Technical Data

HiCombi™ Dual Performance Salmonella Medium - XLD

LQ030

Intended use

A qualitative test for rapid growth and confirmation of *Salmonella*. Combination of solid (7 ml) media and liquid (20 ml) media in single bottle.

Composition**

Ingredients	g/ L
Solid Phase	**7 ml**
Yeast extract	3.000
L-Lysine	5.000
Lactose	7.500
Sucrose	7.500
Xylose	3.500
Sodium chloride	5.000
Sodium deoxycholate	2.500
Sodium thiosulphate	6.800
Ferric ammonium citrate	0.800
Phenol red	0.080
Agar	15.000
Liquid Phase	**20 ml**
Yeast extract	3.000
L-Lysine	5.000
Lactose	7.500
Sucrose	7.500
Xylose	3.500
Sodium chloride	5.000
Sodium deoxycholate	2.500
Sodium thiosulphate	6.800
Ferric ammonium citrate	0.800
Phenol red	0.080
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Label the ready to use HiCombi ™ Dual performance medium bottle. Do not unscrew the cap. Remove the top seal of the cap. Disinfect the part of the rubber stopper which is now exposed. Transfer the Sample into the culture bottle by puncturing the rubber stopper with the sterile, disposable needle and syringe. Venting may be carried for aerobic cultures as in Hisafe Dual performance culturing system. Incubate the bottle for 4-6 hours at 30 -35°C. For adsorption on solid surface. Tilt the bottle horizontally whereby solid medium is submerged with liquid phase. DO NOT SHAKE OR HOLD MORE THAN 15 SECONDS. Revert into an upright position and incubate for 18-24 hours at 30-35°C or longer if necessary. Venting: Use 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 the contents by gently inverting the bottle 2-3 times. Do not vent the bottle for anaerobic cultures. Recommended volume of blood to be tested in LQ030: 3-5 ml (For Paediatric use).

Principle And Interpretation

Hicombi™ Dual performance Medium contains in a single glass bottle, a combination of 20 ml of broth and one Agar coated surface. Both media are rich in growth factors. The Hicombi™ Dual Culturing system is a fast, efficient and simpler detection and preliminary identification system for microorganism. Two in one step procedure involving inoculation and simultaneous isolation on solid surface. XLD Agar has been recommended for the identification of *Enterobacteriaceae* (1). It is both selective and differential medium. The medium contains yeast extract, which provides nitrogen and vitamins

required for growth. Though the sugars xylose, lactose and sucrose provide sources of fermentable carbohydrates, xylose is mainly incorporated into the medium since it is not fermented by Shigellae but practically by all enterics. This helps in the differentiation of *Shigella* species. Sodium chloride maintains the osmotic balance of the medium. Lysine is included to differentiate the *Salmonella* group from the non-pathogens. Salmonellae rapidly ferment xylose and exhaust the supply. Subsequently lysine is decarboxylate by the enzyme lysine decarboxylase to form amines with reversion to an alkaline pH that mimics the *Shigella* reaction. However, to prevent this reaction by lysine-positive coliforms, lactose and sucrose are added to produce acid in excess. Degradation of xylose, lactose and sucrose to acid causes phenol red indicator to change its colour to yellow. Bacteria that decarboxylate lysine to cadaverine can be recognized by the appearance of a red colouration around the colonies due to an increase in pH. These reactions can proceed simultaneously or successively, and this may cause the pH indicator to exhibit various shades of colour or it may change its colour from yellow to red on prolonged incubation. To add to the differentiating ability of the formulation, an H_2S indicator system, consisting of sodium thiosulphate and ferric ammonium citrate, is included for the visualization of hydrogen sulphide produced, resulting in the formation of colonies with black centers. The non-pathogenic H_2S producers do not decarboxylase lysine; therefore, the acid reaction produced by them prevents the blackening of the colonies (2).

Type of specimen

Clinical samples - Blood

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (3,4).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic Use only. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Slight precipitation in the medium may occur,which is inheritant property of the medium,and does not affect the performance of the medium.
2. Some species may show poor growth due to nutritional variations.
3. Some *Proteus* strains may give red to yellow colouration with most colonies developing black centers, giving rise to false positive reactions.
4. Non-enterics like *Pseudomonas* and *Providencia* may exhibit red colonies.
5. *S.* Paratyphi A, *S.*Choleraesuis, *S.*Pullorum and *S.*Gallinarum may form red colonies without H_2S, thus resembling *Shigella* species.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance
In a sterile glass bottle combination of broth and one agar coated surface.

Colour of Agar medium	Colour of liquid medium
Red coloured Agar media	Red coloured liquid medium

Quantity of medium
7 ml of solid media in glass bottle and 20 ml of liquid medium in glass bottle

pH of Agar medium	pH of liquid medium
7.20- 7.60	7.20- 7.60

Sterility Check
Passes release criteria

Cultural response
Cultural characteristics was observed after incubation at 35-37°C for 18-72 hours.

Organism	Inoculum (CFU)	Growth in liquid medium	Growth on agar medium	Colour of colony
Salmonella Typhimurium ATCC 14028 (00031*)	50 -100	luxuriant	good-luxuriant	red with black
Salmonella Abony NCTC 6017 (00029*)	50 -100	luxuriant	good-luxuriant	red with black centres
Escherichia coli ATCC 8739 (00012*)	50 -100	fair	fair	yellow
Escherichia coli ATCC 25922 (00013*)	50 -100	fair	fair	yellow
## *Proteus hauseri* ATCC 13315	50 -100	luxuriant	good-luxuriant	grey with black centres
Salmonella Paratyphi A ATCC 9150	50 -100	luxuriant	good-luxuriant	red
Salmonella Paratyphi B ATCC 8759	50 -100	luxuriant	good-luxuriant	red with black centres
Salmonella Enteritidis ATCC 13076 (00030*)	50 -100	luxuriant	good-luxuriant	red with black centres
Salmonella Typhi ATCC 6539	50 -100	luxuriant	good-luxuriant	red with black centres
Shigella dysenteriae ATCC 13313	50 -100	luxuriant	good-luxuriant	red
Shigella flexneri ATCC 12022 (00126*)	50 -100	luxuriant	fair-good	red
Shigella sonnei ATCC 25931	50 -100	luxuriant	fair-good	red
# *Klebsiella aerogenes* ATCC 13048 (00175*)	50 -100	fair	fair	yellow
Enterobacter cloacae ATCC 13047 (00083*)	50 -100	luxuriant	fair	yellow
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	>=10^4	inhibited	inhibited	
Staphylococcus aureus subsp. *aureus* ATCC 6538 (00032*)	>=10^4	inhibited	inhibited	
Enterococcus faecalis ATCC 29212 (00087*)	>=10^4	inhibited	inhibited	

Key : (*) Corresponding WDCM numbers.
Formerly known as *Proteus vulgaris* # *formerly known as Enterobacter aerogenes*

Storage and Shelf Life

On receipt store between 2-8°C. Use before expiry date on the label. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

Reference

1. Chadwick P., Delisle G. H and Byer M., 1974, Can. J. Microbiol., 20, 1653-1664.
2. Taylor W. L., 1965, Am. J. Clin. Pathol., 44:471-475.
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
4. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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IVD ***In vitro* diagnostic medical device**

2°C 8°C **Storage temperature**

EC REP

CEpartner4U, Esdoornlaan 13,
3951DB Maarn, NL
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CE Marking

Do not use if package is damaged

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