

For identification and differentiation of *E. coli* and Total coliforms

HiCrome™ Coliform Agar w/ SLS/ Coliform Broth w/ SLS

Recommended for the simultaneous detection of *Escherichia coli* and total coliforms in water and food samples.

M1300/
M1826

| Composition ** | M1300 | M1826 |
|--------------------------------|-------------|-------------|
| Ingredients | Grams/Litre | Grams/Litre |
| Peptone, special | 3.00 | 3.00 |
| Sodium chloride | 5.00 | 5.00 |
| Dipotassium hydrogen phosphate | 3.00 | 3.00 |
| Potassium dihydrogen phosphate | 1.70 | 1.70 |
| Sodium pyruvate | 1.00 | 1.00 |
| L-Tryptophan | 1.00 | 1.00 |
| Sodium lauryl sulphate (SLS) | 0.10 | 0.10 |
| Chromogenic mixture | 0.20 | - |
| Chromogenic substrate | - | 0.30 |
| Agar | 12.00 | - |
| Final pH (at 25°C) | 6.8 ± 0.2 | 6.8 ± 0.2 |

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 27 grams of M1300 or 15.10 grams of M1826 in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. For M1826 dispense into tubes or flasks or as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Add 5mg/ l novobiocin before autoclaving the medium, when a high number of gram-positive accompanying bacteria are expected. Cool to 45-50°C. Mix well and pour into sterile Petri plates for M1300.

Principle and Interpretation

HiCrome™ Coliform Agar / Broth w/ SLS is a selective medium recommended for the simultaneous detection of *Escherichia coli* and total coliforms in water and food samples (5). Peptone special and Sodium pyruvate provides nitrogenous, carbonaceous, compounds, long chain amino acid and other essential growth nutrients. The phosphates buffer the medium well. The medium composition helps even the sublethally injured coliforms to grow rapidly. Sodium lauryl sulphate inhibits gram-positive organisms.

The chromogenic mixture contains two chromogenic substrates. The enzyme β -galactosidase produced by coliforms cleaves one chromogen, resulting in the salmon to red colouration of coliform colonies. The enzyme β -glucuronidase produced by *E. coli*, cleaves X-glucuronide. *E. coli* forms dark blue to violet coloured colonies due to cleavage of both the chromogens (1, 3, 4). The addition of L-Tryptophan improves the indole reaction, thereby increasing detection reliability in combination with the two chromogens. In M1826 chromogenic substrate in the medium help to detect β -glucuronidase positive *E. coli*. Other gram negative bacteria forms colourless colonies, except some organisms which are β -glucuronidase positive. β -glucuronidase positive organisms gives light blue to turquoise colonies. GUD is present in 94-96% of *E. coli* strains and in some *Salmonella*, *Shigella* and *Yersinia* spp (2). To confirm *E. coli*, add a drop of Kovac's reagent (R008) on the dark-blue

to violet colony/Blue colour broth. Formation of cherry-red colour indicates positive reaction.

Type of specimen

Water samples ; Food samples.

Specimen Collection and Handling

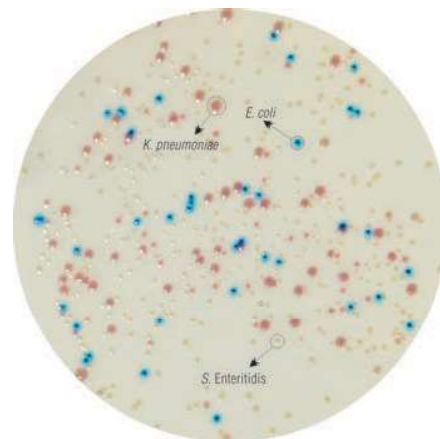
For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (6).

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

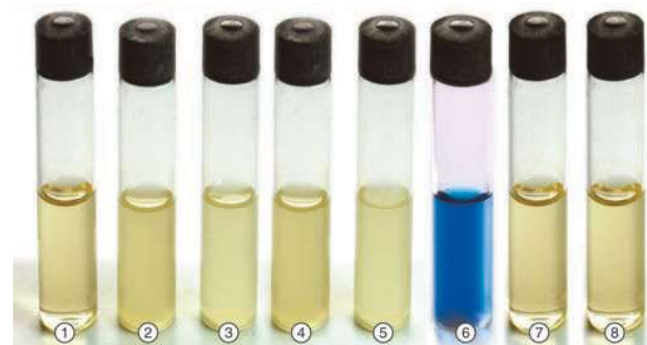
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

Read the label before opening the container. Wear protective gloves/



M1300 HiCrome™ Coliform Agar w/SLS



M1826 Coliform Broth w/SLS

1. Control
2. *Citrobacter freundii* ATCC 8090
3. *Klebsiella pneumoniae* ATCC 13883
4. *Salmonella* Enteritidis ATCC 13076
5. *Shigella flexneri* ATCC 12022
6. *Escherichia coli* ATCC 25922 (00013*)
7. *Enterococcus faecalis* ATCC 29212 (00087*)
8. *Staphylococcus aureus* subsp aureus ATCC 25923 (00034*)

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

HiCrome™ Coliform Agar w/ SLS (M1300) is also available as HiCrome™ Coliform HiVeg™ Agar w/ SLS (MV1300) wherein all the animal origin nutrients have been replaced by vegetable based nutrients.

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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

Limitations

1. β -glucuronidase is present in 97% of *E. coli* strains, however few *E. coli* may be negative.
2. Certain species of *Shigella* and *Salmonella* are β -glucuronidase positive which may appear as light blue.
3. Further biochemical and serological test must be carried out for confirmation of suspected colonies.

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** : Light yellow to beige coloured (M1300)/Cream to yellow coloured (M1826), homogeneous, free flowing powder.
- Gelling** : Firm, comparable with 1.2% Agar gel (M1300).
- Colour and Clarity of prepared medium** : Colourless, clear to very slightly opalescent gel forms in Petri plates (M1300).
Cream, clear to slightly opalescent solution, may have slight precipitate (M1826).
- Reaction** : Reaction of 2.70% w/v (M1300) / 1.51% w/v (M1826) aqueous solution of M1300 at 25°C. pH: 6.8 ± 0.2.
- Cultural Response** : Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours (48 hours if necessary).

| Organisms (ATCC) | Inoculum (CFU) | Growth | Recovery (M1300) | Colour of colony (M1300) | Colour of medium (M1826) |
|--|----------------|------------------|------------------|--------------------------|--------------------------|
| <i>Escherichia coli</i> (25922) (00013*) | 50-100 | good - luxuriant | >=50% | dark blue / violet ● | blue |
| <i>Enterobacter cloacae</i> (23355) (00082*) | 50-100 | good - luxuriant | >=50% | salmon to red | - |
| <i>Citrobacter freundii</i> (8090) | 50-100 | good - luxuriant | >=50% | salmon to red | colourless |
| <i>Escherichia coli</i> (35218) | 50-100 | - | >=50% | - | blue |
| <i>Klebsiella pneumoniae</i> (13883) (00097*) | 50-100 | good - luxuriant | >=50% | light pink | colourless |
| <i>Salmonella</i> Enteritidis (13076) (00030*) | 50-100 | good | 40-50% | colourless | colourless |

| | | | | | |
|---|-------------------|-----------|--------|------------|------------|
| <i>Shigella flexneri</i> (12022) (00126*) | 50-100 | good | 40-50% | colourless | colourless |
| <i>Enterococcus faecalis</i> (29212) (00087*) | >=10 ³ | inhibited | 0% | - | - |
| <i>Staphylococcus aureus</i> subsp <i>aureus</i> (25923) (00034*) | >=10 ³ | - | 0% | - | - |
| <i>Staphylococcus aureus</i> subsp <i>aureus</i> (6538) (00032*) | >=10 ³ | - | 0% | - | - |

Key : ● : positive reaction, confirmation of red colour around the colony by addition of Kovac's reagent (R008) - : negative reaction.

* : corresponding WDCM Numbers

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 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. 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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5. Manafi M. and Kneifel W., (1989), Zentralbl. Hyg., 189:225.
6. 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
7. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
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9. 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.

| Ready Prepared Media | | | |
|---|---|--|---------|
| Code | Product Name | Usage | Packing |
| Category : DriFilter Membrane Nutrient Pad | | | |
| MF026E | #Coliform Medium w/ SLS (Economy Pack)(without Membrane Filter) | for detection and enumeration of total coliforms and <i>E. coli</i> based on chromogenic differentiation | 50 pcs |
| MF026F | Coliform Medium w/ SLS w/ Sterile Membrane Filter | for detection and enumeration of total coliforms and <i>E. coli</i> based on chromogenic differentiation | 50 plts |