FASTIDIOUS ANAREOBE AGAR (FAA)

INSTRUCTION FOR USE READY-TO-USE PLATES

1. Intended use

Fastidious Anareobe Agar (FAA) is used specifically for the susceptibility testing of anaerobic bacteria by the disk diffusion method. This formula conforms to EUCAST.
The function of the medium is to support the diagnostic process by determining the profile of drug susceptibility to antibacterial drugs of microorganisms isolated from clinical samples of human origin or other materials. Information about the drug susceptibility profile and the detected resistance mechanisms of the pathogen detected in clinical materials allows the physician to make the right decision regarding the selection of an appropriate antibiotic therapy.

Ref .	**Type of medium:**	**Packaging:**
201371	ready-to-use medium-plate	1x10 pcs (90 mm)

2. Principle:

The peptones included have been chosen for maximum growth stimulation. Starch and sodium bicarbonate act as de-toxification agents while hemin encourages pigment production in *Porphyromonas melaninogenicus*. Specific growth promoting agents are cysteine for *Fusobacterium necrophorum*, *Propionibacterium acne* and *Bacteriodes fragilis,* arginine for *Eubacterium* spp. soluble pyrophosphate for *Porphyromonas gingivalis* and *Porphyromonas asaccharolyticus*. Pyruvate helps neutralize hydrogen peroxide and is also utilized by *Veillionella* spp. as an energy source. Vitamin K and sodium succinate provide essential growth factors for some anaerobes as does the 0.1% glucose. The low level of glucose prevents the production of high levels of acids and alcohols which would inhibit colonial development.

3.Formula

Formula/Liter:		**Supplements / Liter:**	
Peptones mix	23.0 g	Horse blood	50 ml
Sodium chloride	5.0 g		
Soluble ctarch	1.0 g		
Agar	12.0 g		
Sodium bicarbonate	0.4 g		
Glucose	1.0 g		
Sodium puryvate	1.0 g		
Cysteine HCl monohydrate	0.5 g		
Hemin	0.01 g		
Vitamin K	0.001 g		
L-Arginine	1.0 g		
Soluble pyrophosphate	0.25 g		
Sodium succinate	0.5 g		

pH 7,3 ± 0,2 at25°C.
Prepared Appearance: prepared medium is homogenous and red.

4. Preparation

The medium is ready to use. Before inoculation allow to warm to room temperaturę.

5. Equipment required, not provided

Equipment and reagents necessary to perform the test (e.g. saline, swabs, discs impregnated with antibiotics), sachets and generators for the generation of an anaerobic atmosphere and general laboratory microbiological equipment, including a bacteriological densitometer or a density standard and a laboratory 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 the medium should be handled in accordance with the principles of working with potentially infectious biological material.
• Plates should not be used if the substrate shows signs of microbial contamination, discoloration, drying out, cracks or other signs of deterioration.
• Do not use damaged plates.
• Do not use hemolyzed plates.
• Do not use plates after the expiry date.
• Re-incubation of previously inoculated plates is not allowed.
• Verify that anaerobic conditions have been achieved.
• Bring the antibiotic disks to room temperature before use.
• Do not use poorly stored antibiotic discs or their expiry date.
• Use the appropriate number of antibiotic discs to prevent overlapping zones of growth inhibition.
• Follow the instructions and EUCAST guidelines to ensure correct results.
• In the event that the handling of the medium during the test is different from that described in these instructions, the laboratory is obliged to validate the adopted procedure.

7. Storage
On receipt, store plates at 2-12°C away from direct sun light in an inverted position. Do not overload a refrigerator with excessive amounts of plates to avoid water condensation on the lids during storage. Plates must not come into direct contact with the inner walls of refrigerator, as the media may freeze, invalidating the tests. Prepared plates, stored in their original sleeve wrapping at 2-12°C until just prior to use, may be inoculated up to the expiration date and incubated for recommended incubation times. Plates from opened stacks of 10 plates should be used for two weeks when stored in a clean area at 2 to 12° C. Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking or others signs of deterioration. Allow the medium to warm to the room temperature before inoculation

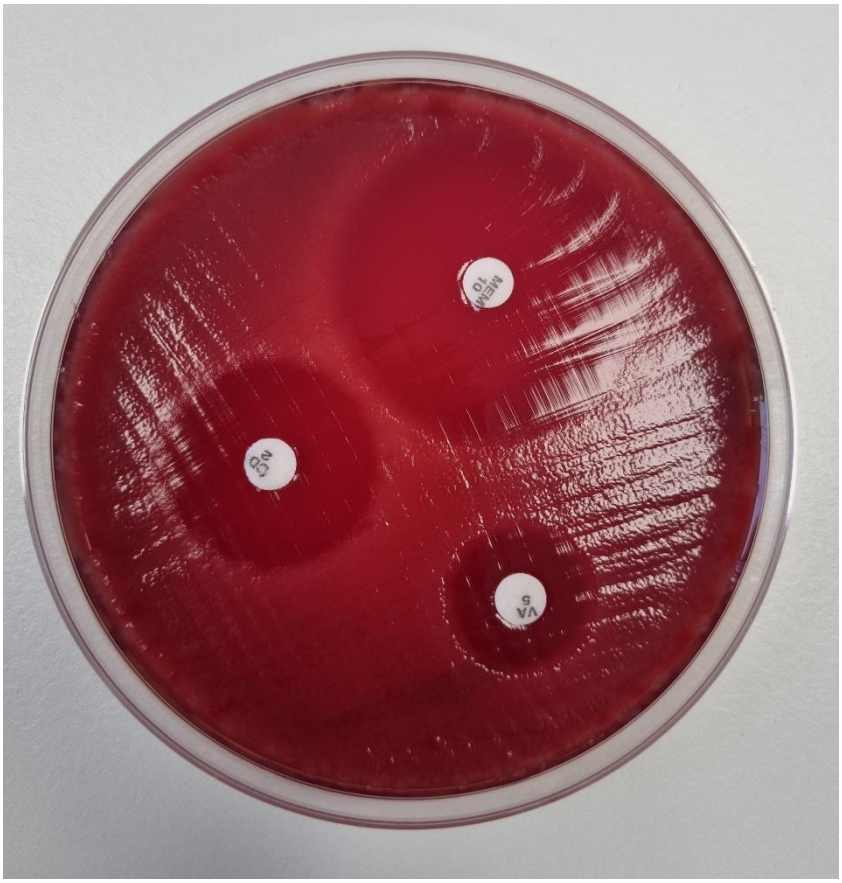
8. Shelf life:
The medium stored at 2–12ºC maintains its properties for up to 45 days from the date of production.

9. Samples
The sample is pure, fresh (approx. 16-24 hours) strain cultures isolated from human clinical specimens or other specimens inoculated onto solid media.

10. Test procedure
If the agar plate has been refrigerated, allow to warm to room temperature before inoculation. Prepare of 1,0 (0,9-1,1) McFarland suspension of tested microorganism. Within 15 minutes, dip a sterile swab into the suspension, squeeze it against the walls of the tube to remove excess liquid, then streak it over the surface of the agar plates to obtain a uniform distribution of the inoculum. Leave the plates to dry then lay the paper discs pressing them onto the surface of the agar. Incubate at 35-37°C for 16-20 hours then read the inhibition zones by taking in to consideration the zones, which are completely free of microbial growth and which have distinct borders.

https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Anaerobic_bacteria/2022_anaerobes/EUCAST_disk_diffusion_methodology_for_rapidly_growing_anaerobic_bacteria_with_QC_v_1.0_2022.pdf

11. Results
After incubation time read the zones around antibiotic discs. Compare the zone sizes obtained to those reported on the tables of the EUCAST.



Inhibition zone using a suspension of the reference strain *Clostridium perfringens* ATCC 13124

12. Quality control

Quality control of the medium should be performed with the frequency and in accordance with the current EUCAST guidelines for the quality control of the disc diffusion method and the procedures in force in the laboratory.
Reference strains ensuring traceability in accordance with the EUCAST guidelines should be used to perform the quality control studies.

13. Precautions:
• Leaving the inoculated plates prior to applying the discs longer at room temperature may cause microbial growth, resulting in a reduced diameter of the zones of inhibition. Therefore, it is important to follow the 15-15-15 rule: use the suspension within 15 minutes of preparation, apply the discs within 15 minutes of inoculating the plate, and start incubating the plates within 15 minutes of applying the discs.
• Improper storage of antibiotic discs can affect the stability of the antibiotics they contain, which can reduce the diameter of the zones of growth inhibition and is a common source of misinterpretation.

14. Characteristics of the method
Refer to EUCAST guidelines and available literature.

15. Disposal of waste
After use, all plates and any other contaminated materials must be sterilized or disposed of in line with appropriate internal procedures and in accordance with local legislations. Plates can be destroyed by autoclaving at 121°C for at least 20 minutes.

16. Reporting of adverse events

In accordance with the applicable regulations, adverse events and incidents that can be directly related to the described substrate should be reported to the producer and competent authorities.

17. Literature

1. EUCAST Disk Diffusion Method for Antimicrobial Susceptibility Testing. Search for latest version at http://www.eucast.org.
2. The European Committee on Antimicrobial Susceptibility Testing. Routine and extended internal quality control for MIC
3. Baker, C.N., Thornsberry, C. and Hawkinson R.W. Inoculum standardization in antimicrobial susceptibility testing: evaluation of overnight agar cultures and the rapid inoculum standardization system. J. Clin. Microbiol. 1983: 17:450-457. 11. The European Committee on Antimicrobial Susceptibility Testing. Reading guide. EUCAST disk diffusion method for antimicrobial susceptibility testing. Search for latest version at http://www.eucast.org.
4. EUCAST Quality Control, http://www.eucast.org

Document change history

Data changes	Section	Description of the change
2022/03/04	Entire document	Adaptation to the requirements of the Regulation UE 2017/746

SYMBOL	SYMBOL NAME	DESCRIPTION	NR REF.
	Manufacturer	Means the manufacturer of a medical device as defined in EU Directives 90/385 / EEC, 93/42 / EEC and 98/79 / EC.	5.1.1
	date of production	Indicates the date of manufacture of the medical device.	5.1.3
	Ref No.	Indicates the product number in the manufacturer's catalog that allows the identification of a medical device.	5.1.6
	Lot number / batch code	Indicates a manufacturer's batch number that allows to identify the batch of products to which the medical device belongs.	5.1.5
	In vitro diagnostic	Means an in vitro diagnostic medical device	5.5.1
	Do not re-use	Means a medical device that is intended for single use or for use in treating a single patient in a single medical procedure.	5.4.2
	Enough to do <n> tests	It means the value given by the manufacturer for how many tests the product is sufficient for.	5.5.5
	Expiry date	Indicates the date after which the medical device should not be used.	5.1.4
	Required storage temperature	Indicates the maximum and minimum temperature value at which an item should be stored, transported or used.	5.3.7
	Safety symbol (Compliance with EU requirements)	The CE marking on the product is the manufacturer's declaration confirming the product's compliance with the	Na.

CE		essential requirements of the relevant EU regulations on health, safety and environmental protection.	
	Consult instructions for use	Indicates the need to read the instructions for use	5.4.3
	Sterilized by aseptic processing techniques	Indicates a medical device that has been manufactured with accepted aseptic techniques.	5.2.2
	Do not use with damaged packaging	Indicates a medical device that should not be used if the packaging is damaged or opened.	5.2.8

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