Instructions for Use

REF - KM0124 **MacConkey Agar with Salt**

Dehydrated Culture Media

1. Intended Use

For *in vitro* diagnostic use. KM0124 MacConkey Agar with Salt is a selective medium for the isolation and differentiation of bile tolerant Gram-negative (enteric) and Gram-positive (*staphylococci* and *enterococci*) organisms in all areas of bacteriology.

2. Composition*

Ingredient	g/L
Peptone	20.0
Lactose	10.0
Bile salts	5.0
Sodium chloride	5.0
Neutral red	0.05
Agar	12.0

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

MacConkey Agar with Salt is based on the formulation by MacConkey in 1900 [1]. Grunbaum and Hume made several significant changes in 1902 [2], and by 1930, 10 modifications of "MacConkey's Basal Bile Salt Peptone" agar were published in a compendium of microbiological media [3]. *Staphylococcus* and *Enterococcus* spp. are able to grow due to the omission of crystal violet from this formulation. This medium is recommended by the World Health Organisation [4] and the Department of Health [5] for the bacteriological examination of water. KM0124 is recommended as a differential primary isolation media by the UK Standards for Microbiology Investigations [6].

4. Principle

The omission of crystal violet permits the growth of *Staphylococcus* and *Enterococcus* species. The peptone is the nitrogen, carbon, and vitamin source in this medium. Lactose is the fermentable carbohydrate. Lactose fermentation causes local pH to drop around the colonies which will react with the pH indicator, neutral red, and hence aids in differentiation [7]. Bile salts function as the selective agent.

5. Preparation Instructions

Suspend 52.0g/L of dried culture media in deionised/purified water and allow to soak whilst mixing for 10 minutes.

Autoclave to sterilise at 121°C for 15 minutes before cooling in a water bath to 45-50°C.

Aseptically dispense the specified volume into appropriate sterile containers and allow to cool.

Prepared media may be kept at between 2 and 8°C for up to 14 days away from direct sunlight.

6. Physical Characteristics

	Dehydrated Medium	Prepared Medium
Appearance and Colour	Beige fine powder	Pale pink/red firm gel
pH	N/A	7.4 ± 0.2

7. Materials Provided

KM0124 can be provided in the formats detailed below. Each plate is ink-jet printed with (abbreviated) product name, product code, lot number and expiry date.

Product Code	Product Format
KM0124-500G-500	1 x 500g Dehydrated Culture Media Tub
KM0124-5KG-5000	1 x 5kg Dehydrated Culture Media Tub
KM0124-10KG-10000	1 x 10kg Dehydrated Culture Media Tub

8. Materials Needed but not Provided

Standard microbiological laboratory materials e.g., sterile loops or swabs, collection containers, incubators, and quality control organisms.

9. Specimens

KM0124 MacConkey Agar with Salt is suitable for the testing of the following specimens:

- Food and Environmental: products for human consumption, animal feed and environmental samples

- Clinical: tissue, blood, urine, spinal fluid, and stool samples, as well as rectal swabs or swabs from other areas of interest
- Water Samples: Potable waters, water from heating systems, cooling towers and air conditioning systems.

Sampling and transport equipment must be used in accordance with the end user's suppliers' recommendations. Refer to appropriate standard method or local guidance on sample collection and subsequent processing.

10. Test Procedures and Interpretation of results

If plates manufactured have been refrigerated, allow the plates to come to room temperature before inoculation.

Based on sample type and information provided, check to see if the specimen needs to be pre-enriched prior to inoculation to the prepared medium.

Food and Environmental Specimens:

For solid samples, prepare a homogenised suspension using a ratio of 1:9 of sample to Maximum Recovery Diluent (E&O BM0760). With homogenised and liquid specimens, media should be inoculated with 0.5ml of the specimen and incubate at 37°C ± 1°C aerobically for 18-24 hours.

Clinical Specimens:

Inoculate specimen, or pre-enriched sample, directly onto the medium and streak across the agar surface using a sterile loop or automated plate streaker. Incubate at 37°C ± 1°C aerobically for 18-24 hours.

Water Specimens:

Filtration: A suitable volume of the sample is passed through a filter membrane of 0.45µm pore and the membrane is then placed on the surface of the medium, ensuring there are no air bubbles underneath, before incubating at 37°C ± 1°C aerobically for 24-48 hours.

Spread plate: 0.5ml of the specimen, or pre-enriched sample, is inoculated directly onto the medium and streak across the agar surface using a sterile loop or automated plate streaker, before incubating at 37°C ± 1°C aerobically for 24-48 hours.

After incubation, examine agar for colonies (typical colony appearance outlined in Quality Control table below). Perform further biochemical, serological, or mass spectroscopy testing to confirm identity of presumptive positive isolates. Refer to relevant local guidelines.

11. Quality Control

Organism	Incubation	Result (Specificity)
E. coli (NCTC 12241)	37 ± 1°C aerobically for 18-24 hours	Growth: Pink/red colonies
P. mirabilis (NCTC 10975)	37 ± 1°C aerobically for 18-24 hours	Growth: Yellow colonies
S. typhimurium (NCTC 12023)	37 ± 1°C aerobically for 18-24 hours	Growth: Pale straw colonies
E. faecalis (NCTC 12697)	37 ± 1°C aerobically for 18-24 hours	Growth: Pink colonies
S. aureus (NCTC 12981)	37 ± 1°C aerobically for 18-24 hours	Growth: Pink colonies
S. sonnei (NCTC 8574)	37 ± 1°C aerobically for 18-24 hours	Growth: Colourless colonies
K. aerogenes (NCTC 10006)	37 ± 1°C aerobically for 18-24 hours	Growth: Red/orange colonies
P. aeruginosa (NCTC12903)	37 ± 1°C aerobically for 18-24 hours	Growth: Straw colonies

It is the responsibility of the user to perform Quality Control testing taking into consideration the intended use of the medium, and in agreement with any local relevant guidelines (e.g., frequency, strains used, atmosphere, incubation temperature).

12. Performance

To fully verify KM0124 MacConkey Agar with Salt performance, dehydrated culture media samples were used to prepare MacConkey Agar with Salt and tested to assess colony morphology and recovery level (where an acceptable range is ≥70% and ≤120%) compared to a non-selective reference medium. Prepared samples were inoculated with 30-150cfu and incubated at 37 ± 1°C aerobically for 18-24 hours. All samples of prepared media produced grew and showed good recovery and the correct morphology of the required test organisms: *Escherichia coli* (NCTC 12241), *Proteus mirabilis* (NCTC 10975), *Salmonella typhimurium* (NCTC 12023), *Enterococcus faecalis* (NCTC 12697), *Staphylococcus aureus* (NCTC 12981), *Shigella sonnei* (NCTC 8574), *Klebsiella aerogenes* (NCTC 10006) and *Pseudomonas aeruginosa* (NCTC 12903). Therefore, it can be concluded that KM0124 MacConkey Agar with Salt, meets performance criteria when used according to the instructions outlined above. Trend analysis data available upon request.

13. Limitations of the Media

- KM0124 MacConkey Agar with Salt has the disadvantage that many strains of *Proteus* spp. will spread on it and for this reason MacConkey Agar without salt and crystal violet may be preferred (E&O PP0470 or KM0011).
- Weak lactose fermenters will form colonies more slowly than others, e.g., *Serratia*, *Citrobacter*
- Slow lactose fermenters may appear as colourless colonies, e.g., *Enterobacter*
- The encapsulated bacteria produce capsules by using lactose. This results in sticky, moist-looking colonies, e.g., *Klebsiella*, *Enterobacter*

- Bacteria with strong lactose fermentation produce enough acid to precipitate bile salts, resulting in a pink halo in the medium surrounding individual colonies or areas of confluent growth.
- To enhance the pigment of suspected *S. aureus*, hold the plates on the bench at ambient temperature for 12-18 hours.

14. Precautions and Warnings

This product is considered hazardous under CLP regulations. Refer to KM0124 Material Safety Data Sheet. Wear such PPE as recommended by laboratory COSHH assessment. During and after use, always handle all materials in a manner conforming to Good Laboratory Practices and consider that material under test should be regarded as a potential biohazard if mishandled.

15. Storage conditions and Shelf life

Store product in the original container with the lid tightly closed at between 10 and 30°C in low humidity conditions away from direct sunlight. Kept under these conditions, the product may be used up to the date of expiry shown on the product label.

Do not use if the product is not free-flowing or displays any sign of colour change, formation of large lumps or hardening of the powder. Additionally, do not use medium if it has been stored inappropriately, the packaging has been damaged or has passed the expiry date.

Dehydrated culture media does not need to be used all at once; replace the cap and ensure that the container is tightly closed and stored according to labelled instructions.

Dispose of in accordance with local and national authority requirements.

16. References

1. MacConkey, A. (1900) A note on a new medium for the growth and differentiation of the bacillus *Coli communis* and the bacillus *Typhi abdominalis*. Lancet, 20. Princeton University: J. Onwhyn.
2. Grunbaum, A.S. and Hume, E.H. (1902) "Note on media for distinguishing *B. Coli*, *B. typhosus* and related species," BMJ, 1(2163), pp.1473-1474.
3. Levine, M. and Schoenlein, H. (1930) MacConkey's Basal Salt Peptone Agar in A compilation of Culture Media for the cultivation of microorganisms. Baltimore: The Williams & Wilkins Company, pp.459.
4. WHO (1963) International Standards for Drinking Water, 2nd ed. Geneva: WHO.
5. Environment Agency (2002) The Microbiology of Drinking Water 2002. Methods for examination of Waters and Associated Materials.
6. Public Health England (Various) UK Standards for Microbiology Investigations. London: Standards Unit, Microbiology Services
7. MacConkey, A. (1908) Bile Salt Media and their advantages in some Bacteriological Examinations. Am J Hyg, 8(3), pp.322-34.

Version History*

001	10/10/2023 - New Document Created
002	25/03/2025 - Update based on new formatting and device file

*Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

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TABLE OF APPLICABLE SYMBOLS

REF o REF Catalogue number	LOT Batch code	IVD *In vitro* Diagnostic Medical Device	Manufacturer	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	Store in a dry place