Specification

Solid selective and differential medium used in isolation and identification of *Salmonella* and coliforms according to the Pharmacopeial Harmonized Method and ISO standard 21150 and 21567.

Formula * in g/L

Pancreatic digest of gelatin	17.000	Sodium chloride	5.000
Meat peptone	1.500	Neutral red	0.030
Casein peptone	1.500	Crystal violet	0.001
Lactose monohydrate	10.000	Agar	13.500
Bile salts	1.500		

Final pH 7.1 ±0.2 at 25 °C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 50 g of powder in 1 L of distilled water. Bring to the boil and sterilize in the autoclave at 121ºC for 15 minutes.

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 phenol 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.

Some enterococci may also grow, but they are easy to distinguish from coliforms, as they form smaller colonies and have no precipitation zone.

Technique

Prepare 10-fold serial dilutions of the sample and take 1 ml aliquots from each dilution, in duplicates, placing them into sterile Petri plates. Pour 15 ml of molten medium at 45 °C into each plate and mix carefully. Once solidified, add a second layer of 5 ml of sterile medium to seal the surface and facilitate colony enumeration. Incubate the plates for 24 hours at 35 °C, then select those with 30 to 150 colonies. Characteristic colonies must be confirmed as coliforms by gas production from lactose in a broth culture.

Quality control

Incubation temperature: 30-35 ºC **Incubation time:** 18-72 h

Inoculum: Practical range 100 ±20 CFU. min. 50 CFU (productivity)/ 10^4-10^6 CFU (selectivity), according to Ph. Eur. and ISO 11133:2014/Amd 1:2018.

Microorganism	Growth	Remarks
Staphylococcus aureus ATCC® 6538	Inhibited	Selectivity
Enterococcus faecalis ATCC® 29212	Inhibited	Selectivity
Escherichia coli ATCC® 8739	Productivity > 0.50	Dark violet colonies w. precipitate zone
Escherichia coli ATCC® 25922	Productivity > 0.50	Dark violet colonies w. precipitate zone
Salmonella typhimurium ATCC® 14028	Productivity > 0.50	Colourless colonies without precipitate
Salmonella abony NCTC® 6017	Productivity > 0.50	Colourless colonies without precipitate
Pseudomonas aeruginosa ATCC® 9027	Good	Colourless colonies without precipitate

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Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 ºC to 30 ºC).