



LÖWENSTEIN-JENSEN COOL

IN STANDARD OR "CELEVER" TUBE
MYCOBACTERIA SOLID CULTURE MEDIUM
Instruction for Use

Catalog No:TL060-065

For In Vitro Diagnostic Use

Product name:**LÖWENSTEIN-JENSEN COOL****Intended use:**

Lowenstein-Jensen (LJ) is a classic egg-based medium used for over a century to grow mycobacteria. When preparing LJ, after adding eggs to the medium, it must be kept at 85°C for 50 minutes to solidify. This heating process causes some substances necessary for bacterial growth in the egg to deteriorate. Although **LÖWENSTEIN JENSEN COOL** has the same basic content as LJ, it solidifies without the need for heating after adding eggs due to its special formula. Thus, the proportion of some substances necessary for growth that deteriorate during cooking is higher than in LJ medium. This accelerates the growth of mycobacteria.

General Information:

After the clinical sample is decontaminated and concentrated, it is inoculated into the medium. Colonies become visible 2-6 weeks after inoculation.

Limitations of the method:

LÖWENSTEIN JENSEN COOL contains glycerol. Adding glycerol to the medium accelerates the growth of *Mycobacterium tuberculosis*, while it has an inhibitory effect on many strains of *M. bovis*. **LÖWENSTEIN JENSEN COOL** without glycerol should be used for the isolation of *M. bovis*.

Scientific Basis of the Method:

LÖWENSTEIN JENSEN COOL is a rich medium containing all the nutrients required for mycobacterial growth. Solidifying the medium without heating after adding eggs adds additional richness to the medium. Egg albumin binds to toxic fatty acids that are produced during mycobacterial growth. Malachite green, which gives the medium its green color, inhibits the growth of many other types of bacteria and creates a selective medium for mycobacteria that are resistant to this dye.^{1,2}

Ingredients:

Potassium dihydrogen phosphate, magnesium sulfate, magnesium citrate, asparagine, malachite green, glycerol, gelling agents and eggs.

Cautions and warnings:

- FOR IN VITRO DIAGNOSTIC USE.
- Laboratory procedures involving mycobacteria require special equipment and techniques to minimize biohazards. Sample processing should be performed in a level II biosafety chamber. People who apply these techniques are recommended to have special training in this area.
- Additional precautions should be taken to reduce the risks of accidental exposure to infectious agents. At a minimum, specimen manipulation should be done in a contained environment having controlled access, which has a tuberculosis exposure control plan. The locations should have surfaces that can be easily decontaminated using an appropriate topical disinfectant.
- Pathogenic microorganisms including Hepatitis B Virus and Human Immunodeficiency Virus (HIV) may be present in specimens. Universal precautions and local laboratory guidelines should be followed in handling all items contaminated with blood or other body fluids. If a container is found to be leaking or is accidentally broken during collection or transport, use the established procedures in your facility for dealing with mycobacterial spills.

General safety precautions:

- Always wear masks and gloves when working with potential biohazard material.
- Work in a laminar flow cabin, biosafety level II, when transferring, homogenizing and pipetting samples.
- Never use mouth pipetting.
- A refrigerated centrifuge with airtight swinging buckets is recommended for sedimenting bacteria to minimize aerosols.
- If spills of the contaminated material occur, disinfect with 2.5% hypochlorite solution.

- Pathogenic microorganisms including Hepatitis B virus and Human Immunodeficiency Virus (HIV) may be present in specimens. Universal precautions and local laboratory guidelines should be followed in handling all items contaminated with blood or body fluids. If a tube leaks or is accidentally broken during collection or transport, use the established procedures in your facility for dealing with mycobacterial spills. At a minimum, universal precautions should be employed.
- Tubes should be discarded in an appropriate manner according to biosafety principles.

Storage instructions:

Store at 2 to 8°C.

Indications of instability or deterioration:

Do not use the medium if there is any discoloration, contamination, drying or cracking.

Sample preparation:

Clinical samples containing microorganisms other than *M. tuberculosis*, such as sputum, require decontamination and concentration to prepare them for inoculation into **LÖWENSTEIN JENSEN COOL** medium.³ Samples taken from sterile body sites, such as cerebrospinal fluid, can be inoculated directly into **LÖWENSTEIN JENSEN COOL** medium.

Recommended procedures:

For decontamination and concentration, we recommend **DECOCENT**, which provides good pH adjustment, or **DECOMICS**, which eliminates the need for centrifugation while ensuring good pH adjustment.

Inoculations should be carried out in a level II biosafety chamber. If you are using **LÖWENSTEIN JENSEN COOL** in a standard tube, leave the tubes on an inclined surface, horizontal to the ground, for 30 minutes after inoculation. This ensures that the liquid is absorbed by the entire surface of the medium and that the mycobacteria adhere to the medium surface.

The "Clever" tube is designed to be tilted both vertically and parallel to the medium surface. The short length of the tube prevents the non-sterile part of the pipette or loop from entering the tube during processes such as inoculation with a pipette or colony collection with a loop, thus reducing the possibility of contamination. The ability to incubate the tubes in an incubator in an inclined position after inoculation eliminates the need to wait for the sample to spread into the medium and for bacteria to adhere. **LÖWENSTEIN JENSEN COOL** in Clever tubes can be placed in the incubator in their cardboard boxes, stacked on top of each other. If you are not using a carbon dioxide incubator and the medium tends to dry out during long incubation times, close the lid tightly.

Materials Provided:

The product in standard tubes is presented in cardboard boxes of 150 pieces.

The product in "Clever" tubes is presented in cardboard boxes of 22 pieces.

Necessary materials that are not provided:

- Level II biosafety chamber.
- Materials and equipment required for microbiological cultivation.

Temperature:

Sample processing and cultivation should be done at room temperature. Incubations should be performed at 37°C.

Time restrictions:

Although the effect of the length of time between processing and inoculation of the samples has not been determined, inoculation of the samples immediately after being processed may increase the chance of recovery of mycobacteria.



TiBO

TRENDS in INNOVATIVE BIOTECHNOLOGY ORGANIZATION

Application:

1. Write down patient information on the tube containing **LÖWENSTEIN JENSEN COOL**.
2. Remove the tube cap.
3. Inoculate 200-500µL sample taken directly from the clinical samples like cerebrospinal fluid, obtained from sterile body parts, or from sputum samples that have undergone decontamination and concentration processes, into medium.
4. Close the lid of the tube without tightening it too much so as not to prevent gas exchange if the tubes will be incubated in a CO₂ incubator. Media that are not placed in a CO₂ incubator tend to dry out for a long time in a standard incubator. In this case, the lid can be closed tightly to promote CO₂ accumulation in the tube and prevent drying.)
5. If you are using **LÖWENSTEIN JENSEN COOL** in a standard tube, after inoculation, tilt the medium in the tubes parallel to the ground and wait for half an hour so that the sample spreads evenly on the surface of the medium and the bacteria can adhere. If you are using **LÖWENSTEIN JENSEN COOL** in a "Clever" tube, you can place the tubes directly in the incubator lying down and perform the entire incubation in this manner.
6. Place the inoculated tube in a standard 37°C incubator or, if possible, in a CO₂ incubator.

Evaluation of results:

The culture medium should be checked every 2-3 days. If any growth is observed, an examination should be made with a microscope after acid-fast staining to determine the presence of mycobacteria. If no growth is observed, incubation must continue for at least 6 weeks to give a negative test result. Due to its modified, improved formula the colonies of *M. tuberculosis* on **LÖWENSTEIN JENSEN COOL** will look white compared to colonies that looks more yellowish cream color on classical Löwenstein Jensen medium. This is perfectly normal expected result.

Quality control:

Mycobacterium tuberculosis H37Ra and *Mycobacterium smegmatis* are used for quality control.

Limitations of the procedure:

The pH of the sample must be carefully adjusted during decontamination and concentration application. If the pH is not adjusted appropriately, mycobacteria may not grow in **LÖWENSTEIN JENSEN COOL** medium.

Performance characteristics:

The time required for detection of *M. tuberculosis* in **LÖWENSTEIN JENSEN COOL** medium is generally between 2 and 6 weeks.

Shelf life:

Six months.

Bibliography:

1. Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn WC Jr. Mycobacteria. In Diagnostic Microbiology 5th Ed. 1997. 892-952.
2. Heifets LB, Good RC. Current laboratory methods for the diagnosis of tuberculosis. In "Tuberculosis" Ed. Bloom BR. ASM Press, Washington D.C. 1994. 85-110.
3. Kubica GP, Dye WE, Cohn ML, Middlebrook G. Sputum digestion and decontamination with N-acetyl-L-cysteine-sodium hydroxide for culture of mycobacteria. 1963. Am. Rev. Respir. Dis. 87:775-779.

Manufacturer:

Trends In Innovative Biotechnology Organization

Ahmet Yesevi Mah. Kerem Sok.

No:9/1-7

Pendik 34903

İSTANBUL, TÜRKİYE

Catalog number:

TL060-065

