Technical Data

Iron Medium Base

M1871

Intended Use:

Recommended for presumptive confirmation of *Clostridium perfringens* from food in accordance with FDA BAM, 1998.

Composition**

Ingredients	Gms / Litre
Ferrous sulphate heptahydrate	1.000

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 1.00 grams in 50ml purified / distilled water. Take 1 liter of whole milk in another flask and sterilize both the solutions separately by autoclaving at 118°C for 12 minutes. After sterilization, slowly add ferrous sulphate solution to milk. Dispense 11ml medium into 16×150 mm culture tubes.

Principle And Interpretation

Clostridium is a large genus of gram-positive spore bearing anaerobes. *Clostridium perfringens* is one of the most common anaerobes found in foods. Small numbers of *C. perfringens* are commonly found in raw meats, poultry, dehydrated soups and sauces, raw vegetables and spices. The spores of these strains are resistant to high temperatures and survive at 100°C for more than one hour (5). Inadequately processed foods and improper storage often leads to proliferation of these organisms. Hence detection of *C. perfringens* become necessary. Iron Milk Medium is one of the medium for presumptive detection of *C. perfringens* in accordance with FDA, BAM (2). On isolation of black colonies from suspected foods on TSC agar, the culture is enriched in Fluid thioglycollate medium. The enriched culture is tested for stormy fermentation in Iron milk Medium Base with added whole milk (M1871).

As per the procedure, the food sample under test; whole portion or representative 25 gms is checked for total bacterial count by inoculating on TSC agar (M1005F). Presumptive Clostridia species grow as black colonies which is cultured and enriched in Fluid Thioglycollate Medium (M009) at 35°C for 18-24 hours. Inoculate modified iron-milk medium with 1 ml of actively growing *C. perfringens* in Fluid Thioglycollate culture and incubate at 46°C in a water bath. Make periodic observations after 2hours for "stormy fermentation", which is characterized by rapid coagulation of milk followed by fracturing of curd into spongy mass which usually rises above medium surface (1). Bigger test tubes are used for the prevention of spillage into the water bath. Cultures that fail to exhibit "stormy fermentation" within 5 h are unlikely to be *C. perfringens*. An occasional strain may require 6 h or more, but this is a questionable result that should be confirmed by further testing. Some strains of *C. baratii* react in this manner, but this species can be differentiated by its inability to liquefy gelatin in lactose-gelatin medium. The rapidity with which the "stormy fermentation" occurs depends on the strain and the initial population. Therefore, only actively growing cultures are appropriate for this test. The presumptive test in iron-milk medium may be sufficient for some purposes. However, the completed test must always be performed with isolates associated with food poisoning outbreaks (2).

Type of specimen

Food samples.

Specimen Collection and Handling

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (5).
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.

Limitations

1. Only actively growing cultures are appropriate for this test.
2. Further biochemical and serological tests must be carried out for further identification.

Performance and Evaluation

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

Quality Control

Appearance

Pale green to green Crystalline granules

Colour and Clarity of prepared medium

Basal medium Colourless to pale green coloured on addition of 1 litre of whole milk to basal medium its Offwhite coloured opaque milky solution

Cultural Response

Cultural characteristics observed after an incubation at 46°C for 6-18hrs under anaerobic condition and record the reactions of various intervals during the incubation.

Organism	Growth	Reaction
Clostridium perfringens ATCC 13124 (00007*)	good-luxuriant	stormy fermentation (gas)

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 15-25 °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. Abeyta, C Jr . and Wetherington, JH. 1994. J AOAC Int., 77(2): 351-6.
2. FDA, U.S. 1998. Bacteriological Analytical Manual. 8 ed. Gaithersburg, MD: AOAC International.
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. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.

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