MCP AGAR (7477)

Intended Use

MCP Agar is used with additives for the enumeration of *Clostridium perfringens* in environmental samples.

Product Summary and Explanation

MCP Agar was described by Bisson & Capbelli[1] and modified by Armon and Payment.[2] This medium is used to enumerate *Clostridium perfringens* spores from surface and drinking water. *C. perfringens* is present in large numbers in human and animal wastes. *C. perfringens* spores are resistant to wastewater treatment practices and environmental stresses. Growth of *C. perfringens* spores is an excellent indicator of present and past fecal contamination.

Principles of the Procedure

Enzymatic Digest of Casein provides nitrogen, amino acids, and carbon required for organism growth in MCP Agar. Yeast Extract supplies essential vitamins. Sucrose is the fermentable carbohydrate. L-Cysteine•HCl is a reducing agent and Magnesium Sulfate ($MgSO_4$) provides trace ions. Bromcresol Purple is the pH indicator and Agar is the solidifying agent. MCP is supplemented with D-Cycloserine, Polymyxin B Sulfate, and $FeCl_3•6H_20$ as selective agents. Phenolphthalein Diphosphate is the substrate used for detection of an acid phosphatase enzyme elaborated by *C. perfringens*. In the presence of this enzyme and ammonium hydroxide fumes, the diphosphate bond is cleaved. This reaction is visible by the absorption of Indoxyl-β-D-Glucoside, producing red to dark pink colonies.

Formula / Liter

Enzymatic Digest of Casein	30 g
Yeast Extract	20 g
Sucrose	5 g
L-Cysteine•HCl	1 g
$MgSO_4•7H_2O$	0.1 g
Bromcresol Purple	0.04 g
Agar	15 g

Final pH: 7.6 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Supplement

D-Cycloserine, 0.4 g
Polymyxin B Sulfate, 0.025 g
$FeCl_3•6H_2O$, 2 mL, 4.5%
Phenolphthalein Diphosphate, 20 mL 0.5%
Indoxyl-β-D-Glucoside, 80 mL, 0.075%

Precaution

1. For Laboratory Use.

Directions

1. Suspend 71.1 g of the medium in 900 mL 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. Cool to 50°C.
4. Aspetically add 0.4 g of D-cycloserine, 0.025 g of polymyxin B sulfate, 2 mL of a filter sterilized 4.5% $FeCl_3•6H_2O$ solution, 20 mL of a filter sterilized 0.5% phenolphthalein diphosphate solution, and 80 mL of a filter sterilized 0.075% indoxyl-β-D-glucoside solution.
5. Mix thoroughly and dispense into appropriate petri dishes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige.

Prepared Appearance: Prepared medium is trace to slightly hazy and purple to dark purple.

Expected Cultural Response: Cultural response on MCP Agar with supplements after 18 – 24 hours incubation at 35°C under anaerobic conditions.

Microorganism	Response	Reactions
Clostridium perfringens ATCC® 13124	growth	yellow colonies
Clostridium sporogenes ATCC® 11437	growth	clear colonies
Clostridium novyi ATCC® 7659	inhibited	---

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

Test Procedure

1. Pass the water sample through a membrane filter.
2. Place the filter on MCP agar and incubate anaerobically for 18 – 24 hours at 44.5°C.
3. The presence of yellow colonies indicates sucrose fermentation and indicative of *C. perfringens*.
4. Expose the filter to ammonium hydroxide fumes for 20 seconds by removing the plate lid and inverting the plate surface over an open container of concentrated ammonia hydroxide. Use proper technique and perform this portion of the test in a hood that will ventilate to the outside.
5. Count red to dark pink colonies.

Results

Red or dark pink colonies (acid phosphatase cleavage of phenolphthalein diphosphate) are counted as presumptive of *C. perfringenes*.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

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. MCP is a presumptive test for the presence of *C. perfringenes*. Biochemical tests are required for complete identification of *C. perfringenes*.

Packaging

MCP Agar	**Code No.**	**7477A**	**500 g**
		7477B	**2 kg**
		7477C	**10 kg**

References

1. **Appl. and Environ. Microbiol.** 1979. **37:**5-56.
2. **Canadian J. Microbiol.** 1988. **31:**78-79.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.