INSTRUCTIONS FOR USE

COLUMBIA BLOOD AGAR

Ready-to-use plates

Columbia Blood Agar:
Group A β-haemolytic *Streptococcus*

1 - INTENDED USE

In vitro diagnostic device. Non selective, general purpose medium for the isolation, cultivation and haemolytic pattern determination of fastidious and non-fastidious microorganisms, from clinical specimens and other materials.

2 - COMPOSITION - TYPICAL FORMULA *

Peptocomplex	10 g
Tryptose	10 g
Peptone	3 g
Maize starch	1 g
Sodium chloride	5 g
Agar	12 g
Defibrinated sheep blood	50 mL
Purified water	1000 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

Columbia Blood Agar was first described in 1966 by Ellner, Stoessel, Drakeford and Vasi[1] of the Columbia University, who combined meat and casein peptones and defibrinated sheep blood into one medium. After 2 years trial this medium showed remarkably improved growth promoting properties and it was found to be superior to blood agar previously used for differentiating β and α haemolytic organisms.[1]
Columbia Blood Agar is a non selective, general purpose medium with defibrinated sheep blood, intended for the isolation, cultivation and haemolytic pattern determination of non-fastidious and fastidious microorganisms, such as *Corynebacterium* spp., *Actinomyces* spp., *S.pneumoniae*, *Staphyloccoccus*, *C. jejuni* from clinical specimens[2,3]. Columbia Blood Agar is recommended for purification of colonies and for the confirmation test with incubation at 25°C in aerobic conditions, by ISO 10272 methods for the isolation and enumeration of *Campylobacter* spp. in food.[4]
Peptones provide carbon, nitrogen and trace elements for bacterial growth, sodium chloride maintains the osmotic balance, maize starch is included to absorb toxic by-products contained in the specimen and is an energy source for bacterial growth. The presence of sheep blood enables the determination of haemolytic pattern, as a useful tool for the orientation of bacterial identification.

4 - PHYSICAL CHARACTERISTICS

Medium appearance	red, opaque
Final pH at 20-25 °C	7.3 ± 0.1

5 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Columbia Blood Agar	Ready-to-use plates	541136	2 x 10 plates ø 90 mm primary packaging: 2 cellophane sachets secondary packaging: cardboard box

6 - MATERIALS REQUIRED BUT NOT PROVIDED

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

7 - SPECIMENS

Columbia Blood Agar plates can be directly inoculated with many clinical specimens collected from various normally sterile and non sterile human sites. Refer to the quoted literature for specimens types related to specific infections. [5-7] Columbia Blood Agar is not suitable for direct inoculation of blood samples. Good laboratory practices for collection, transport and storage of the clinical specimens should be applied; consult appropriate references for further information.[5] For the microbiological examination of food consult the ISO standard.[4]

8- TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium.
Inoculate and streak the specimen with a loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap. Alternatively, if the material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.
Incubate at 35-37°C in aerobic conditions with or without 5 -10% CO_2, and record the results after 24, 48 and if necessary, 72 hours.
The user is responsible for choosing the appropriate incubation time, temperature and atmosphere depending on the processed specimen, the requirements of organisms to be recovered and the local applicable protocols.

9 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological, chromatic, haemolytic characteristics of the colonies.
Here below are summarized the colony characteristics of some microorganisms which can be isolated on Columbia Blood Agar plates.[8]

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- The colonies of Group A streptococci typically are about 0.5-1mm in diameter, transparent or translucent, and domed, having a smooth surface and an entire edge. They are surrounded by a well-defined zone of complete haemolysis, usually two or three times the diameter of the colony.
- The colonies of group B streptococci are typically larger (2-4 mm in diameter) surrounded by a much smaller zone of complete haemolysis and some strains do not lyse the blood at all.
- The appearance of surface or subsurface beta-haemolytic group C and group G streptococcal colonies do not differ sufficiently from that of group A colonies to be of any value in identification
- Group D streptococcal colonies (*S.bovis*) are somewhat larger than other streptococcal colonies, they are less opaque, raised, and grey to grey-white.
- Pneumococcal colonies are round with entire edges, mucoid, and about 1mm in diameter. When the culture has been incubated in CO_2 incubators, the colonies are surrounded by a fairly large zone of alpha- haemolysis.
- The *viridans* streptococcal colonies vary in size from pinpoint to a size equal to, or larger than, that of group A streptococci. The colonies are usually smaller than those of the pneumococci. They may appear mucoidal or translucent or glossy and non- translucent. The colonies may be surrounded by a small zone of alpha-haemolysis (partial destruction of red blood cells) or have no zone of haemolysis
- Staphylococci colonies are yellow or white with or without the beta-haemolysis zone.
- *Listeria monocytogenes* colonies are surrounded by a small beta-haemolytic zone.

Once colonies have grown on Columbia Blood Agar plates, user must differentiate potential pathogens requiring identification and antimicrobial testing from contaminants that represent members of normal microbiota.

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.[9]

CONTROL STRAINS			INCUBATION T°/ T / ATM	EXPECTED RESULTS
S. pyogenes	ATCC	19615	35-37°C / 24H / A or CO_2	good growth, beta haemolysis
S. pneumoniae	ATCC	6305	35-37°C / 24H / A or CO_2	good growth, alpha haemolysis
S. aureus	ATCC	25923	35-37°C / 24H / A or CO_2	good growth
E. coli	ATCC	25922	35-37°C / 24H / A or CO_2	good growth

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 plates of Columbia Blood Agar and of the raw material used for the production of prepared plates (dehydrated Columbia Blood Agar Base REF 401136) are tested for productivity and haemolytic pattern by comparing the results with a previously approved Reference Batch.

Productivity is tested by a quantitative test with 2 strains: *C.jejuni* ATCC 33291 and *C.coli* ATCC 43478: the plates are inoculated with decimal dilutions in saline of a colonies suspension and incubated at 41,5°± 1°C for 44±4 hours in microaerophilic atmosphere . The colonies are enumerated on both batches and the productivity ratio (*Pr*) is calculated. If *Pr* is ≥ 0,7 the results are considered acceptable and conform to the specifications. Furthermore the productivity characteristics are tested by semi-quantitative ecometric technique with the following strains: *S.pyogenes* ATCC 19615*, S.pyogenes* ATCC 12384*, S. pneumoniae* ATCC 6305*, S.agalactiae* ATCC 12386*, S.agalactiae* clinical isolate*, S.aureus* ATCC 25923 and *E.coli* ATCC 25922. After incubation at 35-37°C for 18-24 hours the types of haemolysis and the amount of growth is evaluated and recorded. All strains show a good growth with typical haemolytic pattern.

12 - LIMITATIONS OF THE METHOD

- Due to the carbohydrate (starch) content of Columbia Blood Agar, β-haemolytic streptococci may exhibit an α- haemolytic reaction around a small clear zone of β-haemolysis or may exhibit weak haemolytic reactions.
- Depending on the specimens analyzed and the microorganisms being tested for, it is recommended to use also additional media such as selective media and Chocolate Agar.
- The growth and type of haemolysis depends on the metabolic requirements of organisms; it is possible that some strains do not grow and/or can demonstrate haemolytic models other than expected. *Haemophilus influenzae*, which requires both factor X and factor V, will not grow on this medium[10]; *Neisseria*, *Mycobacterium*, *Bordetella* and other microorganisms with highly specific nutritional requirements do not grow adequately; for the detection of these organisms specific culture media should be used.
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates from pure culture for complete identification. If relevant, perform antimicrobial susceptibility testing.
- This culture medium is intended as an aid in the diagnosis of infectious disease; the interpretation of the results must be made considering the patient's clinical history, the origin of the sample and the results of the 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 plates 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 S.r.l. 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 plate of this culture medium is for single use only.

- Ready-to-use plates 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 plates inoculated with samples or microbial strains in accordance with current local legislation.
- The Certificates of Analysis and the Safety Data Sheet of the product 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 diagnostic.
- 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 plates in their original pack at 2-8°C away from direct light. If properly stored, the plates may be used up to the expiration date. Do not use the plates beyond this date. Plates from opened plastic sachet can be used for 7 days when stored in a clean area at 2-8°C. Do not use the plates if the plastic sachet is damaged or if the dish is broken. Do not use the plates with signs of deterioration (e.g. microbial contamination, dehydration, shrinking or cracking of the medium, atypical colour, excess of moisture).

15 - REFERENCES

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10. Nye KJ, Fallon D, Gee B, Messer S, Warren RE, Andrews N. A comparison of blood Agar supplemented with NAD with plain blood agar and chocolated blood bgar in the isolation of Streptococcus pneumoniae and Haemophilus Influenzae from sputum. Bacterial Methods Evaluation Group J Med Microbiol 48 (12), 1111-1114 Dec 1999

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	IVD *In vitro* Diagnostic Medical Device	Manufacturer	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	For single use only	Fragile, handle with care

REVISION HISTORY

Version	Description of changes	Date
Instructions for Use (IFU) - Revision 1	Updated layout and content in compliance with IVDR 2017/746	2020/05
Revision 2	Removal of obsolete classification	2023/03

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

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