COLETSOS
COLETSOS OSSEIN

REF 53154 - 53164

MYCOBACTERIUM ISOLATION MEDIUM

IVD CE

1- APPLICATION

Coletsos agar to which whole egg and egg yolk have been added, is a selective medium that is extremely rich and has been optimized for the development and growth of all aerophilic mycobacteria such as *Mycobacterium tuberculosis*, atypical mycobacteria and deficient mycobacteria that have been or have not been exposed to antibiotics.

Coletsos medium is perfectly suitable for the isolation and abundant growth of particularly difficult mycobacteria. The medium is more specifically indicated for surface culture under strict aerobic conditions.

Coletsos ossein medium promotes the growth of bacilli derived from extra-pulmonary lesions (typical and atypical) and, especially, growth of *M. bovis*.

2- PRINCIPLE

The selectivity of the medium is based on the presence of malachite green and mineral salts, which inhibit most contaminant microorganisms.

The culture of mycobacteria is promoted by the nutrient substances supplied, among others, by egg, trace elements and sodium pyruvate.

In Coletsos ossein medium, ossein is liquefied during incubation and covers the surface of the agar following inoculation, ensuring micro-anaerobic conditions.

3- PRESENTATION

- Ready-for-use Colestos medium:
  - Twenty-five 7-mL slanted culture tubes (screw-cap) code 53154
- Ready-for-use Colestos ossein medium:
  - Twenty-five 7-mL slanted culture tubes (screw-cap) code 53164

4- THEORETICAL COMPOSITION (per 1.6 l of final medium)

Coletsos agar is prepared using the theoretical formula reported by Coletsos (1).

Potassium dihydrogen phosphate	2.4 g
Magnesium sulfate	0.24 g
Magnesium citrate	0.6 g
Anhydrous asparagine	3.6 g
Sodium pyruvate	1.6 g
Sodium glutamate	1.6 g
Potato starch	16 g
Anthracite powder	0.16 g
Sunflower blue	0.4 g
Malachite green	0.4 g
Whole fresh egg	800 mL
Egg yolk	200 mL
Special gelatin (ossein)	6.4 g for Colestos medium 25.60 g for Colestos + ossein medium

5- STORAGE

- Ready-for-use medium: 2 to 8°C, in darkness, in the horizontal position.

The expiration date and batch number are indicated on the packaging.

6- USE

Materials:

- Material supplied:
  - Colestos medium
  - Colestos ossein medium

- Specific material not supplied:
  - Platinum loop

Precautions for use / Health and safety instructions

The handling of biological specimens liable to contain mycobacteria requires application of technical preventive measures (2, 3) and compliance with the safety standards applicable for class III microorganisms.

- All biological specimens may be inoculated into the medium providing that they have undergone prior **fluidification** and **decontamination**. For the storage of biological specimens, refer to current recommendations (4).
- The quantity of microorganisms in the specimens may be small. The sample is to be concentrated by centrifuging to enhance detection sensitivity.

INOCULATION:

Inoculate aa appropriate, prepared and decontaminated pathological specimen or a pure fresh culture specimen from an agar medium.

INCUBATION:

- Incubate at 37°C in the horizontal position for 28 days.
- Following disappearance of the inoculum (3 to 4 days), hermetically close the tubes either by screw-capping or by use of a plastic cap.

READING:

- *M. bovis* colonies are frequently smooth and damp and do not have the dry, rough appearance that characterizes those of the human bacillus (*M. tuberculosis)* on Löwenstein-Jensen medium.
- A count and a morphological study of the colonies should be conducted between 2 and 8 weeks post-inoculation.
- Colonies of tuberculosis bacilli only have a typical appearance if the medium is correctly oxygenated and the liquid part of the inoculum has fully evaporated. Only close the tubes after complete evaporation.

7- TEST PERFORMANCE / QUALITY CONTROL

- Medium appearance: opaque slope **yellowish green to greenish gray** in color with a small ash deposit at the bottom of the tube.
- The culture performance of Colestos medium is controlled using the following strains:

STRAIN	RESULT OF CULTURE AT 37°C FOR 28 DAYS
Mycobacterium tuberculosis H37RV - CIP 64.31	Good growth
Mycobacterium aureum, Rebuffet strain	Good growth

8- MANUFACTURER'S QUALITY CONTROL

All manufactured reagents are prepared according to our Quality System, starting from reception of raw material to the final commercialization of the product. Each lot is submitted to quality control assessments and is only released to the market, after conforming to pre-defined acceptance criteria. The records relating to production and control of each single lot are kept within Bio-Rad.

9- USE RESTRICTIONS

- Conduct complementary tests for identification of the species of the strain isolated.
- The absence of growth does not necessarily indicate the absence of mycobacterial infection. Certain factors may prevent the growth of mycobacteria.

10- REFERENCES

1. Coletsos, P. 1971. De l'isolement des mycobactéries. Intérêt majeur des cultures parallèles en surface, sous cape et en double couche nutritive. Rev. Tub. et Pneumol. **35**: 601
2. Mesures techniques de prévention, notamment de confinement, à mettre en oeuvre dans les industries et les laboratoires de recherché et d'enseignement où les travailleurs sont susceptibles d'être exposés à des agents biologiques pathogènes – Decree dated August 13, 1996 – Journal Officiel de la République Française.
3. Kent, P. T., and Kubica G. P. 1995. Public health mycobacteriology: a guide for the level III laboratory. USDHMS, Centers for Disease Control, Atlanta.
4. Basic Laboratory Procedures in Clinical Bacteriology. World Health Organization. Geneva. 1991. 1st edition.

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