



Iron Milk Medium Base w/o Whole Milk

M1871

Iron Milk Medium Base w/o Whole Milk is used for the presumptive test of *Clostridium perfringens* in accordance with FDA BAM, 1998.

Composition**

Ingredients	Gms / Litre
Ferrous sulfate, 7H ₂ O	1.000

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 1.00 grams in 50ml 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 1 ml 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 (1). 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 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(3). 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).

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.

Cultural Response

Organism	Growth	Reaction
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Cultural Response

<i>Clostridium perfringens</i>	good-luxuriant	stormy
ATCC 13124		fermentation (gas)

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2 -8°C. Use before expiry date on the label

Reference

1. Downes, F.P. and Ito, K. 2001. Methods For The Microbiological Examination of Foods. APHA, Food 4 ed. Washington, D.C.
2. FDA, U.S. 1998. Bacteriological Analytical Manual. 8 ed. Gaithersburg, MD: AOAC International.
3. Abeyta, C Jr . and Wetherington, JH. 1994. J AOAC Int., 77(2): 351-6.

Revision : 2 / 2015

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