BIOMÉRIEUX

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BACT/ALERT® SN

℞only IVD

Intended Use

BACT/ALERT® SN culture bottles are used with BACT/ALERT® Microbial Detection Systems in qualitative procedures for the recovery and detection of anaerobic and facultative anaerobic microorganisms (bacteria) from blood and other normally sterile body fluids.

Summary and Explanation

BACT/ALERT® Microbial Detection Systems are used to determine if microorganisms are present in blood or other normally sterile body fluid samples taken from a patient suspected of having bacteremia. The BACT/ALERT® System and culture bottles provide both a microbial detection system and a culture medium with suitable nutritional and environmental conditions for organisms commonly encountered in blood infections and other normally sterile body fluid infections. An inoculated bottle is placed into the instrument where it is incubated and continuously monitored for the presence of microorganisms that will grow in BACT/ALERT® SN bottles.

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) 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 from blue-green to yellow.[1] The lighter color results in an increase of reflectance units monitored by the system. Bottle reflectance is monitored and recorded by the instrument every 10 minutes.

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, specimen collection needles, and blood-drawing devices should be decontaminated according to your institution's procedures.[2]

BACT/ALERT® SN (color-coded purple) – BACT/ALERT® SN disposable culture bottles contain 40 mL of medium 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 polyanethol sulfonate (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 medium may be adjusted to meet specific performance requirements.

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

2 *Biosafety in Microbiological and Biomedical Laboratories (BMBL)* 5th Edition. U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes of Health. Fifth Edition. US Government Printing Office. Washington: Feb 2007.

Caution: BACT/ALERT® culture bottles contain polycarbonate. Not all disinfectants are intended for use with polycarbonate surfaces and may cause bottle deterioration. Verify disinfectant compatibility with polycarbonate before use on BACT/ALERT® culture bottle surfaces.

Additional Materials Required

- BACT/ALERT® Microbial Detection Systems
- Blood-drawing device
- Sterile Airway Needle/Subculture Units
- Disposable gloves
- Appropriate biohazard waste containers for materials potentially contaminated with infectious agents
- Alcohol pads or equivalent

Materials Available from bioMérieux

- Blood Collection Adapter Cap
- BACT/ALERT® Microbial Detection Systems
- Sterile Airway Needle/Subculture Units

Storage Instructions

BACT/ALERT® SN 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® SN 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 opalescence 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

Note: BACT/ALERT® SN culture bottles should be utilized by trained healthcare personnel. Correct specimen collection is extremely important when obtaining blood culture specimens. Venipuncture is the technique of choice for obtaining blood cultures. Refer to Cumitech 1C for the proper specimen collection procedure.[3]

Note: Take care to prevent contamination during both bottle preparation and inoculation of the patient sample. Proper skin disinfection is an essential requirement to reduce the incidence of contamination.

Note: Although not recommended by bioMérieux, blood may be drawn directly into collection tubes containing SPS. Tubes containing other anticoagulants should never be used for blood culture.[4]

Note: bioMérieux recommends that inoculated culture bottles be placed into the BACT/ALERT® Microbial Detection System as soon as possible after collection. If there is an unavoidable delay, inoculated bottles may be maintained at room temperature up to 24 hours before loading into the instrument.

[3] Baron EJ, Weinstein MP, Dunne Jr. WM, Yagupsky P, Welch DF, Wilson DM. 2005. Cumitech 1C, Blood Cultures IV. Coordinating ed., Baron EJ. ASM Press, Washington, DC.

[4] CLSI. *Principles and Procedures for Blood Cultures*; Approved Guideline. CLSI document M47-A. Wayne, PA: Clinical and Laboratory Standards Institute; 2007.

Bottle Preparation

1. Label the culture bottle with patient information. The icons on the bottle label (, #,) can be defined by the user.
2. Remove plastic flip-top from the culture bottle. Prior to inoculation, disinfect the culture bottle top with an alcohol swab or equivalent. Allow to air dry.
3. Clean the selected venipuncture site as recommended by your institution's approved procedure.

Venipuncture Direct Draw Inoculation Procedure

Note: Direct draw procedures should never be used for obtaining blood from intravascular devices or hemodialysis ports, due to the potential for catheter lumen collapse or reflux of bottle contents into the patient.

Note: If inoculating more than one type of BACT/ALERT® blood culture bottle using a butterfly blood collection set and direct draw adapter cap, inoculate first the aerobic culture bottle and then the anaerobic culture bottle so that any oxygen trapped in the tubing will not be transferred to the anaerobic bottle.

Note: Although lower sample volumes can be used, recovery may be improved using a sample volume closer to the recommended 10 mL.[5,6]

Note: To prevent over inoculation, monitor the blood volume intake into the culture bottle. The target fill-to line on the bottle label may be used to assist in estimating a sample volume of approximately 10 mL. Alternatively, the 5 mL graduations on the bottle label may be used to assist in estimating sample volume.

Note: Monitor the direct draw process closely at all times during collection to assure proper flow is obtained and to avoid flow of the bottle contents into the adapter tubing. Due to the presence of chemical additives in the culture bottle, it is important to prevent possible backflow and subsequent adverse reactions by following all steps below.

1. Hold the culture bottle at a position below the patient's arm with the bottle in an upright position (stopper uppermost).
2. Collect the blood using a butterfly blood collection set and a blood collection adapter cap as recommended by your institution's approved procedure and inoculate directly into the culture bottle at the patient's bedside. Although lower sample volumes can be used, recovery may be improved using a sample volume closer to the recommended 10 mL. To prevent over inoculation, monitor the blood volume intake into the culture bottle, using the 5 mL incremental markings on the bottle label.
3. Release the tourniquet as soon as the blood starts to flow into the culture bottle, or within 2 minutes of application.
4. Do not allow the culture bottle contents to touch the stopper or the end of the needle during the collection procedure.

   Caution: A contaminated culture bottle could contain positive pressure, and if used for direct draw, may cause reflux into the patient's vein. Culture bottle contamination may not be readily apparent. Monitor the direct draw process closely to avoid reflux. Do not use a bottle that contains medium exhibiting turbidity, a yellow sensor, or excess gas pressure; these are signs of possible contamination.
5. Transfer the inoculated culture bottle promptly to the testing laboratory.

Syringe Draw Inoculation Procedure

Note: If inoculating more than one type of BACT/ALERT® blood culture bottle using syringe draw, inoculate first the anaerobic culture bottle and then the aerobic culture bottle so that any oxygen trapped in the syringe will not be transferred to the anaerobic bottle. Line demarcations on the bottle label should be used to assist in estimating the sample volume.

1. Perform venipuncture and blood transfer to the BACT/ALERT® culture bottle according to your institution's established procedures.

   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.
2. Transfer the inoculated culture bottle promptly to the testing laboratory.

[5] Baron EJ, Weinstein MP, Dunne Jr. WM, Yagupsky P, Welch DF, Wilson DM. 2005. Cumitech 1C, Blood Cultures IV. Coordinating ed. Baron EJ. ASM Press, Washington, DC.

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

BACT/ALERT® SN Culture Bottle Test Procedure

Preliminary Comments and Precautions

1. 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.
2. 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.
3. All inoculated culture bottles, specimen collection needles, and blood-drawing devices should be decontaminated according to your institution's procedures.[7]
4. These 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.

Procedural Notes and Precautions

1. Great care must be taken to prevent contamination of the patient sample during venipuncture and during inoculation into the culture bottles. Contamination could lead to a specimen being determined positive when a clinically relevant isolate is not actually present.
2. Obtain blood samples prior to initiating antibiotic therapy. If this is not possible, draw blood immediately before administering the next antibiotic dose.
3. If inoculated culture bottles have been delayed in their receipt into the laboratory or have been incubated prior to entry into the BACT/ALERT® instrument, visually inspect 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.

Laboratory Procedure

Caution: General caution should be taken when subculturing positive culture bottles as they could have been overfilled or contain high gas-producing organisms. Positive culture bottle contents may be under increased internal pressure. Positive culture bottles should be transiently vented before staining or disposal to release any gas produced during microbial metabolism.

1. Visually inspect bottles before testing. Do not use bottles with evidence of damage, leakage, or deterioration. Consider bottles with hemolysis, turbidity, excess gas pressure, yellow sensors, and/or evidence of growth as positive. Smear and subculture. Do not incubate unless smear is negative.
2. After culture bottles have been loaded into the instrument, incubate five to seven days or until designated positive.
3. Smear and subculture all positive bottles. If the smear is negative, indicating a possible false positive, the bottle should be reloaded into the instrument until growth of the subculture or redesignation as positive. Bottles that were initially determined false positive and were redesignated positive should be smeared and subcultured.
4. Negative cultures may be checked by smear and/or subculture at some point prior to discarding as negative.
5. Procedures for loading and unloading culture bottles into the appropriate BACT/ALERT® instrument are given in the User Manual.
6. **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.[8]
7. Utilization of coring devices (i.e., blunt needle) to puncture the septum may result in bottle leakage.

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® SN culture bottles. Refer to the appropriate BACT/ALERT® User Manual and to CLSI® document M22-A3.[9]

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[7] *Biosafety in Microbiological and Biomedical Laboratories (BMBL)* 5th Edition. U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes of Health. Fifth Edition. US Government Printing Office. Washington: Feb 2007.

[8] *Biosafety in Microbiological and Biomedical Laboratories (BMBL)* 5th Edition. U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes of Health. Fifth Edition. US Government Printing Office. Washington: Feb 2007.

Instrument
A BACT/ALERT® Reflectance Standards kit is provided with each BACT/ALERT® 3D instrument and Reflectance Calibration Standards are included with each BACT/ALERT® VIRTUO® 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 blood and other normally sterile body fluid 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. Patient specimens determined positive by BACT/ALERT® may contain organisms that are positive by smear that will not grow on routine subculturing media. When this is suspected, specimens should be subcultured on special media. 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.
2. Certain strains of *Peptostreptococcus anaerobius* may be sensitive to the anticoagulant SPS which may result in a lack of growth or low production of CO_2 by these strains if an insufficient amount of sample is inoculated into the culture bottles.
3. Infrequently, BACT/ALERT® positives may occur due to a very high number of white blood cells being present in the blood sample. This may result in smear and subculture negative samples.
4. Organisms are often few in numbers and may appear intermittently in the blood stream; therefore, several consecutive blood samples should be collected from each patient.
5. 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.
6. 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.
7. It is possible that certain rare, fastidious microorganisms will not grow or may grow slowly in the BACT/ALERT® SN culture bottle growth medium. If rare, fastidious organisms requiring specialized media and culture conditions are suspected, alternative methods or extended incubation time should be considered for recovery.
8. BACT/ALERT® SN culture bottles used to culture non-blood specimens (normally sterile body fluids) may require added blood or other supplements, such as sterile, defibrinated horse blood (5% v/v) to support growth, particularly for recovery of fastidious organisms.[10]
9. On rare occasions organisms may be encountered that grow in the BACT/ALERT® SN culture bottle growth media but do not produce sufficient carbon dioxide to be determined positive. A factor that may lead to this situation is the presence of active antibiotics in a sample.

Expected Values

For BACT/ALERT® 3D, percent positive cultures were observed to be 3.4% overall and 3.0% for significant isolates from one clinical trial site in BACT/ALERT® SN culture bottles that received ≤ 10 mL of blood. For BACT/ALERT ® 3D, percent positive

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

[10] Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn WC. *Color Atlas and Textbook of Diagnostic Microbiology*, 6th ed. 2006, pp. 446,590.

cultures were observed to be 18.7% overall and 17.3% for significant isolates from one clinical trial site in BACT/ALERT® SN culture bottles that received sterile body fluids.

For BACT/ALERT® VIRTUO®, percent positive cultures were observed to be 3.8% overall and 3.3% for significant isolates from one clinical trial site in BACT/ALERT® SN culture bottles that received ≤ 10 mL of blood. For, BACT/ALERT® VIRTUO®, percent positive cultures were observed to be 18.7% overall and 18.7% for significant isolates from one clinical trial site in BACT/ALERT® SN culture bottles that received sterile body fluids.

Expected percent positives will vary based on factors such as patient population, prevalence of significant organisms, site location, and contamination rates. The expected values provided are based on clinical study data.

Performance Characteristics

BACT/ALERT® 3D Microbial Detection Systems

Analytical Sensitivity: Limit of Detection (LoD)
Data in the following table represent results from in-house seeded studies. A minimum of 60 replicates were tested per species per applicable bottle type. At least 95% detection was achieved at LoD. BACT/ALERT® SN culture bottles inoculated with *B. fragilis* received 4 mL of human blood and bottles inoculated with *S. pneumoniae* received 1 mL of human blood obtained from a healthy adult population.

Table 1: Analytical Sensitivity: Limit of Detection (LoD)

Microorganism	Strain ID	BACT/ALERT® 3D (CFU/bottle)
Bacteroides fragilis	ATCC® 25285™	4
Clostridium perfringens	NCTC 8798	4
Enterococcus faecalis	NCTC 12697	3
Escherichia coli	NCTC 12923	4
Staphylococcus aureus	NCTC 10788	6
Streptococcus pneumoniae	ATCC® 6305™	5

Data in the following table represent the results of in-house seeded studies performed using the following microorganisms at levels of ≤10 CFU/bottle and ≤100 CFU/bottle in human blood from a healthy adult population using the BACT/ALERT® 3D System.

Table 2: BACT/ALERT® SN Culture Bottle Performance

Microorganism	Inoculum (CFU/bottle)	Time to Detection (hours)* BACT/ALERT SN (Plastic)
Gram positive anaerobes (*C. perfringens*, *P. asaccharolyticus*, *P. micros*)	≤100	11.6-41.8
	≤10	13.3-46.6
Gram negative anaerobes (*F. nucleatum*, *B. fragilis*, *B. vulgatus*)	≤100	29.5-35.9
	≤10	33.4-44.7
Gram positive facultative anaerobes (*S. aureus*, *S. pneumoniae*)	≤100	13.8-16.4
	≤10	15.2-19.3
Gram negative facultative anaerobe (*E. coli*)	≤100	10.5
	≤10	12.2

* Each organism was tested in triplicate and averages obtained. Values given are a range of these averages, with the exception of *E. coli*, which was the only Gram negative facultative organism tested.

Delayed Entry
The following table includes results from seeded studies using 6 species (*Bacteroides fragilis*, *Clostridium perfringens*, *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pneumoniae*, and *Enterococcus faecium*), at target concentrations of 100 CFU/bottle. Actual inoculum levels ranged from 4-51 CFU/bottle. All bottles were tested with human blood from healthy volunteers and were held at specified temperatures and times prior to loading into the BACT/ALERT® 3D instrument. Percent recovery reflects bottles flagged positive by the instrument and subculture with colony morphology consistent with the seeded organism.

Table 3: Delayed Entry

Sample Input	Incubation Temperature (°C)	Hold Time (hours)	% Recovery	Time to Detection from Sample Inoculation (Hold Time + Instrument TTD in hours)	
				Mean	Range
Inoculated Test Bottles	Control	No delay	99.3 (143/144)	17.5	11.0-67.2
	35-37	8	100.0 (144/144)	19.9	12.6-105.0
	20-25	24	99.3 (142/143)	37.8	29.3-84.2
	20-25	36	98.6 (142/144)	46.0	39.6-94.8
	2-8	48	93.8 (135/144)	65.8	59.0-122.6
Negative Controls	All conditions		0/33	-	-

Caution: Culture bottles held at room temperature for longer than 24 hours before loading may not detect microorganisms and should be subcultured.

BACT/ALERT® VIRTUO® Microbial Detection Systems

Analytical Sensitivity: Limit of Detection (LoD)
Data in the following table represent results from in-house seeded studies. A minimum of 60 replicates were tested per species per applicable bottle type. At least 95% detection was achieved at LoD. BACT/ALERT® SN culture bottles inoculated with *B. fragilis* received 4 mL of human blood and bottles inoculated with *S. pneumoniae* received 1 mL of human blood obtained from a healthy adult population.

Table 4: Analytical Sensitivity: Limit of Detection (LoD)

Microorganism	Strain ID	BACT/ALERT® VIRTUO® (CFU/bottle)
Bacteroides fragilis	ATCC® 25285™	4
Clostridium perfringens	NCTC 8798	4
Enterococcus faecalis	NCTC 12697	3
Escherichia coli	NCTC 12923	4
Staphylococcus aureus	NCTC 10788	6
Streptococcus pneumoniae	ATCC® 6305™	5

Within-Laboratory Precision (Repeatability)
Data in the following table represent results from in-house seeded studies conducted for 20 days using multiple instruments and tested by multiple operators. A minimum of 60 replicates were tested for each organism and lot tested. Bottles were tested without blood unless otherwise indicated.

Table 5: Within-Laboratory Precision (Repeatability)

Microorganism	Range (CFU/bottle)	% Recovery				Time to Detection (hours)	
		Lot 1	Lot 2	Lot 3	Overall	Mean	Range
*Bacteroides fragilis**	12-33	100.0 (60/60)	100.0 (60/60)	100.0 (60/60)	100.0 (180/180)	26.2	19.7-38.9
Clostridium perfringens	10-15	100.0 (60/60)	100.0 (60/60)	100.0 (60/60)	100.0 (180/180)	7.9	6.4-39.7
Enterococcus faecalis	10-16	100.0 (60/60)	100.0 (60/60)	100.0 (60/60)	100.0 (180/180)	9.9	9.1-11.0
Escherichia coli	8-15	100.0 (60/60)	100.0 (60/60)	100.0 (60/60)	100.0 (180/180)	8.7	7.9-9.5
Staphylococcus aureus	6-11	100.0 (60/60)	100.0 (60/60)	100.0 (60/60)	100.0 (180/180)	12.8	11.3-14.5
*Streptococcus pneumoniae**	6-15	100.0 (60/60)	100.0 (60/60)	100.0 (60/60)	100.0 (180/180)	14.0	11.2-17.7

*Tested with 1 mL blood.

Delayed Entry
The following table includes results from seeded studies using 6 species (*Bacteroides fragilis*, *Clostridium perfringens*, *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pneumoniae*, and *Enterococcus faecium*), at target concentrations of 100 CFU/bottle. Actual inoculum levels ranged from 4-51 CFU/bottle. All bottles were tested with human blood from healthy volunteers and were held at specified temperatures and times prior to loading into the BACT/ALERT® VIRTUO® instrument. Percent recovery reflects bottles flagged positive by the instrument and subculture with colony morphology consistent with the seeded organism.

Table 6: Delayed Entry

Sample Input	Incubation Temperature (°C)	Hold Time (hours)	% Recovery	Time to Detection from Sample Inoculation (Hold Time + Instrument TTD in hours)	
				Mean	Range
Inoculated Test Bottles	Control	No delay	99.3 (143/144)	14.7	7.2-72.7
	35-37	8	100.0 (144/144)	17.6	10.7–79.5
	20-25	24	100.0 (144/144)	34.6	27.2–83.0
	20-25	36	97.9 (141/144)	43.7	37.5–75.4
	2-8	48	100.0 (141/141)	63.5	56.5–143.5
Negative Controls	All conditions		0/50	-	-

Caution: Culture bottles held at room temperature for longer than 24 hours before loading may not detect microorganisms and should be subcultured.

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

Analytical Sensitivity: Growth Performance
Data in the following tables represent results from in-house seeded studies performed with and without blood (4 mL and 10 mL) obtained from healthy human donors. A single strain was tested for each species at target inoculum levels of ≤30 CFU/bottle. The actual inoculum levels ranged from 3-27 CFU/bottle for both BACT/ALERT 3D® and BACT/ALERT® VIRTUO®. Two positive bottles were subcultured per organism to assess purity. The species listed are representatives of clinically prevalent organisms in blood cultures and sterile body fluids.

Table 7: Analytical Sensitivity: Growth Performance on BACT/ALERT® VIRTUO® and on BACT/ALERT® 3D in Bottles Tested with Blood

Microorganism	BACT/ALERT® SN BACT/ALERT® VIRTUO® - Blood				BACT/ALERT® SN BACT/ALERT® 3D - Blood			
	% Recovery (n)	Average CFU/Bottle	Time to Detection		% Recovery (n)	Average CFU/Bottle	Time to Detection	
			Mean	Range			Mean	Range
Bacteroides fragilis	100.0 (11/11)	3	38.5	27.6-54.3	100.0 (14/14)	3	33.7	20.6-54.7
Bacteroides thetaiotaomicron	100.0 (18/18)	21	40.8	36.2-46.7	100.0 (18/18)	21	47.1	40.6-53.5
Bacteroides vulgatus	100.0 (11/11)	14	63.3	46.9-78.8	100.0 (15/15)	14	62.3	43.4-85.7
Clostridium perfringens	100.0 (18/18)	27	8.7	8.3-9.2	100.0 (18/18)	27	11.6	11.0-12.2
Clostridium septicum	100.0 (18/18)	21	9.8	9.0-10.7	100.0 (18/18)	21	12.9	12.0-13.4
Eggerthella lenta	88.2* (15/17)	18	43.6	26.5-91.0	94.4* (17/18)	18	40.8	19.7-57.4
Enterococcus faecalis	100.0 (18/18)	11	9.9	9.3-10.6	100.0 (18/18)	11	12.6	12.2-13.7
Escherichia coli	100.0 (18/18)	9	8.6	8.0-9.1	100.0 (18/18)	9	10.9	9.8-11.5
Fusobacterium nucleatum	100.0 (18/18)	13	32.8	13.8-50.0	100.0 (18/18)	13	39.7	21.4-53.8
Parvimonas micra	100.0 (18/18)	24	45.2	28.0-64.2	100.0 (17/17)	24	49.4	21.6-74.6
Peptoniphilus asaccharolyticus	100.0 (18/18)	7	33.1	27.8-39.3	100.0 (18/18)	7	42.5	36.2-58.3
Staphylococcus aureus	100.0 (18/18)	14	10.3	9.9-11.1	100.0 (18/18)	15	13.3	12.7-13.9
Streptococcus pneumoniae	100.0 (18/18)	22	11.9	11.3-12.6	100.0 (18/18)	22	15.1	14.4-15.8
Streptococcus pyogenes	100.0 (18/18)	16	9.7	9.1-10.3	100.0 (18/18)	16	12.2	11.3-12.7

*Upon subculture of the negative bottles to solid medium, pure growth was observed. Thus, these bottles are false negatives.

Table 8: Analytical Sensitivity: Growth Performance on BACT/ALERT® VIRTUO® and on BACT/ALERT® 3D in Bottles Tested with No Blood

Microorganism	BACT/ALERT® SN BACT/ALERT® VIRTUO® - No Blood				BACT/ALERT® SN BACT/ALERT® 3D - No Blood			
	% Recovery (n)	Average CFU/Bottle	Time to Detection		% Recovery (n)	Average CFU/Bottle	Time to Detection	
			Mean	Range			Mean	Range
Bacteroides fragilis	100.0 (9/9)	3	25.0	23.3-28.5	100.0 (8/8)	3	29.5	28.8-30.0
Bacteroides thetaiotaomicron	100.0 (9/9)	21	25.5	23.7-27.3	100.0 (9/9)	21	29.5	28.3-31.7
Bacteroides vulgatus	100.0 (9/9)	14	27.2	25.6-28.3	100.0 (9/9)	14	31.4	31.0-32.4
Clostridium perfringens	100.0 (9/9)	27	8.0	7.7-8.5	100.0 (9/9)	27	10.9	10.6-11.3
Clostridium septicum	100.0 (9/9)	21	12.0	10.4-15.1	100.0 (9/9)	21	16.5	15.1-18.2
Eggerthella lenta	100.0 (8/8)	18	24.5	23.2-25.7	100.0 (9/9)	18	27.5	27.1-28.3
Enterococcus faecalis	100.0 (9/9)	11	9.4	8.9-10.2	100.0 (9/9)	11	12.6	12.2-13.0
Escherichia coli	100.0 (9/9)	9	8.3	7.9-9.0	100.0 (9/9)	9	11.0	10.6-11.8
Fusobacterium nucleatum	100.0 (8/8)	13	28.3	24.9-31.5	100.0 (9/9)	13	42.0	34.8-49.2
Parvimonas micra	100.0 (8/8)	24	38.0	36.3-40.2	100.0 (9/9)	24	43.8	42.0-45.1
Peptoniphilus asaccharolyticus	100.0 (9/9)	7	29.2	28.1-31.5	100.0 (9/9)	7	37.1	35.5-38.9
Staphylococcus aureus	100.0 (9/9)	14	11.4	11.0-12.0	100.0 (9/9)	15	15.2	14.9-15.6
Streptococcus pneumoniae	100.0 (9/9)	22	14.3	13.3-15.4	100.0 (9/9)	22	17.2	16.3-17.8

Microorganism	BACT/ALERT® SN BACT/ALERT® VIRTUO® - No Blood				BACT/ALERT® SN BACT/ALERT® 3D - No Blood			
	% Recovery (n)	Average CFU/Bottle	Time to Detection		% Recovery (n)	Average CFU/Bottle	Time to Detection	
			Mean	Range			Mean	Range
Streptococcus pyogenes	100.0 (9/9)	16	10.4	9.5-11.7	100.0 (9/9)	16	13.5	13.0-14.4

Clinical Study Results (Blood Cultures)
Results compare BACT/ALERT® VIRTUO® to BACT/ALERT® 3D with BACT/ALERT® SN bottles for blood cultures (for all compliant pairs). A clinical study was conducted at one site in the U.S. comparing the performance of the BACT/ALERT® VIRTUO® and BACT/ALERT® 3D for anaerobic culture pairs in which each bottle was filled with up to 10 mL of blood and in which the blood volume of the bottle with the smallest volume was within 30% of that of the bottle with the largest volume (compliant pairs). A total of 826 bottle pairs were obtained from 394 adult patients suspected of blood stream bacterial/yeast infections. Subcultures of both bottles were performed when either bottle in the set was determined to be positive by the BACT/ALERT® VIRTUO® or BACT/ALERT® 3D System. A pair of bottles was determined to have a positive status if the subculture of either the BACT/ALERT® VIRTUO® or BACT/ALERT® 3D SN culture bottle was positive. A culture bottle was determined to be a “True Positive” if the culture was flagged positive by the BACT/ALERT® VIRTUO® or BACT/ALERT® 3D System and resulted in growth of the isolate upon subculture of this bottle. True positive rates were calculated for the BACT/ALERT® VIRTUO® SN and BACT/ALERT® 3D SN culture bottles, and the ratio of BACT/ALERT® VIRTUO® SN true positives to BACT/ALERT® 3D SN true positives was calculated to compare performance. Clinical isolates recovered were classified as significant, contaminant, or unknown based on determination by the clinical trial sites.

A total of 45 isolates were recovered from all compliant anaerobic blood culture pairs with a positive status. There were a total of 41 bottle pairs that recovered at least one isolate by subculture of BACT/ALERT® VIRTUO® or BACT/ALERT® 3D SN culture bottles. A total of 38 bottle pairs recovered a single isolate, 2 bottle pairs recovered two isolates, and 1 bottle pair recovered three isolates. The total population reported in Table 9 comprises the 45 isolates recovered from positive bottle pairs and 785 negative bottle pairs for a total of 830 results. The BACT/ALERT® VIRTUO® SN culture bottle detected a total of 34 isolates compared to the BACT/ALERT® 3D SN culture bottle that detected 34 isolates. Of the significant isolates, the BACT/ALERT® VIRTUO® SN culture bottle detected a total of 32 isolates compared to the BACT/ALERT® 3D SN culture bottle that detected 30 isolates. No false positives were identified by subculture of positive BACT/ALERT® VIRTUO® SN culture bottles in the study population (0/830). Four false positives were identified by subculture of positive BACT/ALERT® 3D SN culture bottles and comprised 0.48% (4/830) of the study population.

The following tables compare results of the BACT/ALERT® VIRTUO® to BACT/ALERT® 3D blood cultures for all compliant SN blood culture bottles that yielded any number of isolates on subculture (Table 9), a single isolate alone on subculture (Table 10), and multiple isolates on subculture (Table 11).

Table 9: Blood Culture – Compliant – Single And Multiple Isolates

Clinical Determination	BACT/ALERT® VIRTUO® True Positives	% of BACT/ALERT® VIRTUO® True Positives in Population	BACT/ALERT® 3D True Positives	% of BACT/ALERT® 3D True Positives in Population	Ratio of True Positives	95% CI (LCL, UCL)
Significant	32	3.9 (32/830)	30	3.6 (30/830)	1.067	0.797, 1.337
Contaminant	1	0.1 (1/830)	4	0.5 (4/830)	0.250	-
Unknown	1	0.1 (1/830)	0	0.0 (0/830)	-	-
Total	34	4.1 (34/830)	34	4.1 (34/830)	1.000	0.730, 1.270

Table 10: Blood Culture – Compliant – Single Isolates

Clinical Determination	BACT/ALERT® VIRTUO® True Positives	% of BACT/ALERT® VIRTUO® True Positives in Population	BACT/ALERT® 3D True Positives	% of BACT/ALERT® 3D True Positives in Population	Ratio of True Positives	95% CI (LCL, UCL)
Significant	25	3.0 (25/823)	24	2.9 (24/823)	1.042	0.719, 1.365
Contaminant	1	0.1 (1/823)	4	0.5 (4/823)	0.250	-

Clinical Determination	BACT/ALERT® VIRTUO® True Positives	% of BACT/ALERT® VIRTUO® True Positives in Population	BACT/ALERT® 3D True Positives	% of BACT/ALERT® 3D True Positives in Population	Ratio of True Positives	95% CI (LCL, UCL)
Unknown	1	0.1 (1/823)	0	0.0 (0/823)	-	-
Total	27	3.3 (27/823)	28	3.4 (28/823)	0.964	0.649, 1.279

Table 11: Blood Culture – Compliant – Multiple Isolates

Clinical Determination	BACT/ALERT® VIRTUO® True Positives	% of BACT/ALERT® VIRTUO® True Positives in Population	BACT/ALERT® 3D True Positives	% of BACT/ALERT® 3D True Positives in Population	Ratio of True Positives	95% CI (LCL, UCL)
Significant	7	100.0 (7/7)	6	85.7 (6/7)	1.167	0.814, 1.520
Contaminant	0	0.0 (0/7)	0	0.0 (0/7)	-	-
Unknown	0	0.0 (0/7)	0	0.0 (0/7)	-	-
Total	7	100.0 (7/7)	6	85.7 (6/7)	1.167	0.814, 1.520

A comparative yield of microorganisms (number of isolates) from BACT/ALERT® VIRTUO® and BACT/ALERT® 3D recovered on subculture of BACT/ALERT® SN culture bottles is presented in the following table.

Table 12: Comparative Yield of Microorganisms (Number of Isolates) – Blood Cultures

Group	BACT/ALERT® VIRTUO®	BACT/ALERT® 3D
Anaerobes	1	4
Enterobacteriaceae	7	8
Enterococcus spp.	7	7
Yeasts	4	3
Non-Fermentative Gram-Negative Bacilli	1	1
Other Gram-Positive	2	2
Coagulase-Negative *Staphylococcus*	7	5
Staphylococcus aureus	5	2
Streptococcus spp.	0	2

Note: Isolate table includes polymicrobic cultures.

In this clinical study, there were 1005 pairs of BACT/ALERT® VIRTUO® and BACT/ALERT® 3D culture bottles with negative instrument results for both systems after 5 days of incubation. Among these pairs, terminal subcultures were performed for 935 pairs, and four false negative results by both BACT/ALERT® VIRTUO® and BACT/ALERT® 3D were observed; subculture on BACT/ALERT® VIRTUO® bottles alone was performed for 68 pairs, and no false negative result was observed; both subcultures were not performed for 2 pairs of bottles. A culture bottle was determined to be false negative if the bottle result was negative by the instrument and resulted in growth upon subculture of the bottle.

Table 13: Summary of Percent False Negatives from Anaerobic Blood Culture Pairs That Were Flagged Negative by Both Instruments

Subculture Performed BACT/ALERT® VIRTUO®	Subculture Performed BACT/ALERT® 3D	% False Negative BACT/ALERT® VIRTUO®	% False Negative BACT/ALERT® 3D
Yes	Yes	0.43 (4/935)	0.43 (4/935)
Yes	No	0.00 (0/68)	-

Of these four positive subcultures, one subculture yielded an isolate that is a strict aerobe (*Pseudomonas putida*). The SN culture bottle is not intended to detect strict aerobes from blood or other normally sterile body fluids. The overall false negative rate for BACT/ALERT® VIRTUO® based on a subset of terminal subcultures was 0.40% (4/1003) and excluding strict aerobes was 0.30% (3/1003).

Clinical Study Results (Sterile Body Fluid Cultures)
A clinical study was conducted at one site in Canada comparing the performance of the BACT/ALERT® VIRTUO® SN and BACT/ALERT® 3D SN culture bottles with sterile body fluid specimens. A total of 75 bottle pairs were obtained from 71 adult patients suspected of sterile body fluid bacterial/yeast infections. Sterile body fluid types evaluated were continuous ambulatory peritoneal dialysis (CAPD) fluid, cerebrospinal fluid (CSF), pericardial fluid, peritoneal fluid, pleural fluid, and synovial fluid. Clinical isolates recovered were classified as significant, contaminant, or unknown based on determination by the clinical trial sites.

A total of 21 isolates were recovered from all anaerobic sterile body fluid culture pairs with a positive status. There were a total of 15 bottle pairs that recovered at least one isolate by subculture of BACT/ALERT® VIRTUO® SN or BACT/ALERT® 3D SN culture bottles. A total of 10 bottle pairs recovered a single isolate, 4 bottle pairs recovered two isolates, and 1 bottle pair recovered three isolates. The total population reported in the table below comprises the 21 isolates recovered from positive bottle pairs and 60 negative bottle pairs for a total of 81 results. The BACT/ALERT® VIRTUO® SN culture bottle detected a total of 19 isolates compared to the BACT/ALERT® 3D SN culture bottle that detected 18 isolates. Of the significant isolates, the BACT/ALERT® VIRTUO® SN culture bottle detected a total of 17 isolates compared to the BACT/ALERT® 3D SN culture bottle that detected 17 isolates. No false positives were identified by subculture of positive BACT/ALERT® VIRTUO® SN culture bottles in the study population (0/81). No false positives were identified by subculture of positive BACT/ALERT® 3D SN culture bottles in the study population (0/81).

The following table compares results of the BACT/ALERT® VIRTUO® SN to BACT/ALERT® 3D SN sterile body fluid cultures that yielded single or multiple isolates on subculture.

Table 14: Sterile Body Fluids – Single and Multiple Isolates

Clinical Determination	BACT/ALERT® VIRTUO® True Positives	% of BACT/ALERT® VIRTUO® True Positives in Population	BACT/ALERT® 3D True Positives	% of BACT/ALERT® 3D True Positives in Population	Ratio of True Positives	95% CI (LCL, UCL)
Significant	17	21.0 (17/81)	17	21.0 (17/81)	1.000	0.837, 1.163
Contaminant	1	1.2 (1/81)	1	1.2 (1/81)	1.000	-
Unknown	1	1.2 (1/81)	0	0.0 (0/81)	-	-
Total	19	23.5 (19/81)	18	22.2 (18/81)	1.056	0.806, 1.306

A comparative yield of microorganisms (number of isolates) from BACT/ALERT® VIRTUO® and BACT/ALERT® 3D recovered on subculture of BACT/ALERT® SN culture bottles is presented in Table 15 and the number of positive specimens by fluid type is presented in Table 16.

Table 15: Comparative Yield of Microorganisms (Number of Isolates) – Sterile Body Fluid Cultures

Group	BACT/ALERT® VIRTUO®	BACT/ALERT® 3D
Anaerobes	2	2
Enterobacteriaceae	3	4
Enterococcus spp.	3	2
Yeasts	1	0
Non-Fermentative Gram-Negative Bacilli	-	-
Other Gram-Positive	1	1
Coagulase-Negative *Staphylococcus*	4	4
Staphylococcus aureus	3	3
Streptococcus spp.	2	2

Note: Isolate table includes polymicrobic cultures.

Table 16: Number of Positive Specimens – Sterile Body Fluid Cultures

Sterile Body Fluid Type	BACT/ALERT® VIRTUO®	BACT/ALERT® 3D
CAPD	3	3
Peritoneal	5	5
Synovial	6	6

In this clinical study, there were 60 pairs of BACT/ALERT® VIRTUO® and BACT/ALERT® 3D culture bottles with negative instrument results for both systems after 5 days of incubation. Among these pairs, terminal subcultures were performed for 60 pairs, and no false negative result by either BACT/ALERT® VIRTUO® or BACT/ALERT® 3D was observed. A culture bottle was determined to be false negative if the bottle result was negative by the instrument and resulted in growth upon subculture of the bottle.

Table 17: Summary of Percent False Negatives from Anaerobic Sterile Body Fluid Culture Pairs That Were Flagged Negative by Both Instruments

Subculture Performed BACT/ALERT® VIRTUO®	Subculture Performed BACT/ALERT® 3D	% False Negative BACT/ALERT® VIRTUO®	% False Negative BACT/ALERT® 3D
Yes	Yes	0.0 (0/60)	0.0 (0/60)

Summary of False Positive Results
A culture bottle was determined to be a false positive if the culture was flagged positive by the BACT/ALERT® VIRTUO® or BACT/ALERT® 3D System and was negative upon subculture of the bottle. The study population consisted of culture pairs that received specimen volumes ≤10 mL. The false positive results were identified by subculture of positive BACT/ALERT® VIRTUO® bottles and positive BACT/ALERT® 3D bottles, comprising proportions of the study populations based on the total numbers of corresponding blood cultures or sterile body fluid cultures.

Table 18: Summary of False Positive Results

Bottle Type – Specimen Type	% False Positive BACT/ALERT® VIRTUO®	% False Positive BACT/ALERT® 3D
BACT/ALERT® SN – Blood	0.00 (0/1061)	0.47 (5/1061)
BACT/ALERT® SN – Sterile Body Fluid	0.00 (0/75)	0.00 (0/75)

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® SN	100/case	REF 259790

For technical assistance in the USA, contact bioMérieux Customer Service at 1-800-682-2666. 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
	Authorized Representative in the European Community
	This way up
	*In Vitro* Diagnostic Medical Device
	Do not reuse
	Does not contain latex
	For US Only: Caution: US Federal Law restricts this device to sale by or on the order of a licensed practitioner
	Fill-to

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
2020-08	043260-01	Administrative change	Migration to CMS, including standardization of content; no technical changes.
2017-04	9313397 E	Technical change	Addition of VIRTUO® information throughout, including **Expected Values** and **Performance Characteristics of the Test** sections
			Limitations of the Test - Addition of limitations (7-9)
2016-03	9311983 D	Technical change	**Limitations of the Test** - Addition of delayed entry limitation
			Addition of Rx-only caution for US customers
		Administrative	**Limited Warranty** - Addition of statement
			Index of Symbols - Update Rx-only symbol definition
2015-05	9307043 C	Technical Change	**Reagents** - Clarification of expiration date
			Specimen Collection and Preparation • Addition of Caution regarding bottle pressure • Addition of Note regarding bottle label fill-to information • Addition of Notes regarding venipuncture information
		Administrative	**Index of Symbols**: Update to reflect new symbols on product
			Addition of technical library address

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