HiSafe™
Blood Culturing System
safe, efficient & easy

HiMedia Laboratories Pvt. Limited
For life is precious
Blood is one of the most important specimens received by the laboratory. Since blood is normally sterile, blood cultures are of great importance in diagnosing blood-stream related infections such as endocarditis, typhoid fever, pneumonia, suppurative thrombophlebitis, infections of vascular grafts etc. (1). In most cases of bacteremia the organisms are not numerous, hence a relatively large amount of blood has to be used as inoculum. The blood’s natural bactericidal or bacteriostatic action as well as antibiotics used in therapy may delay or reduce the chances of obtaining a positive culture (2). Hence substances such as liquoid (Sodium polyanethol sulfonate, SPS) may be used as a non-toxic anticoagulant which enables bacterial growth and prevents the action of natural bacterial inhibitors of blood (3,4,5). SPS inhibits the activity of streptomycin (6), polymyxin B (7), kanamycin and gentamycin (8). Van Haebler and Miles (9) first demonstrated the usefulness of SPS in Blood Culture Medium. The Hi-Safe Blood Culturing system is a fast, efficient and simpler detection and preliminary identification system for microorganisms from blood. All HiSafe Blood Culture media BHI - Brain Heart Infusion, BHI - Supplemented w/ 0.05% SPS, Columbia Broth, Fluid Thioglycollate Medium w/0.05% SPS, Glucose Broth Supplemented w/ 0.05 % SPS, Hartley Broth, Hartley Broth w/ 0.05% SPS, Modified Wilkins Chalgren Broth, Thiodiglycollate Broth, Tryptone Soya Broth, Tryptone Soya Broth Supplemented w/ 0.05%SPS, Tryptone Soya Broth w/ 10% Sucrose have been developed to support the growth of a variety of clinically significant pathogenic microorganisms and also fastidious ones.

**Procedure**

Blood is collected from the patient (before commencing antibiotic therapy preferably) using a sterile blood collection set and transferred aseptically to HiSafe Blood Culture Bottle containing a suitable growth medium (depending on the suspected pathogen). The bottle is then incubated and observed for turbidity, colour change, hemolysis, gas production.

**HiSafe Blood Culturing System**

Combination of Solid (20 ml) and Liquid (40 ml) media in single bottle.
Specimen collection and handling

- 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.
- Blood samples should be obtained before meals, as hyperlipemia may mask growth in the liquid medium.
- Collection of blood samples should be done at intervals specially at the first sign of fever.
- Multiple blood cultures (1 to 3) may have to be performed at prefixed time intervals.
- 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.
- Clean the puncture site thoroughly with a swab soaked in 70% isopropanol or ethanol and disinfect with a 2% iodine solution. Allow the skin to dry before puncture.
- Using a sterile (preferably disposable) needle and syringe to withdraw patient's blood as follows:

<table>
<thead>
<tr>
<th>Volume of Blood</th>
<th>Volume of Liquid Medium</th>
<th>Volume of Solid Medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>8-10 ml (adults)</td>
<td>70 ml</td>
<td>—</td>
</tr>
<tr>
<td>40 ml (adults)</td>
<td>20 ml</td>
<td>—</td>
</tr>
<tr>
<td>3-5 ml (paediatrics)</td>
<td>20 ml</td>
<td>7 ml</td>
</tr>
<tr>
<td>1-3 ml (paediatrics)</td>
<td>20 ml</td>
<td>—</td>
</tr>
</tbody>
</table>

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

Test Protocol

1. Label the ready to use blood culture bottle.
2. Do not unscrew cap. Remove the top of the screw cap.
3. Disinfect the part of the rubber stopper which is now exposed.
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.

Venting

- Use a sterile venting needle (LA038). Keep the bottle in an upright position preferably in a biological safety cabinet, place an alcohol swab over the rubber stopper and insert the venting needle with filter through it. Insertion and withdrawal of the needle should be done in a straight line. Discard the needle and mix contents by gently inverting the bottle 2-3 times. Do not vent the culture bottle for anaerobic cultures.
6. Incubate at 35±2°C for 18-24 hours and further for seven days.

Results and Interpretation

Bacterial growth is usually evident by 48 hours. The bottles should be incubated further for 7 days to confirm negative results.

Subculturing on suitable media is necessary for identification.

Note: For further identification of microorganisms in positive blood cultures users may follow the scheme suggested in Essential Procedures in Clinical Microbiology (10) or any suitable Handbook of Clinical Methods.

Drawbacks of the procedure

Various factors affect the recovery of microorganisms from blood:

- Antimicrobial therapy before sample collection
- Bacteremia of transient nature
- Contamination of patient's blood by external microflora
- Volume of blood drawn
- Selection of improper medium
- Discarding negative cultures prematurely.

Important Instructions: For in vitro diagnostic use only.

Do not use bottles showing cracks or defects or signs of contamination. Decontaminate all inoculated bottles prior to discarding by autoclaving at 121°C for 15 minutes (15 psi pressure). Loosen the cap and rubber stopper prior to autoclaving.

References

HiSafe™
Blood Culturing System
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List of Products

<table>
<thead>
<tr>
<th>Code</th>
<th>Type</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>LQ003A</td>
<td>Adults 70 ml, LQ003 (Paediatrics) 20ml</td>
<td>BHI - Brain Heart Infusion. A qualitative test for detection of microorganisms in blood.</td>
</tr>
<tr>
<td>LQ004A</td>
<td>Adults 70 ml, LQ004 (Paediatrics) 20ml</td>
<td>BHI - Supplemented w/ 0.05% SPS*. A qualitative test for detection of microorganisms in blood.</td>
</tr>
<tr>
<td>LQ005A</td>
<td>Adults 70 ml, LQ005 (Paediatrics) 20ml</td>
<td>Columbia Broth. For cultivation of fastidious organisms from blood.</td>
</tr>
<tr>
<td>LQ006A</td>
<td>Adults 70 ml, LQ006 (Paediatrics) 20ml</td>
<td>Columbia Broth. For cultivation of fastidious organisms from blood.</td>
</tr>
<tr>
<td>LQ007A</td>
<td>Adults 70 ml, LQ007 (Paediatrics) 20ml</td>
<td>Columbia Broth. For cultivation of fastidious organisms from blood.</td>
</tr>
<tr>
<td>LQ008A</td>
<td>Adults 70 ml, LQ008 (Paediatrics) 20ml</td>
<td>Columbia Broth. For cultivation of fastidious organisms from blood.</td>
</tr>
<tr>
<td>LQ009A</td>
<td>Adults 70 ml, LQ009 (Paediatrics) 20ml</td>
<td>Columbia Broth. For cultivation of fastidious organisms from blood.</td>
</tr>
<tr>
<td>LQ010A</td>
<td>Adults 70 ml, LQ010 (Paediatrics) 20ml</td>
<td>Columbia Broth. For cultivation of fastidious organisms from blood.</td>
</tr>
<tr>
<td>LQ011A</td>
<td>Adults 70 ml, LQ011 (Paediatrics) 20ml</td>
<td>Columbia Broth. For cultivation of fastidious organisms from blood.</td>
</tr>
<tr>
<td>LQ012A</td>
<td>Adults 70 ml, LQ012 (Paediatrics) 20ml</td>
<td>Columbia Broth. For cultivation of fastidious organisms from blood.</td>
</tr>
<tr>
<td>LQ013A</td>
<td>Adults 70 ml, LQ013 (Paediatrics) 20ml</td>
<td>Hartley Broth. For the recovery of anaerobic and facultative microorganisms.</td>
</tr>
<tr>
<td>LQ014A</td>
<td>Adults 70 ml, LQ014 (Paediatrics) 20ml</td>
<td>Modified Wilkins Chalgren Broth. A qualitative test for detection of strict or facultative anaerobic microorganisms in blood.</td>
</tr>
<tr>
<td>LQ095A</td>
<td>Adults 70 ml, LQ095 (Paediatrics) 20ml</td>
<td>Hartley Broth w/ 0.05% SPS*. For the recovery of anaerobic and facultative microorganisms.</td>
</tr>
<tr>
<td>LA038</td>
<td>HiAerator Needles</td>
<td>Sterile metal needles for venting blood culture bottles. Available from the HiMedia range.</td>
</tr>
</tbody>
</table>

Accessories

- **Racks for Blood Culture Bottles**
  - LA278 Combi System S.S. Rack
  - LA279 Safe Blood Culture Bottle S.S. Rack (for 8 bottles)

- **Venting Metal Needles**
  - LA038 HiAerator Needles

Open the lid
Clean the surface of stopper
Collect patient's blood
Inoculate the medium w/ blood
Incubate at appropriate condition
(Venting required for aerobic culture)

*LQ media's available sterile in packing of glass bottles only.*
HiCombi Range of Products

A TWO in ONE STEP procedure involving inoculation and simultaneous isolation on solid surface. Achieved in a HiCombi Dual Performance Medium.

Available in two sizes;
Solid phase (7ml) and Liquid (20 ml) medium and
Solid phase (20ml) and Liquid (40 ml) medium

HiCombi Media for growth and confirmation of *Salmonella* species

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>LQ029</td>
<td>HiCombi Dual Performance Salmonella Medium - SS</td>
<td>A qualitative test for growth and confirmation of <em>Salmonella</em>. Combination of solid (7 ml) and liquid (20 ml) media in single bottle.</td>
</tr>
<tr>
<td>LQ030</td>
<td>HiCombi Dual Performance Salmonella Medium - XLD</td>
<td>A qualitative test for growth and confirmation of <em>Salmonella</em>. Combination of solid (7 ml) and liquid (20 ml) media in single bottle.</td>
</tr>
<tr>
<td>LQ031</td>
<td>HiCombi Dual Performance Salmonella Medium - DCA</td>
<td>A qualitative test for growth and confirmation of <em>Salmonella</em>. Combination of solid (7 ml) and liquid (20 ml) media in single bottle.</td>
</tr>
<tr>
<td>LQ032</td>
<td>HiCombi Dual Performance Salmonella Medium - HEA</td>
<td>A qualitative test for growth and confirmation of <em>Salmonella</em>. Combination of solid (7 ml) and liquid (20 ml) media in single bottle.</td>
</tr>
<tr>
<td>LQ033</td>
<td>HiCombi Dual Performance Selective Medium - HEA</td>
<td>A qualitative test for growth and confirmation of <em>Salmonella</em>. Combination of solid (7 ml) and liquid (20 ml) media in single bottle.</td>
</tr>
<tr>
<td>LQ034</td>
<td>HiCombi Dual Performance Selective Medium - SS</td>
<td>A qualitative test for growth and confirmation of <em>Salmonella</em>. Combination of solid (7 ml) and liquid (20 ml) media in single bottle.</td>
</tr>
</tbody>
</table>

HiCombi Media for yeast and moulds

<table>
<thead>
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</tr>
</thead>
<tbody>
<tr>
<td>LQ034</td>
<td>HiCombi Dual Performance Fungal Medium Kit</td>
<td>Recommended for the detection of yeasts and moulds from pathological specimens. Combination of solid (7 ml) and liquid (20 ml) media in single bottle. For fungal selectivity, CC supplement (FD169A) is recommended. (Kit contains 10 bottles and 10 vials of CC Supplement, Modified (FD169A)).</td>
</tr>
<tr>
<td>LQ034A</td>
<td>HiCombi Dual Performance Fungal Medium Kit</td>
<td>Recommended for the detection of yeasts and moulds from pathological specimens. Combination of solid (20 ml) and liquid (40 ml) media in single bottle. For fungal selectivity, CC supplement, Modified (FD169A) is recommended. (Kit contains 10 bottles and 10 vials of CC Supplement, Modified (FD169A)).</td>
</tr>
</tbody>
</table>

LQ media’s available sterile in packing of glass bottles only.

For life is precious
HiCombi Media for Enterobacteria, Pseudomonas, Staphylococci and Candida

LQ033 HiCombi Dual Performance Medium

For growth of Enterobacteria, Pseudomonas, Staphylococci and Candida.
Combination of solid (7 ml) and liquid (20 ml) media in single bottle.
(1pk contains 10 bottles)

LQ012 HiCombi Dual Performance Medium

For growth of Enterobacteria, Pseudomonas, Staphylococci and Candida.
Combination of solid (20 ml) and liquid (40 ml) media in single bottle.

Description

HiCombi Dual Performance Medium contains in a single glass bottle, a combination of 40 ml of broth and one agar-coated surface. Both the media are rich in growth factors enabling detection of the obligate aerobes and facultative anaerobes which cause septicemia. The special peptone used provides a variety of amino acids, yeast extract provides vitamins, hemin and NAD are growth factors for fastidious bacteria such as Haemophilus. The combination medium is strongly recommended for rapid growth of Enterobacteriaceae, Pseudomonas species, Staphylococci, Streptococci, Candida species.

Test Protocol

1. Inoculate HiCombi Dual Performance medium as for HiSafe Blood Culturing System.
2. Incubate for 4-6 hours at 35 ± 2°C. Then tip the system to allow complete flooding of the agar surface. DO NOT SHAKE OR HOLD FOR MORE THAN 15 SECONDS.
3. Revert into an upright position and incubate for 18-24 hours at 35 ± 2°C or longer if necessary.
4. Venting may be carried out for aerobic cultures as in HiSafe Blood Culturing System.

* : Same Protocol is applicable for all HiCombi Dual Performance Media.

LQ media's available sterile in packing of glass bottles only.

\[\text{(1)}\text{ Solid and liquid phases of the biphasic medium as seen before inoculation.}\]
\[\text{(2)}\text{ Colonies appearing on surface of solid phase after addition of blood sample from a patient, and incubation for 24 hrs.}\]