GELATIN MEDIUM (NUTRIENT GELATIN)

INTENDED USE
Remel Gelatin Medium (Nutrient Gelatin) is a solid medium recommended for use in qualitative procedures to determine the ability of an organism to liquefy gelatin.

SUMMARY AND EXPLANATION
Levine and Carpenter incorporated gelatin into nutrient medium in their studies of gelatin liquefaction by gram-negative bacilli.[1] Some bacteria produce gelatinase, a proteolytic enzyme that hydrolyzes gelatin into its constituent amino acids with a loss of its gelling characteristics.[2] The production of gelatinase is a characteristic which is used to differentiate and identify certain genera of enteric gram-negative bacilli and nonfermentative gram-negative bacilli. Gelatin Medium (Nutrient Gelatin) is recommended as a standard method for use in taxonomic work, because the rate of gelatin liquefaction is important in the characterization of the *Enterobacteriaceae.*[3]

PRINCIPLE
Peptone and beef extract supply amino acids and other essential nutrients to support the growth of nonfastidious bacteria. Gelatin is incorporated in the medium to determine an organism's ability to produce gelatinase, a proteolytic enzyme. Gelatinase decomposes gelatin into smaller components, resulting in liquefaction of the gelatin.

REAGENTS (CLASSICAL FORMULA)*

Gelatin	120.0 g	Beef Extract	3.0 g
Peptone	5.0 g	Demineralized Water	1000.0 ml

pH 6.8 ± 0.2 @ 25°C

*Adjusted as required to meet performance standards.

PROCEDURE
1. Keep gelatin tubes in the refrigerator until just prior to inoculation. The medium should be solidified. Gelatin changes from a gel (solid state) to a liquid at about 28°C.[4]
2. Using a heavy inoculum from a pure, 18-24-hour culture, stab the medium with an inoculating needle to a depth of ½ to 1 inch from the bottom of the tube.
3. An uninoculated tube of Gelatin Medium should be incubated simultaneously with the test isolate as a negative control.
4. Incubate in ambient air at 22-25°C or 33-37°C for 24 hours and up to 14 days.
5. Observe for growth (turbidity) and liquefaction at various intervals during the incubation process by placing test and control tubes in a refrigerator or ice bath for approximately 2 hours or until the control tube solidifies. Do not shake the tubes when transferring from incubator to refrigerator. When reading results, invert tubes to test for liquefaction or solidification.
6. Check tubes periodically for up to two weeks. Occasionally, prolonged incubation (30 days to 6 weeks) may be required.[5] For routine testing, liquefaction results are determined at the end of 2 weeks incubation at 33-37°C.
7. The control tube must be solid for the test to be valid.

INTERPRETATION OF THE TEST
Positive Test - Liquefaction after refrigeration
Negative Test - Solidification after refrigeration

QUALITY CONTROL
All lot numbers of Gelatin Medium (Nutrient Gelatin) have been tested using the following quality control organisms and have been found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

CONTROL	INCUBATION	RESULTS
Serratia marcescens ATCC® 8100	Ambient, up to 10 days @ 33-37°C	Positive (liquefaction)
Morganella morganii ATCC® 25830	Ambient, up to 10 days @ 33-37°C	Negative (no liquefaction)

LIMITATIONS
1. An uninoculated tube of Gelatin Medium should be incubated simultaneously with the test isolate tube(s) for use as a negative control.[4]
2. Do not shake gelatin tubes while they are warm. Growth and liquefaction frequently occur only on the surface layer and if the gelatin mixes with the warm fluid of the medium, a positive result could be overlooked.[4]
3. Some bacteria will not grow in Gelatin Medium.[4]

BIBLIOGRAPHY

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4. MacFaddin, J.F. 1985. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Williams & Wilkins, Baltimore, MD.
5. Forbes, B.A., D.F. Sahm, and A.S. Weissfeld. 2007. Bailey and Scott's Diagnostic Microbiology. 12th ed. Mosby Elsevier, St. Louis, MO.

Refer to the front of Remel *Technical Manual of Microbiological Media* for **General Information** regarding precautions, product storage and deterioration, specimen collection, storage and transportation, materials required, quality control, and limitations.

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IFU 60950, Revised July 23, 2014 Printed in U.S.A.

remel
12076 Santa Fe Trail Drive, Lenexa, KS 66215, USA
General Information: (800) 255-6730 Website: www.remel.com Email: remel@remel.com
Local/International Phone: (913) 888-0939 International Fax: (913) 895-4128