Instructions for Use

REF - KM0026 ***Edwardsiella ictaluri* Medium (EIM)**

Dehydrated Culture Media

1. Intended Use

KM0026 *Edwardsiella ictaluri* Medium (EIM) is used for the isolation and differentiation of *Edwardsiella* species.

2. Composition*

Ingredient	g/L
Peptones	20
Phenylalanine	1.25
Ferric ammonium citrate	1.2
Sodium chloride	5
Mannitol	3.5
Bile salts	1
Colistin	0.01
Bromothymol blue	0.03
Agar	13

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

This medium was developed by Shotts *et al.* for the isolation of *Edwardsiella* spp. [1]. *Edwardsiella ictaluri* is an intracellular pathogen causing enteric septicaemia of freshwater fish. The bacterium can survive in sediment and surviving fish can become carriers. They can be found in the kidneys of fish and are thought to be shed in the faeces. *E. ictaluri* can be separated from *E. tarda* because it is indole-negative and does not produce H_2S on triple sugar iron (TSI) agar.

Schotts noted that *Proteus* species may swarm on mixed culture plates. To overcome this, p-nitrophenylglycerol (PNPG) Selective Supplement (E&O LS0021), which restricts Proteus spp. swarming, may be added to the medium [1].

4. Principle

The peptones supply the required carbon, nitrogen, and other essential growth factors. Sodium chloride maintains the osmotic balance. Colistin and bile salts are selective agents to inhibit most Gram-negative and Gram-positive organisms. Mannitol is a fermentable carbohydrate and bromothymol blue is a pH indicator. Phenylalanine and ferric ammonium citrate are used to detect phenylalanine deaminase activity in *Proteus* species. Agar is a solidifying agent.

5. Preparation Instructions

Suspend 45.0g of dehydrated culture medium in 1 litre of deionised/purified water and allow to mix for 10 minutes.

Autoclave to sterilise at 121°C for 15 minutes before cooling in a water bath to 45-50°C. At this stage, the appropriate volume of PNPG (E&O LS0021) may be added.

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

Prepared media may be kept at between 2 and 8°C for up to 14 days away from direct sunlight.

6. Physical Characteristics

	Dehydrated Medium	Prepared Medium
Appearance and Colour	Straw fine powder	Green firm gel
pH	N/A	7.1 ± 0.2

7. Materials Provided

KM0026 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**
KM0026-500G-500	1 x 500g Dehydrated Culture Media Tub
KM0026-5KG-5000	1 x 5kg Dehydrated Culture Media Tub
KM0026-10KG-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, supplements, and quality control organisms.

9. Specimens

KM0026 *Edwardsiella ictaluri* Medium (EIM) is suitable for the testing of the following specimens:

- Veterinary Specimens: Blood, faeces, and tissue samples (particularly from the kidney) samples from potentially infected fish. Domestic hosts include white, walking, channel and sutchi catfish species and, under experimental setting, rainbow trout and chinook salmon.

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.

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 aerobically at 30 ± 2°C for 24-48 hours.

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

11. Quality Control

Organism	Incubation	Result (Specificity)
E. ictaluri (NCTC 12122)	30 ± 2°C aerobically for 24-48 hours	Growth: Green colonies
E. tarda (NCIMB 2056)	30 ± 2°C aerobically for 24-48 hours	Growth: Green colonies with faint blue halo
P. mirabilis (NCTC 11938)	30 ± 2°C aerobically for 24-48 hours	Growth: Dark blue with brown halo, swarming around colony
S. marcescens (NCTC 13382)	30 ± 2°C aerobically for 24-48 hours	Growth: Yellow/Red colonies
E. coli (NCTC 12241)	30 ± 2°C aerobically for 24-48 hours	Inhibited
L. monocytogenes (NCTC 11994)	30 ± 2°C aerobically for 24-48 hours	Inhibited
P. aeruginosa (NCTC 12903)	30 ± 2°C aerobically for 24-48 hours	Inhibited
A. hydrophila (NCTC 8049)	30 ± 2°C aerobically for 24-48 hours	Inhibited
S. iniae (ATCC 29178)	30 ± 2°C aerobically for 24-48 hours	Inhibited
S. agalactiae (NCTC 8181)	30 ± 2°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 KM0026 *Edwardsiella ictaluri* Medium (EIM) performance, dehydrated culture medium samples were used to prepare *Edwardsiella ictaluri* Medium poured plates 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 aerobically at 30 ± 2°C for 24-48 hours. All samples of prepared medium showed expected recovery and morphology of the required test organisms: *Edwardsiella ictaluri* (NCTC 12122), *Edwardsiella tarda* (NCIMB 2056), *Proteus mirabilis* (NCTC 11938)and *Serratia marcescens* (NCTC 13382), and no recovery of the non-target test organisms: *Escherichia coli* (NCTC 12241), *Listeria monocytogenes* (NCTC 11994), *Pseudomonas aeruginosa* (NCTC 12903), *Aeromonas hydrophila* (NCTC 8049), *Streptococcus iniae* (ATCC 29178) and *Streptococcus agalactiae* (NCTC 8181). Therefore, it can be concluded that KM0026 *Edwardsiella ictaluri* Medium (EIM), meets performance criteria when used according to the instructions outlined above. Trend analysis data available upon request.

13. Limitations of the Medium

- Further testing using pure cultures may be necessary for complete identification.
- Due to natural variation, some strains may grow poorly on this medium.

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 KM0026 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 do 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. Shotts, E. B., and Waltman, W. D., 2nd (1990). A medium for the selective isolation of Edwardsiella ictaluri. Journal of Wildlife Diseases, 26(2), 214–218

Version History*

001 18/09/23 - 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		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