



2015/05

1- INTENDED USE

Columbia agar with sheep blood supplemented with the CNA inhibitory mixture (Colistin + Nalidixic Acid) is a selective medium for Gram (+) bacteria. It is also used to demonstrate haemolytic reactions.

This medium is used for the analysis of mixed flora specimens, such as genital tract or oropharyngeal specimens.

2- PRINCIPLE

The selectivity of this medium is based on the presence of the CNA antibiotic mixture (colistin + nalidixic acid), which inhibits the growth of Gram (-) bacteria.

The growth of most Gram (+) bacteria is promoted by the nutrients and sheep blood present in the medium.

3- HOW SUPPLIED

Ready to use medium:

- box of 20 Petri dishes (90 mm) (**CSB CNA**) code 63954

4- THEORETICAL COMPOSITION (g/l of distilled water)

The Columbia CNA + 5% Sheep Blood agar medium is prepared according to the formula described by Ellner *et al.* (1)

Special mixture of peptones	23
Starch	1
Sodium chloride	5
Nalidixic acid	0.015
Colistin	0.010
Agar	10

Columbia agar is supplemented with 5% defibrinated sheep blood.

5- WARNING AND PRECAUTIONS

For hazard and precaution recommendations related to some chemical components in this test kit, please refer to the pictogram(s) mentioned on the labels and the information supplied at the end of instruction for use. The Safety Data Sheet is available on www.bio-rad.com.

6- STORAGE

- Ready to use medium: at +2-8°C.

The expiry date and batch number are indicated on the packaging.

7- INSTRUCTIONS**Material:**

- Material provided: Columbia CNA + 5% Sheep Blood.

Inoculation:

Inoculate by streaking directly from the specimen to be examined.

Refer to current recommendations for storage of biological specimens (2).

Incubation:

Incubate for 24 to 48 hours at 37°C in normal atmosphere or CO₂.

Reading:

Note the haemolytic characteristics of the colonies:

- **no haemolysis:** no colour change of the medium around the colony.
- **α haemolysis:** greenish zone with blurred contours around the colony.
- **β haemolysis:** clear zone with well defined contours around the colony.

8- PERFORMANCE/QUALITY CONTROL OF THE TEST

- Appearance of the ready to use medium: **cherry red** agar.
- The growth performances of Columbia CNA agar + 5% Sheep Blood are verified with the following strains:

STRAINS	CULTURE RESULT AFTER 24 to 48 HOURS AT 37°C
<i>Streptococcus pyogenes</i> ATCC® 19615™	Good growth, β haemolysis
<i>Streptococcus pneumoniae</i> ATCC® 6303™	Good growth, α haemolysis
<i>Enterococcus faecalis var zymogenes</i> ATCC® 29212™	Good growth, no haemolysis
<i>Neisseria meningitidis</i> ATCC® 13090™ (CO ₂)	No growth
<i>Escherichia coli</i> ATCC® 25922™	No growth
<i>Pseudomonas aeruginosa</i> ATCC® 27853™	No growth

9- QUALITY CONTROL OF THE MANUFACTURER

All manufactured reagents are prepared according to our Quality System, starting from reception of raw material to the final commercialization of the product. Each lot is submitted to quality control assessments and is only released to the market, after conforming to pre-defined acceptance criteria. The records relating to production and control of each single lot are kept within Bio-Rad.

10- LIMITS OF USE

- Some strains may not grow on this medium due to their nutritional requirements.
- Some *Enterobacteriaceae*, such as *Proteus*, *Serratia*, *Pseudomonas*, *Klebsiella*, are resistant to the CNA antibiotic mixture and may not be inhibited on this medium.
- The inhibitory effect is decreased in the presence of an excessively heavy inoculum.
- The colony diameter is generally less than that observed on Columbia agar with sheep blood but without CNA.
- Complementary tests must be performed to identify the species of the strain isolated.

11- REFERENCES

1. ELLNER, D., STOESSEL, C. J., DRAKEFORD, E., VASI, F. 1966. *A new culture medium for medical bacteriology*. American Journal of Clinical Pathology. **45** : p. 502-504.
2. Basic Laboratory Procedures in clinical Bacteriology. World Health Organization. Geneva. 1991. 1st edition.
3. HERMANN G.J., MOORE M.S., PARSONI I. 1958. American Journal of Clinical Pathology. **29** : p. 181-183.
4. THAYER J.D., MARTIN J.E. 1966. *Improved medium selective for Neisseria gonorrhoeae and Neisseria Meningitidis*. Public Health Report. **81** : p. 559-562

Certificates of analysis available on <http://www.bio-rad.com/certificate>



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