HiCombi™ XLD - MacConkey Agar Plate HB004

Intended Use

Recommended for selective isolation and enumeration of *Salmonella* species and differentiation of enteric pathogens.

Composition**

Xylose-Lysine Deoxycholate Agar (XLD Agar)

Ingredients	g/ L
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
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

MacConkey Agar

Ingredients	g / L
Gelatin peptone	17.000
Tryptone	1.500
Peptone	1.500
Lactose	10.000
Bile salts	1.500
Sodium chloride	5.000
Neutral red	0.030
Crystal violet	0.001
Agar	15.000
Final pH (at 25°C)	7.1±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Streak the test inoculum (50-100 CFU) aseptically.

Principle And Interpretation

XLD Agar

XLD Agar has been recommended for the identification of *Enterobacteriaceae* (1) and for the microbiological testing. XLD Agar was formulated by Taylor (2-6) for the isolation and differentiation of enteric pathogens including *Salmonella* Typhi from other *Salmonella* species of foods, water and dairy products (7,8,9,10). XLD Agar exhibits increased selectivity and sensitivity as compared to other plating media e.g. SS Agar (M108), EMB Agar (M022) and Bismuth Sulphite Agar (M027) (10,8,11,3,5,12,13). The media formulation does not allow the overgrowth of other organisms over *Salmonella* and *Shigella* (14). Samples suspected of containing enteric pathogens, along with other mixed flora, are initially enriched in Modified Semisolid RV Medium Base (M1482) (15).

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 nonpathogens. *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 (9).

XLD Agar is both selective and differential medium. It utilizes sodium deoxycholate as the selective agent and therefore it is inhibitory to gram-positive microorganisms. Some Proteus strains may give red to yellow colouration with most colonies developing black centers, giving rise to false positive reactions. Non-enterics like *Pseudomonas* and *Providencia* may exhibit red colonies. *S.* Paratyphi A, *S*. Choleraesuis, S. Pullorum and *S*. Gallinarum may form red colonies without H_2S, thus resembling *Shigella* species (16).

MacConkey Agar

MacConkey agars are slightly selective and differential plating media mainly used for the detection and isolation of gram-negative organisms from clinical (17), dairy (18), food (19,20), water (7), pharmaceutical (21,22) and industrial sources (23). It is also recommended for the selection and recovery of the *Enterobacteriaceae* and related enteric gram-negative bacilli. USP recommends this medium for use in the performance of Microbial Limit Tests (22).

These agar media are selective since the concentration of bile salts, which inhibit gram-positive microorganisms, is low in comparison with other enteric plating media. The medium M081, which corresponds with, that recommended by APHA can be used for the direct plating of water samples for coliform bacilli, for the examination of food samples for food poisoning organisms (20) and for the isolation of *Salmonella* and *Shigella* species in cheese (24). Other than that this medium is also used for count of coli-aerogenes bacteria in cattle and sheep faeces (25), the count of coli-aerogenes and nonlactose fermenters in poultry carcasses (25), bacterial counts on irradiated canned minced chicken and the recognition of coli-aerogenes bacteria during investigations on the genus *Aeromonas* (26).

MacConkey Agar is the earliest selective and differential medium for cultivation of enteric microorganisms from a variety of clinical specimens (24,27). The original medium contains protein, bile salts, sodium chloride and two dyes. The selective action of this medium is attributed to crystal violet and bile salts, which are inhibitory to most species of gram-positive bacteria. Gram-negative bacteria usually grow well on the medium and are differentiated by their ability to ferment lactose. Lactose-fermenting strains grow as red or pink colonies and may be surrounded by a zone of acid precipitated bile. The red colour is due to production of acid from lactose, absorption of neutral red and a subsequent colour change of the dye when the pH of medium falls below 6.8. Lactose non-fermenting strains, such as *Shigella* and *Salmonella* are colourless, transparent and typically do not alter appearance of the medium.

Peptone, Tryptone and gelatin peptone are sources of nitrogen, carbon, long chain amino acids and other nutrients. Lactose is a fermentable carbohydrate,Sodium chloride maintains the osmotic equilibrium. Bile salts and crystal violet are selective agents that inhibit growth of gram-positive organisms. Neutral red is the pH indicator dye.

Type of specimen

Clinical samples - faeces, urine and other pathological material, Food and dairy samples, water samples, pharmaceutical samples.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (9,11). For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (8,19,29). For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards(2,20).

For pharmaceutical samples, follow appropriate techniques for sample collection, processing as per guidelines(4,27). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic use. 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

XLD Agar

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.This medium is general purpose medium and may not support the growth of fastidious organisms.

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.

Mac Conkey Agar

1. Though the medium is recommended for selective isolation, further biochemical and serological testing must be carried out for further confirmation.
2. The surface of the medium should be dry when inoculated.

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
Sterile XLD Agar and MacConkey Agar in 90mm disposable biplates.
Colour of XLD Agar
Red coloured medium
Colour of MacConkey Agar
Red coloured medium with purplish tinge
Quantity of medium
10ml of each medium in biplate
pH of XLD Agar
7.20- 7.60
pH of MacConkey Agar
6.90 -7.30
Sterility Check
Passes release criteria
Cultural response
Cultural characteristics observed after incubation at 35-37°C for 18-48 hours.

Organism	Growth on XLD Agar	Colour of colony on XLD Agar	Growth on MacConkey Agar	Colour of colony on MacConkey Agar
# *Klebsiella aerogenes* ATCC 13048 (00175*)	Fair	Yellow	luxuriant	pink to red
Escherichia coli ATCC 25922 (00013*)	Fair	Yellow	luxuriant	pink to red with bile precipitate
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	Good-luxuriant	Yellow	inhibited	-
Proteus vulgaris ATCC 13315	Good-luxuriant	Yellow	luxuriant	colourless
Salmonella Paratyphi A ATCC 9150	Good-luxuriant	Red	luxuriant	colourless
Salmonella Paratyphi B ATCC 8759	Good-luxuriant	Red w/ black centres	luxuriant	colourless
Salmonella Typhi ATCC 6539	Good-luxuriant	Red w/ black centres	luxuriant	colourless
Salmonella Typhimurium ATCC 14028 (00031*)	Good-luxuriant	Red w/ black centres	luxuriant	colourless
Salmonella Enteritidis ATCC 13076 (00030*)	Good-luxuriant	Red w/ black centres	luxuriant	colourless
Salmonella Typhi ATCC 6539	Good-luxuriant	Red w/ black centres	luxuriant	colourless
Salmonella Typhimurium ATCC 14028 (00031*)	Good-luxuriant	Red w/ black centres	luxuriant	colourless

Salmonella Enteritidis ATCC 13076 (00030*)	Good-luxuriant	Red w/ black centres	luxuriant	colourless
Salmonella Typhi ATCC 6539	Good-luxuriant	Red w/ black centres	luxuriant	colourless
Salmonella Typhimurium ATCC 14028 (00031*)	Good-luxuriant	Red w/ black centres	luxuriant	colourless
Salmonella Enteritidis ATCC 13076 (00030*)	Good-luxuriant	Red w/ black centres	luxuriant	colourless
Shigella dysenteriae ATCC 13313	Good-luxuriant	red	fair to good	colourless-pink
Shigella flexneri ATCC 12022 (00126*)	Good	red	fair to good	colourless-pink
Shigella sonnei ATCC 25931	Good-luxuriant	red	fair-good	red
Staphylococcus aureus subsp.*aureus* ATCC 25923 (00034*)	inhibited	-	inhibited	-
Enterococcus faecalis ATCC 29212 (00087*)	inhibited	-	none - poor	colourless-pink

Key :- * Corresponding WDCM numbers # Formerly known as *Enterobacter aerogenes*

Storage and Shelf Life

On receipt store between 20-30°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 (28,29).

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.Taylor W. L. and Harris B., 1965, Am. J. Clin. Pathol., 44:476.
4.Taylor W. L. and Harris B., 1967, Am. J. Clin. Pathol., 48:350.
5.Taylor W. L. and Schelhart B., 1967, Am. J. Clin. Pathol., 48:356.
6.Taylor W. L. and Schelhart B., 1968, Am. J. Clin. Pathol., 16:1387.
7.Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.
8.MacCarthy M. D., 1966, N. Z. J. Med. Lab. Technol., 20, 127-131.
9.Taylor W. L., 1965, Am. J. Clin. Pathol., 44:471-475.
10.Dunn C. and Martin W. J., 1971, Appl. Microbiol., 22, 17-22.
11.Rollender M. A., Beckford O., Belsky R. D and Kostroff B. 1969, Am. J. Clin. Pathol., 51, 284-286.
12. Taylor W. L. and Schelhart B., 1969, Appl. Microbiol., 18.393-395.
13. Taylor W. L. and Schelhart B., 1969, Appl. Micro. 18, 1387-1392.
14. Isenberg H. D., Kominos S., and Sigeal M., 1969, Appl Microbiol., 18, 656-659.
15. Aspinall S. T., Hindle M. A. and Hutchinson D. N., 1992, Eur. J. Clin. Microbiol., Inf. Dis. 11, 936-939.
16.MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore.
17.Murray P. R, Baron E, J., Jorgensen J. H., Pfaller M. A., Yolken R. H., (Eds.), 8th Ed., 2003, Manual of Clinical Microbiology, ASM, Washington, D.C.
18. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.
19. FDA Bacteriological Analytical Manual, 2005, 18th Ed., AOAC, Washington, D.C.
20.Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.

21.British Pharmacopoeia, 2016, The Stationery office British Pharmacopoeia.
22.The United States Pharmacopoeia, 2019, The United States Pharmacopeial Convention, Rockville, M.D.
23.Williams H., (Ed.), 2005, Official Methods of Analysis of the Association of Official Analytical Chemists, 19th Ed., AOAC, Washington, D.C.
24.MacConkey A., 1905, J. Hyg., 5:333.
25. Medrek T. F and Barnes Ella M., 1962, J. Appl. Bacteriol., 25(2),159-168
26.Eddy B. P., 1960, J. Appl. Bacteriol., 23(2).216-249.
27.MacConkey A., 1900, The Lancet, ii:20.
28.Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
29.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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