INSTRUCTIONS FOR USE

CNA ANTIMICROBIC SUPPLEMENT

Freeze-dried selective supplement

1 - INTENDED USE

In vitro diagnostic. Mixture of antimicrobials to be added to Columbia Agar Base for the isolation of Gram-positive cocci from clinical and non-clinical specimens.

2 - COMPOSITION - VIAL CONTENTS FOR 500 mL OF MEDIUM

Nalidixic acid	7.5 mg
Colistin	5 mg

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

CNA Antimicrobic Supplement is a freeze-dried mixture of antimicrobials to be used as a supplement of Columbia Agar Base.
The complete medium Columbia CNA with defibrinated sheep or horse blood corresponds to the medium described in 1966 by Ellner, Stoessel, Drakeford and Vasi[1] and is 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]
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.

4- DIRECTIONS

Aseptically reconstitute the contents of one vial with 5 mL of sterile purified water and mix gently to dissolve.
Prepare 500 mL of Columbia Agar Base (REF 401136), autoclaved and cooled to 47-50°C and add the contents of one vial of CNA Antimicrobic Supplement, together with 5% of defibrinated sheep or horse blood, under aseptic conditions. Mix well and distribute into sterile Petri dishes.

5 - PHYSICAL CHARACTERISTICS

Freeze-dried supplement appearance	short, dense, white pastille
Reconstituted supplement appearance	limpid, colourless

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
CNA Antimicrobic Supplement	Freeze-dried supplement	4240018	10 vials, each for 500 mL of medium

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Columbia Agar Base (REF 401136), autoclave, water-bath, incubator and laboratory equipment as required, sterile loops and swabs, Petri dishes, Erlenmeyer flasks, ancillary culture media and reagents for the identification of the colonies.

8 - SPECIMENS

Columbia Agar Base supplemented with defibrinated sheep blood and CAN Antimicrobic Supplement can be directly inoculated with clinical specimens collected from various normally non-sterile human sites such as ear, upper respiratory tract, genital tract, pus and exudates.[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.

10 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological, chromatic, haemolytic characteristics of the colonies.
Consult the cited bibliography for the interpretation of the haemolytic characteristics of the colonies.[6]

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 testing 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

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12 - PERFORMANCES CHARACTERISTICS

Prior to release for sale a representative sample of all lots of CNA Antimicrobic Supplement added to dehydrated Columbia Agar Base together with 5% of defibrinated sheep blood, is tested for productivity and selectivity by comparing the results with previously approved Reference Batch.
Productivity characteristics are tested by semi-quantitative ecometric technique with the following target strains *S.pyogenes* ATCC 19615, *S. pneumoniae* ATCC 6305, *S.aureus* ATCC 25923, *S.epidermidis* ATCC 12228, *E.faecalis* ATCC 19433. 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 12453, *P.aeruginosa* ATCC 27853, *C.albicans* ATCC 60193. After incubation at 35-37°C for 18-24 hours in an aerobic atmosphere, the growth of *E. coli* is completely inhibited and the growth of the other non-target strains is partially inhibited.

13 - LIMITATIONS OF THE METHOD

- Due to the carbohydrate (starch) content of Columbia CNA Blood Agar Base, some β-haemolytic streptococci may exhibit an α-haemolytic reaction around a small clear zone of β-haemolysis or may exhibit weak haemolytic reactions.[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 Sheep.
- 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

- The supplement is a qualitative *in vitro* diagnostic, for professional use only; it must be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- The supplement is classified as dangerous according to current European legislation; consult the Safety Data Sheet before use.
- The supplement and the medium base shall be used in association according to the directions described above. Apply Good Manufacturing Practice in the production process of prepared media.
- The supplement is sterilized by autoclaving.
- Be careful when opening the metal ring to avoid injury.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as medium powder and supplements or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused supplements and the sterilized media inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use the supplement as active ingredients for pharmaceutical preparations or as production materials 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 the product in the original package at 2-8°C away from direct light. If properly stored, the product may be used up to the expiry date printed on the label; do not use beyond this date. Once the vial has been opened and the lyophilised product has been reconstituted, the resulting solution should be used immediately. Before use, examine the lyophilized and reconstituted product and discard if there are obvious signs of deterioration (e.g., contamination, atypical colour or other abnormal characteristics).

The user is responsible for the manufacturing and quality control processes of prepared media and the validation of their shelf life, according to the type (plates/tubes) and the applied storage conditions (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 H.D. 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

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Hazardous ingredient: nalidixic acid sodium salt, colistin

Classification

Acute toxicity, category 3	H301	Toxic if swallowed.
Acute toxicity, category 4	H332	Harmful if inhaled.
Specific target organ toxicity - single exposure, category 3	H335	May cause respiratory irritation

Labelling

Pictogram

Signal word	Danger
Hazard statement(s):	
H301	Toxic if swallowed.
H332	Harmful if inhaled.
H335	May cause respiratory irritation.
Precautionary statements:	
P261	Avoid breathing dust / fume / gas / mist / vapours / spray.
P312	Call a POISON CENTRE / doctor / . . . if you feel unwell.
P403+P233	Store in a well-ventilated place. Keep container tightly closed.
P264	Wash . . . thoroughly after handling.

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	IVD *In vitro* Diagnostic Medical Device	Manufacturer	This side up	
Temperature limitation	Content sufficient for <n> tests	Consult Instructions for Use	Use by	Keep away from direct light	Fragile

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2022/02
Revision 2	Removal of obsolete classification	2023/04

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

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