MacConkey Agar without Crystal Violet and Salt (NCM0072)

Revision: 2 Effective Date: 3/3/2022

Intended Use

MacConkey Agar without Crystal Violet and Salt is used for the isolation and differentiation of Gram-negative enteric bacilli from specimens containing swarming strains of *Proteus* spp, and is not intended for use in the diagnosis of disease or other conditions in humans.

Description

MacConkey Agar is based on the bile salt-neutral red-lactose agar of MacConkey. The original MacConkey medium was used to differentiate strains of *Salmonella typhosa* from members of the coliform group. Formula modifications improved growth of *Shigella* and *Salmonella* strains. These modifications include the addition of 0.5% sodium chloride, decreased agar content, altered bile salts, and neutral red concentrations. The formula modifications improved differential reactions between enteric pathogens and coliforms.

MacConkey Agar without Crystal Violet and Salt is a differential medium that restricts swarming of *Proteus* spp., aiding in the detection and isolation of enteric microorganisms. Sodium Chloride is deleted from the medium to provide an electrolyte deficient medium preventing *Proteus* spp. from spreading. In addition, this medium does not contain crystal violet allowing *Staphylococcus*, *Enterococcus,* and *Mycobacterium* spp. to grow.

Typical Formulation

Enzymatic Digest of Casein	10.0 g/L
Enzymatic Digest of Gelatin	10.0 g/L
Lactose	10.0 g/L
Bile Salts	5.0 g/L
Neutral Red	0.04 g/L
Agar	12.0 g/L

Final pH: 7.4 ± 0.2 at 25°C
Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

Refer to SDS

Preparation

1. Suspend 47 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Test Procedure

Refer to appropriate references using MacConkey Agar without Crystal Violet and Salt for the isolation and identification of enteric organisms.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and trace to light pink-beige.

Prepared Appearance: Prepared medium is trace to slightly hazy, and pink to rose-orange.

Expected Cultural Response: Cultural response on MacConkey Agar without Crystal Violet & Salt incubated at the appropriate atmosphere and temperature and examined for growth after 18 - 24 hours.

620 Lesher Place • Lansing, MI 48912
800-234-5333 (USA/Canada) • 517-372-9200
foodsafety@neogen.com • foodsafety.neogen.com

Microorganism	Approx. Inoculum (CFU)	Expected Results	
		Growth	Reaction
Enterococcus faecalis ATCC® 29212	50-200	May show partial inhibition	Pink colonies
Escherichia coli ATCC® 25922	50-200	>70%	Bright pink colonies
Proteus mirabilis ATCC® 12453	50-200	>70%	Colorless colonies, no swarming
Proteus mirabilis ATCC® 29906	50-200	>70%	Colorless colonies, swarming +/-
Salmonella typhimurium ATCC® 14028	50-200	>70%	Colorless colonies
Shigella sonnei ATCC® 25931	50-200	>70%	Colorless colonies
Staphylococcus aureus ATCC® 25923	~10^4	Growth; suppressed size	Colorless to pink colonies
Streptococcus pneumonia ATCC® 6305	>10^5	Complete Inhibition	--
Streptococcus pyogenes ATCC® 19615	>10^5	Complete Inhibition	--

The organisms listed are the minimum that should be used for quality control testing.

Results

Lactose-fermenting organisms grow as pink to brick-red colonies with or without a zone of precipitated bile. Non-lactose fermenting organisms grow as colorless or clear colonies. Swarming by *Proteus* spp. is reduced.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. Although MacConkey Agar without Crystal Violet & Salt is a selective medium, it is less inhibitory than MacConkey Agar, allowing gram-positive organisms to grow. Biochemical and serological testing using pure cultures are recommended for complete identification.
3. Incubation of MacConkey Agar without Crystal Violet & Salt under increased CO_2 has been reported to reduce growth and recovery of certain Gram-negative bacilli.

Storage

Store dehydrated culture media at 2 – 30°C away from direct sunlight. Once opened and recapped, place the container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

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References

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2. Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (eds.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
3. Mazura-Reetz, G. T. Neblett, and J. M. Galperin. 1979. MacConkey Agar: CO2 vs. ambient incubation. Abst. Ann. Mtg. American Society for Microbiology. C179.

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