Instructions for Use

REF - KM0076 ***Burkholderia cepacia* Agar**

Dehydrated Culture Media

1. Intended Use

For *in vitro* diagnostic use only. KM0076 *Burkholderia cepacia* Agar is a selective medium for the isolation of *Burkholderia cepacia* from cystic fibrosis patients.

2. Composition*

Ingredient	g/L
Magnesium sulphate	0.2
Ferrous ammonium sulphate	0.01
Potassium dihydrogen phosphate	4.35
Disodium hydrogen phosphate	1.42
Bile salts	0.5
Ammonium sulphate	1.0
Peptone mixture	8.9
Crystal violet	0.001
Phenol red	0.02
Sodium pyruvate	5.0
Agar	12.0

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

This medium is based on the *Pseudomonas cepacia* medium described by Gilligan *et al*. [(1)].

Burkholderia cepacia is a gram-negative bacterium found in soil and moist environments that is known to cause various types of infections, including catheter-associated infections and respiratory tract infections, in hospitalized patients. *B. cepacia* can cause pneumonia in immune-compromised patients, especially those with cystic fibrosis. This infection can lead to a rapid decline in those with lung problems and strict isolation is necessary. This is an important opportunistic pathogen in cystic fibrosis patients and can lead to fatal infection in approximately 20% of individuals that have been colonised with *B. cepacia* complex organisms.

KM0076 is recommended by the UK Standards for Microbiology Investigations [(2)] and tested in accordance with the principals of ISO 11133:2014 [(3)].

4. Principle

Magnesium sulphate, ammonium sulphate and ferrous ammonium sulphate supports the growth of *B. cepacia*. Potassium di-hydrogen phosphate and di-sodium hydrogen phosphate are buffering agents, used to maintain the pH the medium. The phenol red is used as a pH indicator. If the sodium pyruvate in the medium is metabolised by *B. cepacia* alkaline by-products are produced which raises the pH. This causes the colour of the medium to turn pink/red around sections of heavy growth on the medium. Bile salts and crystal violet are selective agents. The associated selective supplement for this medium, E&O LS0125 *Burkholderia cepacia* Selective Supplement, contains ticarcillin and polymyxin B to further improve a media's selectivity particularly against *Pseudomonas* species.

5. Preparation Instructions

Suspend 33.4g of dehydrated culture medium in 1 litre of deionised/purified water and allow to soak whilst mixing for 10 minutes.

Autoclave to sterilise at 121°C for 15 minutes before cooling in a water bath to 45-50°C and aseptically add the appropriate volume of selective supplement E&O LS0125.

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

6. Physical Characteristics

	Dehydrated Medium	Prepared Medium
Appearance and Colour	Beige fine powder	Pale yellow firm gel
pH	N/A	6.2 ± 0.2

7. Materials Provided

KM0076 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
KM0076-500G-500	1 x 500g Dehydrated Culture Media Tub
KM0076-5KG-5000	1 x 5kg Dehydrated Culture Media Tub
KM0076-10KG-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.

Ingredients for preparation of media supplied by E&O Laboratories e.g., supplement E&O LS0125 *Burkholderia cepacia* Selective Supplement.

9. Specimens

KM0076 *Burkholderia cepacia* Agar is suitable for the testing of the following specimens:

- Clinical Specimens: Samples for the testing and isolation of *B. cepacia* may include sputum, specialised swabs from the infected areas, biopsy material, pus, and blood.

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.

Clinical Specimens [2]

- Inoculate specimen directly onto prepared KM0076 medium and streak across the agar surface using a sterile loop or automated plate streaker.
- Incubate at 35-37°C aerobically for 48-72 hours (additional incubation at 30°C aerobically for a further 5 days may be required).

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)
B. cepacia (NCTC 10661)	37 ± 1°C aerobically for 24-48 hours	Growth: Green colonies with pink halo
E. coli (NCTC 12241)	37 ± 1°C aerobically for 24-48 hours	Inhibited
P. aeruginosa (NCTC 12903)	37 ± 1°C aerobically for 24-48 hours	Inhibited
S. aureus (NCTC 12981)	37 ± 1°C aerobically for 24-48 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 KM0076 *Burkholderia cepacia* Agar performance, dehydrated culture media samples were used to prepare *Burkholderia cepacia* Agar and tested to assess colony morphology and recovery level (where an acceptable range is ≥50% and ≤120%) compared to a non-selective reference medium. Prepared samples were inoculated with 30-150cfu for the target organisms and 10^4-10^5 cfu for the non-target organisms then incubated at 37 ± 1°C aerobically for 24-48 hours. All samples of prepared media produced showed expected recovery and morphology of the required test organism: *Burkholderia cepacia* (NCTC 10661), and no recovery of the non-target test organisms: *Escherichia coli* (NCTC 12241), *Pseudomonas aeruginosa* (NCTC 12903) and *Staphylococcus aureus* (NCTC 12981). Therefore, it can be concluded that KM0076 *Burkholderia cepacia* Agar, meets performance criteria when used according to the instructions outlined above. Trend analysis data available upon request.

13. Limitations of the Media

- Due to variation in nutritional requirements, some strains may grow poorly on this medium.
- Further biochemical or mass spectroscopy testing to confirm identity of presumptive positive isolates as other bacterial isolates may be able to form colonies on this medium.

14. Precautions and Warnings

Refer to KM0076 Material Safety Data Sheet prior to use.

This product is considered 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.

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 the 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. Gilligan, P., Gage, P., Bradshaw, L., Schidlow, D., and DeCicco, B. (1985) Isolation medium for the recovery of *Pseudomonas cepacia* from respiratory secretions of patients with cystic fibrosis. J. Clin. Microbiol., 22(1), pp.5-8.
2. Public Health England (2015) Identification of *Pseudomonas* species and other Non-Glucose Fermenters. UK Standards for Microbiology Investigations. Bacteriology – Identification, ID 17(3). London: Standards Unit, Microbiology Services.
3. 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*

001 11/09/23 - New Document Created

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

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TABLE OF APPLICABLE SYMBOLS

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