Instructions for Use

REF - KM0096 ***Clostridium difficile* Agar**

Dehydrated Culture Media

1. Intended Use

For *in vitro* diagnostic use only. KM0096 *Clostridium difficile* Agar is used, when supplemented, for the isolation of *C. difficile* from samples.

2. Composition*

Ingredient	g/L
Proteose peptone	40.0
Fructose	6.0
Disodium hydrogen phosphate	5.0
Potassium dihydrogen phosphate	1.0
Magnesium sulphate	0.1
Sodium chloride	2.0
Agar	14.9

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

This formulation is a modification of Cycloserine Cefoxitin Fructose Agar developed by George *et al.*[1]. *C. difficile* bacteria usually live harmlessly in your bowel along with lots of other types of bacteria. But sometimes when a patient takes antibiotics, the balance of bacteria in their bowel can change, causing an infection.

4. Principle

The proteose peptone function as a carbon, nitrogen, and vitamin source in this medium. Fructose is a fermentable carbohydrate. Sodium chloride maintains the osmotic balance of the medium. Disodium hydrogen phosphate and potassium dihydrogen phosphate are buffering agents. The magnesium sulphate acts as a source of inorganic ions, and agar is the solidifying agent. This media requires the addition of defibrinated horse blood and *Clostridium difficile* selective supplement (E&O LS0022).

5. Preparation Instructions

Suspend 69.0g 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 then aseptically add 7% horse blood and the appropriate volume of E&O LS0022 Clostridium difficile selective supplement.
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	Straw fine powder	Straw firm gel
pH	N/A	pH = 7.4 ± 0.2

7. Materials Provided

KM0096 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
KM0096-500G-500	1 x 500g Dehydrated Culture Media Tub
KM0096-5KG-5000	1 x 5kg Dehydrated Culture Media Tub
KM0096-10KG-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

KM0096 *Clostridium difficile* Agar can be used with the following specimens:

- Clinical: rectal swabs and stool specimens

Sampling and transport equipment must be used in accordance with the end user's suppliers' recommendations for the

conservation of *Clostridium difficile*. Refer to appropriate standard method or local guidance on sample collection and subsequent processing.

10. Test Procedures and Interpretation of results

Clinical Specimens:

Inoculate specimen, or pre-enriched sample, directly onto the medium and streak across the agar surface using a sterile loop or automated plate streaker. Incubate anaerobically at 37 ± 1°C for 24-48 hours.

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. Typical Gram stain morphology of *C. difficile* may not be evident in colonies picked from this medium because of antibiotics present. Subculture suspected colonies to blood agar to obtain characteristic morphology. Refer to relevant local guidelines.

11. Quality Control

Organism	Incubation	Result (Specificity)
C. difficile (NCTC 11204)	Anaerobically at 37 ± 1°C for 24-48 hours	Growth: Grey colonies
E. coli (NCTC 12241)	Anaerobically at 37 ± 1°C for 24-48 hours	Inhibited
C. perfringens (NCTC 8237)	Anaerobically at 37 ± 1°C for 24-48 hours	Inhibited
C. sporogenes (NCTC 532)	Anaerobically at 37 ± 1°C for 24-48 hours	Inhibited
B. fragilis (NCTC 9343)	Anaerobically at 37 ± 1°C for 24-48 hours	Inhibited
*S. aureus (*NCTC12981)	Anaerobically at 37 ± 1°C 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 KM0096 *Clostridium difficile* Agar performance, dehydrated culture media samples were used to prepare *Clostridium difficile* 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 30-150 cfu for the target organisms and 10^4-10^5 cfu for the non-target organisms then incubated at 37 ± 1°C for 24-48 hours. All samples of prepared media showed expected recovery and morphology of the required test organism: *Clostridium difficile* (NCTC 11204), and no recovery of the non-target test organisms: *Escherichia coli* (NCTC 12241), *Clostridium perfringens* (NCTC 8237), *Clostridium sporogenes* (NCTC 532), *Bacteroides fragilis* (NCTC 9343) and *Staphylococcus aureus (*NCTC12981). Therefore, it can be concluded that KM0096 *Clostridium difficile* 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 natural variation, some strains may grow poorly on this medium.
- *Clostridium Difficile* Agar does not contain neutral red indicator as it is designed for use with horse blood.

14. Precautions and Warnings

This product is considered non-hazardous under CLP regulations. Wear such PPE as recommended by laboratory COSSH 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 KM0096 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 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. George, W., Sutter, V., Citron, D., and Finegold, S. (1979) Selective and differential medium for isolation of Clostridium difficile. J. Clin. Microbiol. 9(2), 214-219.

Version History*

001 14/04/23 - 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	Σ Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	Store in a dry place