INSTRUCTIONS FOR USE

COLUMBIA AGAR BASE

Dehydrated culture medium

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

1 - INTENDED USE

In vitro diagnostic. 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 * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Peptocomplex	10 g
Tryptose	10 g
Peptone	3 g
Maize starch	1 g
Sodium chloride	5 g
Agar	12 g

*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 was found to be superior to blood agar previously used for differentiating β and α haemolytic organisms.[1]
Columbia Agar Base is a non-selective, general purpose medium, 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 with 5% sheep blood addition is recommended for purification of colonies and for confirmation tests with incubation at 25°C in aerobic conditions, by ISO 10272 methods for the isolation and enumeration of *Campylobacter* spp. in food.[4]
Columbia Agar Base supplemented with 5-10% (v/v) horse or sheep blood, Campylobacter Growth Supplement (REF 4240021) and a suitable antimicrobial mixture is used for the preparation of plating media for the isolation of *Campylobacter* spp.: Skirrow medium,[5] Blaser Wang medium[6].
Columbia Agar Base supplemented with 5% defibrinated sheep, horse or human blood and a specific antimicrobial mixture is used for the isolation of *Gardnerella vaginalis*. [7,8]
Columbia Agar Base with added 5% defibrinated sheep blood and CNA Antimicrobic Supplement (REF 4240018) is used for the isolation of gram-positive cocci.[1]
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 addition of sheep blood enables the determination of haemolytic pattern, as a useful tool for the orientation of bacterial identification.

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 41 g in 1000 mL of cold purified water; heat to boiling stirring constantly and sterilise by autoclaving at 121°C for 15 minutes. Cool to 47-50°C and add 5-7 % of defibrinated sheep or horse blood. Mix well and pour into sterile Petri dishes.
Columbia Agar Base may be used also for the preparation of the following media:
Columbia CNA blood agar: add 5 % sterile defibrinated sheep or horse blood and the content of one vial of CNA Antimicrobic Supplement (REF 4240018); mix well and pour into sterile Petri dishes.
Campylobacter Skirrow medium: to 500 mL of sterilised and pre-cooled base, add 50 mL of defibrinated sheep blood or 25 mL of lysed horse blood, the content of one vial of Campylobacter Growth Supplement (REF 4240021) and the content of one vial of Skirrow Antimicrobic Supplement (REF 4240016); mix well and pour into sterile Petri dishes.
Gardnerella vaginalis agar: to 500ml of sterilised and pre-cooled base, add 25 mL of human, sheep or horse blood and the content of one vial of Gardnerella Selective Supplement (REF 4240019); mix well and pour into sterile Petri dishes.

4 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	pale yellow, fine, homogeneous, free-flowing powder
Solution appearance	pale yellow, slightly opalescent
Prepared plates (with animal blood) appearance	red, opaque
Final pH at 20-25 °C	7,3 ± 0,2

5 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Columbia Agar Base	Dehydrated culture medium	4011362	500 g (12.2L)
		4011364	5 kg (122 L)

6 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile loops and swabs, incubator and laboratory equipment as required, controlled atmosphere generators and jars, animal blood, selective supplements, ancillary culture media and reagents for the identification of the colonies.

7 - SPECIMENS

Columbia Agar Base supplemented with blood and, if necessary, with the suitable antimicrobial mixtures, can be directly inoculated with many clinical specimens collected from various normally sterile and non-sterile human sites. Refer to the quoted literature for specimen types related to specific infections. [10-12] Plates prepared with Columbia Agar Base are not suitable for direct inoculation of blood samples. Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of the

clinical specimens should be applied; consult appropriate references for further information.[10] 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 plates prepared with Columbia Agar Base with added 5% defibrinated sheep blood.[13] For other applications of Columbia Agar Base consult the suitable literature.

- 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 sub-surface 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 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* 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 of Columbia Agar Base with addition of 5% defibrinated sheep blood.[14]

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 dehydrated Columbia Agar Base supplemented with 5% defibrinated sheep blood is 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 colony suspensions 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[15]; 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 diseases; 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.

14 - 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.
- Dehydrated media must be handled with suitable protection. Before use, consult the Safety Data Sheet.
- 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 this product doesn't contain any transmissible pathogen. Therefore, it is recommended that the culture medium 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.
- Apply Good Manufacturing Practice in the preparation process of plated or tubed or bottled media.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the sterilized plates inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium as active ingredient for pharmaceutical preparations or as production material intended for human and animal consumption.
- 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.

15 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store at +10°C /+30°C away from direct light in a dry place. If properly stored, it may be used up to the expiration date. Do not use beyond this date. Avoid opening the bottle in humid places. After use, the container must be tightly closed. Discard the product if the container and/or the cap are damaged, or if the container is not well closed, or in case of evident deterioration of the powder (colour changes, hardening, large lumps. The user is responsible for the manufacturing and quality control processes of prepared media and for the validation of the period of validity of the finished products, according to the type (plates/tubes/bottles), the added supplements and the storage method applied (temperature and packaging).

16 - REFERENCES

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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	Keep away from direct light	Store in a dry place

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2020/05
Revision 2	Modification of "precautions and warnings", "storage conditions and shelf life".	2022/01
Revision 3	Removal of obsolete classification	2023/04

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

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