BACTEROIDES BILE ESCULIN AGAR

INSTRUCTIONS FOR USE THE READY-TO-USE PLATED MEDIUM

1. Intended use

Bacteroides Bile Esculin Agar with bile, esculin and gentamicin is a selective medium intended for qualitative detection and preliminary identification of *Bacteroides fragilis* in human clinical specimens and other specimens.
It is a selective medium to support diagnosis of suspected anaerobic bacterial infections. It is typically in the process of diagnosing infections located in organs characterized by rich anaerobic flora such as intra-abdominal infections, gastrointestinal infections, reproductive tract infections or skin and soft tissue infections.
Bacteroides fragilis species are a natural component of the gastrointestinal microbiome, and also occur in genitourinary organs and in the mouth, throat and upper respiratory tract. They are most often isolated from endogenous infections. They can affect virtually any organ or tissue which provides suitable conditions for these anaerobes.

Cat. no:	**Medium type:**	**Packaging:**
1039PD90; 201039	Solid medium on a plate	1x10 pcs. (90 mm)

2. Principle of the procedure

Brucella Agar and proteose peptone provide a source of nitrogen and vitamins that are necessary for the growth of fastidious anaerobic microorganisms such as *Bacteroides* spp. Esculin and ferric ammonium citrate are used to determine esculin hydrolysis. Bile salts stimulate the growth of *Bacteroides spp. fragilis* while inhibiting the growth of other anaerobic bacteria. Gentamicin inhibits the growth of most facultative anaerobic bacteria.

3. Medium composition

In g/l distilled water:	
Brucella Agar	45,5 g
Pepton proteose	10,0 g
Esculin	1,0 g
Ferric ammonium citrate	0,5 g
Oxbile	20,0 g
Gentamicin	0,1 g

pH: 7.0± 0.2 at 25° C.

Appearance of the medium - Clear, straw.

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 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 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 appearance of 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 2-12ºC retains its properties up to 3 months from the date of production.

9. Specimens type

Human clinical specimens and other specimens
Collect test samples in accordance with current guidelines. Store samples until delivery to the laboratory, in accordance with current guidelines for storage and transport of biological material implemented by the laboratory. Store swabs, aspirates, pus, serum fluids and other materials collected in transport media at room temperature, following the manufacturer's recommendations. Inoculate the sample as soon as possible after delivery to the laboratory.

10. Test procedure

- Allow the medium to warm to room temperature before inoculation.
- Inoculate the specimen by spreading it directly on the agar surface.
- 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.
- Incubate the inoculated plates under anaerobic conditions at 35 ± 2°C.
- Examine for growth after 24-48 hours of incubation.

11. Reading and inetrpretation

After incubation observe:
- the presence of bacterial colony growth,
- colony morphology,
- colony coloration.

Typical morphology of colonies grown on Bacteroides Bile esculin Agar:

Microorganism	**Typical colony morphology/medium colour**
Bacteroides fragilis	grey, circular, entire edged, black or dark brown coloration of surrounding medium (esculin hydrolysis)
Bacteroides ovatus	grey, circular, entire edged, black or dark brown coloration of surrounding medium (esculin hydrolysis)
Bacteroides thetaiotaomicron	grey, circular, entire edged, black or dark brown coloration of surrounding medium (esculin hydrolysis)
Clostridium perfringens	No growth
Escherichia coli	No growth or very weak growth
Proteus mirabilis	No growth or very weak growth

Colony morphology and growth pattern of *Bacteroides fragilis* on Bacteroides Bile esculin Agar

12. Quality control

The nutritional properties and selectivity of the medium should be checked using reference strains giving the expected positive and negative 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 medium quality control:

Reference strain:	**Colonies morphology:**	**Growth intensity:**
Bacteroides fragilis ATCC 25285	grey, circular, entire edged, black colour of medium around of colony	good growth
Proteus mirabilis ATCC 12453	-	no growth

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 Bacteroides Bile Esculin Agar.

14. Characteristics of the method

Bacteroides Bile Esculin Agar is designed for the isolation and presumptive identification of *Bacteroides fragilis* Group. Growth in the presence of 20% bile and the ability to hydrolyse esculin, expressed by the blackness of the medium, allows for the presumptive identification of *Bacteroides fragilis* Group. Gentamicin and bile salts inhibit the growth of most organisms other than *Bacteroides* that can tolerate the presence of bile in the medium.
Livingston S.J. et al. in J. Clin. Microbiol. examined 160 isolates of *Bacteroides fragilis* Group. 159 of them grew well and 157 blackened the medium. Other anaerobes, *Enterobacterales* or enterococci, did not grow on the medium or did not show the characteristic morphology and blackening of the medium typical of *Bacteroides fragilis* Group. 687 patient samples were tested in clinical trials. From 81 (11.8%), *Bacteroides fragilis* Group was cultured after 24 and 48 hours of incubation. The use of Bacteroides Bile Esculin Agar allows for rapid cultivation and identification of *Bacteroides fragilis* Group.

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 must be reported to the manufacturer and to the competent authorities.

17. References

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History of document changes

Date of change	Section	Description of the change
2023/03/03	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 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