



## Gelatin Iron Agar

M686

### Intended use

Gelatin Iron Agar is used for detecting gelatin liquefaction and hydrogen sulphide production.

### Composition\*\*

Ingredients	Gms / Litre
Peptone	25.000
HM extract #	7.500
Sodium chloride	5.000
Gelatin	120.000
Ferrous chloride	0.500
Agar	1.000
Final pH ( at 25°C)	7.0±0.2

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

# - Equivalent to Meat extract

### Directions

Suspend 15.9 grams in 100 ml warm purified/distilled water. Heat to boiling to dissolve the medium completely. Dispense in test tubes as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

### Principle And Interpretation Composition\*\*

Gelatin liquefaction along with the production of hydrogen sulphide is one of the characteristics used in the classification of bacteria. Hydrogen sulphide can be produced in small amounts from sulphur containing amino acids by a large number of bacteria. Methods to detect hydrogen sulphide production by suspending strips of paper impregnated with lead acetate above cultures are of variable sensitivity and are of limited value. The hydrogen sulphide production test combined with gelatin liquefaction test is useful for group differentiation within the *Enterobacteriaceae* species (3). Few *Clostridia* exhibit gelatinase activity as well as H<sub>2</sub>S production. *Escherichia coli* grow well on this medium but show neither gelatinase activity nor H<sub>2</sub>S production.

The medium consists of peptone, HM extract and gelatin, which provide nitrogen compounds and also the carbon compounds for the growing organisms. Gelatin acts as solidifying agent and is the substrate for the organisms producing gelatinase enzyme. Ferrous chloride aids in the detection of hydrogen sulphide indicated by black precipitate. Gelatin is usually liquefied by *Clostridium perfringens* within 24 to 48 hours.

### Type of specimen

Isolated Microorganism

### Specimen Collection and Handling

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,6).

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(2)

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. Due to nutritional variations, some strains may show poor growth.

## 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

Cream to yellow homogeneous free flowing powder

### Gelling

Semisolid, comparable with 0.1% Agar gel and 12.0% Gelatin gel.

### Colour and Clarity of prepared medium

Light yellow coloured, clear to slightly opalescent gel forms in tubes as butts

### Reaction

Reaction of 15.9% w/v aqueous solution at 25°C. pH : 7.0±0.2

### pH

6.80-7.20

### Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 24-48 hours .

Organism	Inoculum (CFU)	Growth	Gelatinase reaction	H2S production
<i>Bacillus subtilis subsp. spizizenii</i> ATCC 6633 (00003*)	50-100	luxuriant	positive reaction	negative, no blackening of medium
<i>Clostridium perfringens</i> ATCC 12924	50-100	luxuriant	positive reaction	positive, blackening of medium
<i>Escherichia coli</i> ATCC 25922 (00013*)	50-100	luxuriant	negative reaction	negative, no blackening of medium

\* - Corresponding WDCM numbers

## Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°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 (4,5).

## Reference

1. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
2. 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.
3. Collee J. G., Fraser A. G., Marmion B. P., Simmons A., (Eds.), Mackie and McCartney, Practical Medical Microbiology, 1996, 14th Edition, Churchill Livingstone
4. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
5. 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.
6. 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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