

**DEKLARACJA ZGODNOŚCI UE/
EU DECLARATION OF CONFORMITY**



DANE DOTYCZĄCE PRODUCENTA/ MANUFACTURER DETAILS	
Producent/ Manufacturer	Graso Zenon Sobiecki
Adres producenta/ Adress	Krąg 4A 83-200 Starogard Gdański
Numer rejestracyjny producenta/ Manufacturer's registration number	PL-MF-000022437
DANE DOTYCZĄCE WYROBU/ DEVICE DETAILS	
Nazwa produktu/ Product name	Chocolate Agar
Numer katalogowy/Article number	1080PD90, 201080
Basic-UDI-DI	590470801080PD90MS
Klasa ryzyka wyrobu, wg reguły/ Risk class and classification rule	klasa A wg Załącznika VIII reguła 5a / class A acc. Annex VIII rule 5a
Ocena zgodności/ Conformity assessment	wg Załącznika IX acc. to Annex IX
OŚWIADCZENIE PRODUCENTA/ MANUFACTURER STATEMENT	
<p>Producent Graso Zenon Sobiecki z siedzibą firmy w miejscowości Krąg 4A, 83-200 Starogard Gdański na swoją wyłączną odpowiedzialność oświadcza, że:</p> <p>wyżej wymienione produkty spełniają wymagania Rozporządzenia Parlamentu Europejskiego i Rady (UE) 2017/746 z 5 kwietnia 2017r. w sprawie wyrobów medycznych do diagnostyki in vitro oraz uchylecia dyrektywy 98/79/WE i decyzji Komisji 2010/227/UE oraz wymagania Ustawy o wyrobach medycznych z dnia 7 kwietnia 2022 r. Dokumentacja techniczna potwierdzająca zgodność wyrobu z powyższymi wymaganiami przechowywana jest w siedzibie producenta./</p> <p>The manufacturer Graso Zenon Sobiecki located in Krąg 4A, 83-200 Starogard Gdański declares on his own responsibility that:</p> <p>the above-mentioned products meet the requirements of the Regulation of the European Parliament and of the Council (EU) 2017/746 of April 5, 2017. on in vitro diagnostic medical devices and repealing Directive 98/79 / EC and Commission Decision 2010/227/ EU and the requirements of the Medical Devices Act of April 7, 2022. . The technical documentation confirming the compliance of the product with the requirements of the Regulation is kept at the manufacturer's premises.</p>	
ZASTOSOWANE WSPÓLNE SPECYFIKACJE, Z KTÓRYMI DEKLARUJE SIĘ ZGODNOŚĆ/	

REFERENCES TO ANY COMMON SPECIFICATION USED AND IN RELATION TO WHICH CONFORMITY IS DECLARED
Producent posiada zintegrowany System Zarządzania Jakością zgodny z: The manufacturer implemented integrated quality management system in accordance with:
<u>PN-EN ISO 9001</u> Systemy Zarządzania Jakością. Wymagania. / Quality Management System. Requirements.
<u>PN-EN ISO 13485</u> Wyroby medyczne. Systemy zarządzania jakością. Wymagania do celów przepisów prawnych. / Medical devices. Quality management systems. Requirements for regulatory purposes
DATA I MIEJSCE WYDANIA DEKLARACJI ZGODNOŚCI/ PLACE AND DATE OF ISSUE OF THE DECLARATION OF CONFORMITY
17.06.2025r. Krąg 4A, 83-200 Starogard Gdański

Imię i nazwisko / Name : Zenon Sobiecki

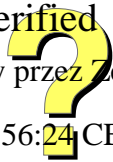
Stanowisko / Position : Prezes firmy/ Company President

Signature Not Verified

Dokument podpisany przez Zenon Sobiecki;

GRASO

Data: 2025.06.25 12:56:24 CEST



CHOCOLATE AGAR

INSTRUCTIONS FOR USE THE READY-TO-USE PLATED MEDIUM

1. Intended use

Chocolate Agar is a medium used for qualitative detection and isolation of pathogenic fastidious microorganisms in samples of human and non-human clinical specimens.

The medium is designed to support diagnosis and can be used for any type of specimens but its main purpose is to isolate fastidious microorganisms such as: *Haemophilus*, *Neisseria*, *Streptococcus* from human clinical specimens. The composition of the medium, especially the content of denatured sheep blood, is conducive to culturing *Haemophilus* but also other pathogenic microorganisms of the genus or *Streptococcus*.

Haemophilus influenzae, especially those producing coating, are responsible for severe invasive infections (meningitis, epiglottitis, severe pneumonia with bacteremia, osteoarthritis, cellulitis). The non-contagious strains cause non-invasive infections such as chronic bronchitis, pneumonia, acute otitis media, sinusitis and conjunctivitis, as well as exacerbations of POChP.

Two species from the genus *Neisseria*, are pathogenic to humans. *Neisseria gonorrhoeae* is the etiological agent of gonorrhea, a sexually transmitted disease, and *Neisseria meningitidis* is responsible for meningitis and septicemia known as invasive meningococcal disease.

Streptococci including *Streptococcus pneumoniae* are the most common agents of respiratory tract inflammation and meningitis and septicemia.

Cat. no:	Substrate type:	Packaging:
1080PD90	Solid medium on a plate	1x10 pcs (90 mm)

2. Principle of the procedure

Enzymatic digest of animal tissues and casein provide nutrients necessary for bacterial growth. Monopotassium phosphate and dipotassium phosphate prevent the pH changes that occur during the amine production process. Corn starch absorbs toxic substances found in the test sample and at the same time stimulates bacterial growth. Hemoglobin provides a source of hemin (factor X), which is essential for the growth of *Haemophilus* spp. The growth supplement in the form of Biovitex provides factor V (NAD) needed for the growth of *Haemophilus* spp. as well as vitamins, amino acids, coenzymes, glucose, iron ions and other factors promoting growth of pathogenic *Neisseria* spp.

3. Medium composition

In g/l distilled water:	Supplements/liter of medium:		
Enzymatic digest of casein	7,5 g	Growth supplement (BIOVITEX)	2.0 ml
Enzymatic digest of animal tissues	7,5 g	2% of hemoglobin	100.0 ml
Corn starch	1,0 g		
Agar	10,0 g		
Sodium chloride	5,0 g		
Monopotassium phosphate	1,0 g		
Dipotassium phosphate	4,0 g		

pH 7.2± 0.2 at 25° C.

Appearance of the medium – Homogeneous, brown.

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 microbiology equipment necessary for testing, including an 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 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 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, do not store plates close to the refrigerator walls. To avoid water condensation on the plate lid, do not open the refrigerator more than necessary and do not store plates in an overfilled refrigerator.

8. Expiration date

The medium stored at 2-12°C retains its properties up to 3 months from the date of production.

9. Specimen types

Human clinical specimens, or other samples. Collect samples for testing in accordance with current guidelines. Store test specimens until delivery to the laboratory in accordance with the the guidelines for storage and transport of biological material. Inoculate as soon as possible after delivery of the test material to the laboratory. Respiratory tract products such as alveolar-bronchial lavage, sputum, aspirates should be stored at room temperature. Swabs collected for transport media should be stored at room temperature according to the manufacturer's recommendations. Specimens of cerebrospinal fluid or blood should be stored at 37 degrees. Specimens should not be refrigerated. Materials collected for isolation of gonorrhea germs should be inoculated immediately after collection.

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.
4. If the specimen is collected on a swab - gently rotate the tip of the swab on a small area of agar just around the edges of the plate, and then inoculate specimen using streak plate method with a sterile loop.
5. Incubate the inoculated plates under aerobic conditions or in CO₂ enriched atmosphere, at 35 ±2°C.
6. Examine for the growth after 18-24 hours of incubation.

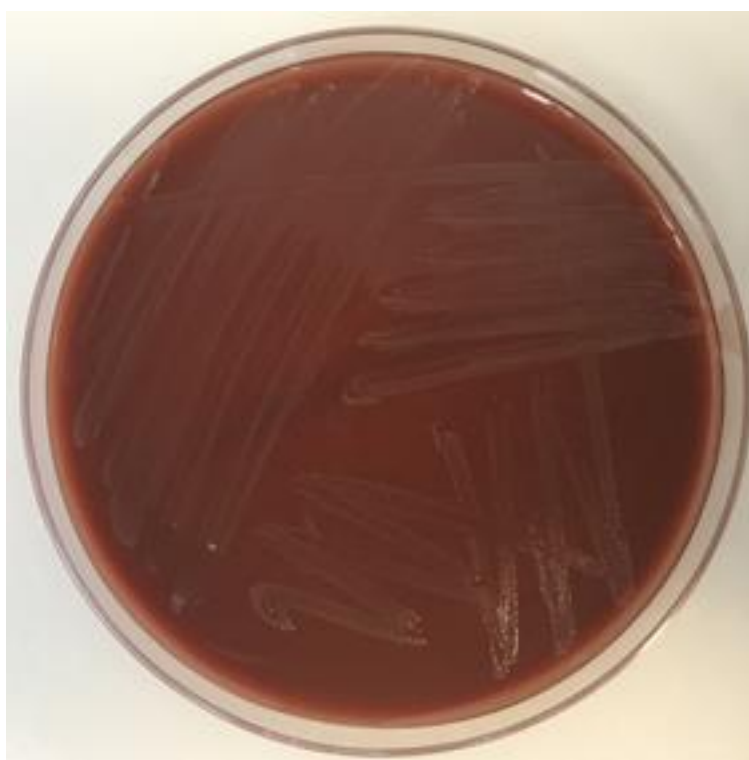
11. Reading and interpretation

After incubation, observe:

- the presence of bacterial colony growth,
- colony morphology.

Typical morphology of bacterial colonies grown on Chocolate Agar:

Microorganism	Typical colony morphology
<i>Haemophilus influenzae</i>	Colonies small, mucoid pearly with a characteristic odor
<i>Neisseria gonorrhoeae</i>	Colonies small, grayish-white, mucoid
<i>Neisseria meningitidis</i>	Medium to large, blue-gray, mucoid colonies
<i>Streptococcus pneumoniae</i>	Colonies small, flat, slimy, greenish, the substrate around the colony may have a greenish coloration



Colony morphology and growth pattern of *Haemophilus influenzae* on Chocolate Agar

For the final identification of cultured microorganisms, additional tests and/or confirmatory tests should be carried out using other methods used in the laboratory.

12. Quality control

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

Reference strain:	Growth intensity:	Colonies morphology:
<i>Haemophilus influenzae</i> ATCC 49766	good growth	small, mucoid, pearly
<i>Streptococcus pneumoniae</i> ATCC 49619	good growth	small, flat, mucous, greenish with discoloration of the substrate around the colony
<i>Neisseria meningitidis</i> ATCC 13090	good growth	medium to large, blue-gray, mucoid
<i>Neisseria gonorrhoeae</i> ATCC 43069	good growth	small, grayish-white, mucoid

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 the nutritional value of the medium, some strains may grow poorly or not at all on Chocolate Agar.
- Depending on the type of sample collected and the stability of the pathogen sought, appropriate conditions for transporting the specimen to the laboratory should be used.
- Depending on the type of tested specimen and the pathogen being sought, it is recommended that the specimen be inoculated onto another additional pathogen-specific selective medium.
- For materials suspected of having *Neisseria gonorrhoeae* bacteria, sampling with cotton swabs is not recommended.
- If the test specimen may contain gonococci, it should be inoculated immediately after collection.

14. Characteristics of the method

Chocolate Agar is a basic medium for cultivating fastidious bacteria such as *Neisseria*, *Haemophilus*, *Streptococcus* or others. Currently, there are many variants of the medium available on the market, however, it is still used in routine microbiological diagnostics. The basic version of the medium is very often enriched with a growth-promoting factor (Isovitalex, Biovitex, etc.).

Saha S.K. et al. in the pages of Indian J. Med. Res. studied the effect of the addition of growth-promoting factor to chocolate medium. The reason for the study was the low detection rate of infections caused by *Haemophilus influenzae* type b. Errors in the detection of these microorganisms were cited as the reason for the low isolation rate. We analyzed 194 *Haemophilus influenzae* type b isolates, which were inoculated separately on plates containing chocolate medium variants with and without Isovitalex. The resulting average colony size was only 0.1 cm larger in the medium with an added growth-promoting factor. It was concluded that the addition of substances promoting bacterial growth is of negligible importance for the culture and isolation of this microorganism.

Chocolate Agar is often utilized for isolation of *Neisseria gonorrhoeae*, but in such cases, it is best used with other media to increase the sensitivity of the test. Bonin P. et al. compared three media for the isolation of *Neisseria gonorrhoeae*: Chocolate Agar (CA), modified Thayer-Martin medium (MTM) and selective vancomycin-free medium (VFSM). Of the 326 gonococcal infections of the cervical canal, 92% were detected using CA medium compared to 98.2% using MTM medium. Similarly, of 306 gonococcal infections of the cervical canal, 95.8% of infections were detected using VFSM medium compared to 98.4% using MTM medium. For 1,632 cases of male urethral infections, all three substrates were equivalent and detected more than 98% of infections.

15. Disposal of used material

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

16. Reporting of adverse events

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

17. References






1. Martin, J.E., T.E. Billings, J.F. Hackney, and J.D. Thayer. 1967. primary isolation of *N. gonorrhoeae* with a new commercial medium. *Public Health Rep.* 82:361-363.
2. Vastine, D.W., C.R. Dawson, I. Hoshiwara, C. Yonega, T. Daghfous, and M. Messadi. 1974. Comparison of media for the isolation of *Haemophilus* species from cases of seasonal conjunctivitis associated with severe endemic trachoma. *Appl. Microbiol.* 28:688-690.
3. Ruoff, L.R. 2003. *Aerococcus*, *Abiotrophia*, and other infrequently isolated aerobic catalasenegative, gram-positive cocci. In: Murray, P. R., E. J. Baron, J. H. Jorgensen, M. A. Pfaller, and R. H. Tenover (ed.). *Manual of clinical microbiology*, 8th ed. American Society for Microbiology, Washington, D.C..
4. Thomson, R.B., and J.M. Miller. 2003. specimen collection, transport, and processing: bacteriology. In: Murray, P. R., E. J. Baron, J.H. Jorgensen, M. A. Pfaller, and R. H. Tenover (ed.). *Manual of clinical microbiology*, 8th ed. American Society for Microbiology, Washington, D.C..
5. Forbes, B.A., and P.A. Granato. 1995. processing specimens for bacteria. In P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover, and R.H. Tenover (ed.), *Manual of clinical microbiology*, 6th ed. American Society for Microbiology, Washington, D.C..
6. Murray, P. R., E. J. Baron, J. H. Jorgensen, M. A. Pfaller, and R. H. Tenover (ed.). 2003 *Manual of clinical microbiology*, 8th ed. American Society for Microbiology, Washington, D.C..
7. Saha S.K., Baqui A.H., Darmstadt G.L., Islam M., Arifeen S.E., Santosham M., Nagatake T., Black R.E., Addition of isolvitalax in chocolate agar for the isolation of *Haemophilus influenzae*, *Indian J. Med. Res.*, Jan 2009, 99-101
8. Bonin P., Tanino T.T., Handsfield H.H., Isolation of *Neisseria gonorrhoeae* on selective and nonselective media in a sexually transmitted disease clinic, *J. Clin. Microbiol.*, Feb 1984, 218-20










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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**DEKLARACJA ZGODNOŚCI UE/
EU DECLARATION OF CONFORMITY**



DANE DOTYCZĄCE PRODUCENTA/ MANUFACTURER DETAILS	
Producent/ Manufacturer	Graso Zenon Sobiecki
Adres producenta/ Adress	Krąg 4A 83-200 Starogard Gdański
Numer rejestracyjny producenta/ Manufacturer's registration number	PL-MF-000022437
DANE DOTYCZĄCE WYROBU/ DEVICE DETAILS	
Nazwa produktu/ Product name	Columbia Agar + 5% Sheep Blood
Numer katalogowy/Article number	1190PD90, 1190PD140, 201190
Basic UDI-DI	590470801190PD90NQ
Klasa ryzyka wyrobu, wg reguły/ Risk class and classification rule	klasa A wg Załącznika VIII reguła 5a / class A acc. Annex VIII rule 5a
Ocena zgodności/ Conformity assessment	wg Załącznika IX acc. to Annex IX
OŚWIADCZENIE PRODUCENTA/ MANUFACTURER STATEMENT	
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ZASTOSOWANE WSPÓLNE SPECYFIKACJE, Z KTÓRYMI DEKLARUJE SIĘ ZGODNOŚĆ/ REFERENCES TO ANY COMMON SPECIFICATION USED AND IN RELATION TO WHICH CONFORMITY IS DECLARED
Producent posiada zintegrowany System Zarządzania Jakością zgodny z: The manufacturer implemented integrated quality management system in accordance with:
<u>PN-EN ISO 9001</u> Systemy Zarządzania Jakością. Wymagania. / Quality Management System. Requirements.
<u>PN-EN ISO 13485</u> Wyroby medyczne. Systemy zarządzania jakością. Wymagania do celów przepisów prawnych. / Medical devices. Quality management systems. Requirements for regulatory purposes
DATA I MIEJSCE WYDANIA DEKLARACJI ZGODNOŚCI/ PLACE AND DATE OF ISSUE OF THE DECLARATION OF CONFORMITY
09.11.2023r. Krąg 4A,83-200 Starogard Gdański

Imię i nazwisko / Name :

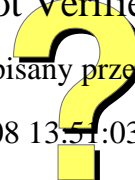
Stanowisko / Position :

Podpis / Signature : (-)

Signature Not Verified

Dokument podpisany przez Zenon Sobiecki;
GRASO

Data: 2023.12.08 13:51:03 CET



COLUMBIA AGAR +5% SHEEP BLOOD

INSTRUCTIONS FOR USE THE READY-TO-USE PLATED MEDIUM

1. Intendent Use

Columbia Agar +5 % Sheep Blood is a non-selective medium used for the qualitative detection of fastidious and non-fastidious bacteria in human clinical specimens and other specimens. Columbia Agar +5% Sheep Blood is the primary medium used for microbiological testing of infections caused by most human pathogens.

The function of the Columbia Agar +5% Sheep Blood medium is to support diagnosis in patients with symptoms indicating potential infections with various pathogenic microorganisms.

Human pathogenic microorganisms belong to various groups of bacteria that cause local infections of tissues and organs, as well as systemic infections. Due to its properties, the medium is used for the detection of most pathogenic microorganisms belonging to different taxonomic groups. These microorganisms include Gram-positive cocci (*Staphylococcus*, *Enterococcus*, *Streptococcus*), Gram-negative bacilli (*Enterobacterales*, *Pseudomonas*, *Acinetobacter*), as well as Gram-positive bacilli (*Corynebacterium*). The presence of blood in the medium makes it possible to determine the type of hemolysis, which is used in the preliminary identification of certain groups of microorganisms, especially members of the genus *Streptococcus*.

Cat. no:	Medium type:	Packaging:
1190PD90	Solid medium on a plate	1x10 pcs (90 mm)

2. Principles of the procedure

The medium contains high-value protein hydrolysates that enable abundant and rapid growth of fastidious microorganisms. Corn starch is energy source that stimulates bacterial growth, absorbs toxic components present in the test specimens and enhances the hemolytic response of some streptococci. Yeast enriched peptone is a source of B vitamins. The presence of sheep blood is a necessary factor for growth of many bacteria. It also allows to determine the type of hemolysis, and enables a preliminary identification of bacteria present in the test specimen.

3. Medium composition

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

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 65 days from the date of production.

9. Specimen type

Human clinical specimens taken mainly from the ears, upper respiratory tract, genital tract, as well as pus and exudative fluids.

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 CO_2 (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

11. Reading and interpretation

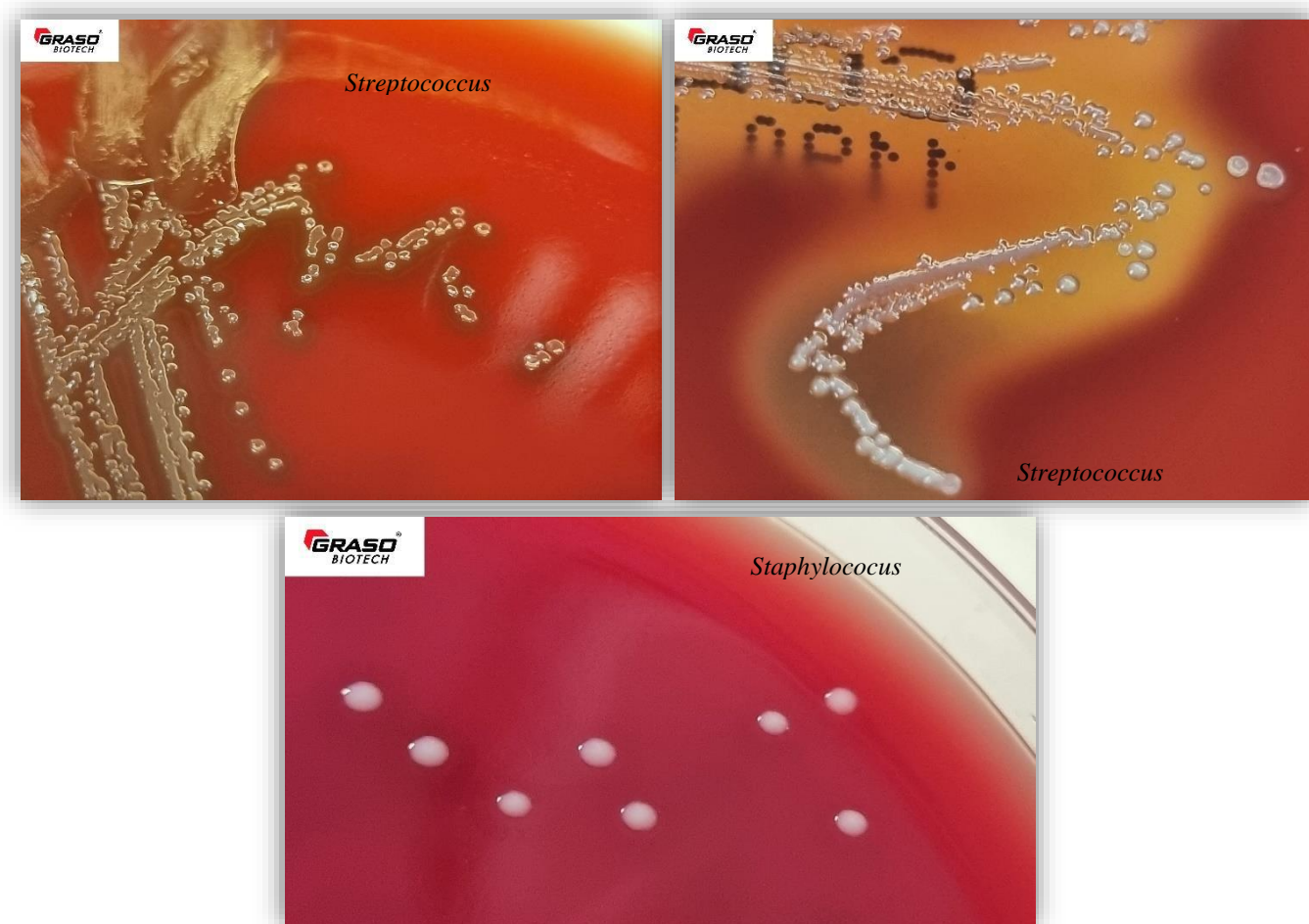
After incubation, observe:

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

Typical morphology of bacterial colonies grown on Columbia 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
Enterobacterales	Medium to large, gray colonies	Colonies with or without hemolysis zone
<i>Candida</i> spp.	Small, white colonies	-

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 microorganisms on Columbia 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>Escherichia coli</i> ATCC 25922	good growth	colonies large, flat, gray, smooth, shiny	possible β -type hemolysis

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 nutritional requirements, some strains may grow poorly or not at all on Columbia Agar +5% Sheep Blood.
- Depending on the origin of the blood used, group D streptococci can exhibit different hemolytic reactions. On media containing horse, rabbit and human blood, they produce β -type hemolysis, while on media with sheep blood they produce α -type 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 procedures specified by the laboratory.
- 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.
- The medium does not contain factor V (nicotinamide adenine dinucleotide, NAD) because sheep blood contains NADase, which destroys NAD. For this reason, *Haemophilus influenzae*, which requires both factor X and factor V to grow, will not grow on this medium.
- Yeast and fungi can grow on the substrate

14. Characteristics of the method

In 1966, Ellner and co-workers presented a multicomponent medium containing blood, which due to the presence of casein hydrolysate and peptones, yielded faster and abundant microbial growth, a stronger and more unambiguous hemolysis reaction, and a more typical colony morphology with better staining. Columbia Agar with blood and vitamin K and hemin is a universal medium used for the isolation and cultivation of all clinically relevant anaerobes and facultative anaerobes. This medium is a recommended medium for the detection of less common bacteria, such as *Bartonella bacilliformis* causing Carrion's disease. 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 those 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.

15. 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.

16. 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.

17. References











1. MiQ - Qualitätsstandards in der mikrobiologisch-infektiologischen Diagnostik, edited by Mauch, H., R. Lüttken, and S. Gatermann for the Deutsche Gesellschaft für Hygiene und Mikrobiologie (DGHM). Volumes 3, 6, and 7. Urban & Fischer, Munich, Germany.
2. Murray, P. R., E. J. Baron, J. H. Jorgensen, M. A. Pfaller, and R. H. Tenover (ed.). 2003 Manual of clinical microbiology, 8th ed. American Society for Microbiology, Washington, D.C..
3. Chapin, K.C., and T.-L. Lauderdale. 2003. reactants, stains, and media. In: Murray, P. R., E. J. Baron, J. H. Jorgensen, M. A. Pfaller, and R. H. Tenover (ed.). Manual of clinical microbiology, 8th ed. American Society for Microbiology, Washington, D.C..
4. Isenberg, H. D. (ed.). 1992. interpretation of aerobic bacterial growth on primary culture media, Clinical microbiology procedures handbook, vol.1, p. 1.6.1-1.6.7. American Society for Microbiology, Washington, D.C..
5. Baron, E. J, L. R. Peterson, and S. M. Finegold. 1994. bailey & Scott's diagnostic microbiology, 9th ed., p. 415. mosby-Year Book, Inc. St. Louis, MO.
6. MacFaddin, J. F. 1985 Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, p. 86-92. Williams & Wilkins, Baltimore, MD.





History of document changes

Date of change	Section	Description of the change
2023/04/13	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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