BRUCELLA MEDIUM BASE

For the cultivation of *Brucella*, *Campylobacter* and other microorganisms

Typical formula (g/l)

Peptone	10
Beef Extract	5
Glucose	10
Sodium Chloride	5
Agar	15

Directions

Suspend 45 g in 1000 ml of cold distilled water. Heat to boiling and autoclave at 121°C for 15 minutes. Cool to 50°C and add 5 % of horse serum, inactivated by heating at 56°C for 80 minutes. To obtain a selective medium, various antibiotics and dyes can be added: the addition of 10mg cycloheximide, 2500 I.U. bacitracin, 600 IU. polymyxin B to 100 ml of medium is recommended. In addition, as suggested by Renoux, ethyl violet at a final concentration of 1:800,000 can be added. Brucella Medium Base may be used for the preparation of *Campylobacter* selective plating media by adding the suitable supplements: defibrinated or lysed blood, Campylobacter Growth Supplement (cat. N° 4240021), Skirrow Supplement (cat. N° 4240016) or Blaser Wang Supplement (cat. N° 4240015). See the relevant technical sheets.
Final pH 7.5 ± 0.2

Description

Brucella Medium Base can be used to prepare the glucose serum antibiotics medium described by Jones and Brinley Morgan, and is 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. *Brucella* grows on the medium, with incubation in a 10% CO_2 atmosphere at 37°C, after 3 days. However, examination of the plates is recommended on the fourth day, when the colonies have a diameter of 2-3mm. Cultures considered negative after four days of incubation should be re-examined on the eighth and tenth day, and then eliminated. Examined in indirect sunlight, the colonies appear translucent, with a slightly amber tinge. To check that the colonies are *Brucella,* a specific antiserum agglutination test is suggested. The WHO recommends the use of thionine and basic fuchsin resistance tests to differentiate *Brucella melitensis, Brucella abortus* and *Brucella suis.* Prepare 0.1 % solution of the dyes in distilled water and boil in water baths for one hour; add the dyes (final concentrations from 1:25,000 to 1:100,000) to the medium (with added serum) and pour into plates. Optimal working concentrations must be established using standard fuchsin and thionine.
Dry the dishes with covers off by incubation at 37°C for 1-2 hours. Divide the dishes into four squares, and inoculate each quadrant with a different microbial suspension, tracing five streaks, without reloading the loop. In addition, inoculate all the suspensions to be examined onto plates without the addition of dyes. Incubate in CO_2 atmosphere for four days.
Brucella abortus grows in the presence of fuchsin and does not grow in the presence of thionine.
Brucella melitensis grows in the presence of dyes.
Brucella suis only grows in the presence of thionine.
Brucella Medium Base is recommended as a base medium for the preparation of the selective media for the isolation of *Campylobacter jejuni.* For media preparation see the technical sheets of *Campylobacter* Culture Media.

Storage

Dehydrated medium: 10-30°C

References

• Alton, G.G. & Jones L.M. (1968) - La brucellose tecniques de laboratoire. Genéve: OMS.
• Kuzdas, C.D. & Morse, E.V. (1953) - J. Bact., **56**, 502.
• Jones, Lois M. & Brinley Morgan, W.J. (1958) - Bull. Wld. Hlth. Org., **19**, 200.
• Renoux, G. (1954) - Ann. Inst. Pasteur, **87**, 25.

Packaging

4012752 Brucella Medium Base, 500 g (11 l)