BRUCELLA MEDIUM BASE

Dehydrated culture medium

1 - INTENDED USE

General purpose basal medium for the cultivation of Brucella.

2- COMPOSITION

TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER) *

Peptone	10 g
Beef extract	5 g
Glucose	10 g
Sodium chloride	5 g
Agar	15 g

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

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Brucellosis is a widespread zoonotic disease, transmitted mainly from ruminants to humans. It is a disease of major public health importance, animal welfare and economic significance worldwide.[1]

Brucella Medium Base can be used to prepare the glucose serum antibiotics medium described by Jones and Brinley Morgan, and recommended by the WHO for the selective isolation of *Brucella*, including fastidious strains, and *Brucella abortus* type II, which is very difficult to grow on common media.[2] Brucella Medium Base may be used for the preparation of Farrell's medium (FM)[3] for the isolation of *B.abortus* from contaminated samples by supplementation with foetal bovine serum 5%, polymyxin B 5 mg/L, bacitracin 25 mg/L, natamycin 50 mg/L, nalidixic acid 5 mg/L, vancomycin 20 mg/L and nystatin 17,7 mg/L.

Since nalidixic acid and bacitracin contained in various selective media can inhibit the growth of some *Brucella* strains[4] a medium with foetal bovine serum 5%, natamycin 20 mg/L, amphotericin B 4 mg/L, vancomycin 20 mg/L, nystatin 17.7 mg/L, colistin 7,5 mg/L and nitrofurantoin 10 mg/L has been proposed.[5]

Peptone and beef extract provide nitrogen, vitamins, minerals and amino acids for microbial growth. Glucose is the fermentable carbohydrate providing carbon and energy. Sodium chloride maintains the osmotic balance, agar is the solidifying agent. The addition of foetal calf serum enhances the productivity properties of the medium while the addition of antibiotics makes the medium inhibitory for yeasts, moulds and common Gram-positive and Gram-negative bacteria.

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 45 g in 1000 ml of cold purified water. Heat to boiling with frequent agitation and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C and add 5% of inactivated horse serum (horse serum held at 56°C for 30 minutes) or 5% of foetal bovine serum. For specific uses, add the required antimicrobials mix.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	white, fine, homogeneous, free-flowing powder
Solution and prepared tubes appearance	yellow, limpid
Final pH at 20-25°C	7.5 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Brucella Medium Base	Dehydrated medium	4012752	500 g (11 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Sterile loops, needles and swabs, incubator and laboratory equipment as required, ancillary culture media and reagents for the identification of the cultures.

8 - SPECIMENS

Brucella are excreted in large numbers by animals at parturition and can be cultured from a range of material including vaginal mucus, placenta, foetal stomach contents, and milk.[1] Brucella Medium Base may be inoculated with a variety of specimens for the isolation/cultivation of *Brucella* spp. Good laboratory practices for collection, transport and storage of the samples should be applied.[1]

9 - TEST PROCEDURE

Milk samples should be allowed to stand overnight at 4°C before lightly centrifuging.

The cream and the deposit are spread on to the surface of at least three plates of solid selective medium.

Inoculate the surface of the plated medium with 10 µl of the initial suspension and/or diluted sample.

Streak the inoculum with a loop or with a bent sterile glass rod over the four quadrants of the plate to obtain well isolated colonies

Incubate the plates in an inverted position at a temperature of 35 ± 2ºC in an atmosphere of 10% CO_2 and examine every two days for ten days.

10 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.

Examined in indirect sunlight, *Brucella* colonies appear translucent, with a slightly amber tinge. Bacterial colonies may be provisionally identified as Brucella on the basis of their cultural properties and appearance, Gram staining, and agglutination with positive antiserum.

11 - USER QUALITY CONTROL

All manufactured lots of the product 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 testing in accordance with the local applicable regulations, in

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compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control

CONTROL STRAINS	INCUBATION T°/ t / ATM	EXPECTED RESULTS
B.brochispetica ATCC 4617	35-37°C / 18-24H / C	good growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

12 - LIMITATIONS OF THE METHOD

- The nutritional requirements of microorganisms can be different, it is therefore possible that some microbial strains do not grow or grow scantily.
- Sub-cultures onto suitable solid media are necessary for purification of the culture and to perform identification tests.
- The preparation of selective diagnostic media with the addition of specific compounds must be validated by the user.
- Biochemical, immunological, molecular, or mass spectrometry testing should be performed on isolates, from pure culture, for complete identification.

13 - PRECAUTIONS AND WARNINGS

- This product is for microbiological control and for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- Dehydrated media must be handled with suitable protection. Before use, consult the Safety Data Sheet.
- Apply Good Manufacturing Practice in the production process of prepared media.
- 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 doesn't contain any transmissible pathogen. Therefore, it is recommended that the culture medium 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.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the sterilized media inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium as active ingredient for pharmaceutical preparations or as production material intended for human and animal consumption.
- The Certificates of Analysis and the Safety Data Sheet of the product 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 at +10°C /+30°C away from direct light in a dry place. If properly stored, it may be used up to the expiration date. Do not use beyond this date. Avoid opening the bottle in humid places. After use, the container must be tightly closed. Discard the product if the container and/or the cap are damaged, or if the container is not well closed, or in case of evident deterioration of the powder (colour changes, hardening, large lumps).
The user is responsible for the manufacturing and quality control processes of prepared media and for the validation of the shelf life of the finished products, according to the type (plates/tubes/bottles) and the storage method (temperature and packaging).

15 – REFERENCES

1. Corbel MJ, Elberg SS, Cosivi O. Brucellosis in Humans and Animals. World Health Organization Press; Geneva: 2006.]
2. Jones LM, Brinley Morgan WJ. A preliminary report on a selective medium for the culture of Brucella, including fastidious types. Bull Wld Hlth Org 1958; 19:200.
3. Farrell ID.The development of a new selective medium for the isolation of Brucella abortus from contaminated sources', Research in Veterinary Science 1974; 16(3): 280–286
4. Marin CM, Alabart JL, Blasco JM. Effect of antibiotics contained in two Brucella selective media', J Clin Microbiol 1966; 34(2), 426–428
5. Ledwaba MB et al. Investigating selective media for optimal isolation of Brucella spp. in South Africa. Onderstepoort J Vet Res v.87(1); 2020.

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	Manufacturer	Store in a dry place	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2022/05

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

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