IFU

Instructions for Use

LOWENSTEIN JENSEN DEEP

Cat. no. C27	Lowenstein Jensen Medium, 20x125mm Tube, 5ml Deep	20 tubes/box

INTENDED USE

Hardy Diagnostics Lowenstein Jensen Deep is recommended for use in the semiquantitative catalase test to aid in differentiation of *Mycobacterium* species.

SUMMARY

The original formulation of Lowenstein Jensen media was developed by Lowenstein who incorporated congo red and malachite green to inhibit unwanted bacteria.[9,10] The present formulation, a glycerated egg-based medium, is based upon Jensen's modification. Jensen's version eliminates congo red and uses a moderate concentration of malachite green to prevent growth of the majority of contaminants surviving decontamination of the specimen. A tubed deep of Lowenstein Jensen Media was developed by Wayne and is useful for the classification of mycobacteria.

Nitrogen, fatty acids, and proteins are supplied by egg and asparagine. Glycerol serves as a carbon source and is favorable to the growth of the human type tubercle *Bacillus* while being unfavorable to the bovine type. Malachite green acts as an inhibitory agent toward microorganisms other than mycobacteria.[5]

Acid-fast bacilli produce catalase, an intracellular, soluble enzyme. This enzyme is capable of splitting hydrogen peroxide into water and oxygen. The production of gas, apparent by the presence of oxygen bubbles, indicates catalase activity. The semiquantitative catalase test divides the mycobacteria into two groups, those that produce less than 45mm of bubbles and those that produce more than 45mm of bubbles. *M. kansasii, M. simiae* , most scotochromogens, the non-photochromogenic saprophytes and the rapid growers usually produce more than 45mm of bubbles. *M. tuberculosis, M. marinum, M. avium complex, M. xenopi and M. gastri* are among the mycobacteria that produce less than 45mm of bubbles.[2,3,8]

FORMULA

Ingredients per liter of deionized water:*

Potato Flour	30.0gm
Asparagine	3.6gm
Monopotassium Phosphate	2.4gm
Magnesium Citrate	0.6gm
Malachite Green	0.4gm
Magnesium Sulfate	0.24gm
Glycerol	7.5ml

Egg Base	625.0ml

* Adjusted and/or supplemented as required to meet performance criteria.

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8ºC. away from direct light. Media should not be used if there are any signs of deterioration, discoloration, contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

The expiration date on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended incubation times as stated below.

Refer to the document "Storage" for more information.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual Universal Precautions for blood. Do not ingest, inhale, or allow to come into contact with skin.

This product is for *in vitro* diagnostic use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "Guidelines for Isolation Precautions" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M29: *Protection of Laboratory Workers from Occupationally Acquired Infections.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

PROCEDURE

Specimen Collection: Infectious material should be submitted directly to the laboratory without delay and protected from excessive heat and cold. Consult listed references for information on specimen collection.[1-3,6,7,12]

Method of Use:

1. Inoculate the medium with either 0.1ml of a 7-day liquid culture or a loopful of growth from an actively growing slant.

2. Inoculate test organism(s) as well as a strong catalase producer (*M. kansasii*) and a weak catalase producer (*M. intracellulare*) as controls.

3. Incubate tubes aerobically or in 5-10% CO_2, with loose caps, at 35-37ºC. for two weeks.

4. Prepare a fresh 1:1 mixture of 10% Tween 80 and 30% hydrogen peroxide.

5. Add 1ml of the Tween® 80-peroxide mixture to each culture.

6. Allow the tubes to stand at room temperature for 5 minutes before measuring the height of the columns of bubbles.

Record, in millimeters, the height of the column of bubbles above the medium surface.

LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification of bacteria and/or fungi.

Protect the media from all sources of light, as malachite green is very photo-sensitive.

Refer to the document "Limitations of Procedures and Warranty" for more information.

INTERPRETATION OF RESULTS

An uninoculated tube of medium should be used as a negative control.

In the semiquantitative catalase test, most mycobacteria fall into two groups:[2,3,6,7,12]

1. Column of bubbles >45mm height above medium surface

M. chelonae	*M. scrofulaceum*	*M. simiae*
M. fortuitum	*M. ulcerans*	*M. triviale*
M. gordonae	*M. terrae* complex	*M. smegmatis*
M. kansasii	*M. marinum*	

2. Column of bubbles <45mm in height above medium surface

M. avium complex	*M. bovis*	*M. gastri*
M. haemophilum	*M. intracellulare*	*M. malmoense*
M. tuberculosis	*M. xenopi*	

Refer to listed references for additional information.[2,3,6,7,12]

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, other culture media, swabs, applicator sticks, incinerators, and incubators, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificate of Analysis (CofA) and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*. The following microorganisms are routinely used for testing at Hardy Diagnostics:

Lowenstein Jensen Deeps are tested for growth performance only.

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Mycobacterium tuberculosis H37Ra ATCC® 25177	G	21 days	35°C	CO_2**	Growth; colonies seen in 2 weeks, mature in 3 weeks

Mycobacterium kansasii Group I ATCCATCC® 12478	G	21 days	35°C	CO_2**	Growth; colonies seen in 2 weeks, mature in 3 weeks
Mycobacterium scrofulaceum Group II ATCC® 19981	G	21 days	35°C	CO_2**	Growth; colonies seen in 2 weeks, mature in 3 weeks
Mycobacterium intracellulare Group III ATCC® 13950	G	21 days	35°C	CO_2**	Growth; colonies seen in 2 weeks, mature in 3 weeks
Mycobacterium fortuitum Group IV ATCC® 6841	G	21 days	35°C	CO_2**	Growth; colonies visible in 4 days

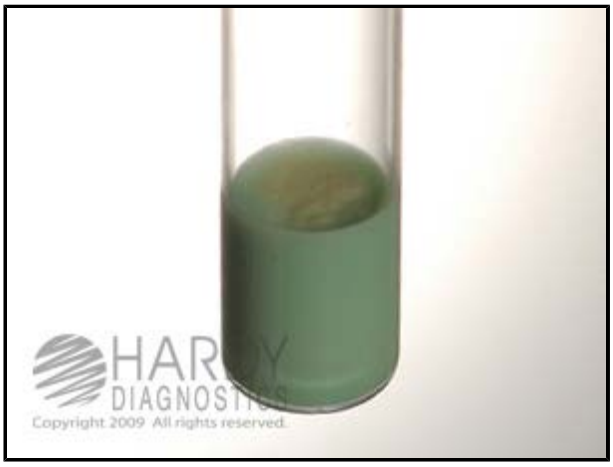
* Refer to the document "Inoculation Procedures for Media QC" for more information.

USER QUALITY CONTROL

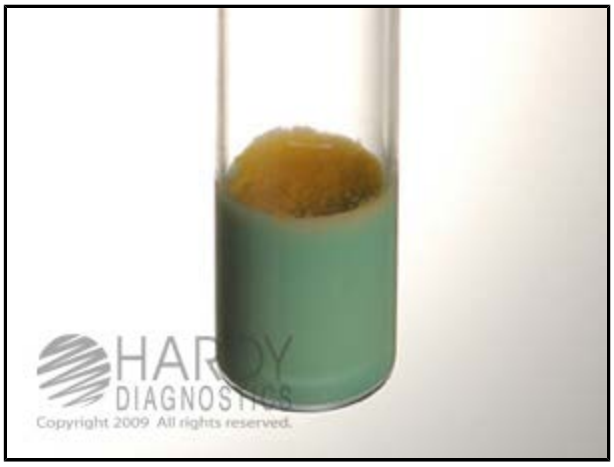
End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificate of analysis (CofA) available from Hardy Diagnostics Certificate of Analysis website. Also refer to the document "Finished Product Quality Control Procedures," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

PHYSICAL APPEARANCE

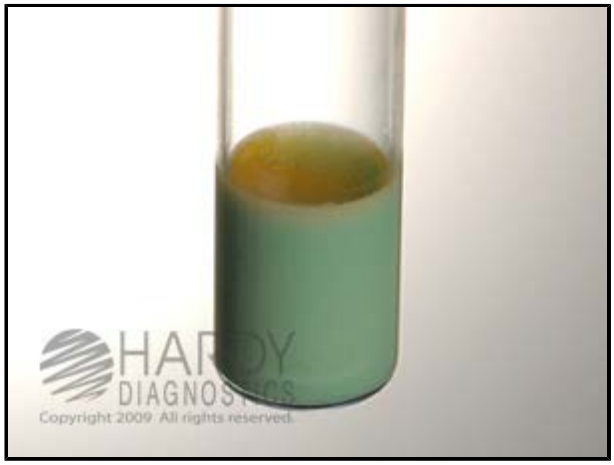
Lowenstein Jensen Media should appear opaque, and pale green in color.



Mycobacterium tuberculosis H37Ra (ATCC® 25177) growing on Lowenstein Jensen Deep (Cat. no. C27). Incubated in CO_2 for 21 days at 35ºC.



Mycobacterium kansasii Group I (ATCC® 12478) growing on Lowenstein Jensen Deep (Cat. no. C27). Incubated in CO_2 for 21 days at 35ºC.



Mycobacterium scrofulaceum Group II (ATCC® 19981) growing on Lowenstein Jensen Deep (Cat. no. C27). Incubated in CO_2 for 21 days at 35ºC.

Mycobacterium intracellulare Group III (ATCC® 13950) growing on Lowenstein Jensen Deep (Cat. no. C27). Incubated in CO_2 for 21 days at 35ºC.

Mycobacterium fortuitum Group IV (ATCC® 6841) growing on Lowenstein Jensen Deep (Cat. no. C27). Incubated in CO_2 for 21 days at 35ºC.

Semiquantitative Catalase Test for *Mycobacterium fortuitum* ATCC® 6841. Incubated in CO_2 for two weeks at 35ºC. in a Lowenstein-Jensen Deep (Cat. no. C27). Equal parts of 10%

Close up of the tube on the left. Showing that the height of the bubbles was greater than 45mm.

Tween® 80 (Cat. no. Z101) and 30% hydrogen peroxide were mixed and a 1mL aliquot was added to the tube. The tube was allowed to stand at room temperature for five minutes before the bubbles were measured.

Semiquantitative Catalase Test for *Mycobacterium intracellulare* ATCC® 13950. Incubated in CO_2 for two weeks at 35ºC. in a Lowenstein-Jensen Deep (Cat. no. C27). Equal parts of 10% Tween® 80 (Cat. no. Z101) and 30% hydrogen peroxide were mixed and a 1mL aliquot was added to the tube. The tube was allowed to stand at room temperature for five minutes before the bubbles were measured.

Close up of the tube on the left. Showing that the height of the bubbles was less than 45mm.

REFERENCES

1. Anderson, N.L., et al. *Cumitech 3B; Quality Systems in the Clinical Microbiology Laboratory*, Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.

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3. Tille, P., et al. *Bailey and Scott's Diagnostic Microbiology*, C.V. Mosby Company, St. Louis, MO.

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5. Jensen, F. 1932. *Zentralbl. Bakteriol. Parasetenkd. Infektionskr*., Abt. 1 Orig.; 125:222.

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9. Lowenstein, E. 1933. *Ann. Inst. Pasteur*.; 50:161.

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11. *Quality Assurance for Commercially Prepared Microbiological Culture Media*, M22. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA.

12. Vestal, A.L. 1975. *Procedures of the Isolation and Identification of Mycobacteria*. DHEW (CDC 75-8230). Centers for Diseases Control. Atlanta, GA.

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The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.

HDQA 2207F [D]