

Specification

Selective and differential medium used in the detection, isolation and enumeration of *Salmonella* and coliforms in clinical specimens according to the Pharmacopoeial Harmonized Methodology and in foodstuffs specimens according to ISO standard 21150.

Presentation

	Packaging Details	Shelf Life	Storage
10 Prepared bottle Bottle 125 ml with: 100 ± 3 ml	1 box with 10 bottles 125 ml Non injectable cap	12 months	8-25°C

Composition

Composition (g/l):

Gelatin Peptone.....	17.0
Casein and Meat Peptone.....	3.00
Lactose.....	10.0
Bile Salts	1.50
Sodium chloride.....	5.00
Crystal violet.....	0.001
Neutral red.....	0.03
Agar.....	15.0

Description /Technique

Description

At the beginning of the last century, MacConkey made the original formulation and included ox bile as inhibitor of Gram positive bacteria and litmus as an indicator of acid production from lactose sugar. More recently litmus has been substituted by a neutral red indicator making interpretations easier and more precise. Advancements in the understanding of bacterial physiology has meant that the medium has now been adapted to facilitate the detection of coliforms. The two most significant modifications to the original formulation are as follows:

- The substitution of ox bile by purified bile salts that improves the selectivity and avoids the inherent turbidity, which is due to the fat composition of bile. The efficiency of the inhibition due to bile salts is variable and depends on the relative concentration of cholate and taurocholate.
- The inclusion of supplementary inhibitors such as crystal violet and/or brilliant green. A popular formulation in America, but not in Europe where lower selectivity is preferred.

Lactose positive bacteria grown on this medium form red colonies due to acid production resulting from lactose fermentation and thus *Escherichia coli* colonies can be easily distinguished as they also form a small precipitation zone of bile salts around them. Bile salts and violet crystal inhibit gram-positive microorganisms. Some enterococci may also grow, but they are easy to distinguish from coliforms, as they form smaller colonies and have no precipitation zone.

Technique

Collect, dilute and prepare samples and volumes as required according to specifications, directives, official standard regulations and/or expected results.

Melt the medium contained in the bottles in a water bath (100°C) or in a microwave oven, avoiding overheating. Before melting any medium loosen the screwcap of the container to avoid breaking the container. Pour Petri dishes when cooled to room temperature. Once solidified on a flat surface, spread the plates by streaking methodology or by spiral method. Incubate the plates right side up aerobically at 30-35°C for 18-72h.

(Incubation times longer than those mentioned above or different incubation temperatures may be required depending on the sample or specifications. This medium can be inoculated directly or after enrichment broth).

After incubation, enumerate all the colonies that have appeared onto the surface of the agar.

Coliforms will develop reddish colonies. Reddish colonies on plates incubated at 44 ± 1°C will indicate faecal coliforms presence on the sample.

Presumptive isolation of *E.coli* must be confirmed by further microbiological and biochemical tests.

Quality control**Physical/Chemical control**

Color : Pink

pH: 7.1 ± 0.2 at 25°C

Microbiological control

Melting - pour plates - inoculation Practical range 100±20 CFU; Min. 50 CFU (Productivity) / 10⁴-10⁶ CFU (Selectivity)
Aerobiosis. Incubation at 30-35°C. Reading at 24 hours

Microorganism

Staphylococcus aureus ATCC® 6538, WDCM 00032
Escherichia coli ATCC® 8739, WDCM 00012
Escherichia coli ATCC® 25922, WDCM 00013
Enterococcus faecalis ATCC® 19433, WDCM 00009
Salmonella typhimurium ATCC® 14028, WDCM 00031
Ps. aeruginosa ATCC® 9027, WDCM 00026

Growth

Inhibited
Good - Red purple colonies - Biliar precipitate
Good - Red purple colonies - Biliar precipitate
Inhibited
Colourless colonies without biliar precipitate
Colourless colonies without biliar precipitate

Sterility Control

Incubation 48 hours at 30-35°C and 48 hours at 20-25°C: NO GROWTH
Check at 7 days after incubation in same conditions

Bibliography

- ATLAS, R.M., L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press, Inc. London.
- CLESCERI, L.S., A.E. GEENBERG & A.D. EATON (1998) Standard Methods for the Examination of Water and Wastewater. 20th ed. APHA-AWWA-WEF. Washington. DC. USA.
- DOWNES, F.P. & K. ITO (2001) Compendium of Methods for the Microbiological Examination of Foods. 4th ed. APHA. Washington.
- EUROPEAN PHARMA COEIA 8.0 (2014) 8th ed. § 2.6.13. Microbiological examination of non-sterile products: Test for specified microorganisms. Harmonised Method. EDQM. Council of Europe. Strasbourg.
- HITCHINS, A.D., P. FENG, W.D. WATKINS, S.R. RIPEY & C.A. CHANDLER (1998) E. coli and coliform bacteria. Bacteriological Analytical Manual. 8th ed. AOAC International. Gaithersburg. MD. USA.
- HORWITZ, W. (2000) Official Methods of Analysis. AOAC Intl. Gaithersburg. MD. USA.
- ISO 11133:2014/ Adm 1:2018. Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media.
- ISO 21150: 2015 Standard. Cosmetics - Detection of E. coli.
- MacCONKEY, A.T. (1905) Lactose-fermenting Bacteria in faeces. J. Hyg 5:333.
- MURRAY, P.R., E.J. BARON, M.A. PFALLER, F.C. TENOVER, & R.H. YOLKEN (Eds) (1995) Manual of Clinical Microbiology. 6th ed. A.S.M. Washington. DC. USA.
- RAPPAPORT, F. & E. HENING (1952) Media for the isolation and differentiation of pathogenic E. coli (serotypes O111 and O55) J. Clin. Pathology 5:361-362.
- USP 33 - NF 28 (2011) <62> Microbiological examination of non-sterile products: Test for specified microorganisms. Harmonised Method. USP Corp. Inc. Rockville. MD. USA.
- VARNAM, A.H. & M.G. EVANS (1991) Foodborne pathogens. Manson Publishing Ltd. London. UK.
- WHO (1963) International Standards for Drinking Waters. 7th ed. Churchill Ltd. London.