



Nutrient Gelatin

M060

Nutrient Gelatin is recommended for detection of gelatin liquefaction by proteolytic microorganisms.

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	5.000
Beef extract	3.000
Gelatin	120.000
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 128 grams in 1000 ml of warm (50°C) distilled water. Heat to boiling to dissolve the medium completely. Dispense into test tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Allow the tubed medium to cool in an upright position.

Principle And Interpretation

Nutrient Gelatin is prepared as per the formulation formerly used in the examination of water, sewage and other materials of sanitary importance (1). Gelatin liquefaction is one of the essential test for the differentiation of enteric bacilli (2). This medium can also be used for the microbial plate counts of water.

Peptic digest of animal tissue and beef extract supply nutrients for the growth of non-fastidious organisms. Gelatin is the substrate for the determination of the ability of an organism to produce gelatinase, a proteolytic enzyme active in the liquefaction of gelatin.

An 18-24 hours old pure culture from Triple Sugar Iron Agar (M021) or Kligler Iron Agar (M078) is stab-inoculated in Nutrient Gelatin with an inoculating needle directly down the centre of the medium to a depth of approximately one half an inches from the bottom of the tube. Incubate the tubes including an un-inoculated control at 35±2°C for 24-48 hours. Many species require prolonged incubation (3, 4) for gelatin liquefaction. Gelatin is solid at 20°C or less temperature and liquid at 35°C or higher temperature. Gelatin liquefies at about 28°C, so incubation is carried out at 35°C but kept in a refrigerator for about 2 hours before interpretation of the results (3). Liquefaction of gelatin occurs on the surface layer, so care should be taken not to shake the tubes (5). Control is run along with every testing as gelling ability of gelatin varies (3) and also the gelatin concentration should not exceed 12% as it may inhibit growth (6). For plate counts of water, the incubation is carried out at 20-22°C for upto 30 days.

Nutrient Gelatin Medium is not recommended for determination of gelatin liquefaction by fastidious species and obligate anaerobes. At various intervals during the incubation process, examine the tubes for growth and liquefaction. At each interval, tighten the caps and transfer the tubes to refrigerator for sufficient time period to determine whether liquefaction has occurred or not.

Quality Control

Appearance

Cream to yellow homogeneous free flowing slightly coarse powder

Gelling

Semisolid, comparable with 12.0% Gelatin gel

Colour and Clarity of prepared medium

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

Reaction

Reaction of 12.8% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH

Please refer disclaimer Overleaf.

6.60-7.00

Cultural Response

M060: Cultural characteristics observed after an incubation at 35-37°C for 1 to 7 days, (Incubated anaerobically for *Cl.perfringens*). (For gelatinase test, cool below 20°C)

Organism	Inoculum (CFU)	Growth	Gelatinase
Cultural Response			
<i>Clostridium perfringens</i> ATCC 12924	50-100	good-luxuriant	positive reaction
<i>Bacillus subtilis</i> ATCC 6633	50-100	good-luxuriant	positive reaction
<i>Escherichia coli</i> ATCC 25922	50-100	good-luxuriant	negative reaction
<i>Proteus vulgaris</i> ATCC 13315	50-100	good-luxuriant	positive reaction
<i>Staphylococcus aureus</i> ATCC 25923	50-100	good-luxuriant	positive reaction

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. American Public Health Association, 1975, Standard Methods for the Examination of Water and Wastewater, 14th Ed., APHA, Washington, D.C.
2. Ewing, 1986, Edwards and Ewings Identification of Enterobacteriaceae, 4th Ed., Elsevier Science Publishing Co., Inc. New York.
3. Cawan S. and Steel K., 1966, Manual for the Identification of Medical Bacteria, Cambridge University Press, Pg. 19, 27-28, 116 and 156.
4. Lautrop H., 1956, Acta. Pathol. Microbiol. Scand., 39:357.
5. Frobisher M., 1957, Fundamentals of Microbiology, 6th Ed., W.B. Saunders Co., Philadelphia, p. 239.
6. Branson D., 1972, Methods in Clinical Bacteriology, Springfield, III, pg. 21.

Revision : 1 / 2011

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