)	8.9 (8.8 - 9.0) 9.7 (9.3 - 10.1)	9.7 (9.5 - 9.8) 10.9 (10.7 - 11.1)	10.4 (9.7 - 10.7) 10.8 (10.2 - 11.1)
Escherichia coli	25922™	215 5	9.9 (9.7 - 10.1) 11.1 (10.9 - 11.2)	10.8 (10.6 - 11.1) 12.0 (11.9 - 12.1)	9.3 (9.1 - 9.4) 10.3 (10.2 - 10.4)	10.2 (10.1 - 10.3) 11.0 (10.9 - 11.1)
Enterobacter cloacae	Clinical Isolate	300 21	9.9 (9.8 - 10.0) 11.0 (10.9 - 11.2)	10.7 (10.7 - 10.8) 11.8 (11.7 - 11.8)	9.6 (9.5 - 9.7) 10.3 (10.2 - 10.4)	10.5 (10.4 - 10.5) 11.5 (11.4 - 11.5)
Klebsiella oxytoca	Clinical Isolate	32 5	10.1 (9.9 - 10.3) 10.9 (10.7 - 11.0)	10.8 (10.8 - 10.8) 11.5 (11.5 - 11.5)	10.4 (10.3 - 10.5) 11.1 (11.0 - 11.2)	11.2 (11.1 - 11.3) 12.1 (12.0 - 12.2)
Propionibacterium acnes	Clinical Isolate	130 16	Negative‡ Negative‡	Negative‡ Negative‡	64.0 (62.4 - 64.8) 72.8 (72.0 - 74.4)	80.8 (76.8 - 86.4) 90.4 (88.8 - 93.6)
Serratia marcescens	43862™	50 5	11.4 (11.1 - 11.6) 12.6 (12.4 - 12.8)	11.9 (11.8 - 12.1) 13.0 (12.8 - 13.3)	11.8 (11.8 - 11.9) 12.8 (12.8 - 12.8)	12.8 (12.8 - 12.8) 13.8 (13.8 - 13.9)
Staphylococcus aureus	27217™	140 4	10.4 (10.3 - 10.5) 11.4 (11.1 - 11.7)	11.0 (10.8 - 11.3) 12.0 (12.0 - 12.1)	11.5 (11.2 - 11.8) 12.9 (12.7 - 13.0)	12.6 (12.3 - 12.8) 13.5 (13.0 - 14.4)
Staphylococcus epidermidis	49134™	40 1	15.6 (15.5 - 15.7) 17.3 (17.0 - 17.5)	16.9 (16.8 - 17.0) 18.9 (18.8 - 19.2)	19.6 (19.5 - 19.7) 21.6 (21.3 - 21.8)	36.9 (19.3 - 47.8) 40.1 (23.7 - 50.4)
Streptococcus viridans	Clinical Isolate	110 3	15.3 (15.1 - 15.4) 17.9 (17.2 - 18.4)	18.9 (18.6 - 19.1) 22.4 (21.7 - 23.2)	14.7 (14.0 - 15.2) 18.0 (17.6 - 18.4)	18.2 (17.4 - 19.1) 21.4 (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 7	11.4§ (8.7 - 15.7) 12.7§ (9.1 - 18.4)	12.5§ (8.8 - 19.1) 13.9§ (9.3 - 23.2)	17.8 (9.1 - 64.8) 20.1 (10.2 - 74.4)	22.6 (9.7 - 86.4) 25.0 (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 *Propionibacterium 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
Propionibacterium 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 Table 2 for results.

Table 2. Recovery of Organisms in Single Units of Whole Blood Platelet Concentrates

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

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

			Number of Positive Cultures			
Microorganism	**ATCC® #**	**Inoculum in pooled unit (CFU/mL)***	**BacT/ALERT BPA†**	**BacT/ALERT BPN†**	**BacT/ALERT BPA + BacT/ALERT BPN‡**	**Solid Media**
Bacillus cereus	11778™	<2 8	10 10	10 10	10 10	6 10
Escherichia coli	25922™	<2 6	10 10	10 10	10 10	6 10
Enterobacter cloacae	Clinical Isolate	2 24	10 10	10 10	10 10	10 10
Klebsiella pneumoniae	Clinical Isolate	<2 2	10 10	8 10	10 10	0 9
Propionibacterium acnes	11827™	<2 2.5	0§ 0§	10 10	10 10	2 10
Serratia marcescens	43862™	<2 <2	4 10	4 10	7 10	1 6
Staphylococcus aureus	27217™	2 10	10 10	10 10	10 10	4 10
Staphylococcus epidermidis	49134™	2 11	10 10	10 10	10 10	6 10
Streptococcus viridans	Clinical Isolate	<2 <2	9 10	9 10	10 10	1 10
Clostridium perfringens	13124™	<2 <2	0§ 0§	3 10	3 10	2 1
Positive			153	184	190	124
Total % Recovery 95% Confidence Interval (CI)			76.5% 70.0 - 82.2	92.0% 87.3 - 95.4	95.0% 91.0 - 97.6	62.0% 54.9 - 68.8
% Recovery of Facultative Organisms and Strict Aerobes 95% CI			95.6% 91.2 - 98.2	–	–	–
% Recovery of Facultative Organisms and Strict Anaerobes 95% CI			–	92.0% 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

			Number of Positive Cultures			
Microorganism	**ATCC® #**	**Inoculum in pooled unit (CFU/mL)***	**BacT/ALERT BPA†**	**BacT/ALERT BPN†**	**BacT/ALERT BPA + BacT/ALERT BPN‡**	**Solid Media**
Bacillus cereus	11778™	3 11	10 10	10 10	10 10	6 10
Escherichia coli	25922™	3 10	10 10	10 10	10 10	6 10
Enterobacter cloacae	Clinical Isolate	2 14	10 10	10 10	10 10	10 10
Klebsiella pneumoniae	Clinical Isolate	<2 3	5 10	6 10	7 10	0 3
Propionibacterium acnes	11827™	<2 18	0§ 1	0 10	0 10	0 10
Serratia marcescens	43862™	4 31	10 10	10 10	10 10	8 10
Staphylococcus aureus	27217™	<2 17	10 10	10 10	10 10	9 10
Staphylococcus epidermidis	49134™	2 19	10 10	10 10	10 10	8 10
Streptococcus viridans	Clinical Isolate	1 33	10 10	10 10	10 10	10 10
Clostridium perfringens	13124™	<2 15	0§ 0§	2 10	2 10	8 10
Positive			156	178	179	162
Total % Recovery 95 % Confidence Interval (CI)			78.0% 71.6 - 83.5	89.0% 83.8 - 93.0	89.5% 84.4 - 93.4	81.0% 74.9 - 86.2
% Recovery of Facultative Organisms and Strict Aerobes 95% CI			96.9% 92.9 - 99.0	–	–	–
% Recovery of Facultative Organisms and Strict Anaerobes 95% CI				89.0% 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 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.

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. [9] 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 BTA 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

Organism*	BacT/ALERT Bottle	Platelet Age (Days)	N	Mean TTD (Hours)	Median TTD (Hours)	Median Test χ^2 Exact P-value
Bacillus cereus NCTC7464	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*** NCTC8789	BacT/ ALERT BPN	3	3	13.67	10.30	0.5772
		4	10	19.83	10.70	
		5	6	11.78	10.30	
Escherichia coli NCTC12241	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*** NCTC12924	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 NCTC10788	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 NCTC12696	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 BPN and 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 Table 7.

Table 7: Organism(s) by Prevalence

	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)

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 Table 8.

Table 8. Instrument False Positive (FP) Results

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

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AVAILABILITY

bioMérieux
BacT/ALERT® BPA
100/case REF 279018

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
REF	Catalogue number
	Legal Manufacturer
	Date of Manufacture
	Temperature limit
	Use by date
LOT	Batch code
	Consult Instructions for Use
	Contains sufficient for <n> tests
EC REP	Authorized representative in the European Community
	This way up
IVD	In Vitro Diagnostic Medical Device
	Do not reuse
	Does not contain latex
Rx only	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 TABLE

This section contains a summary of changes made to the BacT/ALERT BPA Instructions for Use (9306999 B).

Revision Date	Revision Number	Change Type	Change Summary
2017-10	9306999 B	Content Change:	**Reagents** - Clarification of expiration date
			Specimen Test/Inoculation Procedure - Addition of Caution regarding bottle pressure
			Quality Control – Addition of Caution regarding LIS and bottle type abbreviations
			Summary and Explanation, Platelet Specimen Collection and Preparation, Culture Bottle Test Procedure, and **Performance Characteristics –** Addition of secondary safety measure test information
		Administrative:	**Index of Symbols** - update to reflect new symbols on product

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

Change Type categories:

- **Correction** = Correction of documentation anomalies.
- **Content Change** = Implementation of new and modified (updated) intended use and performance characteristics.
- **Administrative** = Implementation of non-technical changes noticeable to the user.

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