

# Instructions for Use

## LOWENSTEIN JENSEN (LJ) MEDIA

<a href="#">Cat. no. C21</a>	Lowenstein Jensen, 20x125mm Tube, Slant	20 tubes/box
<a href="#">Cat. no. X22</a>	Lowenstein Jensen, 50ml Hardy Flask™, Slant	20 flasks/box
<a href="#">Cat. no. C23</a>	LJ, Gruft Modification, 20x125mm Tube, Slant	20 tubes/box
<a href="#">Cat. no. X23</a>	LJ, Gruft Modification, 50ml Hardy Flask™, Slant	20 flasks/box
<a href="#">Cat. no. C25</a>	LJ, Selective, 20x125mm Tube, Slant	20 tubes/box
<a href="#">Cat. no. C28</a>	LJ with Pyruvate, 20x125mm Tube, Slant	20 tubes/box
<a href="#">Cat. no. X19</a>	LJ with Pyruvate, 50ml Hardy Flask™, Slant	20 flasks/box
<a href="#">Cat. no. C37</a>	LJ with Iron, 20x125mm Tube, Slant	20 tubes/box
<a href="#">Cat. no. X21</a>	LJ with Iron, 50ml Hardy Flask™, Slant	20 flasks/box

## INTENDED USE

Hardy Diagnostics Lowenstein Jensen Media are recommended for use in the cultivation and isolation 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.<sup>(8,9)</sup> 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.<sup>(14)</sup> Malachite green acts as an inhibitory agent toward microorganisms other than mycobacteria.<sup>(6)</sup>

The Lowenstein Jensen, Gruft Modification formula is the same as the LJ formulation except for the addition of penicillin, nalidixic acid and ribonucleic acid (RNA).<sup>(4)</sup> Nalidixic acid and penicillin inhibit most of the gram-negative bacteria. RNA serves as a growth stimulant for the increased isolation rate of mycobacteria.

Lowenstein Jensen, Selective media, in addition to LJ ingredients, contains cycloheximide, lincomycin, and nalidixic acid. Cycloheximide suppresses saprophytic fungi while lincomycin inhibits gram-positive bacteria.

Lowenstein Jensen with Pyruvate incorporates pyruvic acid into the LJ basal medium to stimulate the growth of *M. bovis* and mycobacteria spp. other than *M. tuberculosis*.

Lowenstein Jensen with Iron is used to determine iron uptake as a means of differentiation between slow and rapid growing *Mycobacterium* species.<sup>(3,7)</sup> This is done by using an aqueous solution of 20% ferric ammonium citrate. The ability of certain species, such as *M. fortuitum*, to take up soluble iron salts from the culture media results in the growth of colonies with a rusty brown color.

## FORMULA

Ingredients per 367.5ml of deionized water:\*

### Lowenstein Jensen Medium:

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

### Lowenstein Jensen, Gruft Modification is the same as LJ Medium with the addition of:

Nalidixic Acid	56.0mg
Penicillin	52.8mg
Ribonucleic Acid	0.05mg

### Lowenstein Jensen, Selective is the same as LJ Medium with the addition of:

Nalidixic Acid	35.0mg
Lincomycin	2.0mg
Cycloheximide	0.4gm

### Lowenstein Jensen with Pyruvate is the same as LJ Medium with the addition of:

Pyruvic Acid	2.5mg
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### Lowenstein Jensen with Iron is the same as LJ Medium with the addition of:

Ferric Ammonium Citrate	10.0gm
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Final pH 7.2 +/- 0.3 at 25°C (except Cat. Nos. C28 and X19 for which the final pH is 7.1 +/- 0.3 at 25°C).

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

## STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8°C. 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.<sup>(1,3,5,7,11,15)</sup>

Method of Use:

1. Inoculate the Lowenstein Jensen Media with specimen after decontamination and neutralization, according to test procedures recommended by the Centers for Disease Control. Consult listed references for methods.<sup>(1,3,5,7,11,15)</sup>
2. Incubate medium in a CO<sub>2</sub> atmosphere at 35-37°C. Protect from light. Tubed media should be incubated for one week with loosened caps to allow the circulation of CO<sub>2</sub> for the initiation of growth. Caps should be tightened after one week in order to prevent dehydration of media.
3. Examine the media within five to seven days, and weekly thereafter for up to eight weeks.
4. Examine plates under light for the appearance of macroscopic growth.
5. Examine tubes under light and magnifying mirror for macroscopic growth. Record and describe colony morphology on the first day growth is observed.
6. Consult appropriate references for recording the number of colonies and for aid in the biochemical identification of acid-fast bacilli.<sup>(1,5,11,15)</sup>

## INTERPRETATION OF RESULTS

Consult listed references for the interpretation of growth of *Mycobacterium* species on this medium.<sup>(1,3,5,7,11,15)</sup>

Examine and record each type of colony morphology, pigment, and growth rate. Biochemical testing is required for definitive identification.

## 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.

Lowenstein Jensen Media require incubation in a 5-10% CO<sub>2</sub> atmosphere in order to recover mycobacteria. Mycobacteria, for unknown reasons, are not recovered well from candle extinction jars.<sup>(2)</sup>

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, slides, decontamination supplies, applicator sticks, pipets, incinerators, CO<sub>2</sub> incubators, microscopes, 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	
<b>LJ Media and LJ Media with Iron:</b>					
<i>Mycobacterium tuberculosis</i> H37Ra ATCC® 25177	G	21 days	35°C	CO <sub>2</sub> **	Growth; colonies seen in 2 weeks, mature in 3 weeks
<i>Mycobacterium kansasii</i> Group I ATCC® 12478	G	21 days	35°C	CO <sub>2</sub> **	Growth; colonies seen in 2 weeks, mature in 3 weeks
<i>Mycobacterium scrofulaceum</i> Group II ATCC® 19981	G	21 days	35°C	CO <sub>2</sub> **	Growth; colonies seen in 2 weeks, mature in 3 weeks
<i>Mycobacterium intracellulare</i> Group III ATCC® 13950	G	21 days	35°C	CO <sub>2</sub> **	Growth; colonies seen in 2 weeks, mature in 3 weeks
<i>Mycobacterium fortuitum</i> Group IV ATCC® 6841	G	21 days	35°C	CO <sub>2</sub> **	Growth; colonies visible in 4 days

Additionally, the following organisms are tested on LJ, Gruft Modification and LJ, Selective:					
<i>Escherichia coli</i> ATCC® 25922	B	24hr	35°C	Aerobic	Partial to complete inhibition
<i>Staphylococcus aureus</i> ATCC® 25923	B	24hr	35°C	Aerobic	Partial to complete inhibition

\* Refer to the document "[Inoculation Procedures for Media QC](#)" for more information.

\*\* Atmosphere of incubation is enriched with 5-10% CO<sub>2</sub>.

## 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

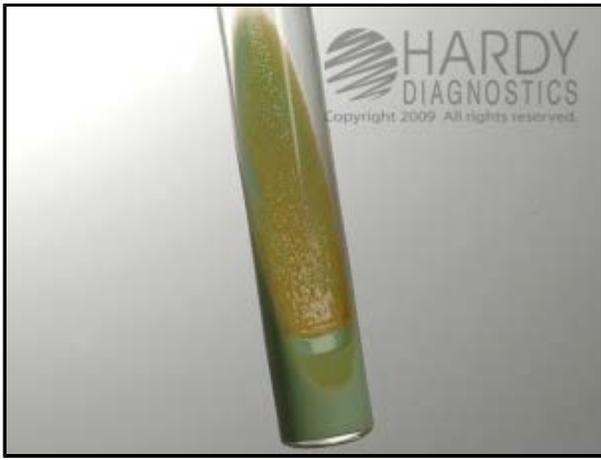
All Lowenstein Jensen Media should appear opaque, and pale green in color. LJ Media with iron may contain a precipitate settled at the bottom of the flask or tube.



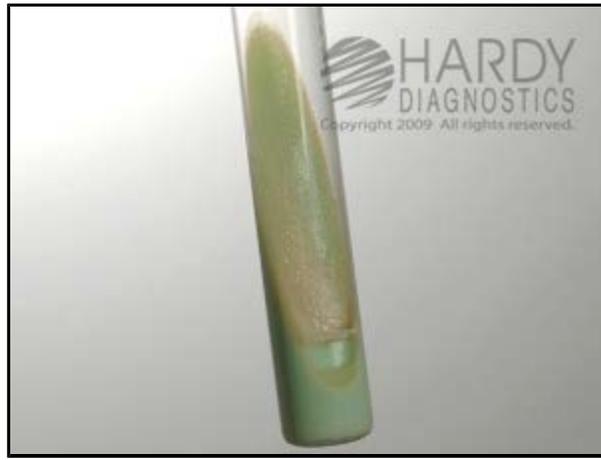
*Mycobacterium tuberculosis* H37Ra (ATCC® 25177) colonies growing on Lowenstein Jensen Medium (Cat. no. C21). Incubated in CO<sub>2</sub> for 21 days at 35°C.



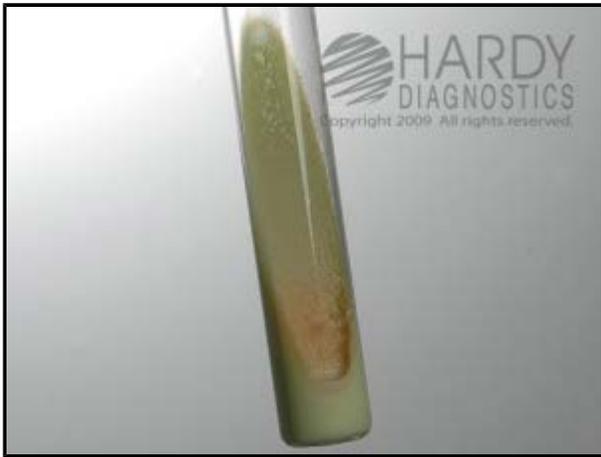
*Mycobacterium kansasii* Group I (ATCC® 12478) colonies growing on Lowenstein Jensen Medium (Cat. no. C21). Incubated in CO<sub>2</sub> for 21 days at 35°C.



*Mycobacterium scrofulaceum* Group II (ATCC® 19981) colonies growing on Lowenstein Jensen Medium (Cat. no. C21). Incubated in CO<sub>2</sub> for 21 days at 35°C.



*Mycobacterium intracellulare* Group III (ATCC® 13950) colonies growing on Lowenstein Jensen Medium (Cat. no. C21). Incubated in CO<sub>2</sub> for 21 days at 35°C.



*Mycobacterium fortuitum* Group IV (ATCC® 6841) colonies growing on Lowenstein Jensen Medium (Cat. no. C21). Incubated in CO<sub>2</sub> for 21 days at 35°C.



Uninoculated tube of Lowenstein Jensen Medium (Cat. no. C21).

## REFERENCES

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