

Instructions for Use

REF - PP0540

CLED Double Indicator Agar (Bevis)

Pre-Poured Media

1. Intended Use

For *in vitro* diagnostic use only. PP0540 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*

<u>Ingredient</u>	<u>g/L</u>
Peptone	8.0
Beef extract	3.0
Lactose	10.0
L-cystine	0.128
Bromothymol blue	0.02
Acid fuchsin	0.08
Agar	15.0

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

PP0540 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⁾.

PP0540 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 alkalization. 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. Physical Characteristics

	Medium
Appearance and Colour	Green/blue firm gel
pH	7.5 ± 0.2

6. Materials Provided

PP0540 CLED Double Indicator Agar (Bevis) is supplied in packs of 10 x 90mm P090 Petri dishes (18-22ml fill) (product code: PP0540-P090). Each plate is ink-jet printed with (abbreviated) product name, product code, lot number and expiry date.

7. Materials Needed but not Provided

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

8. Specimens

PP0540 CLED Double Indicator Agar (Bevis) is suitable for the for the testing of the following samples:

- According to multiple UKHSA SMI's, PP0540 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.

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.

9. 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 $36 \pm 1^\circ\text{C}$ for 16-24 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.

10. Quality Control

Organism	Incubation	Result (Specificity)
<i>S. aureus</i> (NCTC 12981)	$37 \pm 1^\circ\text{C}$ for 18-24 hours	Growth: Yellow/orange with red halo
<i>E. coli</i> (NCTC 12241)	$37 \pm 1^\circ\text{C}$ for 18-24 hours	Growth: Orange colonies with pale red halo
<i>P. mirabilis</i> (NCTC 10975)	$37 \pm 1^\circ\text{C}$ for 18-24 hours	Growth: Blue colonies

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).

11. Performance

To fully verify PP0540 CLED Double Indicator Agar (Bevis) performance, samples were tested to assess colony morphology and recovery level (where an acceptable range is $\geq 70\%$ and $\leq 120\%$) compared to a non-selective reference medium. Samples were inoculated with 30-150cfu and incubated at $37 \pm 1^\circ\text{C}$ for 18-24 hours. All samples showed expected recovery and morphology of the required test organisms: *Staphylococcus aureus* (NCTC 12981), *Escherichia coli* (NCTC 12241) and *Proteus mirabilis* (NCTC 10975). Therefore, it can be concluded that PP0540 CLED Double Indicator Agar (Bevis), meets performance criteria when used according to the instructions outlined above. Trend analysis data available upon request.

12. 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.

13. 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 the E&O Poured Plate Material Safety Data Sheet.

14. Storage conditions and Shelf life

Store PP0540 in the original packaging between 2 and 8°C in the dark. Kept under these conditions it may be used up to date of expiry shown on product label.

Dispose of in accordance with local and national authority requirements. Do not use media if it is contaminated, discoloured, out of date or if cracks in the agar medium are present. Additionally, do not use media if it has been stored inappropriately or the packaging has been damaged.

15. 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 23/05/2023 - New Document Created
- 002 18/09/2023 - Add additional details in Section 8
- 003 27/02/2025 - Typographical errors corrected

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



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TABLE OF APPLICABLE SYMBOLS

 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