Technical Data

Rapid HiColiform™ HiCynth™ Agar

MCD1465

Intended use

Recommended for detection and confirmation of *Escherichia coli* and total coliforms on the basis of enzyme substrate reaction from water samples, using a combination of chromogenic and fluorogenic substrates. It can also be used for clinical samples.

Composition**

Ingredients	Gms / Litre
HiCynth™ Peptone No.1#	5.000
Sodium chloride	5.000
Sorbitol	1.000
Dipotassium hydrogen phosphate	2.700
Potassium dihydrogen phosphate	2.000
Sodium lauryl sulphate (SLS)	0.100
Chromogenic substrate	0.080
Fluorogenic substrate	0.050
IPTG(1-Isopropyl-b-D-1-thiogalactopyranoside	0.100
Agar	15.000
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Chemically defined peptones

Directions

Suspend 31.03 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

The Rapid HiColiform™ Agar is modification of LMX Broth described by Manafi and Kneifel (5). Rapid HiColiform™ HiCynth™ Agar is used on same principle by replacing animal and vegetable peptones with synthetic peptones to avoid BSE/TSE and GMO risk, for the simultaneous detection of total coliforms and *Escherichia coli*. This media is useful for the detection and confirmation of *Escherichia coli* and total coliforms in water samples on the basis of chromogenic and fluorogenic substrates (2,5-9). It can also be used for clinical samples.

HiCynth™ Peptone No.1 which is rich in tryptophan content, provides essential growth nutrients and is useful for the simultaneous detection of indole production. Sorbitol provides the carbon source. The phosphate salts provide buffering action for rapid growth of coliforms. Sodium lauryl sulphate makes the medium selective by inhibiting accompanying microflora, especially the gram-positive organisms. The fluorogenic substrate, is split by enzyme ß-D-glucuronidase, which is specifically found in *Escherichia coli.* The reaction is indicated by a blue fluorescence under UV light. The presence of total coliforms is indicated by a blue-green colour of the colonies due to the cleavages of the chromogenic substrate. IPTG amplifies enzyme synthesis and increases the activity of ß-D-galactosidase. To confirm presence of E. coli, add 2-3 drops of Kovacs reagent over the suspected colony. The colony turns red within 2 minutes in case of positive reaction.

Type of specimen

Clinical samples- urine, Water samples.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (3,4).

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(1)

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic use. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. 97% of the *E.coli* strains are ß-D-glucuronidase positive, however few strains may show negative fluorescence.

2.Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within expiry period when stored at the recommended temperature.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Light yellow coloured, clear to slightly opalescent gel forms in Petri plates

Reaction
Reaction of 3.1% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH
6.60-7.00

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours

Organism	Inoculum (CFU)	Growth	Colour of medium/ Colony	Fluorescence (under uv)	Indole production
# *Klebsiella aerogenes* ATCC 13048 (00175*)	50-100	luxuriant	blue-green	negative reaction	negative reaction
Escherichia coli ATCC 25922 (00012*)	50-100	luxuriant	blue-green	positive reaction	positive reaction
Klebsiella pneumoniae ATCC 13883 (00097*)	50-100	luxuriant	blue-green	negative reaction	negative reaction
Salmonella Typhimurium ATCC 14028 (00031*)	50-100	luxuriant	yellow	negative reaction	negative

Formerly known as *Enterobacter aerogenes*

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 15-25°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

Reference

1. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.
2. Hahn G. and Wittrock E., (1991), Acta Microbiologica Hungarica 38(3-4):265-271.
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
4. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
5. Manafi. M. and Kneifel W., (1989), Zbl. Hygiene and Umweltmedizin 189:225-234.
6. Manafi M., (1990), Forum Stadte-Hygiene 41:181-184.
7. Manafi M., (1991), Ernahrung / Nutrition, 15, Nr. 10.
8. Manafi M. and Kneifel W., (1991), Acta Microbiologica Hungarica 33(3-4):293-304.
9. Manafi M., Kneifel B. and Bascon S., (1991), Microbiol. Rev., 55:335-348.

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IVD	In vitro diagnostic medical device
CE	CE Marking
25°C 15°C	Storage temperature
	Do not use if package is damaged
	HiMedia Laboratories Pvt. Limited, 23 Vadhani Industrial Estate, LBS Marg,Mumbai-86,MS,India
EC REP	CE Partner 4U ,Esdoornlaan 13, 3951 DB Maarn The Netherlands, www.cepartner 4u.eu