INSTRUCTIONS FOR USE

SUPPLEMENTED BRUCELLA AGAR

Ready-to-use plates

Supplemented Brucella Agar:
Bacteroides fragilis and cefoxitin gradient strip

1 - INTENDED USE

In vitro diagnostic device. Culture medium for quantitative determination of susceptibility of anaerobes to antimicrobial agents by gradient-based strips.

2 - COMPOSITION - TYPICAL FORMULA *

Tryptone	10.0 g
Peptone	10.0 g
Yeast extract	2.0 g
Glucose	1.0 g
Sodium chloride	5.0 g
Sodium bisulphite	0.1 g
Agar	15.0 g
Vitamin K1	1.0 mg
Haemin	5.0 mg
Lysed horse blood	50.0 mL
Purified water	1000 mL

* The formula may be adjusted and/or supplemented to meet the required performances criteria.

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

The reference tests for antimicrobial susceptibility testing of anaerobic bacteria are the agar dilution and the broth microdilution methods devised by the Clinical and Laboratory Standards Institute (CLSI).[1]
According to the international guidelines, the susceptibility testing of anaerobic bacteria is very expensive, time consuming and requires experienced laboratory staff.[2] To overcome these limitations some methods based on gradient strip testing have been developed and are commercially available for the laboratory. Such tests are based on the diffusion of a stable concentration gradient of an antimicrobial agent from a plastic or paper strip onto an agar medium.
The medium recommended for the detection of MIC's of anaerobes with gradient based strips, is Brucella Agar supplemented with vitamin K1, haemin and lysed horse or sheep blood.[3] Supplemented Brucella Agar derives from the formulation recommended by CLSI for the broth microdilution assays[1] and contains lysed horse blood.
In Supplemented Brucella Agar, peptones and yeast extract, together with glucose, supply nitrogen, carbon and vitamins for microbial growth. Lysed horse blood provides additional nutrients. Sodium bisulphite lowers the redox potential to values suitable for strict anaerobes. Both haemin and vitamin K1 increase growth of certain anaerobic bacteria.
Comparative studies of the gradient strip method performances against CLSI agar dilution method have shown generally high categorical agreement across many anaerobic species with a variety of antibiotics.[4-8] Gradient strip method can be also used for the detection of hetero-resistance (e.g., in the case of imipenem resistance of *B. fragilis* or in the case of metronidazole resistance of *C.difficile* and *B.fragilis*).[2,9,10]

4 - PHYSICAL CHARACTERISTICS

Medium appearance	red, transparent
Final pH at 20-25 °C	7.2 ± 0.2

5 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Supplemented Brucella Agar	Ready-to-use plates	549850	2 x 10 plates ø 90 mm primary packaging: 2 cellophane sachets secondary packaging: cardboard box

6 - MATERIALS REQUIRED BUT NOT PROVIDED

Sterile loops and swabs, incubator and laboratory equipment as required, gradient-based strips with antimicrobial agents.

7 - SPECIMENS

Supplemented Brucella Agar must be used with pure culture of anaerobes isolated from clinical specimens. Supplemented Brucella Agar is not intended for microbial isolation directly from clinical specimens.

8- TEST PROCEDURE

Allow plates and antimicrobial gradient strips to equilibrate to room temperature. The surface of the agar should be dry before use.
Guidelines for inoculums preparation:
Prepare a bacterial suspension in Brucella Broth or Mueller Hinton Broth with a turbidity equivalent to 1 McFarland.
Dip a sterile cotton swab into the suspension and remove excess fluid by pressing and turning the swab against the inside of the tube.
Plates can be inoculated either by swabbing in three directions or by using an automatic plate rotator. Spread the inoculum evenly over the entire agar surface ensuring that there are no gaps between streaks.
As soon as the inoculum has been absorbed and the agar surface is dry, apply the gradient-strips. Make sure that the strips are in complete contact with the agar surface and must not be moved once they have been applied as the initial diffusion of antimicrobial agents

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from strips is very rapid. The strips should be placed on the agar plate in a manner which does not result in overlapping zones of inhibition.
Guidelines for incubation:
Incubate at 35 ± 2°C for 24-48-72 hours (depending on the species) in anaerobic atmosphere (80-85% N_2/10% CO_2/10% H_2).
For the details of inoculation and incubation procedures consult the gradient strips manufacturer's package insert.

9 - READING AND INTERPRETATION

After incubation, read the plates from the front with the lid removed and with reflected light.
A correct inoculum and satisfactorily streaked plates should result in a confluent lawn of growth. If individual colonies can be seen, the inoculum is too light and the test must be repeated.
The growth should be evenly distributed over the agar surface to achieve a uniformly inhibition ellipse.
Check that inhibition zones for quality control strain are within acceptable range.
Determination of the MIC is at the point at which the lower part of the bacterial growth ellipse intersects with the corresponding number on the test strip.
For specific reading and interpretation instructions consult the gradient strips manufacturer's package insert.

10 - USER QUALITY CONTROL

All manufactured lots of Supplemented Brucella Agar plates are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. For QC organisms testing, details are described in the strips manufacturer's package insert. At a minimum, at least one QC strain should be tested to ensure proper product functionality.[4]
A strain and the gradient strip useful for the quality control is: *B.fragilis* ATCC 25285 / metronidazole.
Refer to interpretation guidelines provided by the strips manufacturer's package insert.

ATCC is a trademark of American Type Culture Collection.

11 - PERFORMANCES CHARACTERISTICS

Prior to release for sale a representative sample of all lots of ready-to-use plates of Supplemented Brucella Agar is tested by Antimicrobial Sensitivity Testing, performed according to the gradient strips manufacturer's package insert with the following strains and gradient strips: *B.fragilis* ATCC 25285 / metronidazole, cefoxitin, benzylpenicillin, imipenem.
After incubation at 35-37°C in anaerobic atmosphere for 48 hours, MICs are read where the inhibition ellipse intersects the MIC scale zones and the measures are recorded and evaluated to be within the quality control ranges reported by strips manufacturer's package insert.

12 - LIMITATIONS OF THE METHOD

- Incorrect inoculum concentration, improper storage of antimicrobial strips, improper storage of the plates resulting in an agar depth and pH out of the specifications, excessive moisture, improper measurement of endpoints, may produce incorrect results.
- The inoculation, incubation and reading methods here described are to be considered as guidelines; strict adherence to the protocol suggested by the gradient strips manufacturer is required to ensure reliable results.
- Pre-reduction of test plates in an anaerobic environment overnight prior to use decreases the likelihood of false resistance.[4]
- Reading and interpretation require expertise and close adherence to the gradient strips manufacturer's instructions; Occasionally, certain antibiotic/bacterium combinations may give unusual results. In these cases, judgement of the MIC endpoint may be difficult for inexperienced personnel. [3]
- This culture medium is intended as an aid in the treatment of infectious diseases; the interpretation of the results must be made considering the patient's clinical history, the origin of the sample and the results of other diagnostic tests.

13 - PRECAUTIONS AND WARNINGS

- Supplemented Brucella Agar is a qualitative *in vitro* diagnostic, for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- Supplemented Brucella Agar is not classified as dangerous according to current European legislation.
- This culture medium contains raw materials of animal origin. The *ante* and *post mortem* controls of the animals and those during the production and distribution cycle of the raw materials, cannot completely guarantee that this product does not contain any transmissible pathogen. Therefore, it is recommended that the ready-to-use plates be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes. Download the TSE Statement from the website www.biolifeitaliana.it, describing the measures implemented by Biolife Italiana for the risk reduction linked to infectious animal diseases.
- All laboratory specimens should be considered infectious.
- Each plate of this culture medium is for single use only.
- Ready-to-use plates are not to be considered a "sterile product" as they are not subject to terminal sterilization but a product with controlled bio contamination, within the limits of defined specifications reported on the Quality Control Certificate.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the plates inoculated with samples or microbial strains in accordance with current local legislation.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- The Certificates of Analysis and the Safety Data Sheet are available on the website www.biolifeitaliana.it.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

14 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store plates in their original pack at 2-8°C away from direct light. If properly stored, the plates may be used up to the expiration date. Do not use the plates beyond this date. Plates from opened plastic sachet can be used for 7 days when stored in a clean

area at 2-8°C. Do not use the plates if the plastic sachet is damaged or if the dish is broken. Do not use the plates with signs of deterioration (e.g., microbial contamination, dehydration, shrinking or cracking of the medium, atypical colour, excess of moisture).

15 – REFERENCES

1. Clinical and Laboratory Standards Institute. Methods for antimicrobial susceptibility testing of anaerobic bacteria; approved standard, 8th ed. CLSI document M11-A9, . Clinical and Laboratory Standards Institute, Wayne, Pa. 2012.
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8. Poulet P, Duffaut D, Lodter JP. Evaluation of the Etest for determining the in-vitro susceptibilities of Prevotella intermedia isolates to metronidazole J Antimicrob Chemother 1999;43(4):610-1.
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TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	IVD *In vitro* Diagnostic Medical Device	Manufacturer	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	For single use only	Fragile, handle with care

REVISION HISTORY

Version	Description of changes	Date
Instructions for Use (IFU) - Revision 1	Updated layout and content in compliance with IVDR 2017/746	2021/01
Revision 2	Removal of obsolete classification	2023/03

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

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