Rapid HiColiform™ Agar / Broth

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.

M1465 / M1453

Composition **	M1465	M1453
Ingredients	Grams/Litre	Grams/Litre
Peptone, special	5.00	5.00
Sodium chloride	5.00	5.00
Sorbitol	1.00	1.00
Dipotassium hydrogen phosphate	2.70	2.70
Potassium dihydrogen phosphate	2.00	2.00
Sodium lauryl sulphate (SLS)	0.10	0.10
Chromogenic substrate	0.08	0.08
Fluorogenic substrate	0.05	0.05
IPTG (Isopropyl-β-D-thiogalactopyranoside)	0.10	0.10
Agar	15.00	—

Final pH (at 25°C) 6.8 ± 0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 31.03 grams of M1465 and 16.03 grams of M1453 in 1000 ml distilled water. For double strength broth use 32.06 grams of M1453 in 1000 ml 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 dispense as desired (for M1453) or pour into sterile Petri plates (for M1465).

Principle and Interpretation

The Rapid HiColiform™ Agar is modification of LMX Broth described by Manafi and Kneifel (2). These media are useful for the detection and confirmation of *Escherichia coli* and total coliforms in water samples on the basis of chromogenic and fluorogenic substrates (1-6).

Peptone special, which is rich in tryptophan 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 β-D-glucuronidase, which is specifically found in *E. 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 broth due to cleavage of chromogenic substrate. IPTG, a highly stable synthetic analog of lactose induces synthesis of β-D-glucuronidase. In agar medium, 2-3 drops of Kovac's reagent is added over the suspected colonies. Change in the colour of colony to red confirms E. coli. Broth medium is overlayed with Kovac's reagent and formation of red ring confirms *E. coli*. If fluorescence is negative after 24 hours of incubation, continue incubation for another 24 hours without performing the indole test.

Type of specimen

Water samples

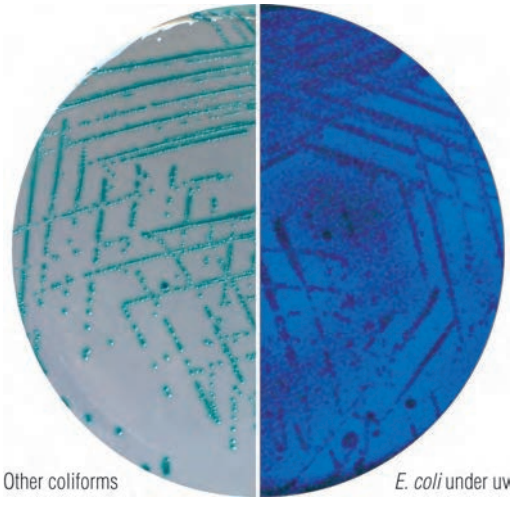
Specimen Collection and Handling

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (7).

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

Warning and Precautions

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 specimens. Safety guidelines may be referred in individual safety data sheets



M1465 Rapid HiColiform™ Agar

HiCromeVeg™ Single Streak Rapid Differentiation Series *Freedom from BSE / TSE worries*

Rapid HiColiform™ Agar / Broth (M1465 / M1453) is also available as Rapid HiColiform™ HiVeg™ Agar / Broth (MV1465 / MV1453) wherein all the animal origin nutrients have been replaced by vegetable based nutrients.

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Limitations

1. β-glucuronidase especially present in 97% of *Escherichia coli,* however few *E. coli* may be negative.
2. Slight colour variation may be observed depending upon the utilization of the substrate by the organism.
3. Further confirmation of *E.coli* must be confirmed by addition of Kovacs reagent to the suspected colony after visualization of fluorescence

Performance and Evaluation

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

Quality Control

Appearance of powder : Cream to yellow coloured, homogeneous, free flowing powder.

Gelling : Firm, comparable with 1.5% Agar gel of M1465

Colour and Clarity of prepared medium : Light yellow coloured, clear to slightly opalescent gel forms in Petri plates (M1465)/ clear solution having slight precipitate in tubes (M1453).

Reaction : Reaction of 3.1% w/v of M1465 or 1.6 % w/v of M1453 aqueous solution at 25°C. pH:6.8 ± 0.2

Cultural Response : M1453 : Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organisms (ATCC)	Inoculum (CFU)	Growth	Colour change in medium	Fluorescence (Under UV light)	Indole reaction
Escherichia coli (25922) (00013*)	50-100	luxuriant	blue-green	+	+
#*Klebesiella aerogenes* (13048) (00075*)	50-100	luxuriant	blue-green	-	-

M1465 : Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

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#*Klebesiella aerogenes* (13048) (00075*)	50-100	luxuriant	blue-green	-	-
Klebsiella pneumoniae (13883) (00097*)	50-100	luxuriant	blue-green	-	-
Salmonella Typhimurium (14028) (00031*)	50-100	luxuriant	yellow	-	-

Key : * : Corresponds to WDCM number
: + : positive reaction, - : negative reaction.
: Formerly known as *Enterobacter aerogenes*

Storage and Shelf-life

Store between 2-8°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 inorder 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. Use before expiry date on the label.

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 (8, 9).

References

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