DIAGNOTIS

TK LJRed®

AUTOMATED, SELECTIVE «Löwenstein–Jensen» MEDIUM FOR CULTURING MYCOBACTERIA

Catalogue #: LJ030 **Instructions for Use** **For In Vitro Diagnostic Use**

Product name:

TK LJRed®

Product's intended use:

TK LJRed® is an improved Löwenstein-Jensen (LJ) medium used for determination of mycobacterial growth. Culture results support the diagnosis of tuberculosis. It is intended for in vitro diagnostic use.

TK LJRed® is used for primary isolation of mycobacteria from samples. ***TK LJRed®*** contains antibacterials and an antifungal, which makes it more selective for mycobacteria, by inhibiting the growth of other types of microorganisms.

Summary and explanation of the test:

TK LJRed® is a selective culture medium with multiple dye indicators that permit detection of mycobacterial growth earlier than classical LJ. ***TK LJRed®*** has the ability to differentiate mycobacterial growth from contamination. The original red color of the medium turns yellow with the growth of mycobacteria and green in the presence of many other bacterial or fungal species. The color change occurs long before the colonies become visible. The color change is easily evaluated by the naked eye or using a low cost but very advanced automated incubator reader, ***MYCOLOR TK®***. Clinical samples, like sputum, contain many microorganisms of normal flora that overgrow mycobacteria in many types of culture media including ***TK LJRed®***. The samples should be decontaminated and concentrated before being inoculated to ***TK LJRed®***. We recommend the use ***DECOCENT®*** which provides an improved Kubica method or ***DECOMICS®*** which eliminates the need for centrifugation thus lowering the processing time from 45 to 23 minutes. Some microorganisms other than mycobacteria may survive after application of this procedure. The antimicrobials included in ***TK LJRed®*** inhibit the growth of many of these microorganisms reducing the contamination rate and improve the probability that mycobacteria will be isolated.[1-3]

Limitations:

Some microorganisms other than mycobacteria may be resistant to antimicrobials included in ***TK LJRed®*** and may grow in this medium. Although most contaminant organisms (fungi and gram negative bacteria) turn the color of ***TK LJRed®*** from red to green, gram positive bacteria (e.g. streptococci) may change the color to yellow as with mycobacteria. For that reason, the type of microorganism growing in ***TK LJRed®*** should always be evaluated by microscopy for the presence of acid-fast staining microorganisms.

Principles of the procedure:

The color change in ***TK LJRed®*** is based on multiple dye indicators and depends on the metabolites and enzymes produced by different species of microorganisms. ***TK LJRed®*** does not contain any radioactive material or fluorescent dye and does not require any scintillation counter, UV light, or other specialized detection systems for evaluation of culture tubes.

Ingredients:

TK LJRed® contains egg suspension, polypeptides, carbohydrates, salts, dye indicators, vitamins and antimicrobials.

Cautions and warnings:

- FOR *IN VITRO* DIAGNOSTIC USE.
- The tubes should only be opened just before use.
- The caps of the tubes should be closed tightly after inoculation in order to monitor the change in gas content. The change in gas content will lead to a color change in the medium.
- ***TK LJRed®*** is a pH sensitive medium. Therefore the pH of the inoculum should be carefully adjusted (to approximately 7.4±0.2) before inoculation. This can easily be achieved using the standard sodium hydroxide-N- acetyl-L-cysteine (NaOH-NALC) decontamination and concentration procedure. It is recommended that the decontamination and concentration kits, ***DECOCENT®*** or ***DECOMICS®*** be used in the processing of samples, before the samples are inoculated to ***TK LJRed®***. These kits are available separately for this purpose.
- Laboratory procedures involving mycobacteria require special equipment and techniques to minimize biohazards. People who apply these techniques are recommended to have special training in this area. Specimen preparation must be done in a biological safety cabinet. 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.

General safety precautions:

- Always wear masks and gloves when working with potential biohazard material.
- Work in a laminary flow cabin, biosafety level II, when pipetting the samples.
- Never mouth pipette.
- A refrigerated centrifuge with airtight swinging buckets is recommended for sedimenting bacteria.
- If spills of the contaminated material occur, disinfect with 2.5% hypo-chlorite 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 is leaking 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 8ºC.

Shelf life:

9 months.

Indications of instability or deterioration:

Do not use the medium if a color change to yellow or green is observed prior to inoculation.

Specimen collection and preparation for analysis:

The final pH of the sample before inoculation to ***TK LJRed®*** should be 7.4±0.2. It is recommended that ***DECOCENT®*** or ***DECOMICS®*** be used to process the samples. This process

TK LJRed®

guarantees the appropriate pH for inoculation. Samples, like cerebrospinal fluid, obtained from sterile body compartments, can be directly inoculated to ***TK LJRed®***.

Recommended procedures:

Inoculations should be performed in a biosafety level II cabinet.

Application:

1. Write down patient information on the tube containing ***TK LJRed®***.
2. Remove the tube cap.
3. Inoculate 500µL of decontaminated and concentrated sample or a sample obtained directly from a sterile body compartment. Put the inoculum at the top end of the medium and allow it to slowly migrate downward, wetting the entire surface of the medium.
4. Replace the cap making sure that it is closed tightly (this is very important since the color change in the medium is partly dependent on the consumption of oxygen and production of CO_2. This process can only be monitored in an airtight closed tube. However, very extensive screwing may create cracks in the caps leading to gas leaks).
5. Place the tube into a regular 37°C incubator or ***MYCOLOR TK®***.
6. If using ***MYCOLOR TK®***, enter the necessary information related to the patient and the sample.

Evaluation of *TK LJRed®*:

Visual evaluation:

TK LJRed® can be easily evaluated visually if an automated incubator-reader, ***MYCOLOR TK®***, is not available and the culture tubes are kept in a regular 37°C incubator. The color of the media should be checked visually on a daily basis.

A change in color from red to orange and then to yellow will indicate mycobacterial growth. Orange indicates possible growth and yellow indicates definite growth. The color change will usually occur before colonies become visible. This is especially likely when the number of colony-forming units is high in the inoculated sample. A color change to yellow within 48 hours may indicate contamination with gram-positive bacteria. A color change to yellow between day 3 and day 5 may indicate a rapid-growing species of mycobacteria. A color change to yellow after the 5th day will usually indicate slow-growing mycobacteria including *M. tuberculosis*.

A change in color from red to green will indicate contamination with either fungi or gram-negative bacteria. In mixed cultures containing both contaminants and mycobacteria, the color will first change to green due to the activity of rapid-growing contaminants and then to yellow due to the activity of mycobacteria.

Evaluation using ***MYCOLOR TK®***

The evaluation of color changes in culture tubes is automatically followed by ***MYCOLOR TK®*** and predictions for growing microorganisms are made accordingly. The color of all culture tubes and growth curves can be easily visualized on the screen.

Important:

Color change in ***TK LJRed®*** only allows early detection and prediction of the type of organism growing in the culture. When any type of color change occurs, a smear should be prepared from the surface of the medium. After acid-fast staining, the smear should be examined under the microscope for the presence of acid-fast bacilli and other contaminating organisms. **The final diagnosis should only be made after examination by experienced personnel.**

Materials provided:

Sterile ready-to-use ***TK LJRed®*** in screw capped pet tubes (16mm in diameter).

Necessary materials that are not provided:

Biosafety level II cabinet.

Materials and equipment needed for microbiological culture inoculations.

Temperature:

The processing of the samples and inoculation should be done at room temperature.

The incubation of the culture tubes should be done 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.

Quality control:

The following organisms are used for quality control and the following color changes are obtained after incubation at 37°C:

Mycobacterium tuberculosis H37Ra: .. Yellow
Mycobacterium smegmatis:..... Yellow
Escherichia coli: ..... Red
Candida albicans: ..... Red
Uninoculated medium: ..... Red

Limitations of the procedure:

At the end of the decontamination and concentration procedure, the pH of the inoculum should be 7.4±0.2. Inability to neutralize alkaline pH, created by NaOH may change the color of ***TK LJRed®*** from red to purple and inhibit or slow down the growth of mycobacteria (it is recommended that ***DECOCENT®*** or ***DECOMICS®*** be used to process the clinical samples to obtain the required pH for inoculation).

Performance characteristics:

The special formula of ***TK LJRed®*** lowers contamination rate 10 times as compared to the classical LJ with better mycobacteria recovery rates.[3]

The monitoring of mycobacterial growth may be done automatically by ***MYCOLOR TK®***. Therefore, bacterial growth can be predicted within two weeks reducing dramatically the detection time and workload.

Bibliography:

1. Migliori G.B., Matteelli A., Cirillo D., Pai M. Diagnosis of multidrug-resistant tuberculosis and extensively drug-resistant tuberculosis: Current standards and challenges. Can. J. Infect. Dis. Med. Microbiol. 2008, 19(2):169-172
2. Pai M., Kalantri S., Dheda K. New tools and emerging technologies for the diagnosis of tuberculosis: Part II. Active tuberculosis and drug resistance. Expert Rev. Mol. Diagn. 2003. 6(3):423-432.
3. Kocagöz T., Altın S., Türkyılmaz Ö., Taş İ., Yuca P., Bolaban D., Yeşilyurt E., Öktem S., Aytekin N., Şınık G., Mozioğlu E., Silier T. The efficiency of TK Culture System in the diagnosis of tuberculosis. Diagn Microbiol Infect Dis. 2012 72(4):350-357.

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