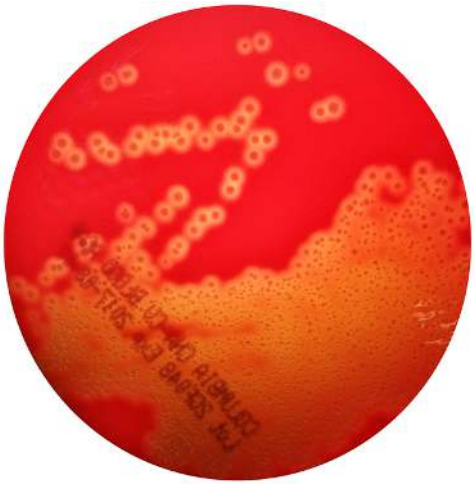
INSTRUCTIONS FOR USE

COLUMBIA CNA AGAR BASE

Dehydrated culture medium



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

1 - INTENDED USE

In vitro diagnostic. Selective medium for the isolation of Gram-positive cocci from clinical and non-clinical specimens containing mixed flora and for the determination of bacterial haemolytic pattern.

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
Nalidixic acid	15 mg
Colistin	10 mg

*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 with 10 mg/L of colistin and 15 mg/L of nalidixic acid was first described in 1966 by Ellner, Stoessel, Drakeford and Vasi[1] of the Columbia University, who combined meat and casein peptones, antibiotics and defibrinated sheep blood into one medium for the isolation of Gram-positive cocci. 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 CNA Blood Agar is a selective medium intended for the isolation and haemolytic properties determination of Gram-positive cocci (*Staphylococcus* and *Streptococcus*) particularly when Gram-negative bacteria (e.g. *Pseudomonas*, *Proteus*, *Klebsiella*) are present in the specimens and tend to overgrow on conventional blood agar plates.[2,3]
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. Colistin, a polypeptide antibiotic of the polymyxin group, and nalidixic acid, a first-generation quinolone, are primarily active against Gram-negative bacteria rendering the medium selective for Gram-positive cocci. The presence of defibrinated 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 with frequent agitation and sterilise by autoclaving at 121°C for 15 minutes. Cool to 47-50°C and add 5-7 % sterile defibrinated sheep or horse blood, mix well and pour into sterile Petri dishes.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution appearance	yellow, opalescent
Prepared plates appearance	red, opaque
Final pH at 20-25 °C	7.3 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Columbia CNA Blood Agar Base	Dehydrated medium	40113612 40113614	500 g (12.2 L) 5 kg (122 L)

7 - 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.

8 - SPECIMENS

Columbia CNA Blood Agar Base supplemented with defibrinated sheep or horse blood can be directly inoculated with clinical specimens collected from various normally non-sterile human sites.[4,5] Good laboratory practices for collection, transport and storage of the clinical specimens should be applied; consult appropriate references for further information.[4]

9- 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 18-24 and 48 hours.

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10 - 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 colonies' characteristics of some microorganisms which can be isolated on Columbia CNA Agar supplemented with defibrinated sheep blood.[6]

- The colonies of Group A streptococci typically are about 0.5 mm 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 (1-2 mm in diameter) surrounded by a much smaller zone of complete haemolysis. Some strains do not lyse the blood at all.
- The appearance of surface or sub-surface β-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, grey to grey-white and non-haemolytic.
- Pneumococcal colonies are round with entire edges, mucoid, and about 0.5-1 mm in diameter. When the culture has been incubated in CO_2 incubators, the colonies are surrounded by a fairly large zone of α-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 α-haemolysis (partial destruction of red blood cells) or have no zone of haemolysis.
- Staphylococci colonies are yellow or white with or without a β-haemolysis zone.

The use of defibrinated blood other than sheep's blood (e.g. horse) may give rise to haemolytic types other than those described above.

11 - 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.[7]

CONTROL STRAINS			INCUBATION T°/ T / ATM	EXPECTED RESULTS
S. pyogenes	ATCC	19615	35-37°C / 18-24H / A or CO_2	growth, beta haemolysis
S. pneumoniae	ATCC	6305	35-37°C / 18-24H / A or CO_2	growth, alpha haemolysis
S. aureus	ATCC	25923	35-37°C / 18-24H / A or CO_2	growth
P. mirabilis	ATCC	12453	35-37°C / 44-48H / A or CO_2	totally or partially inhibited

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

12- PERFORMANCES CHARACTERISTICS

Prior to release for sale, a representative sample of all lots of dehydrated Columbia CNA Agar Base supplemented with defibrinated sheep blood is tested for productivity, selectivity and haemolytic pattern by comparing the results with a previously approved Reference Batch.

Productivity is tested by semi-quantitative ecometric technique with the following target strains: *S. pyogenes* ATCC 19615, *S. pyogenes* ATCC 12384, *S. pneumoniae* ATCC 6305, *S. agalactiae* ATCC 12386, *S. agalactiae* ATCC 13813, Group C *Streptococcus* ATCC 12388, *S. aureus* ATCC 25923. After incubation at 35-37°C for 18-24 hours in aerobic atmosphere the types of haemolysis and the amount of growth is evaluated and recorded. All strains show a good growth with typical haemolytic pattern. The selectivity is evaluated with modified Miles-Misra surface drop method by inoculating the plates with decimal dilutions in saline from 10^{-1} to 10^{-4} of a 0.5 McFarland suspension of the non-target organisms *E. coli* ATCC 25922, *P. mirabilis* ATCC 10005, *P. aeruginosa* ATCC 14207. After incubation at 35-37°C for 44-48 hours in aerobic atmosphere, the growth of all non-target strains is inhibited at the dilution 10^{-1}.

13 - LIMITATIONS OF THE METHOD

- Due to the carbohydrate (starch) content of Columbia CNA Blood Agar, some β-haemolytic streptococci may exhibit an α-haemolytic reaction around a small clear zone of β-haemolysis or may exhibit weak haemolytic reactions with defibrinated sheep blood.[3]
- The growth and type of haemolysis depend on the metabolic requirements of the organisms; it is possible that some strains do not grow and/or can demonstrate haemolytic patterns other than expected.
- The colony diameter is generally smaller than that observed on Columbia Blood Agar.
- Some Gram-negative bacteria and yeasts could be resistant to the CNA antibiotic mixture and may not be inhibited on this medium.
- Since some pathogens required carbon dioxide for growing, it is preferable to incubate the plates with 5 -10% CO_2.
- 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 required and 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 microscopic and/or 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 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/bottles), the added supplements and the storage method applied (temperature and packaging).

16 - REFERENCES

1. Ellner PD, Stoessel CJ, Drakeford E, Vasi, F. A new culture medium for medical bacteriology. Am. J. Clin. Path 1966; 45: 502-504.
2. Atlas D, Snyder J. Media Reagents and Stains. In Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington,DC: American Society for Microbiology; 2015. p.345.
3. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
4. Baron EJ, Specimen Collection, Transport and Processing:Bacteriology. *In* Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington,DC: American Society for Microbiology; 2015. p.270.
5. Public Health England- UK Standards for microbiology investigations (UK SMI): searchable index. 9 January 2019
6. Balows, A., Hausler, W.J., Herrmann, K.L., Isenberg HD and Shadomy, H.J. (ed) (1991) In Manual of Clinical Microbiology, 5th edition, Washington, DC: American Society for Microbiology; 1991.
7. CLSI (formerly NCCLS) Quality Control of Commercially Prepared Culture Media. Approved Standard, 3rd edition. M22 A3 vol. 24 n° 19, 2004

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	2020/06
Revision 2	Update of “precautions and warnings” and “storage conditions and shelf life”	2022/01
Revision 3	Removal of obsolete classification	2023/04
Revision 4	Inclusion of horse blood in the plate preparation method	2024/04

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

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