Instructions for Use

REF - KM0005 CLED Double Indicator Agar (Bevis)

Dehydrated Culture Media

1. Intended Use

For *in vitro* diagnostic use only. KM0005 CLED Double Indicator Agar (Bevis) is a differential culture medium used clinically for isolation and enumeration of bacteria in urine, from the suspected cases of urinary tract infection (UTI) [1].

2. Composition*

Ingredient	g/L
Peptone	8.0
Beef extract	3.0
Lactose	10.0
L-cystine	0.0128
Anrade's Indicator	0.08
Bromothymol Blue	0.02
Agar	15.0

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

KM0005 CLED Double Indicator Agar (Bevis) is a differential culture medium which contains cystine, lactose, bromothymol blue, acid fuchsin and is electrolyte deficient to prevent swarming of *Proteus* species [2]. Bevis changed the original medium by Mackey and Sandys by introducing a second pH indicator to enhance the differentiation of colony characteristics of lactose and non-lactose fermenting organisms [3]. The medium can allow for quantitative determination of urinary pathogens, including Proteus species, when automated systems or calibrated loops are used for inoculation [4].

KM0005 is recommended as a differential primary isolation media by the UK Standards for Microbiology Investigations [4] and tested in accordance with ISO 11133:2014 [5].

4. Principle

The peptone and beef extract are the source of the required nitrogen, carbon, and vitamins. L-Cystine is present to benefit those organisms that have a specific need for cystine such as coliforms. Lactose is included to provide an energy source for organisms capable of utilizing it by a fermentative mechanism. Bromothymol blue and acid fuchsin are used as a pH indicator system to differentiate lactose fermenters from lactose non-fermenters. Lactose-positive bacteria form yellow-red colonies due to acid production during lactose fermentation and lactose-negative bacteria turn blue due to alkalinization. Bacteria that decarboxylate L-cystine also cause an alkaline reaction and form dark blue colonies. Electrolyte sources are reduced to minimize swarming of *Proteus* species.

5. Preparation Instructions

Suspend 36.2g of dehydrated culture medium in 1 litre of deionised/purified water and heat with frequent agitation and boil for one minute to completely dissolve the medium.

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

Aseptically dispense the specified volume into appropriate sterile containers and allow to cool.

6. Physical Characteristics

	Dehydrated Medium	Prepared Medium
Appearance and Colour	Pale straw fine powder	Green-blue firm gel
pH	N/A	7.5 ± 0.2

7. Materials Provided

KM0005 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
KM0005-500G-500	1 x 500g Dehydrated Culture Media Tub
KM0005-5KG-5000	1 x 5kg Dehydrated Culture Media Tub
KM0005-10KG-10000	1 x 10kg Dehydrated Culture Media Tub

8. Materials Needed but not Provided

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

9. Specimens

KM0005 CLED Double Indicator Agar (Bevis) can be used with the following specimens:

- According to multiple UKHSA SMI's, KM0005 may be used in the testing of many types of clinical specimens including (as Standard Medium) infections of ear, skin, pus, bile, deep sites and organs, urine, bronchoalveolar, and organ transport fluid. It may also be used as primary isolation medium for *Enterobacteriaceae*, *Pseudomonas* spp., *Salmonella* spp., streptococci/enterococci, staphylococci, micrococci, and *Rothia* spp.

The laboratory transport of specimens must be in accordance with the end user's and suppliers' recommendations for the conservation of microorganisms. Refer to appropriate standard method or local guidance on sample collection and processing.

10. Test Procedures and Interpretation of results

Allow the plates to come to room temperature.

Clinical Specimens [2 3 4]

- 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 37 ± 1°C aerobically for 18-24 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. Refer to relevant local guidelines.

11. Quality Control

Organism	Incubation	Result (Specificity)
S. aureus (NCTC 12981)	37 ± 1°C aerobically for 18-24 hours	Growth: yellow/orange colonies with red halo
E. coli (NCTC 12241)	37 ± 1°C aerobically for 18-24 hours	Growth: orange colonies with red halo
P. mirabilis (NCTC 10975)	37 ± 1°C aerobically for 18-24 hours	Growth: blue colonies (no swarming)
P. aeruginosa (NCTC 12903)	37 ± 1°C aerobically for 18-24 hours	Growth: blue colonies
K. pneumoniae (NCTC 9633)	37 ± 1°C aerobically for 18-24 hours	Growth: yellow colonies
E. faecalis (NCTC 12697)	37 ± 1°C aerobically for 18-24 hours	Growth: pale yellow colonies
P. vulgaris (ATCC 8427)	37 ± 1°C aerobically for 18-24 hours	Growth: blue colonies (no swarming)

ATCC is a trademark of the American Type Culture Collection.

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 KM0005 CLED Double Indicator Agar (Bevis) performance, dehydrated culture media samples were used to prepare CLED DI poured plates and tested to assess colony morphology and recovery level (where an acceptable range is ≥70% and ≤120%) compared to a non-selective reference medium. Prepared samples were inoculated with 30-150cfu and incubated at 35-37°C for 18-24 hours. All samples of prepared media showed expected recovery and morphology of the required test organisms: *Staphylococcus aureus* (NCTC 12981), *Escherichia coli* (NCTC 12241), *Proteus mirabilis* (NCTC 10975), *Pseudomonas aeruginosa* (NCTC 12903), *Klebsiella pneumoniae* (NCTC 9633), *Enterococcus faecalis* (NCTC 12697) and *Proteus vulgaris* (ATCC 8427). Therefore, it can be concluded that KM0005 CLED Double Indicator Agar (Bevis), meets performance criteria when used according to the instructions outlined above. Trend analysis data available upon request.

13. Limitations of the Media

- Some streptococci and other organisms requiring blood or serum for growth may be scarcely recovered on this medium or may need extended incubation. Therefore, an additional blood agar plate should also be inoculated if such organisms are suspected.
- Not suitable for recovery of several genitourinary pathogens such as *Neisseria gonorrhoeae*, *Gardnerella vaginalis*, *Chlamydia*, *Mycoplasma* or other fastidious organisms as these organisms do not grow on this medium.
- *Shigella* species may not grow on this medium.
- 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 KM0005 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. Mackey, J. and Sandys, G. (1966) Diagnosis of Urinary Infections. British Medical Journal, 1(5496), pp.1173-1173.
2. Sandys, G. (1960) A new method of preventing swarming of Proteus species with a description of a new medium suitable for use in routine laboratory practice. Journal Medical Laboratory Technology, 17, pp.224-233.
3. Bevis, T. (1968) Modified electrolyte deficient culture medium. Journal of Medical Laboratory Technology, 25(1), pp.38-41.
4. Public Health England. UK Standards for Microbiology Investigations. Bacteriology. London: Standards Unit, Microbiology Services.
5. 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	06/04/2023 - New Document Created
002	18/09/2023 - Add additional details in Section 8
003	10/03/2025 - Update based on new formatting and device file.

*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