

INSTRUCTIONS FOR USE

PERGOLA MEDIUM

Ready-to-use tubes

 Pergola Medium: un-inoculated tube and colonies of *Corynebacterium diphtheriae*
1 - INTENDED USE

In vitro diagnostic device. For the cultivation and isolation of *Corynebacterium diphtheriae* from clinical specimens.

2 - COMPOSITION -TYPICAL FORMULA *

Horse serum	600 mL
Saline solution	184 mL
Egg yolk	200 mL
Potassium tellurite 1% solution	16 mL

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Pergola medium is one of the media that can be used for the cultivation and isolation of *C. diphtheriae* from clinical specimens, together with Loeffler's medium, Serum Tellurite Agar, Tinsdale agar and blood agar. Pergola Medium is coagulated by exposure at 80°C for 60 minutes in a slanted position. The medium contains horse serum and egg yolk to stimulate and support the growth of corynebacteria; potassium tellurite inhibits the growth of most normal Gram-negative bacteria of the upper respiratory tract¹; it is reduced by corynebacteria and other microorganisms with the formation of grey or black colonies.

4 - PHYSICAL CHARACTERISTICS

Medium appearance	pale yellow, opaque
Final pH at 20-25°C	Not applicable

5 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Pergola Medium	Ready-to-use tubes	554002	20 glass tubes with slanted medium, 17x125 mm, flat bottom, aluminium screw-cap. Packaging: cardboard box

6 - MATERIALS REQUIRED BUT NOT PROVIDED

Sterile loops and swabs, incubator and laboratory equipment as required, ancillary culture media and reagents for the identification of the colonies.

7 - SPECIMENS

Pergola Medium can be directly inoculated with clinical specimens; in case of respiratory diphtheria, material for culture should be obtained on a swab (either cotton or polyester tipped swab) from the inflamed area of nasopharynx; if membranes are present and can be removed, they should also be sent to the laboratory.² Good laboratory practices for collection, transport and storage of the clinical specimens should be applied; consult appropriate references for further information. Collect specimens before antimicrobial therapy where possible.

8 - TEST PROCEDURE

Inoculate the sample directly onto the surface of the slope. Incubate in aerobic conditions at 35-37°C for 18-24 hours. If negative re-incubate for a total of 4 days before reporting as negative.

9 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies. The typical colonies of *C. diphtheriae* are raised, shiny, of a light ashy colour, while the colonies of the generally associated cocci are of an intense black colour. Colonies of *C. diphtheriae* also tend to darken with aging.

10 - USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T° / T / ATM	EXPECTED RESULTS
<i>C. diphtheriae</i> ATCC 11913	35- 37°C / 18-24H / A	growth with light ashy colonies

A: Aerobic incubation; ATCC is a trademark of American Type Culture Collection



**11 - PERFORMANCES CHARACTERISTICS**

Prior to release for sale a representative sample of all lots of ready to use tubes of Pergola Medium is tested for productivity by inoculating the slopes with pure culture of the following target strains: *C. diphtheriae* ATCC 11913, *C. diphtheriae* ATCC 13812. After incubation at 35-37°C for 18-24 hours *C. diphtheriae* grows with typical colonies.

12 - LIMITATIONS OF THE METHOD

- Tellurite inhibits the growth of many non-coryneform bacteria but even a few *C. diphtheriae* strains are sensitive to potassium tellurite and therefore do not grow on Pergola Medium.²
- The growth on Pergola Medium and the reduction of tellurite are not specific for *C. diphtheriae* since many other coryneforms and other Gram-positive bacteria may also produce black colonies.²
- The medium is not inhibitory to Gram-positive bacteria: pseudodiphtheria, staphylococci, streptococci, micrococci, listeriae can grow with white-grey-black colonies. *Candida* grows with small greyish-white colonies.³
- It is advisable to inoculate, together with Pergola Medium, other plated or tubed media such as Blood Agar and Loeffler's Medium.¹⁻³
- It is recommended that suitable identification and susceptibility tests be performed on isolates. For the detailed procedures consult the appropriate reference.²
- This culture medium is intended as an aid in the diagnosis of infectious diseases; the interpretation of the results must be made considering the patient's clinical history, the origin of the sample and the results of other diagnostic tests.

13 - PRECAUTIONS AND WARNINGS

- This product is a qualitative *in vitro* diagnostic, for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- This product is not classified as dangerous according to current European legislation.
- This culture medium contains raw materials of animal origin. The *ante* and *post mortem* controls of the animals and those during the production and distribution cycle of the raw materials, cannot completely guarantee that these products do not contain any transmissible pathogen. Therefore, it is recommended that the ready-to-use tubes be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes. Download the TSE Statement from the website www.biolifeitaliana.it, describing the measures implemented by Biolife Italiana for the risk reduction linked to infectious animal diseases.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- Each tube is for single use only.
- Ready-to-use tubes of Pergola Medium are not to be considered a "sterile product" as they are not subject to terminal sterilization, but a product with controlled bio-contamination, within the limits of defined specifications reported on the Quality Control Certificate.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the tubes inoculated with samples or microbial strains in accordance with current local legislation.
- The Certificates of Analysis and the Safety Data Sheet are available on the website www.biolifeitaliana.it.
- Notify Biolife Italiana Srl (complaint@biolifeitaliana.it) and the relevant Authorities of any serious incident occurring in connection with the use of the *in vitro* diagnostics.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

14 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store tubes in their original pack at 2-8°C away from direct light. If properly stored, the tubes may be used up to the expiration date. Do not use the tubes beyond this date. Before use, check the integrity of the screw cap. Do not use tubes with signs of deterioration (e.g. microbial contamination, atypical colour).

15 - REFERENCES

1. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
2. Bernard KA. Coryneform Gram-positive rods. In Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology, 12th ed. Washington, DC: American Society for Microbiology; 2019.
3. Comitato Regionale per l'Ordinamento dei Servizi di Patologia (1977) Manuale di Tecniche Batteriologiche. Giunta Regionale della Lombardia Ass. Sanità.

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	IVD <i>In vitro</i> Diagnostic Medical Device	Manufacturer	Do not reuse	Recyclable pack This side up
Temperature imitation	Content sufficient for <n> tests	Consult Instructions for Use	Use by	Keep away from direct light	Fragile

REVISION HISTORY

Version	Description of changes	Date
Instructions for Use (IFU) - Revision 0	First publication	2021/04
Revision 1	Removal of obsolete classification	2023/04

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

