

COLUMBIA CNA AGAR +5% SHEEP BLOOD

INSTRUCTIONS FOR USE THE READY-TO-USE PLATED MEDIUM

1. Intended use

Columbia CNA Agar +5% Sheep Blood is a selective medium used for the qualitative detection and isolation of Gram-positive cocci of the genus *Staphylococcus*, *Streptococcus* and *Enterococcus* from human clinical specimens and other specimens.

The function of the medium is to support diagnosis in patients exhibiting symptoms of potential infections, especially those occurring in locations rich in mixed bacterial flora, such as respiratory system, reproductive system and abdomen.

Many species of the genus *Streptococcus* play an important role as pathogens of humans and animals or are a part of the microbiome of the oral cavity and the upper respiratory and gastrointestinal tracts of humans. The species most commonly isolated from clinical specimens are: *S. pneumoniae*, *S. pyogenes*, *S. agalactiae*, *S. dysgalactiae* as well as some species of alpha-hemolytic streptococci. They are responsible for a wide range of infections, from severe invasive infections to respiratory infections or skin and soft tissue infections. *Streptococcus pneumoniae* is one of the main agents of pneumonia and meningitis and *Streptococcus agalactiae* is responsible for severe perinatal infections in newborns.

The *Staphylococcus* genus includes many species, more than half of which are associated with humans, constituting human microbiome but also potentially causing a number of diseases. Staphylococci, especially *Staphylococcus aureus*, can cause local infections affecting almost all tissues and organs, as well as generalized infections, often life-threatening. The most common ones are boils, barley, impetigo, abscesses and osteomyelitis, septic arthritis, endocarditis, pneumonia and, less commonly, meningitis. In addition, *Staphylococcus aureus* and *Staphylococcus epidermididis* have remained at the top of the list of microorganisms causing hospital-acquired infections for years.

The *Enterococcus* genus are opportunistic pathogens that can cause infections outside their physiological habitat, especially in people with compromised immune systems. They can cause urinary tract infections, bacteremia and endocarditis. They are often isolated from patients with intra-abdominal multi-microbial infections. *Enterococcus faecalis* is more likely to cause abdominal infections and *Enterococcus faecium* causes urinary tract and wound infections.

Cat. no:	Medium type:	Packaging:
1191PD90; 201191	Solid medium on a plate	1x10 pcs (90 mm)

2. Principle of the procedure

The medium contains high-value protein hydrolysates that allow abundant and rapid growth of microorganisms with high nutritional requirements. Corn starch is a source of energy that stimulates bacterial growth, absorbs toxic components present in the test sample and enhances the hemolytic response of some streptococci. Yeast extract is a source of B vitamins. The addition of sheep's blood provides the factor X necessary for the growth of many bacteria, and allows to determine the type of hemolysis, and enables a preliminary identification of the bacteria present in the test specimen.

Nalidixic acid and colistin are supplements that inhibit the growth of Gram-negative bacteria (*Enterobacterales* and *Pseudomonas* spp.) without affecting the growth of Gram-positive bacteria, i.e. staphylococci, streptococci, enterococci. Colistin damages the cell membrane of Gram-negative bacteria, thus inhibiting their growth, especially the growth of *Pseudomonas* spp. Nalidixic acid is an antibacterial agent, having an inhibitory effect on the growth of Gram-negative bacteria, blocks DNA replication in susceptible Gram-negative bacteria.

3. Medium composition

In g/l distilled water:		Supplements/liter of medium:	
Enzymatic digest of casein	5,0 g	Sheep blood	50 ml
Enzymatic digest of animal tissue	8,0 g	Colistin	0,015 g
Yeast extract	10,0 g	Nalidixic acid	0,01 g
Agar	14,0 g		
Sodium chloride	5,0 g		
Corn starch	1,0 g		

pH 7.3 ± 0.2 at 25° C.

Appearance of the medium - Homogeneous red

4. Medium preparation

The medium is ready to use. Bring the medium to room temperature immediately before use.

5. Equipment required, not provided

Standard laboratory equipment necessary to perform microbiological tests, including an incubator, or an atmosphere controlled incubator.

6. Precautions

- The product is intended for professional use only.
- Non-automated product.
- The medium contains components of animal origin, which may be associated with the presence of biological pathogens, therefore must be handled in accordance with the principles of handling potentially infectious biological material.
- Do not use plates if the medium shows signs of microbial contamination, discoloration, drying, cracking or other signs of deterioration.
- Do not use damaged plates.
- Do not use hemolyzed plates
- Do not use plates after the expiration date.
- Re-incubation of previously inoculated plates is not allowed.
- To ensure correct test results, follow these instructions.
- If the handling of the medium differs from that described in this manual, the laboratory is obliged to validate the procedure adopted.

7. Storage

Store plates at 2-12°C until the expiration date. Store plates in their original packaging, in an inverted position (agar side up), away from direct light sources. To avoid freezing of agar, do not store plates close to the refrigerator walls. To avoid the appearance of water condensation on the plate lid do not open the refrigerator more often than necessary and do not store plates in an overfilled refrigerator..

8. Expiration date

The medium stored at 2-12°C retains its properties for up to 55 days from the date of production..

9. Specimen types

Collect samples for testing in accordance with current guidelines. Store specimens for testing until delivery to the laboratory in accordance with the laboratory's specimens storage policy. Store urine and stool samples in a refrigerator. Swabs, aspirates, specimens from the respiratory tract, as well as pus and exudate fluids and other specimens collected for transport media should be stored at room temperature in accordance with the recommendations of the media manufacturer. Inoculate the specimens as soon as possible after delivery of the material to the laboratory.

10. Test procedure

1. Allow the medium to warm to room temperature before inoculation.
2. Inoculate the specimen by spreading it directly on the agar surface.
3. If the specimen is collected on a swab - gently rotate the tip of the swab on a small area of agar just at the edges of the plate, and then specimen by streak plate method using a sterile loop.
4. Incubate the inoculated plates under aerobic conditions at $35 \pm 2^\circ\text{C}$.
5. In order to obtain the growth of bacteria with different growth requirements, the medium can be incubated under aerobic conditions supplemented with CO₂ (5 - 10%) for 18-24 or up to 48 hours, depending on the type of specimen to be tested and the microorganism sought.
6. Examine for growth result after 18-24 or 48 hours of incubation

1. Reading and interpretation

After incubation, observe:

- the presence of bacterial colony growth,
- colony morphology,
- changes in the colour of the medium and the presence of hemolysis

Typical morphology of colonies grown on Columbia CNA Agar +5% Sheep Blood medium:

Microorganism	Typical colony morphology	Presence and type of hemolysis
Group A streptococci	Transparent or semi-transparent colonies, about 0.5 mm in diameter, round, entire-edged with a smooth surface	A distinct zone of hemolysis β around the colony
Group B streptococci	Large colonies about 1-2 mm in diameter	Small zone of β -hemolysis or no hemolysis around the colony
Group C and G beta-hemolytic streptococci	Colony morphology similar to that of group A streptococci	A distinct zone of hemolysis β around the colony
Group D streptococci	Colonies larger than other groups of streptococci, slightly opalescent, gray to gray-white	Hemolysis type α or no hemolysis
Pneumococci	Colonies 0.5-1 mm in diameter, round, entire-edged, mucous	Incubated under CO ₂ conditions show a large zone of α -type hemolysis
Viridans streptococci	Colonies from small (the size of a pin head) to equal or larger colonies produced by group A streptococci, usually smaller than pneumococci. Mucous, semi-transparent, or shiny	Colonies surrounded by a small zone of α -type hemolysis or no hemolysis.
Staphylococci	Large yellow or white to gray colonies	β -type hemolysis or no hemolysis
Corynebacteria	Colonies small to large, white to gray or yellow	Colonies, with or without hemolysis zone

For the final identification of cultured microorganisms, additional tests and/or identification tests must be performed using other methods used in the laboratory.



Colony morphology and growth pattern of *S.pyogenes*, *S. agalactiae*, *S. pneumoniae* bacteria on Columbia CNA Agar +5% Sheep Blood

12. Quality control

The nutritional properties of the medium should be checked using reference strains giving the expected positive reactions. The test should be performed using pure, 18-24 hour cultures of reference strains giving the desired reactions. Use the following reference strains to perform the medium quality control:

Reference strain:	Growth intensity:	Colonies morphology:	Type of hemolysis:
<i>Staphylococcus aureus</i> ATCC 25923	good growth	large, white to gray or cream to yellow	β -type
<i>Streptococcus pyogenes</i> ATCC 19615	good growth	small, white to gray,	β -type
<i>Streptococcus pneumoniae</i> ATCC 49619	good growth	very fine, flat, whole-edged	α -type
<i>Proteus vulgaris</i> ATCC 8427	no growth	-	-
<i>Escherichia coli</i> ATCC 25922	no growth	-	-

Other reference strains may be used in accordance with the laboratory's procedures and instructions. Quality control procedures should meet the requirements of applicable regulations and guidelines/recommendations.

13 Limitations of the method

- Due to variability in nutrient requirements, some strains may grow poorly or not at all on Columbia CNA Agar +5% Sheep Blood medium.
- Depending on the blood used, group D streptococci can exhibit different hemolytic reactions. On horse, rabbit and human blood they produce β -type hemolysis, on sheep blood α -hemolysis.

- The hemolytic response of β -hemolytic streptococci can be affected by incubation conditions. It is recommended to incubate under conditions of increased CO₂ (5-10%) according to the rules and procedures specified by the laboratory.
- Some Gram-negative bacteria showing resistance to the mixture of antibiotics in the medium may show growth.
- Fungi can grow on the medium.
- The medium is characterized by a relatively high carbohydrate content, which means that β -hemolytic streptococci can cause a viridans hemolysis, sometimes misinterpreted as alpha-type hemolysis.
- Some Gram-negative bacteria such as *Gardnerella vaginalis* and some *Bacteriodes* spp. can grow very well on Columbia CNA Agar +5% Sheep Blood.
- Some aerobic Gram-positive bacteria that produce spores, such as *Bacillus*, can be inhibited on this medium

1. Characteristics of the method

In 1966, Ellner and colleagues presented a multicomponent medium with blood, which, thanks to casein hydrolysate and peptones, achieved faster and more abundant microbial growth, a stronger and less ambiguous hemolysis, and a more typical colony morphology with better staining.

Columbia Agar with blood is the base medium to which various supplements are added to increase its selectivity.

Columbia CNA Agar +5% Sheep Blood is a common selective medium used for the isolation and culture of clinically relevant Gram-positive microorganisms. Gram-positive cocci of the genus *Staphylococcus*, *Streptococcus* and *Enterococcus* can grow on it, as well as corynebacteria and other cocci. Culture conducted under anaerobic conditions makes it possible to obtain growth of anaerobic cocci α . Antibiotics in the medium inhibit the growth of *Proteus*, *Klebsiella* and *Pseudomonas*.

This medium is also used to determine the type of hemolysis of microorganisms, which is important in the preliminary identification of some groups of pathogenic bacteria, especially of the genus *Streptococcus*. Some diagnostic tests can be performed on this medium. However, in order to properly identify cultured microorganisms, appropriate identification tests must be performed using pure cultures.

2. Disposal of used material

Used and unused materials should be disposed of in accordance with current medical waste regulations and laboratory procedures for the disposal of infectious and potentially infectious materials.

3. Reporting of adverse events

According to current regulations, adverse events and incidents that can be directly linked to the described medium must be reported to the manufacturer and to the competent authorities.

4. References

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7. Diffco & BBD Manual, Manual of Microbiology Culture Media, Second Edition, 2009

History of document changes

Date of change	Section	Description of the change
2023/02/03	Entire document	Adaptation to the requirements of EU Regulation 2017/746

NOTE

The revision history of the document does not include editorial changes.

SYMBOL	NAME OF SYMBOL	DESCRIPTION	REF.
	Manufacturer	Indicates the medical device manufacturer.	5.1.1
	Date of manufacture	Indicates the date after which the medical device is not to be used.	5.1.3
	Catalogue number	Indicates the manufacturer's catalogue number so that the medical device can be used..	5.1.6
	Batch code	Indicates the manufacturer's batch code so that the batch or lot can be identified.	5.1.5
	In vitro diagnostic medical device	Indicates a medical device that is intended to be used as an invitro diagnostic medical device.	5.5.1
	Do not re-use	Indicates a medical device that is intended for one single use only.	5.4.2
	Contains sufficient for <n> tests	Indicates the total number of tests that can be performed with the medical device.	5.5.5
	Use -by date	Indicates the date after which the medical device is not to be used	5.1.4
	Temperature limit	Indicates the temperature limits of temperature shall be indicates adjacent to the upper and lower horizontal lines.	5.3.7
	Safety symbol (Compliance with EU requirements)	The CE marking on a product is a manufacturer's declaration that the product complies with the essential requirements of the relevant European Union health, safety and environmental regulations.	nd.

	Consult instructions for use or consult electronic instructions for use	Indicates the need for the user to consult the instructions for use.	5.4.3
	Sterilized using aseptic processing techniques	Indicates a medical device that has been manufactured using accepted aseptic techniques.	5.2.2
	Do not use if package is damaged and consult instructions for use	Indicates that a medical device that should not be used if the package has been damaged or opened and that the user should consult the instructions for use for additional information.	5.2.8
	Contains biological material of animal origin	Indicates a medical device that contains biological tissue, cells, or their derivatives, of animal origin	5.4.8



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