ISO
USDA

Brucella Agar Matheson, Colman a. Bell, Norwood (Cincinnati) Ohio, USA

Modified medium according to WUNDT (1957) for the isolation and cultivation of Brucella (especially for the pathogenic strains Bruc. melitensis, Bruc. abortus and Bruc. suis) from clinical specimens and foodstuffs of animal origin.

General Information

This culture medium can be utilized as it is or as a base for the preparation of special culture media. It complies with the recommendations of WHO (1953) and HAUSLER and KOONITZ in Diagnostic Procedures (1970).

Mode of Action

KUZDAS and MORSE (1953), RENOUX (1954) and WEED (1957) demonstrated that, in the case of heavily contaminated sample material, the growth of accompanying microbial flora can be suppressed by addition of bacitracin, polymyxin, cycloheximide and possibly ethyl violet. Circulin, which also has been recommended originally, is no longer used (ALTON and JONES 1967).

The various Brucella species can be differentiated by exploiting the fact that they show different sensitivities towards the dyes thionine and fuchsin. Differential culture media can be prepared by adding these two compounds to Brucella agar.

Typical Composition (g/litre)

Peptone from meat 10.0; peptone from casein 10.0; yeast extract 2.0; D(+)glucose 1.0; sodium chloride 5.0; agar-agar 13.0.

Preparation

Suspend 41 g/litre, autoclave (15 min at 121 °C), pour plates.

pH: 7.0 ± 0.2 at 25 °C.

The plates are clear and yellowish-brown.

Preparation of Brucella differential agar: Sterilize Brucella agar, cool, adjust pH to 6.7 ± 0.1. To 1 litre add 1 ml (1:100,000), 2 ml (= 1:50,000) or 4 (=1:25,000) of an aqueous 1 % thionine or basic fuchsin solution, mix. The solutions should first be heated for 20 minutes in a boiling water bath.

Preparation of Brucella selective agar: Sterilize the Brucella agar, cool to 45-50 °C, add filter-sterilized solutions of the following compounds:

Bacitracin	25.000 IU/litre
Polymyxin B sulfate	6.000 IU/litre
Cycloheximide	100 mg/litre
and if required ethyl violet	1.25 mg/litre

Experimental Procedure

Spread the sample material or material from an enriched culture e.g. in Tryptose Broth thinly over the surface of the Brucella agar. If the specimen is heavily contiminated with other bacteria, inoculate selective Brucella agar, too.

Incubation: For the primary culture, incubate in a 10 % carbon dioxide atmosphere for 4-5 days at 35 °C until growth can be seen. If there is no growth, renew the carbon dioxide atmosphere and incubate for up to 21 days.

Prepare subcultures on Brucella agar from individual colonies and incubate as directed above.

Brucella colonies have a diameter of 2-7 mm, are spheroid in shape, pale amber in colour, moist, slightly opalescent and translucent. These characteristics may vary due to changes in pH or moisture content. Examination of gram-stained smears under the microscope shows the presence of short, rod-shaped bacteria.

Further tests should be performed to differentiate between the Brucella species (WUNDT 1958, CRUICKSHANK 1948, FAO/WHO 1964, JONES and WUNDT 1971).

Literature

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Ordering Information

Product	Ordering No.	Pack size
Brucella Agar	1.10490.0500	500 g
Anaeroclip®	1.14226.0001	1 x 25
Anaerocult® C	1.16275.0001	1 x 10
Anaerocult® C mini	1.13682.0001	1 x 25
Thionine (acetate) Certistain®	1.15929.0025	25 g
Tryptose Broth	1.10676.0500	500 g
Bacitracin	1951	
Polymyxin	5291	

Manufacturer	Product
Matheson, Colman a. Bell, Norwood (Cincinnati) Ohio, USA	Ethyl violet

Quality control

Test strains	Growth
Brucella abortus	good
Brucella melitensis	good
Brucella suis	good
Escherichia coli ATCC 25922	good
Listeria monocytogenes ATCC 19118	good