IFU

Instructions for Use

LOWENSTEIN-JENSEN MEDIUM WITH 5% SODIUM CHLORIDE

Cat. no. C29	Lowenstein-Jensen with 5% NaCl, 20x125mm Tube, 10ml Slant	20 or 100 tubes/box

INTENDED USE

Hardy Diagnostics Lowenstein-Jensen with 5% NaCl is used to test *Mycobacterium* species for salt tolerance.

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. This formulation also encourages the earliest possible growth of mycobacteria.

When heated, the egg albumin coagulates, thus providing a solid surface for inoculation. 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.[11]

The addition of 5% NaCl to the Lowenstein-Jensen formulation allows differentiation of slowly growing mycobacteria from rapidly growing mycobacteria based on salt tolerance. This medium supports the growth of most rapid growers as well as the slower growing *Mycobacterium triviale* . Some strains of *Mycobacterium flavescens* may also grow. Distinction between other members of the *Mycobacterium fortuitum* complex from *Mycobacterium chelonae* subsp. *chelonae* is facilitated by the inability of the latter to grow on this salt containing medium.[15]

FORMULA

Ingredients per liter of deionized water:*

Sodium Chloride	50.0gm
Potato Flour	30.0gm
Asparagine	3.6gm
Monopotassium Phosphate	2.4gm
Magnesium Sulfate	0.24gm
Magnesium Citrate	0.6gm

Malachite Green	0.4gm
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

Method of Use:

1. From a pure culture, prepare a suspension that is equivalent to a 1.0 McFarland Standard.[15]

2. Inoculate the Lowenstein-Jensen with NaCl slant with 100ul from the suspension.[15]

3. Incubate medium at 35ºC. in ambient air.[15]

4. Examine the media weekly for up to four weeks.

INTERPRETATION OF RESULTS

A positive test for sodium chloride tolerance is the growth of 50 or more colonies on the slant. A negative test is the appearance of less than 50 colonies.[15]

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 photosensitive.

Selective media often inhibit, to some extent, specific strains of organisms for which they are designed to select.

The color of LJ media may range from a pale-green to a dark blue-green. Do not use media that has turned yellow, as it will interfere with the interpretation of the pigmentation of mycobacteria. Most contaminating bacteria will turn the media blue.

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

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, decontamination supplies, 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:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Mycobacterium fortuitum Group IV ATCC® 6841	**	21 days	35°C	Aerobic	Growth; 50 or more colonies
Mycobacterium kansasii Group I ATCC® 12478	**	21 days	35°C	Aerobic	Inhibited
Mycobacterium scrofulaceum Group II ATCC® 19981	**	21 days	35°C	Aerobic	Inhibited
Mycobacterium intracellulare Group III ATCC® 13950	**	21 days	35°C	Aerobic	Inhibited
Mycobacterium tuberculosis H37Ra ATCC® 25177	**	21 days	35°C	Aerobic	Inhibited

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

USER QUALITY CONTROL

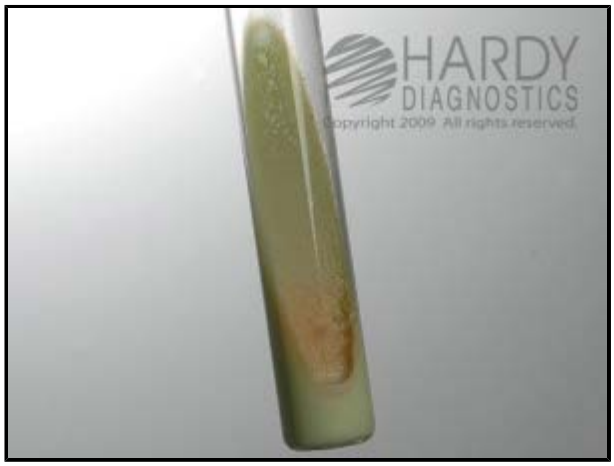
End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory 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.

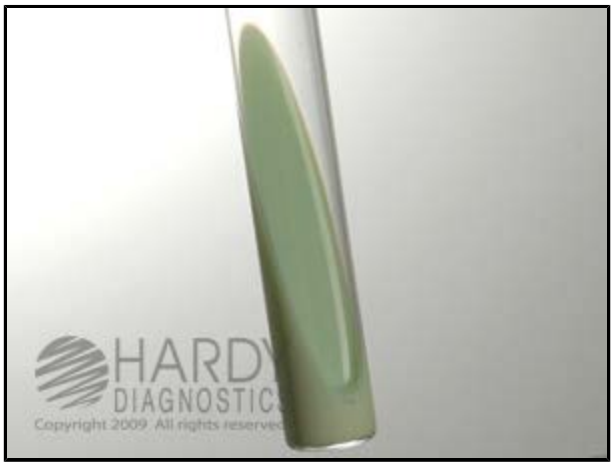
** Inoculate LJ with 5% NaCl with 100ul of a suspension equivalent to a 1.0 McFarland Standard.

PHYSICAL APPEARANCE

Lowenstein-Jensen Medium with 5% NaCl should appear opaque, and pale green in color.



Mycobacterium fortuitum Group IV (ATCC® 6841) colonies growing on Lowenstein-Jensen Medium with 5% NaCl (Cat. no. C29). Incubated aeobically for 21 days at 35ºC.



Mycobacterium kansasii Group I (ATCC® 12478) growth inhibited on Lowenstein-Jensen Medium with 5% NaCl (Cat. no. C29). Incubated aerobically for 21 days at 35ºC.

REFERENCES

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