LOWENSTEIN-JENSEN BUTT (CATALASE TEST)

INTENDED USE
Remel Lowenstein-Jensen Butt is a solid medium recommended for use in the semiquantitative catalase test to aid in differentiation of *Mycobacterium* species.

SUMMARY AND EXPLANATION
In 1931, Lowenstein formulated a medium for cultivation of mycobacteria containing congo red and malachite-green dyes to partially inhibit a number of bacterial species.[1] In 1932, Jensen modified the medium by altering the citrate and phosphate contents, eliminating congo red dye, and increasing the malachite green concentration.[2] Lowenstein-Jensen Medium in a tubed deep (butt) is used to perform the semiquantitative catalase test. This procedure was developed by Wayne in 1962.[3]

PRINCIPLE
Lowenstein-Jensen Medium is a glycerated egg-potato medium which provides fatty acids and protein necessary for the metabolism of mycobacteria. Malachite-green dye is a pH indicator and partially inhibits bacteria other than mycobacteria. Acid-fast bacilli produce catalase, an intracellular, soluble enzyme, which is capable of splitting hydrogen peroxide into water and oxygen. The subsequent ebullition of gas (oxygen bubbles) indicates catalase activity. The semiquantitative catalase test divides the mycobacteria into 2 groups, those producing less than 45 mm of bubbles and those producing more than 45 mm of bubbles. Usually, *Mycobacterium kansasii, Mycobacterium simiae,* most scotochromogens, the nonphotochromogenic saprophytes, and the rapid growers produce more than 45 mm of bubbles. Those that produce less than 45 mm of bubbles include *Mycobacterium tuberculosis, Mycobacterium marinum, Mycobacterium avium* complex, *Mycobacterium xenopi,* and *Mycobacterium gastri.*[4]

REAGENTS (CLASSICAL FORMULA)*

Potato Flour	30.0	g	Malachite Green	0.4	g
Asparagine	3.6	g	Magnesium Sulfate	0.24	g
Monopotassium Phosphate	2.4	g	Glycerol	12.0	ml
Magnesium Citrate	0.6	g	Whole Eggs	1000.0	ml
			Demineralized Water	600.0	ml

*Adjusted as required to meet performance standards.

PROCEDURE
Follow established laboratory safety procedures when working with acid-fast cultures and specimens. Consult appropriate references when necessary for detailed procedural information on specimen processing, media inoculation, and identification of test isolates.[4,5]

1. Inoculate Lowenstein-Jensen Butt with 0.1 ml of a 7 day liquid culture of the test isolate or a loopful of growth from an actively growing slant.
2. Include positive and negative control organisms.
3. Incubate tubes in 8-10% CO_2 at 33-37°C with caps loosened for 2 weeks.
4. After incubation, add 0.5 ml of catalase reagent (1:1 mixture of 10% Polysorbate® 80 (REF R21275) and 30% hydrogen peroxide) to the culture.
5. Place tubes upright in a rack which is standing on paper towels soaked with disinfectant. The column of bubbles produced may overflow the tube if the cap is not replaced and tightened quickly enough.
6. Allow tubes to stand at room temperature for 5 minutes before measuring. Measure in millimeters the height of the column of bubbles above the medium surface.

INTERPRETATION OF THE TEST
Positive - A column of bubbles greater than 45 mm
Negative - A column of bubbles less than 45 mm

QUALITY CONTROL
All lot numbers of Lowenstein-Jensen Butt 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
Mycobacterium flavescens ATCC® 14474	CO_2, 2 weeks @ 33-37°C	Positive

LIMITATIONS
1. Malachite green is a photosensitive dye; therefore, media should not be stored in exposed light.[6]
2. Moisture is required for growth of mycobacteria. Keep tubes tightly sealed prior to inoculation.[6]

BIBLIOGRAPHY

1. Lowenstein, E. 1931. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. 120:127.
2. Jensen, K.A. 1932. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. 125:222.
3. Wayne, L.G. 1962. Am. Rev. Respir. Dis. 86:651.
4. Kent, P.T. and G.P. Kubica. 1985. Public Health Mycobacteriology-A Guide for the Level III Laboratory. U.S. Dept. of H.H.S., CDC, Atlanta, GA.
5. Isenberg, H.D. 2004. Clinical Microbiology Procedures Handbook. 2nd ed., Vol. 2. ASM Press, Washington, D.C.
6. MacFaddin, J.F. 1985. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Vol. 1. Williams & Wilkins, Baltimore, MD.

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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