**Reference:** 5003 **Technical Data Sheet**

**Product:** **MACCONKEY AGAR (MACCONKEY AGAR Nº3)**

CE IVD

## 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 21150:2006.

## Presentation

| | Packaging Details | Shelf Life | Storage |
|---|---|---|---|
| 10 Prepared bottle<br>Bottle 125 ml<br>with: 100 ± 3 ml | 1 box with 10 bottles 125 ml<br>Non injectable cap | 12 months | 8-25ºC |

## 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 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.

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 (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.

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## Quality control

### Physical/Chemical control

Color : Pink pH: 7.1 ± 0.2 at 25ºC

### Microbiological control

Melting - pour plates - inoculation Practical range 100±20 CFU; Min. 50 CFU (Productivity) / $10^4$-$10^6$ CFU (Selectivity)
Aerobiosis. Incubation at 30-35ºC. Reading at 24 hours

| Microorganism | Growth |
|---|---|
| *Staphylococcus aureus ATCC® 6538* | Inhibited |
| *Escherichia coli ATCC® 8739* | Good - Red purple colonies - Biliar precipitate |
| *Escherichia coli ATCC® 25922* | Good - Red purple colonies - Biliar precipitate |
| *Enterococcus faecalis ATCC® 19433* | Inhibited |
| *Salmonella typhimurium ATCC® 14028* | Colourless colonies without biliar precipitate |
| *Pseudomonas aeruginosa ATCC® 9027* | Colourless colonies without biliar precipitate |

### Sterility Control

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

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