Dehydrated Culture Media

CHARCOAL AGAR

Code: CM0119

A medium for the cultivation and isolation of Bordetella pertussis and Haemophilus influenzae

Typical Formula*

	gm/litre
`Lab-Lemco' powder	10.0
Peptone	10.0
Starch	10.0
Charcoal bacteriological	4.0
Sodium chloride	5.0
Nicotinic acid	0.001
Agar	12.0

pH 7.4 ± 0.2 @ 25°C

*** Adjusted as required to meet performance standards**

Directions
Suspend 51g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C, add 10% of defibrinated blood and mix gently. The medium is made selective for the isolation of *Bordetella pertussis* and *Bordetella parapertussis* by the addition of Bordetella Selective Supplement SR0082.

To one vial add 2ml of sterile distilled water and dissolve the contents completely. Add this solution to 500ml of sterile, molten Charcoal Agar, cooled to 50°C, together with 10% v/v defibrinated horse blood SR0050. Mix well before pouring into sterile Petri dishes.
For *Haemophilus influenzae*, omit the selective agents and convert to `chocolate' agar.

Transport Medium for *Bordetella pertussis*
The vial contents may be added to 500ml of half-strength Charcoal Agar + 10% v/v defibrinated horse blood SR0050 for use as a transport medium for *B. pertussis*.

Description
Charcoal Agar was developed by Oxoid to provide a non-blood containing medium for the cultivation of *Bordetella pertussis* and *Haemophilus influenzae*. Proom[1] showed that nicotinic acid was an essential growth factor for the bordetellae. Ensminger *et al*.[2] used a charcoal medium for the growth of *Bordetella pertussis* in vaccine production and found that the medium could replace Bordet-Genou. Mishulow *et al*.[3] used charcoal agar for *Bordetella pertussis* cultivation.

Haemophilus influenzae is cultivated on the medium containing 10% `chocolated' blood but no antibiotics. The inoculated plate is incubated for 2 to 3 days at 37°C. The colonies are usually small, transparent and droplet-like, but some transformation to the `rough' type colony may occur. Species differentiation is performed by examination of the need for X and V growth factors, on Blood Agar Base CM0055.
The greatest problem in the isolation of *Bordetella* species from naso-pharyngeal secretions is the suppression of unwanted flora during the long incubation period on very nutritious media.

Fleming's first *in vitro* demonstration of penicillin was to show that it could help isolate *Bordetella pertussis* on media[4]. Lacey[5] confirmed this but found that the penicillin-resistant flora still caused problems. He supplemented penicillin with 2µg/ml 4,4' diamidino-diphenylamine dihydrochloride (M & B 938) thereby increasing the selectivity of this medium.

Broome *et al*.[6] found methicillin to be superior to penicillin in suppressing unwanted naso-pharyngeal flora but the earlier publication of Sutcliffe and Abbott[7] where cephalexin (40µg/ml) was shown to be superior to penicillin, has proved to be the most significant advance.

The benefits of cephalexin as a selective agent for *Bordetella pertussis* have been confirmed [8,9,10,11]. The ability to recover stressed cells and the much longer shelf life (6-8 weeks) are added benefits to its superiority at suppressing unwanted naso- pharyngeal growth.

Regan and Lowe[8] showed that half-strength Oxoid Charcoal Agar, supplemented with 40µg/ml cephalexin SR0082 v/v lysed, defibrinated horse blood was an excellent enrichment and transport medium.

The efficacy of this transport medium has been confirmed by other workers[12].

Technique

The following technique for the laboratory diagnosis of *Pertussis* is recommended[11].
1. Collect pernasal swabs in the early stage of the illness and place in tubes of half-strength Charcoal Agar supplemented with 10% v/v lysed, defibrinated horse blood and 40mg/ml cephalexin.
2. Generously inoculate the swabs on to thick layers of Charcoal Agar containing 10% v/v defibrinated horse blood and 40µg/ml cephalexin (SR0082).
A non-selective medium in which the cephalexin is omitted may be used in addition.
3. Perform direct fluorescent antibody (DFA) tests on the secretions, using *Bordetella pertussis* and *Bordetella parapertussis*-conjugated antisera, to help make an earlier diagnosis.
4. Replace the swabs in the original transport medium and hold at room temperature. If the culture plates become overgrown with commensal flora or fungi, use the swabs to inoculate fresh plates of medium.
5. Incubate the plates at 35°C in a moist atmosphere (60-70% humidity) for up to six days. Examine the plates after 40 hours incubation and twice-daily thereafter.
6. Look for small, shiny, greyish-white, round convex colonies. Suspicious colonies should be Gram stained, using a two- minute safranin counterstain. Some pleomorphic cells may be seen, caused by the cephalexin in the selective medium.
7. Confirm the identification with DFA tests on the suspicious colonies.

Precautions
Stuart's transport medium or similar formulation media should not be used for Bordetella-containing specimens[13].

Two pernasal swabs should be taken from each patient, one through each nostril[14].
Make sure the charcoal remains in suspension when dispensing the medium by gently swirling the flask.
Lysed horse blood is used in the transport medium but whole blood is used in the isolation medium.
Most naso-pharyngeal flora are inhibited by cephalexin but *Pseudomonas aeruginosa* and some fungi may grow through. Amphotericin B can be added (12mg/ml) as an antifungal agent to prevent the growth of filamentous fungi. However, this level of amphotericin B can be inhibitory to *Bordetella pertussis* and should not be used routinely.

METRONIDAZOLE SUSCEPTIBILITY TEST FOR

Helicobacter pylori
Charcoal agar supplemented with a concentrate of essential growth factors has been reported to be a reliable testing medium for determining metronidazole resistance in *Helicobacter pylori*[15].

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.
Store the prepared plates of medium at 2-8°C.

Appearance
Dehydrated medium: Black, free-flowing powder
Prepared medium: Black gel

With Cephalexin:
Positive controls:
Expected results
Bordetella pertussis ATCC® 8467 Good growth; grey coloured colonies
Bordetella parapertussis NCTC 10521
Good growth; grey coloured colonies
Negative controls:

Staphylococcus aureus ATCC® 25923* inhibited
Klebsiella pneumoniae ATCC® 13883* inhibited
Without Antibiotics:
Positive control:
Haemophilus influenzae ATCC® 35056* Good growth; grey coloured colonies
Negative control:
Uninoculated medium
No change

*** This organism is available as a Culti-Loop®**

References
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