DEKLARACJA ZGODNOŚCI UE/

EU DECLARATION OF CONFORMITY

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Location: Moldova
MOLDOVA EUROPEANĂ

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	5904708011 90PD90NQ
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

Producent Graso Zenon Sobiecki z siedzibą firmy w miejscowości Krąg 4A, 83-200 Starogard Gdański na swoją wyłączną odpowiedzialność **oświadcza, że:**

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 uchylenia 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./ **The manufacturer Graso Zenon Sobiecki** located in Krąg 4A, 83-200 Starogard Gdański **declares** on his own responsibility that:

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.

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 Zarzadzania Jakością zgodny z: The manufacturer implemented integrated quality management system in accordance with:
PN-EN ISO 9001 Systemy Zarządzania Jakością. Wymagania. / Quality Management System. Requirements.
PN-EN ISO 13485 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

DEKLARACJA ZGODNOŚCI UE/

EU DECLARATION OF CONFORMITY

CE

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	**Mueller Hinton II Agar + 5% Horse Blood + 20 mg/l NAD**
Numer katalogowy/Article number	**1370PD90, 201370**
Basic UDI-DI	590470801370PD90NY
Klasa ryzyka wyrobu, wg reguły/ Risk class and classification rule	klasa B wg Załącznika VIII, reguła 6 / class B acc. to the Annex VIII, rule 6
Ocena zgodności/ Conformity assessment	wg załącznika IX / acc. to Annex IX
DANE DOTYCZĄCE JEDNOSTKI NOTYFIKOWANEJ/NOTIFIED BODIES DETAILS	
Nazwa jednostki notyfikowanej/ Name of the Notified Bodies	-
Numer identyfikacyjny jednostki notyfikowanej/ Notified Bodies ID number	-
Numer wydanego certyfikatu/ Certyficate of conformity number	obowiązek spełnienia wymagania od 26.05.2027 r. obligation to meet the requirement since 26.05.2027 r.
Data ważności wydanego certyfikatu/ Certificate expiration date	obowiązek spełnienia wymagania od 26.05.2027 r. obligation to meet the requirement since 26.05.2027 r.
OŚWIADCZENIE PRODUCENTA/ MANUFACTURER STATEMENT	
Producent Graso Zenon Sobiecki z siedzibą firmy w miejscowości Krąg 4A, 83-200 Starogard Gdański na swoją wyłączną odpowiedzialność **oświadcza, że:** 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 uchylenia 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./	

The manufacturer Graso Zenon Sobiecki located in Krąg 4A, 83-200 Starogard Gdański **declares** on his own responsibility that:

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

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 Zarzadzania Jakością zgodny z:

The manufacturer implemented integrated quality management system in accordance with:

PN-EN ISO 9001 Systemy Zarządzania Jakością. Wymagania. / Quality Management System. Requirements.

PN-EN ISO 13485 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

30.11.2023r. Krąg 4A,83-200 Starogard Gdański

DATA WAŻNOŚCI DEKLARACJI ZGODNOŚCI/

EXPIRATION DATE OF THE DECLARATION

26.05.2027r.

Imię i nazwisko / Name :

Stanowisko / Position :

Podpis / Signature : (-)

Signature Not Verified

Dokument podpisany przez Zenon Sobiecki; GRASO
Data: 2023.12.08 13:32:19 CET

POLISH CENTRE FOR
TESTING AND CERTIFICATION

CERTIFICATE

No. J - 2603/6/2023

This is to certify that:

GRASO Zenon Sobiecki
Krąg 4A, 83-200 Starogard Gdański
in location:
Zakład Produkcji Podłoży Mikrobiologicznych
Owidz, ul. Leśna 1, 83-211 Jabłowo

is in conformance with

PN-EN ISO 9001:2015-10

in the following scope of activities:

- **production, packaging and sales of culture media**
- **distribution of reagents, diagnostic tests and laboratory equipement**

The audit carried out by the Polish Centre for Testing and Certification has afforded evidence of the above.

This Certificate shall remain valid provided
that above standard are respected by the Organization.

This certificate is valid:

from 20.11.2023 to 26.10.2026

MEMBER OF IQNET

PCA Polskie Centrum Akredytacji – Certyfikacja Systemów Zarządzania
AC 019

Member of Multilateral Recognition Arrangement IAF

Aleksandra Kostnerva
President

Issued under the Contract No. 2964/JM/5/2023
Date of certification decision: 20.11.2023
Certificate bears a qualified signature.
Warsaw, 20.11.2023

POLISH CENTRE FOR
TESTING AND CERTIFICATION

CERTIFICATE

No. M - 41-a/6/2023

This is to certify that:

GRASO Zenon Sobiecki
Krąg 4A, 83-200 Starogard Gdański
in location:
Zakład Produkcji Podłoży Mikrobiologicznych
Owidz, ul. Leśna 1, 83-211 Jabłowo

is in conformance with

PN-EN ISO 13485:2016-04

in the following scope of activities:

production and placing on the market of culture media for in-vitro diagnostics

The audit carried out by the Polish Centre for Testing and Certification has afforded evidence of the above.

This Certificate shall remain valid provided
that above standard are respected by the Organization.

This certificate is valid:

from 20.11.2023 to 26.10.2026

MEMBER OF IQNET

PCA Polskie Centrum Akredytacji – Certyfikacja Systemów Zarządzania
AC 019

Member of Multilateral Recognition Arrangement IAF

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Date of certification decision: 20.11.2023
Certificate bears a qualified signature.
Warsaw, 20.11.2023

Polish Centre for Testing and Certification 469 Puławska Street, 02-844 Warsaw phone: +48 22 45 46 200, pcbc@pcbc.gov.pl

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	Sheep blood	50 ml
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		

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 2-12ºC 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 2-12ºC 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 ± 2°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

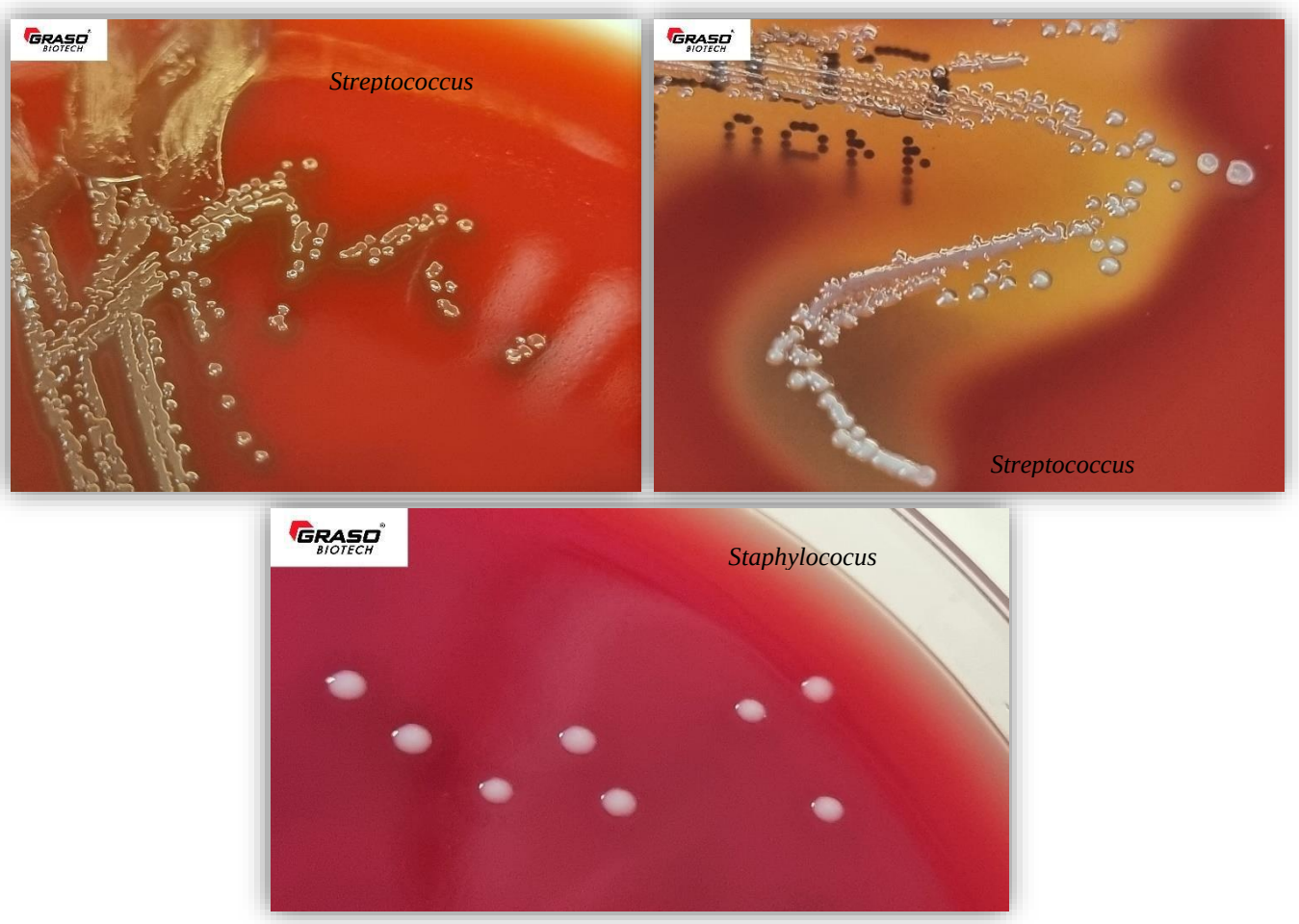
After incubation, observe:

- 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 αor 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
Candida 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:**
Staphylococcus aureus ATCC 25923	good growth	large, white to gray or cream to yellow	β-type
Streptococcus pyogenes ATCC 19615	good growth	small, white to gray,	β-type
Streptococcus pneumoniae ATCC 49619	good growth	very fine, flat, whole-edged	α-type
Escherichia coli 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 ß-type hemolysis, while on media with sheep blood they produce α-type hemolysis.
- The hemolytic response of ß-hemolytic streptococci can be affected by incubation conditions. It is recommended to incubate under conditions of increased CO_2 (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 Carrión'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. Lüttiken, 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. Yolken (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. Yolken (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 culturemedia, 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 useed.	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 addictional 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

GRASO®

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Production Department
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83-211 Jabłowo

IVD CE

MUELLER HINTON II AGAR + HORSE BLOOD + 20mg/L NAD (MH-F).

INSTRUCTIONS FOR USE THE READY-TO-USE PLATED MEDIUM

1. Intended use

Mueller Hinton II Agar + Horse Blood +20mg/L NAD is a medium designed to test drug susceptibility according to EUCAST procedures by the diffusion-agar method, as well as resistance mechanisms of fastidious aerobic bacteria (streptococci) isolated from human clinical specimens.

The function of the medium is to support diagnosis by determining the antimicrobial susceptibility/resistance profile of fastidious bacteria isolated from human clinical specimens.

Information on the drug susceptibility profile and determination of the resistance mechanism of the pathogen detected in the patient's clinical specimen allows to make an appropriate, effective antibiotic therapy, suited to an individual patient.
The EUCAST disk-diffusion method is based on a method described by the International Collaborative Study of Antimicrobial Susceptibility Testing in 1972. Due to its simplicity of implementation, it is the most widely used method of testing bacterial drug susceptibility in medical laboratories. Correct, standardized performance of the test according to the EUCAST method and obtaining reliable results requires the use of this method without modification, including the use of the medium specified by EUCAST.
According to EUCAST guidelines, Mueller Hinton II Agar + Horse Blood + 20mg/L NAD is a medium designed for determining the drug susceptibility profile of fastidious bacteria, especially *Streptococcus* spp. (including *S. pneumoniae*), *Haemophilus influenzae, Moraxella catarrhalis, Listeria monocytogenes, Campylobacter jejuni and coli, Pasteurella multocida, Corynebacterium spp, Aerococcus sanguinicola and urinae and Kingella kingae.*

Cat. no:	**Medium type:**	**Packaging:**
1370PD90; 201370	Solid medium on a plate	1x10 pcs (90 mm)

2. Principle of the procedure

Beef extract and acid casein hydrolysate are sources of nitrogen, vitamins, carbon and amino acids. Corn starch absorbs toxic metabolic products. Defibrinated horse blood and NAD enable the growth of fastidious bacteria. Agar is a solidifying agent. Drug susceptibility testing of microorganisms should be performed by the disk-diffusion method in accordance with the current EUCAST guidelines.
The disk-diffusion method that uses the phenomenon of a concentration gradient of an antimicrobial drug forming in a solid medium, as a result of its diffusion from a blotting paper disk, which inhibits the growth of microorganisms around the disk. The diameter of the zone of inhibited growth, measured in millimeters, is compared with the corresponding limit value specified in the relevant EUCAST guidelines. The result of this comparison allows the tested microorganism to be classified into the appropriate susceptibility category to the specific antimicrobial drugs used in the test. In order to determine the mechanism of resistance, disks containing specific antimicrobial drugs are arranged on the medium. The characteristic size and shape of the zones inhibition of bacterial growth on the medium allows to determine the presence and type of resistance mechanism of the tested pathogen.

3. Medium composition

In g/l distilled water:		**Supplements/Liter:**	
Casein peptone	17,5 g	Horse blood	50 ml
Corn starch	1,5 g	NAD	0.02 g
Beef extract	2,0 g		

Agar	17,0 g	

pH 7.3± 0.1 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

Equipment and reagents necessary for the test (e.g., saline, sterile swabs, antibiotic-soaked blotting paper disks) and standard microbiological laboratory equipment, including a bacteriological densitometer or density standard, an incubator and a ruler or another devices for measuring the zones of inhibited growth.

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 and the EUCAST procedure.
- 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 2-12ºC until the expiration date. Store plates in their original packaging in an inverted position, away from direct light sources. To avoid freezing of agar, do not store plates close to the refrigerator walls. To avoid 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 2-12ºC retains its properties for up to 45 days from the manufacture date.

9. Specimen type

The material for the tests are pure (about 16-24 hours) culture of pathogenic strain of fastidious bacteria isolated from human clinical specimens, or other samples inoculated onto solid medium.

10. Test procedure

Current EUCAST procedures and guidelines must be strictly followed, to ensure correct, reliable disk-diffusion susceptibility testing results

1. Allow the medium to warm to room temperature before inoculation.

2. Inoculum preparation

Prepare a 0.5 McFarland suspension of the test strain by suspending colonies of the strain in saline solution.
Collect colonies with a sterile loop or swab from non-selective medium, after 18-24 hours of incubation.

Select a few morphologically similar colonies. Determine the density of the inoculum using a bacteriological densitometer. The density of the suspension can also be determined by macroscopically comparing the density of the test strain's suspension with a 0.5 McFarland density standard. In this case, the turbidity of the test strain's suspension to the density standard should be compared on a white background with black stripes.

The prepared suspension of the test strain should be used within 15 minutes, and no later than 60 minutes after preparation.

3. **Preparation of bacterial lawn**

Dip a sterile cotton swab into the prepared suspension of the test strain. For Gram-negative bacteria, to avoid excessive inoculation, remove the excess suspension from the swab by pressing it against the inside of the tube. For Gram-positive bacteria, there is no need to press the swab against the inside of the tube. Media can be inoculated manually or with an automatic inoculator. Spread the suspension evenly over the entire agar surface, making sure there are no gaps between each band, which is especially important for Gram-positive bacteria.

4. **Apply antibiotic disks**

Apply antibiotic paper disks to the agar surface. The disks should be applied to the medium within 15 minutes of inoculation. Press the disks lightly, as they should completely adhere to the agar surface. Once applied, the disks must not be moved, due to the rapid diffusion of the antibiotic from the disk into the medium. The number of disks on the plate should be limited, so that the resulting zones of inhibition do not overlap and individual antibiotics do not interact with each other. A maximum of 6 antibiotic disks can be applied to a 90 mm diameter plate.

5. Incubation

Incubate plates under aerobic conditions at the temperature and for the time specified in EUCAST manual, depending on the type of tested microorganism. The plates should be in an inverted position (agar side up), while making sure that the antibiotic disks have not fallen off the agar surface. Incubation of the plates should begin within 15 minutes of applying the disks. The plates should not be incubated for a period longer than recommended.

Detailed guidelines for the selection of antibacterial drugs and the performance of drug susceptibility testing by the disk-diffusion method are available in current EUCAST manuals.

11. Reading and interpretation

After incubation, measure the size of the inhibition zones using a calibrated instrument such as a ruler or caliper, or use an automatic system to measure the size of the inhibiting zone.
Interpret the obtained results based on current EUCAST guidelines.

After incubation:

- Examine plates for the bacterial lawn.
- Measure the diameter or inhibition zones according to current EUCAST guidelines using a ruler or other measuring instrument in millimeters.
- Interpret the obtained measurements based on current EUCAST guidelines, assigning the appropriate drug susceptibility category for each antimicrobial drug used.

When testing a pathogen for resistance mechanisms, assess the size and characteristic shape of inhibited bacterial growth should also be assessed.

12. Quality control

Perform medium quality control at a frequency and in a manner consistent with current EUCAST procedures for quality control of the disk-diffusion method and laboratory procedures.
Reference strains that ensure measurement consistency in accordance with EUCAST procedures should be used to perform quality control tests.

13. Limitations of the method

- Numerous factors can affect the propriety of the size of zones of inhibited growth and the results of the tests.

- Numerous factors can affect the size of inhibition zones and the results of drug susceptibility testing, such as bacterial suspension density, growth rate, medium composition and pH.
- Drug susceptibility testing by the disk-diffusion method should be performed only with pure bacterial cultures, around 16-24 hours old.
- If the inoculum density is too high, it can reduce the diameter of inhibition zones, and if it is too low, it can increase the size of growth inhibition zones and cause difficulties in measuring them.
- Leaving inoculated plates at room temperature for longer than the recommended period before applying the antibiotic disks may cause microbial proliferation, resulting in a decrease in the diameters of the zones of inhibition. Therefore, it is important to follow the 15-15-15 rule: the suspension should be used within 15 minutes of preparation, the disks should be applied within 15 minutes of inoculation, and plate incubation should begin within 15 minutes of disk application.
- Improper storage of antibiotic disks can affect the stability of the tested antibiotics in them, which can reduce the diameter of the zones of inhibition and can be a source of interpretive errors in assessing the drug susceptibility of the pathogen under study.
- An important factor affecting the test result is the arrangement of stacks of inoculated plates in the incubator and if it allows the heat to speed evenly. A maximum recommended number of plates in a stack is 5.
- Excessive shrinkage of the medium, due to improper storage can lead to false results.
- Improper arrangement of antibiotic disks to test on the medium may result in false results

14. Characteristics of the method

Presented in EUCAST documents and available literature.

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 medium described in this manual must be reported to the manufacturer and to the competent authorities.

17. References

History of document changes

Date of change	Section	Description of the change
2023/02/15	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 date when the medical device was manufactured.	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 addictional 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