

Instructions for Use

REF - KM0185

Charcoal Agar

Dehydrated Culture Media

1. Intended Use

For *in vitro* diagnostic use only. KM0185 Charcoal Agar is a selective medium, when supplemented with blood and any necessary selective agents, for the isolation of *Bordetella pertussis*.

2. Composition*

<u>Ingredient</u>	<u>g/L</u>
Beef extract	10.0
Peptone	10.0
Starch	10.0
Charcoal	4.0
Sodium chloride	5.0
Nicotinic acid	0.001
Agar	12.0

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

Whooping cough is caused by *Bordetella pertussis* and *Bordetella parapertussis* which are among the most fastidious bacteria known and require special culturing techniques ⁽¹⁾. A charcoal-based medium was found to be an efficient substitute for Bordet Gengou agar in the production of *B. pertussis* vaccines ⁽²⁾ and can be used as a maintenance medium for stock cultures of *Bordetella* species ⁽³⁾. Proom demonstrated nicotinic acid is an essential growth factor for *Bordetella* spp. ⁽⁴⁾. Sutcliffe and Abbott added 40 µg/ml of cephalixin to charcoal agar to suppress the growth of commensal nasopharyngeal flora and compared its inhibitory effect with that of penicillin ⁽⁵⁾. Stauffer *et al.* compared the inhibitory effects on *Bordetella* spp. of charcoal agar with cephalixin to that of charcoal agar with penicillin and methicillin ⁽⁶⁾. Both groups reported agar supplemented with cephalixin was superior to supplementation with other antibiotics for selective recovery of *B. pertussis*. In 1989, Hoppe *et al.* reported charcoal agar supplemented with horse blood was superior to supplementation with sheep blood or human blood for recovery of *Bordetella* spp. ⁽⁷⁾.

Charcoal Agar with the addition of 10% Horse Blood and Cephalixin (40mg/L) is recommended as a primary isolation media by the UK Standards for Microbiology Investigations ^(8 9).

4. Principle

Beef extract and peptone provide the required nitrogen, carbon and vitamins and sodium chloride maintains the osmotic balance within the medium. Starch and charcoal help neutralise toxic substances in the medium. Nicotinic acid is an essential growth factor for *Bordetella* species. The addition of cephalixin (E&O LS0018) inhibits accompanying nasopharyngeal flora in the specimen.

NB: KM0185 Charcoal Agar is a basic medium only and contains no additional supplements.

5. Preparation Instructions

Suspend 51.0g/L in 900ml of deionised/purified water. Heat with frequent agitation and boil for 1 minute to completely dissolve the medium.

Sterilise at 121°C for 15 minutes before cooling to 45-50°C. Aseptically add 100 ml/L of defibrinated horse blood.

At this point, various selective supplements, such as cephalixin (E&O LS0018), may also be added aseptically to create a variety of selective products.

Gently mix the final medium before aseptically dispensing the specified volume into appropriate sterile containers and allowing to cool.

NB: Continuously mix media during dispensing to ensure the charcoal is kept in suspension.

6. Physical Characteristics

	Dehydrated Medium	Prepared Medium	
Appearance and Colour	Black fine powder	Black firm gel	
pH	N/A	7.4 ± 0.2	

7. Materials Provided

KM0185 can be provided in the formats detailed below. Each tub is labelled with product name, product code, lot number, expiry date, instructions, and appropriate warnings.

Product Code	Product Format
KM0185-500G-500	1 x 500g Dehydrated Culture Media Tub
KM0185-5KG-5000	1 x 5kg Dehydrated Culture Media Tub
KM0185-10KG-10000	1 x 10kg Dehydrated Culture Media Tub

8. Materials Needed but not Provided

Standard microbiological laboratory materials e.g., autoclave, sterile loops or swabs, collection containers, incubators, and quality control organisms.

9. Specimens

KM0185 Charcoal Agar is suitable for the testing of the following specimens:

- Clinical: nasopharyngeal or perinasal swab and nasopharyngeal aspirates.

Sampling and transport equipment must be used in accordance with the end user's suppliers' recommendations. Refer to appropriate standard method or local guidance on sample collection and subsequent processing.

10. Test Procedures and Interpretation of results

Allow the plates to come to room temperature.

Based on sample type and information provided, check to see if the specimen needs to be pre-enriched prior to inoculation to the prepared medium.

Clinical Specimens:

- Process the clinical specimen as required by local guidelines.
- Inoculate specimen directly onto the medium and streak across the agar surface using a sterile loop or automated plate streaker.
- Incubate the plates at $36 \pm 1^\circ\text{C}$ in 5% CO_2 for 7 days (further incubation up to 12 days has shown to improve recovery of clinical *Bordetella* species). It is recommended to check plates at the 4- and 7- day marks.

After incubation, examine agar for colonies (typical colony appearance outlined in Quality Control table below). Perform further biochemical or mass spectroscopy testing to confirm identity of presumptive positive isolates. Refer to relevant local guidelines.

11. Quality Control

Organism	Incubation	Result (Specificity)
<i>B. pertussis</i> (NCTC 10739)	$37 \pm 1^\circ\text{C}$ in 5% CO_2 for 72-120 hours	Growth: Grey colonies
<i>S. aureus</i> (NCTC 12981)	$37 \pm 1^\circ\text{C}$ in 5% CO_2 for 72-120 hours	Inhibited
<i>K. pneumoniae</i> (NCTC 9633)	$37 \pm 1^\circ\text{C}$ in 5% CO_2 for 72-120 hours	Inhibited

It is the responsibility of the user to perform Quality Control testing taking into consideration the intended use of the medium and in agreement with any local relevant guidelines (e.g., frequency, strains used, atmosphere, incubation temperature).

12. Performance

To fully verify KM0185 Charcoal Agar performance, dehydrated culture media samples were used to prepare Charcoal Agar with 10% Defibrinated Horse Blood and Cephalixin and tested to assess colour change and turbidity/colony morphology and recovery level (where an acceptable range is $\geq 50\%$ and $\leq 120\%$) compared to a non-selective reference medium. Prepared samples were inoculated with 30-150cfu and 10^4 - 10^5 cfu for the non-target organisms and incubated at $37 \pm 1^\circ\text{C}$ in 5% CO_2 for 72-120 hours. All samples of prepared media produced grew and showed good recovery and the correct morphology of the required test organism: *Bordetella pertussis* (NCTC 10739), and no recovery of the non-target test organisms: *Staphylococcus aureus* (NCTC 12981) *Klebsiella pneumoniae* (NCTC 9633). Therefore, it can be concluded that KM0185 Charcoal Agar meets performance criteria when used according to the instructions outlined above. Trend analysis data available upon request.

13. Limitations of the Media

- Additional oxidase, serological, or molecular tests should be performed to confirm identity.
- Selective media may not support the growth of all circulating strains of *Bordetella* spp.
- The addition of cephalixin will provide good inhibition of non-target nasopharyngeal flora but *Pseudomonas aeruginosa* and fungi may still grow.

14. Precautions and Warnings

This product is considered non-hazardous under CLP regulations. Wear such PPE as recommended by laboratory COSHH assessment. During and after use, always handle all materials in a manner conforming to Good Laboratory Practices and consider that material under test should be regarded as a potential biohazard if mishandled.

Refer to KM0185 Material Safety Data Sheet.

15. Storage conditions and Shelf life

Store product in the original container with the lid tightly closed at between 10 and 30°C in low humidity conditions away from direct sunlight. Kept under these conditions, the product may be used up to date of expiry shown on the product label.

Do not use if the product is not free-flowing or displays any sign of colour change, formation of large lumps or hardening of the powder. Additionally, do not use medium if it has been stored inappropriately, the packaging has been damaged or has passed the expiry date.

Dehydrated culture media does not need to be used all at once; replace the cap and ensure that the container is tightly closed and stored according to labelled instructions.

Dispose of in accordance with local and national authority requirements.

16. References

1. Isenberg, H. (2004) Clinical Microbiology Procedures Handbook. 2nd ed. ASM Press, Washington, D.C.
2. Ensminger, P., Culbertson, C. and Powell, H. (1953) Antigenic Hemophilus Pertussis Vaccines Grown on Charcoal Agar. J. Infect Dis, 93(3): 266-268.
3. Mishulow, L., Sharpe, L. and Cohen, L. (1953) Beef-heart charcoal agar for the preparation of pertussis vaccines. Am. J. Public Health, 43(11): 1466-1472.
4. Proom, H. (1955) The minimal nutritional requirements of organisms of the genus Bordetella López. J. Gen. Microbiol., 12(1):63-75.
5. Sutcliffe, E. and Abbott, J. (1972) Selective medium for the isolation of Bordetella pertussis and parapertussis. J. Clin. Pathol., 25(8): 732-733.
6. Stauffer, L. R., Brown, D.R. and Sandstrom, R.E. (1983) Cephalixin-supplemented Jones-Kendrick charcoal agar for selective isolation of Bordetella pertussis: comparison with previously described media. J. Clin. Microbiol., 17(1):60-62.
7. Hoppe, J.E. and Schlagenhauf, M. (1989) Comparison of three kinds of blood and two incubation atmospheres for cultivation of Bordetella pertussis on charcoal agar. J. Clin. Microbiol. 27(9):2115-2117.
8. Public Health England (2020) Identification of Bordetella species. UK Standards for Microbiology Investigations. Bacteriology – Identification, ID 5(4). London: Standards Unit, Microbiology Services.
9. Public Health England (2018) Investigation of whooping cough. UK Standards for Microbiology Investigations. Bacteriology, B6(9). London: Standards Unit, Microbiology Services.
10. International Organization for Standardization (2014) 11133:2014 Microbiology of food, animal feed and water – Preparation, production, storage and performance testing of culture media. Geneva, ISO.

Version History*

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|----|---------------------------------------|
| 01 | 25/10/22 - New Document Created |
| 02 | 26/10/23 -Updated with SMI procedures |

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



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TABLE OF APPLICABLE SYMBOLS

IFU/KM0185 REV. 002

 REF o 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	 Keep away from direct light	 Store in a dry place