Instructions for Use

REF - KM0052 Contagious Equine Metritis Organism Agar (CEMO)

Dehydrated Culture Media

1. Intended Use

KM0052 Contagious Equine Metritis Organism (CEMO) Agar is used for the cultivation of *Taylorella equigenitalis*, the causative organism of equine metritis.

2. Composition*

Ingredient	g/L
Enzymatic digest of casein	15.0
Soy peptone	5.0
Sodium chloride	5.0
L-cystine	0.3
Sodium sulphite	0.2
Agar	12.0

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

Contagious equine metritis is a venereal disease of horses caused by the bacteria, *T. equigenitalis*. It is spread during breeding or through contact with contaminated objects, it is highly contagious among horses and can be difficult to detect and control. It can have a negative impact on fertility in both mares and stallions.

Contagious Equine Metritis Organism Agar medium is based on the formulation by Atherton [(1)]. It is routinely used for culturing swabs taken from the genitalia of mares and stallions to test for the presence of *T. equigenitalis*.

4. Principle

The enzymatic digest of casein and soy peptone provides the required nitrogen, carbon, and other essential nutrients. Sodium chloride maintains the osmotic balance in the medium and sodium sulphite is a reducing agent. L-cystine acts as a growth factor. The medium is made selective by the addition of CEMO selective supplement (E&O LS0041) to control bacterial and fungal flora from swab samples.

5. Preparation Instructions

Suspend 37.5 g of dehydrated culture medium in 1 litre of deionised/purified water and mix for 10 minutes.

Autoclave to sterilise at 121°C for 15 minutes before cooling in a water bath to 45-50°C.

Aseptically add the appropriate volume of defibrinated horse blood and mix well. Heat medium to 80°C with frequent stirring until the media become a chocolate brown colour.

Cool in a water bath to 45- 50°C and aseptically add the appropriate volume of CEMO selective supplement (E&O LS0041).

Gently mix 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	Brown firm gel
pH	N/A	7.3 ± 0.2

7. Materials Provided

KM0052 is available 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
KM0052-500G-500	1 x 500g Dehydrated Culture Media Tub
KM0052-5KG-5000	1 x 5kg Dehydrated Culture Media Tub
KM0052-10KG-10000	1 x 10kg Dehydrated Culture Media Tub

8. Materials Needed but not Provided

CEMO Selective Supplement (E&O LS0041)

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

9. Specimens

KM0052 Contagious Equine Metritis Organism Agar (CEMO) is suitable for the testing of the following specimens:

- Veterinary Samples: Swabs taken from the genitalia of mares and stallions. Often, veterinarians collect swabs and send to approved laboratories. They require special handling and must be processed within 48 hours of collection.

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

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.

Inoculate plated media directly with the sample, or subculture onto plated media after incubation in enrichment broth where required.

Veterinary 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 at 37°C ± 1°C in 5-10% CO_2 for 24-48 hours.

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

11. Quality Control

Organism	Incubation	Result (Specificity)
T. equigenitalis (NCTC 11184)	37 ± 1°C in CO_2 for 48-72 hours	Growth: Yellow/cream colonies
T. equigenitalis (NCTC 11225)	37 ± 1°C in CO_2 for 48-72 hours	Growth: Yellow/cream colonies
E. coli (NCTC 12241)	37 ± 1°C in CO_2 for 48-72 hours	Inhibited
C. albicans (NCPF 3179)	37 ± 1°C in CO_2 for 48-72 hours	Inhibited
S. aureus (NCTC 12981)	37 ± 1°C in CO_2 for 48-72 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 KM0052 Contagious Equine Metritis Organism Agar (CEMO) performance, dehydrated culture media samples were used to prepare Contagious Equine Metritis Organism 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 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 in CO_2 for 48-72 hours. All samples of prepared media produced expected recovery and morphology of the required test organisms: *Taylorella equigenitalis* (NCTC 11184) and *Taylorella equigenitalis* (NCTC 11225), and no recovery of the non-target test organisms: *Escherichia coli* (NCTC 12241), *Candida albicans* (NCPF 3179) and *Staphylococcus aureus* (NCTC 12981). Therefore, it can be concluded that KM0052 Contagious Equine Metritis Organism Agar (CEMO), 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.

14. Precautions and Warnings

This product is considered hazardous under CLP regulations. Refer to KM0052 Material Safety Data Sheet. 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. Atherton, J. (1978) Inhibition of CEM organism in mixed cultures. Veterinary Record, 103(19), pp.432.

Version History*

001 25/09/22 - 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		Manufacturer	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	Store in a dry place