PRODUCT SPECIFICATION SHEET

Blood Culture Products

Intended Use
Blood Culture Products are recommended for detection of microorganisms in blood samples.

Product Summary and Explanation
Blood is one of the most important specimens received by the laboratory and blood culture is one of the most important and critical procedures performed in the microbiology laboratory. The isolation and identification of an organism has great diagnostic significance, blood being normally sterile. Blood cultures are of great importance in diagnosing such conditions as endocarditis, typhoid fever, pneumonia, supportive thrombophlebitis, infection of vascular grafts and other disease characterized by bacteremia.[1]

The growth of microorganisms in a blood culture medium may be delayed or prevented if an anticoagulant is not used in the culture medium since the organisms may become trapped in the fibrin clot.[2] However, some anticoagulants may be toxic for certain pathogens.[2-4] In addition, blood has natural bactericidal or bacteriostatic action due to the presence of natural bacterial inhibitors such as antibodies, complement, ß-lysin and phagocytes[5, 6] as well as antibiotics used in the therapy may greatly delay or reduce, if not completely eliminate, the chances of obtaining a positive culture.[7]

These obstacles may be overcome by the use of substances such as liquoid / sodium polyanetholsulfonate (SPS), a nontoxic anticoagulant which enables bacterial growth by obstructing the natural bacterial inhibitors of blood.[4,8-12] Since SPS inhibits the activity of streptomycin,[13] polymyxin B,[14] kanamycin and gentamicin,[15] therapy with these antibiotics should not interfere with microbial growth in blood cultures containing this anticoagulant.

In 1938, Van Haebler and Miles[10] were the first who demonstrated the usefulness of SPS in blood culture media. This was later confirmed in a study[11] which demonstrated that both aerobic and anaerobic bacteria survived longer when SPS was added to blood culture media. The effects of various anticoagulants on bacterial growth in blood cultures were compared by some investigators in 1938. SPS was shown to be the most effective agent for inhibiting the bactericidal activity of blood and the least toxic to the organisms involved. SPS has, therefore, been incorporated into the liquid culture media to provide a convenient blood culture system with an effective but non-inhibiting anticoagulant.

All Micromaster Blood Culture media (Brain Heart Infusion, Brain Heart Infusion-Supplemented, Tryptone Soy Broth, Trypticase Soy Broth with 0.05% SPS, Thioglycollate Broth with 0.05% SPS, Glucose broth, Glucose broth supplemented with 0.05% SPS, Hartley's Broth and Hartley's Broth with 0.05% SPS) will support the growth of a wide variety of clinically important pathogenic microorganisms, including fastidious organisms.

BHI, TSB, GB and HB media are generally recommended for the recovery of aerobic and facultative microorganisms, and Thioglycollate Broth is recommended primarily for the recovery of anaerobic and facultative microorganisms.

The use of a biphasic blood culture system has been shown to improve the sensitivity of blood culture over traditional broth media. When using Micromaster Blood Culture Bottles, after their inoculation with blood, the bottle is tilted and further incubated. The agar surface on the side of the bottles allows the subculture of aerobic, facultative and capnophilic microorganisms present in the specimen.

Principles of the Procedure
Blood is collected from the patient (preferably before antibiotic therapy is initiated) by venipuncture with a needle and syringe (or blood collecting set) and immediately transferred aseptically to Micromaster Blood Culture bottle containing

the desired growth medium. The bottle is then incubated and can be observed for turbidity, color change, hemolysis, gas formation or other evidence of microbial growth. Appropriate conventional subculturing methods should be used.

Formula / Lit

Brain Heart Infusion Broth (BHI Broth)

Ingredients	Gms/Liter
Brain Heart Infusion Broth (DM811)	37.00
SPS	0.25
Final pH (at 25°C) 7.4 ± 0.2	

BHI Broth-Supplemented w/0.05%SPS

Ingredients	Gms/Liter
Brain Heart Infusion Broth (DM811)	37.00
SPS	0.50
Yeast Extract	4.00
Pyridoxal HCl	0.005
Menadione Sodium Bisulfite	2.50
Final pH (at 25°C) 7.4 ± 0.2	

Tryptone Soya Broth (TSB)

Ingredients	Gms/Liter
Tryptone Soya Broth (DM277)	30.00
SPS	0.25
pH after sterilization (at 25°C) 7.3 ± 0.2	

Tryptone Soya Broth Supplemented w/0.05%SPS

Ingredients	Gms/Liter
Tryptone Soya Broth (DM277)	30.00
Yeast Extract	2.50
SPS	0.50
Hemin	0.005
pH after sterilization (at 25°C) 7.3 ± 0.2	

Fluid Thioglycollate Medium-Supplemented w/0.05%SPS

Ingredients	Gms/Liter
Fluid Thioglycollate Medium (DM263)	29.75
Yeast Extract	5.00
SPS	0.50
Final pH (at 25°C) 7.1 ± 0.2	

Glucose Broth

Ingredients	Gms/Liter
Glucose Broth (DM321)	20.00
SPS	0.25
Final pH (at 25°C) 7.3 ± 0.2	

Glucose Broth-Supplemented w/0.05%SPS

Ingredients	Gms/Liter
Glucose Broth (DM321)	20.00
SPS	0.50
Final pH (at 25°C) 7.3 ± 0.2	

Hartley's Broth

Ingredients	Gms/Liter
Hartley's Digest Broth	29.00
SPS	0.25
Final pH (at 25°C) 7.3 ± 0.2	

Hartley Broth-Supplemented w/0.05%SPS

Ingredients	Gms/Liter
Hartley's Digest Broth	29.00
SPS	0.50
Final pH (at 25°C) 7.3 ± 0.2	

Biphasic Blood Culture

Ingredients	Gms/Liter
Brain Heart Infusion Agar (DM810)	52.00
Brain Heart Infusion Broth (DM811)	37.00
SPS	0.25
Final pH (at 25°C) 7.4 ± 0.2	

PRODUCT SPECIFICATION SHEET

Note: In case of Fluid **Thioglycollate Medium-Supplemented w/0.05%SPS (pack of 10)-BC005A and BC005P.**If more than upper one-third of the medium has acquired a pink colour, the medium may be restored once by heating in a water bath or in free flowing steam until the pink colour disappears.

Precautions

1. For *In Vitro* Diagnostic Use Only.
2. Do not use bottles that exhibit any cracks or defects or signs of contamination. Discard bottles in the appropriate manner.
3. Before sampling a presumptively positive bottle, it is necessary to release gas which often builds up due to microbial metabolism.
4. Decontaminate all inoculated bottles prior to discarding by autoclaving at 121°C, 15 psi pressure, for 15 minutes / validated cycle. Loosen the cap and rubber stopper prior to autoclaving.
5. Observe aseptic techniques and use established precautions against microbiological hazards, including the use of gloves, throughout all procedures. All specimens should be handled according to CDC/NIH (Centers for Disease Control and Prevention/National Institutes of Health) recommendations, NCCLS (National Committee for Clinical Laboratory Standards) guidelines or local institution guidelines, for any potentially infectious human serum, blood or other body fluids. Prior to discarding, sterilize specimen containers and other contaminated materials by autoclaving.

Physical Indications of Instability:

Indications of deterioration 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.

Quality Control Specifications

The following list of suggested microorganisms may be used for the quality control testing of the **Micromaster** Blood Culture Media.

Procedure	Aerobic Media	Anaerobic Media
Inoculate the broth with a bacterial inoculum containing approximately 300 CFU/ml.		*Streptococcus pneumonia* ATCC 6305
	Pseudomonas aeruginosa ATCC 27853	*Bacteroides fragilis* ATCC 25285
Observe bottles for evidence of microbial growth.	Broth should appear turbid within 48hr on inoculation	
To check for purity of results, subculture all positive growths from the bottle on the following media.	*Streptococcus pneumonia* TSA with 5% sheep blood	*Streptococcus pneumoniae* TSA with 5% sheep blood
	Pseudomonas aeruginosa TSA with 5% sheep blood	*Bacteroides fragilis* Pre-reduced blood agar
Inoculate the Biphasic blood culture with a bacterial inoculum containing approximately 300 CFU/ml.	*Streptococcus pneumoniae* ATCC 6305	--
	Pseudomonas aeruginosa ATCC 27853	--
Observe bottles for evidence of microbial growth.	Growth on the agar surfaces should show usual colony characteristics for the above organisms.	

PRODUCT SPECIFICATION SHEET

Test Procedure

a) Specimen Collection And Handling:

1. Collect the blood samples preferably before commencement of antibiotic therapy. If antibiotics have already been administered, blood should be drawn just before the next dose is given.
2. Blood samples should be obtained before meals, as hyperlipemia may mask visible evidence of growth in the liquid medium.
3. Collection of blood samples should be done at intervals especially at the first sign of fever.
4. Because bacteraemia is intermittent and may precede episodes of fever and chills by about one hour, it is recommended that the collection of blood cultures should be performed at intervals, with samples being obtained at the first sign of fever.
5. In order to detect septicaemia with sufficient accuracy, it may be necessary to set up one to three blood cultures at designated time intervals, depending on the clinical situation.
6. Multiple blood cultures (1 to 3) may have to be performed at prefixed time intervals.
7. The operator's hands must be clean and dry. Sterile gloves may be worn to protect the operator if there is a possibility of specific hazards such as Hepatitis B or AIDS.
8. Clean the puncture site thoroughly with a swab soaked in 70% isopropanal or ethanol and disinfect with a 2% iodine solution. Allow the skin to dry before puncture.
9. Use a sterile disposable needle and syringe to withdraw patient's blood as follows:

Volume of Blood	Volume of Liquid Medium	Volume of Solid Medium
8-10 ml	70 ml (adults)	-
8-10 ml	40 ml (adults)	20 ml
3-5 ml	20 ml (paediatrics)	7 ml
1-3 ml	20 ml (paediatrics)	-

10. Separate needle and syringe should be used for each patient.
11. For best recovery blood should be collected and immediately inoculated into Micromaster Blood Culture System preferably at the patient's bedside.
12. If there is delay in processing, specimens submitted in blood culture vials should be held at room temperature until they can be appropriately processed.

b) Materials Provided:

Micromaster Blood Culture Bottles.

c) Materials Not Provided:

Needle and syringe (or appropriate blood collection unit), isopropyl or ethyl alcohol (70%), iodine solution (2%), incubator (35-37 C), sterile venting units, inversion rack, disposable absorbent pads and autoclave.

d) Performance of Test

1. Label the ready to use blood culture bottle.
2. Remove the protective top of the aluminum cap on the blood culture bottle.
3. Disinfect the part of the rubber stopper which is now exposed with isopropyl or ethyl alcohol (70%) and allow to dry.
4. Draw patient's blood with a sterile disposable needle and syringe as explained in specimen collection and handling column.

5. Transfer the blood sample immediately into the culture bottle by puncturing the rubber stopper with the needle and injecting the blood under aseptic conditions.
6. Incubate at 35±2°C for 18-24 hours and further for seven days.

e) Subculturing Using the Conventional Methods:

1. Subculturing from the culture bottle should be done in a biosafety cabinet class II.
2. Following inoculation and incubation of the blood culture bottle, observe daily for turbidity, hemolysis, gas formation, color changes and other evidence of microbial growth.
3. If growth is detected in either the aerobic or anaerobic bottle, a Gram-stained smear should be prepared and appropriate subculture methods used.
4. Before sampling a presumptively positive bottle, it is necessary to release gases which often build up due to microbial metabolism.

Results

1. If present, bacterial growth usually becomes evident within 48hrs; however, cultures should be incubated for at least 7 days before results are reported as negative. If a biphasic blood culture is NOT used, the presence of microorganisms must be further confirmed by subculturing on suitable media and by performing appropriate identification procedures.
2. If a biphasic blood culture 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 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.

Storage

Store the sealed bottle containing the dehydrated medium at 2 - 8°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

Refer to the expiration date stamped on the container. The medium should be discarded if any turbidity is observed or the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Recovery of clinically significant microorganisms may be affected by various factors. These include: antimicrobial therapy prior to blood collection, transitory bacteraemia or contamination of patient's blood by exogenous flora. Other factors which influence recovery are the volume of blood drawn, frequency or timing of cultures and selection of medium. Although this system does support the growth of organisms which cause most clinically significant bacteraemia, special handling of the specimens may be required in certain disease conditions. SPS inhibits the growth of certain mycoplasmas and should not be used for their isolation.
2. Premature discarding of apparently negative blood cultures or infrequent observations may result in failure to detect the presence of pathogenic microorganisms or in loss of viability.
3. Culture media sometimes contain small numbers of non-viable organisms derived from medium constituents, which may be visible in smears of un-inoculated blood culture media. Other sources of non-viable 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 re-incubated for an additional hour or two and the smear and staining procedure repeated before a report is issued.

PRODUCT SPECIFICATION SHEET

Packaging

Product Name	Product Code	Available Pack sizes
Brain Heart Infusion Broth (BHI Broth) (pack of 10) medium for detection of microorganisms in blood.	**BC002A** **BC002P**	**70ml-10BL** **20ml-10BL**
BHI Broth-Supplemented w/0.05%SPS (pack of 10) medium for enhanced recovery and cultivation of microorganisms in blood.	**BC007A** **BC007P**	**70ml-10BL** **20ml-10BL**
Fluid Thioglycollate Medium-Supplemented w/0.05%SPS (pack of 10) medium for enhanced recovery and cultivation of aerobes, anaerobes and microaerophiles.	**BC005A** **BC005P**	**70ml-10BL** **20ml-10BL**
Glucose Broth (pack of 10) medium for detection of microorganisms in blood.	**BC004A** **BC004P**	**70ml-10BL** **20ml-10BL**
Glucose Broth-Supplemented w/0.05%SPS (pack of 10) medium for enhanced recovery and cultivation of microorganisms in blood.	**BC008A** **BC008P**	**70ml-10BL** **20ml-10BL**
Hartley's Broth (pack of 10) medium for the recovery of anaerobic and facultative microorganisms in blood.	**BC003A** **BC003P**	**70ml-10BL** **20ml-10BL**
Hartley Broth-Supplemented w/0.05%SPS (pack of 10) medium for enhanced recovery and cultivation of anaerobic and facultative microorganisms in blood.	**BC009A** **BC009P**	**70ml-10BL** **20ml-10BL**
Tryptone Soya Broth (TSB) (pack of 10) medium for detection of microorganisms in blood.	**BC006A** **BC006P**	**70ml-10BL** **20ml-10BL**
Tryptone Soya Broth Supplemented w/0.05%SPS (pack of 10) medium for enhanced recovery and cultivation of microorganisms in blood.	**BC0010A** **BC0010P**	**70ml-10BL** **20ml-10BL**

References

1. Manual of Clinical Micro., 1999, 7th Edition, Editor in Chief Patrick R. Murray, ASM Press.
2. Wright, H.D., J. Pathol. 28:541, 1925.
3. Rammell, C.G., J. Bacteriol. 84:1123-1125, 1962.
4. Evans, G.L., T. Cekoric Jr., R.I. Searcy, Am. J. Med. Technol. 34:103-112, 1968.
5. Myrvik, Q.N., R.S. Weiser, J. Immunol. 74:9-16, 1955.
6. Skarnes, R.C., D.W. Watson, Bacteriol. Rev. 21:273-294, 1957.
7. Shaffer, J.G., M. Goldin, in Clinical Diagnosis by Laboratory Methods, Chapter 17, 13th Edition, edited by I. Davidsohn, B.B. Wells, Philadelphia, W.B. Saunders Company, 1963, p. 710-751.
8. Evans, G.L., et al., Clin. Res. 14:484, 1966.
9. Rosner, R., Am. J. Clin. Pathol. 49:216-219, 1968.
10. Van Haebler, T., A. A. Miles, J. Pathol. 46:245, 1938.
11. Ellner, P.D., C.J. Stoessel, J. Infect. Dis. 116:238-242, 1966.
12. Garrod, P.R., J. Pathol. 91:621-623, 1966.
13. May, J.R., A.E., Voureka, A. Fleming, Br. Med. J. 1:627-630, 1947.
14. Jackson, D.M., E.J.L. Lowbury, E. Topley, Lancet 2:137-147, 1951.
15. Evans, G.L., et al., Antimicrob. Agents Chemother. 1967:687-691, 1968.

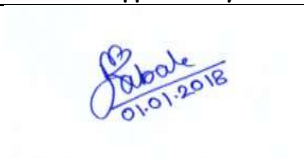
Further Information

For further information please contact your local MICROMASTER Representative.

PRODUCT SPECIFICATION SHEET

MICROMASTER LABORATORIES PRIVATE LIMITED
Unit 38/39, Kalpataru Industrial Estate,
Near Runwal Estate, Behind 'R-Mall' ,Ghodbunder Raod,
Thane (W) – 400607. M.S. INDIA.
Ph: +91-22-25895505, 4760, Cell: 9320126789.
Email: micromaster@micromasterlab.com
sales@micromasterlab.com

BC-AP PSS,QAD/FR/024,Rev.00/01.01.2018

Prepared By	Checked By	Approved By
		
Microbiologist	**Head Quality Control**	**Head Quality Assurance**

Disclaimer :

All Products conform exclusively to the information contained in this and other related Micromaster Publications. Users must ensure that the product(s) is appropriate for their application, prior to use. The information published in this publication is based on research and development work carried out in our laboratory and is to the best of our knowledge true and accurate. Micromaster Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are intended for laboratory, diagnostic, research or further manufacturing use only and not for human or animal or therapeutic use, unless otherwise specified. Statements included herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.