TM 2137 - IRON MEDIUM BASE

INTENDED USE

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

PRODUCT SUMMARY AND EXPLANATION

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. 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. 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.

As per the procedure, the food sample under test; whole portion or representative 25 gm is checked for total bacterial count by inoculating on TSC agar. Presumptive Clostridia species grow as black colonies which is cultured and enriched in Fluid Thioglycollate Medium 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 2 hours 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. 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.

COMPOSITION

Ingredients	Gms / Ltr
Ferrous sulphate heptahydrate	1.000

PRINCIPLE

Ferrous sulphate heptahydrate is used in iron medium base as a source of iron.

INSTRUCTION FOR USE

- Dissolve 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.

QUALITY CONTROL SPECIFICATIONS

Appearance of Powder : Pale green to green Crystalline granules.

Appearance 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.

INTERPRETATION

Cultural characteristics observed after an incubation at under anaerobic condition.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Reaction	Incubation Temperature	Incubation Period
Clostridium perfringens	13124	50-100	Good-luxuriant	Stormy fermentation (gas)	46°C	6-18 Hours

PACKAGING:
In pack size of 500 gm bottles.

STORAGE
Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 2-8°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.
Product Deterioration: Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

DISPOSAL
After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

REFERENCES
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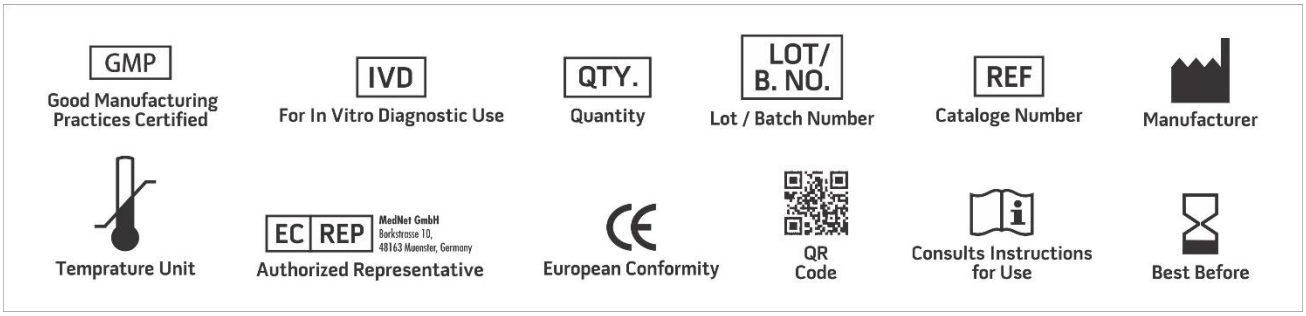
2. FDA, U.S. 1998. Bacteriological Analytical Manual. 8 ed. Gaithersburg, MD: AOAC International.
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
4. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical 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.



NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.
***For Lab Use Only**
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