Reference: FLWR3016 **A.B.E. - Technical Data Sheet**

Product: **MacConkey Agar nº3**

Specification

Selective and differential medium used in the detection, isolation and enumeration of *Salmonella* and coliforms in clinical specimens according to the harmonized chapters of the European and US Pharmacopoeia and in foodstuffs specimens according to ISO standard 21150.

Presentation

	Packaging Details	Shelf Life	Storage
10 Prepared bottles Bottle 125 ml with: 100 ± 3 ml	1 box with 10 bottles 125 ml. Injectable cap: Plastic screw inner cap. The use of syringes needles with a diameter greater than 0.8 mm is not recommended.	12 months	2-25 ºC

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	13,5

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 form 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.

Directions for Use:

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 (100ºC) or in a microwave oven, avoiding overhating. 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 30-35ºC 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 ± 1ºC will indicate faecal coliforms presence on the sample.

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

Note: The solid mediums can be melted in different ways: autoclave, bath and, if the customer considers appropriate, also the microwave. Whenever the microwave option is chosen, it is necessary to take certain safety measures to avoid breaking of the containers, such as loosening the screw cap and putting the bottle or tube in a water bath in the microwave. The fusion temperature and time will depend on the shape of the container, the volume of medium and the heat source. Avoid overheating as both the heating periods.

Precautions

For in vitro diagnostic use. Do not reuse. For professional use only.

Do not use the product if it shows evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration.

Quality control

Physical/Chemical control

Color : Pinkish to red pH: 7.1 ± 0.2 at 25ºC

Microbiological control

Melting- pour plates- Inoculate: 50-100 CFU according to the European and US Pharmacopoeia harmonized chapters and according to ISO 11133 standard.
Analytical methodology according to ISO 11133:2014/A1:2018; A2:2020.

Aerobiosis. Incubation at 30-35 ºC. Reading at 18-24h

S. sonnei incubation at 37°C for 20-24h

Microorganism	Growth
Staphylococcus aureus ATCC® 6538, WDCM 00032	Inhibited
Escherichia coli ATCC® 8739, WDCM 00012	Good (≥ 50%) - Red purple colonies - Biliar precipitate
Escherichia coli ATCC® 25922, WDCM 00013	Good (≥ 50%) - Red purple colonies - Biliar precipitate
Enterococcus faecalis ATCC® 19433, WDCM 00009	Inhibited
Salmonella typhimurium ATCC® 14028, WDCM 00031	Good (≥ 50%) -colourless colonies w/o precipitate
Ps. aeruginosa ATCC® 9027, WDCM 00026	Good -colourless colonies w/o precipitate
Shigella sonnei ATCC® 9290	Good -colourless colonies w/o precipitate

Sterility control

Incubation 48 h at 30-35 ºC and 48 h at 20-25 ºC: 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 PHARMACOPOEIA 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 Standard 21567: 2004. Microbiology of Foods and animal feeding stuffs - Horizontal method for the detection of Shigella spp.
. ISO 4973:2023. Quality control of culture media and diluents used in cosmetics standards
· 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.