

BBL™ SEPTI-CHEK™
Blood Culture with Resins
For Use in the Culture of Microorganisms

I. INTENDED USE

A qualitative test system for the detection of aerobic and facultative microorganisms (bacteria and yeast) in blood.

II. SUMMARY AND EXPLANATION

Blood culture is one of the most important and critical procedures performed in the microbiology laboratory. Since blood is normally sterile, the isolation and identification of an organism has great diagnostic significance. Blood cultures are of great importance in diagnosing such conditions as endocarditis, typhoid fever, pneumonia and other diseases characterized by bacteremia.

The use of a biphasic blood culture system has been shown to improve the sensitivity of blood culture over traditional broth media.¹⁻³ When affixed to **BBL™ SEPTI-CHEK™** Blood Culture Bottles after their inoculation with blood, the agar surfaces on the slide allow the subculture of aerobic, facultative and capnophilic microorganisms present in the specimen after the bottle with the slide attached is tilted and further incubated. The presence of resins in blood culture media has been shown to significantly increase the recovery of clinical pathogens from specimens.^{4,5} This increase in recovery is more pronounced in specimens in which antimicrobials are present, but there have also been significant increases in recovery from specimens which do not contain antimicrobials. The **BBL™ SEPTI-CHEK™** TSB with Resins Culture Bottle, when used together with the **BBL™ SEPTI-CHEK™** Slide, combines both features, and has been shown to be applicable for bacterial isolation in the presence and/or absence of a wide variety of antimicrobials (see “Performance Characteristics”). The presence of both nonselective and selective differential agar media on the slide allows a pre-differentiation of the microorganisms present in the liquid medium of the blood culture bottle.

III. PRINCIPLE OF THE PROCEDURE

Blood is collected by venipuncture and transferred aseptically into a **BBL™ SEPTI-CHEK™** TSB with Resins Culture Bottle. For optimum recovery, the **BBL™ SEPTI-CHEK™** Slide is attached and the system (bottle and slide) inverted 4-6 h post inoculation. The slide/bottle unit is incubated without agitation at $35 \pm 2^\circ\text{C}$ for a 7-day culture period. The slide is examined daily for growth and the system (bottle and slide) inverted periodically. **BBL™ SEPTI-CHEK™** TSB with Resins Culture Bottles are intended for use in recovering organisms in the presence of oxygen, and are not recommended for use in anaerobic culturing. If **BBL™ SEPTI-CHEK™** TSB with Resins Culture Bottles are used without the **BBL™ SEPTI-CHEK™** Slide, they should be transiently vented after inoculation using a sterile venting unit (see “Availability”).

IV. REAGENTS

BBL™ SEPTI-CHEK™ TSB with Resins Culture Bottle

Approximate Formula* per L

TSB (**Trypticase™** Soy Broth) with Resins-70 ml.

Deionized Water.....	1.00 L	Dextrose.....	0.6g
Nonionic Adsorbing Resin.....	64.0g	Sodium Polanetholsulfonate (SPS).....	0.50
Soybean-Casein Digest Broth...	27.5	Pyridoxal HCL (Vitamin B6).....	0.01
Cationic Exchange Resin.....	4.0	Hemin.....	0.005
Yeast Extract.....	2.5	Menadione.....	0.0005
Sucrose.....	0.8		

*Adjusted and/or supplemented as required to meet performance criteria.

Each culture bottle contains an atmosphere of CO₂ |

Comment [JC1]:

Precautions: For In Vitro Diagnostic Use.

SPS inhibits the growth of certain mycoplasmas and should not be used for their isolation.

A number of fastidious and/or SPS sensitive organisms may not grow in media without blood or an appropriate supplement, such as **BACTEC™FOS™**, or **BBL™IsoVitaleX™** Enrichment. Examples of such organisms include *Haemophilus influenzae*, *Neisseria meningitidis* and *Neisseria gonorrhoeae*.

Do not use bottles that exhibit any cracks or defects.

Inoculated culture bottles should be decontaminated before being discarded.

Pathogenic microorganisms including Hepatitis B Virus and Human Immunodeficiency Virus may be present in specimens. “Universal Precautions” and institutional guidelines should be followed in handling all items contaminated with blood or other body fluids.^{9,10} After use, contaminated materials must be sterilized by autoclaving.

Storage: Store culture bottles at 15-25°C. Protect from exposure to light.

Physical Indications of Instability: Indications of instability in an uninoculated bottle are the development of turbidity and/or a change in color. Do not use after the expiration date shown on the bottle label.

BBL™ SEPTI-CHEK™ Slides: Approximate Formula* Per L of Processed Water.

Agar 1: Chocolate Agar	Agar 2:MacConkey Agar	Agar 3: Malt Agar
GC Agar Base.....36.0 g Hemoglobin Powder.....15.0 BBL™ IsoVitaleX™ Enrichment..10.0 ml Granulated Agar.....4.0 g	Peptones.....20.0 g Agar.....18.0 Lactose.....10.0 Sodium Chloride..5.0 Bile Salts.....1.5 Neutral Red.....0.03 Crystal Violet.....0.001	Malt Extract... 30.0 g Agar.....18.0

*Adjusted and/or supplemented as required to meet performance criteria.

Storage: Store slides at 2-8°C. Protect from light; avoid temperature fluctuations during storage as this may cause excessive condensation in the tube of the slide.

Physical Indications of Instability: During inspection of the uninoculated (sealed) slides, any colonies in or on the agar surface are indications of contamination. Shrunken agar surfaces, agar separated from the tray, or strong color differences of the agar surfaces are signs of deterioration. Slides showing any of the signs mentioned previously should not be used and should be disposed of appropriately. Do not use after the expiration date.

V. SPECIMEN COLLECTION AND HANDLING

The resins present in the **BBL™ SEPTI-CHEK™** with Resins Culture Bottles are able to neutralize clinically significant levels of a wide variety of antimicrobials (see “Performance Characteristics”). For optimum results, however, blood samples should be obtained prior to initiating antibiotic therapy. If this not possible, blood should be drawn immediately before administering the next dose.

In order to detect septicemia with sufficient accuracy, it may be necessary to set up one to three blood cultures at designated time intervals depending on the clinical situation.

Skin Preparation: To avoid the potential of false-positive blood cultures (e.g., with *Staphylococcus epidermidis*), the puncture site must be cleaned thoroughly and disinfected. Cleanse the venipuncture site with a swab soaked in 70% isopropyl or ethyl alcohol and disinfect the site with a 2% iodine solution. Let the skin dry before venipuncture. The venipuncture site, once disinfected, should not be touched to avoid renewed contamination. After venipuncture, any residual iodine should be removed.

Collection of Blood: Using a needle and syringe, or the **BBL™ SEPTI-CHEK™** Blood Collection Adaptor (see “Availability”), obtain approximately 8-10 ml of patient’s blood for each 70 ml **BBL™ SEPTI-CHEK™** TSB with Resins Culture Bottle. Due to the concern about contracting infectious disease, consult CDC or NCCLS recommendations on blood collection.^{6,7}

VI. PROCEDURE

Materials Provided: BBL™ SEPTI-CHEK™ TSB with Resins Culture Bottles (see “Availability”).

Materials Required but Not Provided: BBL™ SEPTI-CHEK™ Slides, needle and syringe (or appropriate blood collection unit), isopropyl or ethyl alcohol (70%), iodine solution (2%), incubator (35 ± 2°C), sterile venting units, inversion rack, disposable absorbant pads, *BACTEC™ FOS™* Supplement, BBL IsoVitaleX Enrichment, and autoclave.

Performance of the test:

1. Prepare and label the appropriate culture bottle.
2. DO NOT UNSCREW THE CAP. Remove the protective top of the screw cap on the culture bottle.
3. Disinfect the visible part of the rubber stopper with isopropyl or ethyl alcohol (70%) and allow to dry.
4. Obtain approximately 8-10 ml of patient's blood per bottle using a needle and syringe or blood collection unit.
5. Transfer immediately to the culture bottle under aseptic conditions; mix contents gently by repeated inversion.
6. **If using the BBL™ SEPTI-CHEK™ Slide (recommended use), perform the following:**
Use aseptic technique throughout. It is recommended that these steps be performed in a biosafety cabinet (Class II).

After the inoculated blood culture bottle is incubated for 4-6 h at 35 ± 2°C:

- A. Prepare and label one BBL™ SEPTI-CHEK™ Slide; attach the label in such a way that the agar surfaces are still visible.
 - B. Unscrew the cap of the BBL™ SEPTI-CHEK™ TSB with Resins Culture Bottle.
 - C. Unscrew the unlabelled cap of the BBL™ SEPTI-CHEK™ Slide and screw the slide unit onto the thread of the culture bottle. FINGER TIGHTEN ONLY. CHECK THAT THE CAP OF THE SLIDE IS FIXED TIGHTLY. Place bottles with slides into an inversion rack, if desired (see “Instructions for 70 ml Bottle Inversion Rack”).
 - D. Tip the whole system (combined bottle and slide) and rotate it 180° around the longitudinal axis to allow complete flooding of the agar surfaces. DO NOT HOLD THE SYSTEM INVERTED FOR MORE THAN 15 SEC, AND DO NOT SHAKE. ALSO, DO NOT PLACE THE SYSTEM INVERTED ON THE BENCH SURFACE.
Revert into an upright position (slide up, bottle down).
 - E. Incubate the system for 18-24 h at 35 ± 2°C.
7. **If the BBL™ SEPTI-CHEK™ Slide is NOT used**, the culture bottle must be transiently vented using a sterile venting unit prior to incubation at 35 ± 2°C. Follow the venting procedure described in the following section on “Venting”. After the bottle had been vented, mix contents two or three times by gentle

inversion using an inversion rack, if desired (see “Instructions for 70 ml Bottle Inversion Rack”).

Instructions for 70 ml Bottle Inversion Rack: The rack holds up to 12 bottles with or without **BBL™ SEPTI-CHEK™** Slides. The bottle restraining piece of the rack is moved up and down with a hinged action.

1. Place the rack on bench with hinged piece on top.
2. Grasp the hinged piece at the unhinged end of the frame and pull up, forcefully if necessary.
3. Place each bottle into an individual compartment.
4. Close the hinged piece over the bottles and push down, forcefully if necessary, until the hinged piece is fully closed and secured at the unhinged end of the rack.
5. Invert the rack as described above.

Subculturing using the BBL™ SEPTI-CHEK™ Slide:

- A. After 18-24 h of incubation, check the agar surfaces of the slide from the outside for indication of growth (refer to “Results and Interpretation”). If transparency of the slide is inhibited by condensing water, carefully remove the slide from the tube, as outlined in paragraph D.

NOTE: CARE MUST BE TAKEN NOT TO CONFUSE COLONIES OF MICROORGANISMS WITH RESIN BEADS WHICH MAY BE DEPOSITED ON THE AGAR SURFACES.

- B. If growth has not occurred, inversion and reversion of the system to flood the agar surfaces and subsequent incubation is to be repeated once a day for 7 days. Do not flood the agar surfaces more than once a day. Once colonies have formed on the agar surfaces, further inversion of the system is not recommended.
- C. If growth on any of the agar surfaces is confluent, smeared or mixed, subculturing should be performed.

Use aseptic techniques, preferably in a Class II biosafety cabinet. It is recommended that the following step be performed over a plastic lined absorbant paper sheet or pad to contain any resin beads which may fall out during the procedure. When subculturing is complete, dispose of the sheet or pad as infectious waste.

- D. Carefully unscrew the cap with colored label of the slide and remove the agar tray. Without touching the agar surfaces, hold the tray by the

cap with colored label and using an inoculating loop, remove enough colony mass to perform appropriate procedures for subculturing, identification and susceptibility testing of the isolate(s) as outlined in “Results and Interpretation”. Alternatively, the tray can be inverted and placed with the labeled cap on the bench surface. After subculturing, return the tray into the tube, close and finger-tighten.

- E. If, after the agar tray has been removed, no microbial growth is observed, the tray may be carefully replaced in the chamber and the system returned for further incubation following inversion to flood the agar surfaces.

NOTE: Check to see that no resin beads are in the cap threads, as this may allow the slide chamber to leak during subsequent inversions. Aseptically remove any resin beads that are in the cap threads before returning the slide to the chamber.

Subculturing Using the Conventional Methods: Following inoculation and incubation of the culture bottle, observe daily for turbidity, hemolysis, gas formation, color changes and other evidence of microbial growth. If growth is detected and a **BBL™ SEPTI-CHEK™** slide is NOT attached, it is necessary to vent the bottle prior to subculturing in order to release gas which often builds up due to microbial metabolism. After venting, prepare a Gram-stained smear and use appropriate subculture methods.

Venting: Venting should be performed in a biological safety cabinet. If possible and appropriate, protective clothing should be worn. Place the bottle in an upright position and place an alcohol wipe over the septum. Insert a sterile needle with an appropriate filter or pledget through the alcohol wipe and septum. The insertion and withdrawal of the needle should be done in a straight-line, avoiding any twisting motions. Discard the needle in an appropriate sharps container.

VII. RESULTS AND INTERPRETATION

If present, bacterial growth usually becomes evident within 48 hours; however, cultures should be incubated for at least 7 days before results are reported as negative. If a **BBL™ SEPTI-CHEK™** Slide is NOT used, the presence of microorganisms must be further confirmed by subculturing on suitable media and by performing appropriate identification procedures.

If a **BBL™ SEPTI-CHEK™** Slide is used, growth on the agar surfaces usually becomes visible when organisms in the blood culture have reached approximately 500 CFU/ml. If the concentration of organisms at the time of the first slide subculture (tipping of the system) is above 10^6 CFU/ml, the growth on the slide may be confluent. Typical colony morphology will not be observed with confluent growth, which may appear as a thin film on the agar surface.

The three different agar media in the slide in many cases allow a pre-differentiation of the isolate, provided the specimen is a pure culture.

Agar 1 (Chocolate Agar) is an optimal medium for the recovery of a broad range of microorganisms, including gram-negative and gram-positive bacteria, fastidious organisms such as *Neisseria* and *Haemophilus* spp., and yeasts.

Agar 2 (MacConkey Agar) is a selective differential medium for *Enterobacteriaceae* and certain nonfermenters such as *Pseudomonas aeruginosa*. Gram-positive bacteria are inhibited.

Agar 3 (Malt Agar) is a selective medium for fungi and yeasts. Bacterial growth is usually inhibited. Terminal subculture of the broth onto chocolate agar and incubation for 2 days may be performed to confirm low levels of growth on the slide or to evaluate an unusual appearance of the broth; however, it is not required.

Growth from the slides should be subcultured onto appropriate media, such as Chocolate Agar and TSA Agar with 5% sheep blood, incubated in an aerobic atmosphere enriched with CO₂. Also, a smear with subsequent Gram stain and microscopy can be performed directly from growth on the surface of the slide agar(s). Microscopic examination is also recommended to assure the presence of a pure culture. Appropriate identification tests and usually a susceptibility test of the isolate(s) should be performed.

VIII QUALITY CONTROL

BBL™ SEPTI-CHEK™ TSB with Resins Culture Bottle: The following suggested microorganisms may be used for the quality control testing: *Streptococcus pneumoniae* (ATCC® 6305) and *Pseudomonas aeruginosa* (ATCC 27853). Inoculate the broth with a bacterial inoculum containing approximately 300 CFU/ml. Vent the bottles using a sterile venting unit (see “Venting”) and incubate at 35 ± 2°C. Observe bottles for evidence of microbial growth. Retest any organism which fails to grow within five (5) days. If the retest also fails to show growth, call Technical Services.

BBL™ SEPTI-CHEK™ Slide: For the quality control testing of the slide, inoculate a Chocolate Agar or TSA with 5% sheep blood plate with the test strains. Incubate 18-24 h aerobically in a CO₂-enriched atmosphere at 35± 2°C. Using TSA Broth, prepare serial dilutions of the test strains.

Inoculate a **BBL™ SEPTI-CHEK™** bottle with a bacterial inoculum of 500-1000 CFU/ml. Mix gently. Affix a **BBL™ SEPTI-CHEK™** Slide to the bottle. Tip the system to inoculate the slide completely. Revert the system and incubate for 24 h. After incubation, growth of the following suggested test organisms should be visible on the slides:

Microorganism	Agar 1	Agar 2	Agar 3
<i>Streptococcus pneumoniae</i> ATCC™ 6305	Growth	-	-
<i>Neisseria meningitidis</i> * ATCC 13090	Growth	-	-
<i>Enterococcus faecalis</i> ATCC 29212	Growth	-	-
<i>Escherichia coli</i> ATCC 25922	Growth	Growth	-
<i>Candida albicans</i> ATCC 60193	Growth	-	Growth

*May require 48 h for visible growth.

Properly dispose of all units used in quality control testing.

Patients results should not be reported if positive and negative controls do not yield appropriate results.

IX. LIMITATIONS OF THE PROCEDURE

In order to detect septicemia with sufficient accuracy, it is necessary to prepare 1-3 pairs of blood cultures (aerobic, anaerobic) at consecutive time intervals. No single culture can rule out the presence of microorganisms in blood.

Factors that may affect the growth of clinically significant organisms in the broth and on the slide include antimicrobial therapy prior to blood collection, transitory bacteremias, contamination of the blood by exogenous flora or contamination of the blood culture bottle medium during mounting of the slide. Microaerophilic organisms may grow in the liquid medium of the bottle, but may fail to produce sufficient growth on surfaces of the slide. If the bottle medium is turbid, hemolyzed or shows other signs of microbial growth, but media on the tray fail to recover the organism after the system has been tipped and reincubated, subculture from the bottle medium and incubate the subculture plate in an appropriate gas atmosphere (such as provided by a **BBL™CampyPak™** system). Also, strictly anaerobic organisms will not grow on the agar surfaces of the slide, but may grow in the liquid medium of the bottle in rare cases.

The growth of certain mycoplasmas is inhibited by sodium polyanetholsulfonate (SPS), and the **BBL™ SEPTI-CHEK™** blood culture system should not be used for their isolation.⁸

Neutralization of antimicrobial activity by resins varies depending on dosage level and timing of specimen collection. For information on antimicrobial agents neutralized by resins, contact Technical Services at the toll free number listed below.

Culture media sometimes contain small numbers of nonviable organisms derived from medium constituents, which may be visible in smears of uninoculated blood culture media. Other sources of nonviable organisms visible upon Gram staining include staining reagents, immersion oil, glass slides and the specimens used for inoculation. If there is uncertainty about the validity of the Gram stain, the culture should be reincubated for an additional hour or two and the smear and staining procedure repeated before a report is issued.

X. PERFORMANCE CHARACTERISTICS

The **BBL™ SEPTI-CHEK™** TSB with Resins Culture Bottle and **BBL™ SEPTI-CHEK™** Slide when used together permit the isolation of aerobic and facultative microorganisms.

Seeded culture studies were performed using inoculum levels of 10-100 CFU per bottle. The following is a list of the organisms which grew in **BBL™ SEPTI-CHEK™** TSB with Resins together with the **BBL™ SEPTI-CHEK™** Slide for which growth was observed within a five (5) day period.

<i>Acinetobacter anitratus</i> ATCC 33498	<i>Neisseria gonorrhoeae</i> ** ATCC 43069
<i>Alcaligenes faecalis</i> ATCC 8750	<i>Neisseria meningitidis</i> ** ATCC 13090
<i>Candida albicans</i> ATCC 18804	<i>Providencia stuartii</i> ATCC 33672
<i>Enterobacter cloacae</i> ATCC 35030	<i>Pseudomonas aeruginosa</i> ATCC 27853
<i>Enterococcus faecalis</i> ATCC 29212	<i>Staphylococcus aureus</i> ATCC 25923
<i>Escherichia coli</i> ATCC 25922	<i>Streptococcus pneumoniae</i> ATCC 6305
<i>Haemophilus influenzae</i> * ATCC 10211	<i>Streptococcus pyogenes</i> ATCC 19615
<i>Haemophilus influenzae</i> * ATCC 19418	<i>Xanthomonas maltophilia</i> ATCC 13637
<i>Klebsiella pneumoniae</i> ATCC 33495	

*addition of **BBL™ IsoVitaleX™** Enrichment

Addition of 3.5 ml fresh blood or **BACTEC™ FOS™ Supplement

Internal studies † have shown that antimicrobials are effectively neutralized by the resins used in **BACTEC** resin media. In these tests, antimicrobials were added in clinically relevant concentrations directly to resin media prior to inoculation with susceptible strains. These tests were performed in parallel using non-resin media as controls. Penicillins, cephalosporins (1st, 2nd, and 3rd generation), macrolides, aminoglycosides, fluoroquinolones, lincomycins, tetracycline and chloramphenicol are among the antimicrobial agents neutralized by the resins.

† Data on file, Becton Dickinson Microbiology Systems.

XI. AVAILABILITY

Cat. No.	Description
4343189	BBL™ SEPTI-CHEK™ TSB (Trypticase™ Soy Broth) with Resins Culture Bottle, 10 x 70 ml bottles.
4343181	BBL™ SEPTI-CHEK™ Slide (Chocolate Agar, MacConkey Agar and Malt Agar), Blood Culture/Subculture Slide, 10 slides.
4343500	BBL™ SEPTI-CHEK™ Slide (Chocolate Agar, MacConkey Agar and Malt Agar), Blood Culture/Subculture Slide, 50 slides.
4343563	BBL™ SEPTI-CHEK™ Blood Collection Adaptor, Box of 50.
4343247	Inversion Rack, 70 ml bottle, Pkg. of one.
4371056	Sub/Venting Units for Culture Bottles, Box of 100.
4402153	FOS™ (Fastidious Organism Supplement)
4311875	BBL™ IsoVitaleX™ Enrichment

XII. REFERENCES

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7. National Committee for Clinical Laboratory Standards. 1991. *Protection of Laboratory Workers from Infectious Disease Transmitted by Blood, Body Fluids, and Tissue, 2nd Edition; Tentative Guideline.* NCCLS Document M29-T2. NCCLS, Villanova, Pa.
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9. Recommendations for preventing transmission of Human Immunodeficiency Virus and Hepatitis B. Virus to patients during exposure-prone invasive procedures. MMWR 1991, Vol. 40, No.RR-8.
10. Bloodborne pathogens. Code of Federal Regulations, Title 29, Part 1910.1030, Federal Register, 1991.

TECHNICAL INFORMATION: In the United States, telephone Becton Dickinson Microbiology Systems Technical Services, toll free (800) 638-8663, Prompt 2.

Approved by:

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Reviewed:

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