

For selective isolation and presumptive identification of *Clostridium* species

## HiCrome™ Clostridial Agar Base

Recommended for selective isolation and presumptive identification of *Clostridium* species

M2026

### Composition \*\*

| Ingredients           | Grams/Litre |
|-----------------------|-------------|
| Tryptone              | 15.00       |
| Yeast extract         | 10.00       |
| Dextrose (Glucose)    | 1.00        |
| Sodium chloride       | 5.00        |
| Sodium thioglycollate | 0.50        |
| Chromogenic mixture   | 3.31        |
| Agar                  | 13.00       |

Final pH (at 25°C) 7.1 ± 0.2

\*\* Formula adjusted, standardized to suit performance parameters

### Directions

Suspend 47.81 grams 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. Aseptically add rehydrated contents of one vial of Perfringens Supplement II (FD012). Mix well and pour into sterile Petri plates.

### Principle and Interpretation

One of the major species of anaerobic bacteria to cause disease in humans is *Clostridium*. *Clostridium* species cause tetanus and gas gangrene that ultimately leads to tissue damage. Another *Clostridium* species produces the lethal botulinum toxin, the causative agent of botulism (1). Clostridial Agar formulated by Vera is recommended for the selective isolation of pathogenic Clostridia from mixed flora (2). HiCrome is the modification for chromogenic differentiation.

Tryptone provide the essential nutrients, mainly the nitrogen compounds. Yeast extract serves as source of vitamins especially of the B group. Dextrose acts as fermentable carbohydrate source. Sodium thioglycollate is the reducing agents that help to create low oxidation-reduction potential enabling the growth of Clostridia. Also the media is well supplemented to support luxuriant growth of *Clostridium* species. The selective supplements inhibits other enteric bacteria.

The ideal method of inoculation of Clostridial Agar is direct inoculation of sterile, cooled medium with the specimen (in tubes). Alternatively agar plates of the medium can also be inoculated by streaking.

### Type of specimen

Clinical samples

### Specimen Collection and Handling

For clinical samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (3,4). After use, contaminated materials must be sterilized by autoclaving before discarding.

### Warning and Precautions

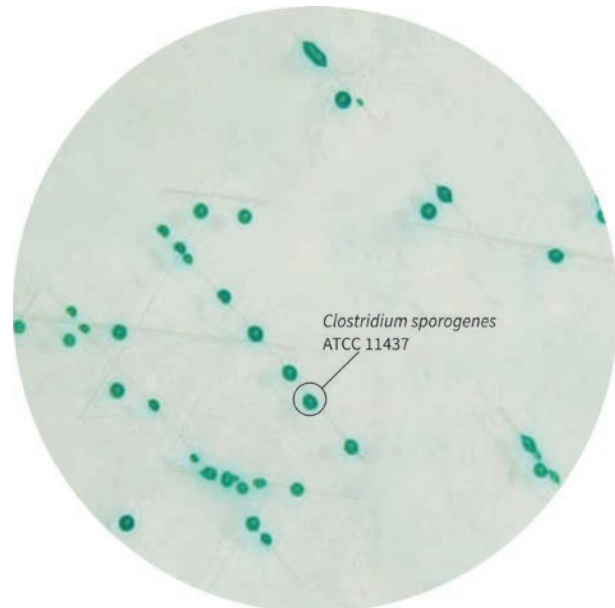
In Vitro diagnostic use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while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

### Limitations

1. Some species may show poor growth due to nutritional variations.
2. Slight colour variation may be observed depending upon strains.

### Performance and Evaluation

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



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### Quality Control

- Appearance of Powder** : Cream to beige homogeneous free flowing powder.
- Gelling** : Firm, comparable with 1.3% Agar gel
- Colour and Clarity** : Yellow coloured, clear to slightly opalescent gel forms in Petri plates
- Reaction** : Reaction of 4.78% w/v aqueous solution at 25°C. pH : 7.1±0.2
- Cultural Response** : Cultural characteristics observed after an incubation at 35-37°C for 24-48hours (under anaerobic condition).

| Organism (ATCC)                                    | Inoculum (CFU)    | Growth    | Recovery | Colour of colony        |
|--|-------------------|-----------|----------|-------------------------|
| <i>Clostridium perfringens</i> ATCC 13124 (00007*) | 50-100            | luxuriant | >=50%    | Pale yellowish green    |
| <i>Clostridium sporogenes</i> ATCC 11437           | 50-100            | luxuriant | >=50%    | Pale green-bluish green |
| <i>Clostridium sporogenes</i> ATCC 19404 (00008*)  | 50-100            | luxuriant | >=50%    | Pale green-bluish green |
| <i>Escherichia coli</i> ATCC 25922 (00013*)        | >=10 <sup>3</sup> | inhibited | 0%       |                         |
| <i>Staphylococcus aureus</i> ATCC 25923 (00034*)   | >=10 <sup>3</sup> | inhibited | 0%       |                         |

### 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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (3, 4).

### References

1. Alcamo E. I., 2001, Fundamentals of Microbiology, 6th Ed., Jones and Bartlett Publishers.
2. Vera, 1962, Presented Pa. Soc. Med. Tech., York, Pa.
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.