WALLENSTEIN MEDIUM

INTENDED USE
Remel Wallenstein Medium is a solid medium recommended for use in qualitative procedures for the isolation of *Mycobacterium* species.

SUMMARY AND EXPLANATION
Wallenstein Medium is a modification of the formulation originally described by Wallenstein in 1941 for the cultivation of tubercle bacilli.[1] It is somewhat more inhibitory than Lowenstein-Jensen Medium which has a lower concentration of malachite green.[2]

PRINCIPLE
The glycerol and egg yolk mixture supply fatty acids and protein required for the metabolism of mycobacteria. Malachite green dye is a selective agent with inhibits the growth of microorganisms other than mycobacteria.

REAGENTS (CLASSICAL FORMULA)*

Egg Yolks	765.0	ml	Malachite Green	15.0	ml
Glycerol	20.0	ml	Demineralized Water	200.0	ml

pH 6.7 ± 0.2 @ 25°C

*Adjusted as required to meet performance standards.

PROCEDURE
Note: Follow established laboratory guidelines and safety procedures when processing specimens for acid-fast cultures. Inoculate media according to test procedures recommended by the Centers for Disease Control and Prevention (CDC) or consult appropriate references.[3-5]

1. After inoculation, allow tubes to stand at room temperature for several hours or until the inoculum dries or is absorbed.
2. Protect tubes from light and incubate at 35-37°C in an atmosphere of 5-10% CO_2. Caps should be loosened for the first week to permit the circulation of CO_2 to initiate growth. Thereafter, tighten caps to prevent dehydration of media.
3. Examine cultures within 5-7 days after incubation and once a week thereafter for a minimum of 8 weeks.
4. Observe cultures for growth rate, pigment production, and colony morphology.
5. Perform an acid-fast stain on growth to confirm isolate is mycobacteria.
6. Initiate identification tests according to established laboratory procedures. Consult appropriate references for further instructions.[3-5]

QUALITY CONTROL
All lot numbers of Wallenstein Medium 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 tuberculosis ATCC® 25177	CO_2, up to 21 days @ 35-37°C	Growth
Mycobacterium kansasii ATCC® 12478	CO_2, up to 21 days @ 35-37°C	Growth

LIMITATIONS
1. Store Wallenstein Medium protected from light; malachite green is photosensitive.[2]
2. This medium is not recommended for the isolation of *Mycobacterium leprae*.[2]

BIBLIOGRAPHY

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2. MacFaddin, J.F. 1985. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Vol. 1. Williams & Wilkins, Baltimore, MD.
3. Kent, P.T. and G.P. Kubica. 1985. Public Health Mycobacteiology, A Guide for the Level III Laboratory. Dept. of H.H.S. and CDC, Atlanta, GA.
4. Winn, W.C., S.D. Allen, W.M. Janda, E.W. Koneman, G.W. Procop, P.C. Schreckenberger, and G.L. Woods. 2006. Koneman's Color Atlas and Textbook of Diagnostic Microbiology. 6th ed. Lippincott Williams & Wilkins, Philadelphia, PA.
5. Garcia, L.S. 2010. Clinical Microbiology Procedures Handbook. 3rd ed. ASM Press, Washington D.C.

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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