BD™ CLED Agar (Bevis)

INTENDED USE

BD CLED Agar (Bevis) is a modified CLED (Cystine-Lactose-Electrolyte-Deficient) Agar. It is a differential culture medium for use in isolating and enumerating bacteria in urine.

PRINCIPLES AND EXPLANATION OF THE PROCEDURE

Microbiological method.

In 1960, Sandys reported on the development of a new method of preventing the swarming of *Proteus* on solid media by restricting the electrolytes in the culture medium which was modified later several times for use in urine culture. [1-3] It was designated as Cystine-Lactose-Electrolyte-Deficient (CLED) medium and reported to be ideal for dip-inoculum techniques and for urinary bacteriology in general. Bevis modified the medium by adding Andrade indicator (acid fuchsin) to the medium.[4] The combination of the two pH indicators, bromthymol blue and acid fuchsin, allows an improved differentiation of the organisms by colony and medium coloration [4,5]

In **BD CLED Agar (Bevis)**, gelatin and casein peptones are nitrogen sources, and beef extract provides additional nutrients. Lactose is included to provide an energy source for organisms capable of utilizing it by a fermentative mechanism. Cystine permits the growth of "dwarf colony" coliforms. Bromthymol blue and acid fuchsin are used as a pH indicator system to differentiate lactose fermenters from lactose nonfermenters. Electrolyte sources are reduced to minimize swarming of *Proteus* species.

REAGENTS

BD CLED Agar (Bevis)

Formula* Per Liter Purified Water

Gelatin Peptone	4.0 g
Casein Peptone	4.0
Beef Extract	3.0
Lactose	10.0
L-Cystine	0.13
Bromthymol Blue	0.02
Andrade Indicator (Acid Fuchsin)	0.1
Agar	15.0

pH 7.5 +/- 0.2

*Adjusted and/or supplemented as required to meet performance criteria.

PRECAUTIONS

IVD . For professional use only.

Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration.

Consult **GENERAL INSTRUCTIONS FOR USE** document for aseptic handling procedures, biohazards, and disposal of used product.

STORAGE AND SHELF LIFE

On receipt, store plates in the dark at 2 to 8° C, in their original sleeve wrapping until just prior to use. Avoid freezing and overheating. The plates may be inoculated up to the expiration date (see package label) and incubated for the recommended incubation times.

Plates from opened stacks of 10 plates can be used for one week when stored in a clean area at 2 to 8° C.

USER QUALITY CONTROL

Inoculate representative samples with the following strains (for details, see **GENERAL INSTRUCTIONS FOR USE** document). Incubate plates at 35 ± 2°C in an aerobic atmosphere. Examine plates at 18 to 24 hours for amount of growth, pigmentation, colony size and inhibition of *Proteus* swarming/spreading.

Strains	**Growth Results**
Escherichia coli ATCC™ 25922	Medium-sized to large orange-red colonies with pink to red halos
Proteus vulgaris ATCC 8427	Medium-sized to large colorless to grey-blue colonies, swarming partially to completely inhibited
Enterococcus faecalis ATCC 29212	Small to medium-sized white to yellow colonies, pink to red halos
Staphylococcus aureus ATCC 25923	Medium-sized golden-yellow colonies with rose halos
Staphylococcus saprophyticus ATCC 15305	Small to medium-sized white to pale rose colonies, pink halos
Uninoculated	Blue

PROCEDURE

Materials Provided

BD CLED Agar (Bevis), provided in 90 mm **Stacker™** plates. Microbiologically controlled.

Materials Not Provided

Ancillary culture media, reagents and laboratory equipment as required.

Specimen Types and Collection of Specimens

This medium is exclusively used for enumerating and differentiating bacteria in urine. Midstream or catheter urine, or urine collected by suprapubic bladder puncture can be used (see also **PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE**). Observe aseptic techniques for collecting urine specimens. Urine must be directly streaked onto the medium not later than 2 hours after collection or must be kept refrigerated (not longer than 24 hours) to avoid overgrowth of the infectious agents or contaminants before inoculation of this medium.[6,7]

Test Procedure

Collect a sample of the undiluted, well-mixed urine using a calibrated loop (0.01 or 0.001 ml). Ensure proper loading of the loop with the specimen. Inoculate the sample down the middle of the plate in a single streak from which additional spreading of the inoculum is performed. [6,7] Incubate plates in ambient air at 35 ± 2°C for 18 to 24 h.

Results

Typical colony appearance on **BD CLED Agar (Bevis)** is as follows:

Organisms	**Growth Results**
Escherichia coli	Orange-red to red colonies with rose to pink halos
Proteus mirabilis	Blue-green, transparent colonies
Klebsiella, Enterobacter	Grey-green or orange to blue, mucoid colonies
Staphylococcus aureus	Smooth, opaque, golden-yellow colonies with rose halos
Staphylococcus saprophyticus	Smooth white to pale rose colonies, pink halos
Enterococcus faecalis	Small opaque yellow to orange colonies, small rose to pink halos

Calculation and Interpretation of Results

Count the number of colonies (cfu) on the plate. If a 0.01 ml loop was used, each resultant colony is representative of 100 CFU/ml; if a 0.001 ml loop was used, each colony corresponds to 1000 CFU/ml of urine.[6,7]

Midstream and catheter urine: Current guidelines indicate that for a single isolate a density of $\geq 10^5$ cfu/ml indicates infection, $<10^5$ cfu/ml indicates urethral or vaginal contamination, and between 10^4 to 10^5 CFU/ml needs to be re-evaluated based on clinical information.[6,7] Contaminant bacteria usually appear in low numbers which vary in colonial morphology.
Urine collected by suprapubic bladder puncture: Since the bladder is sterile in non-infected individuals, any cfu detected indicates an infection.

Urinary pathogens will usually yield high counts having uniform colonial morphology and must be subcultured directly to routine media for identification and susceptibility testing.[6,7,9]

PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE

BD CLED Agar (Bevis) is suitable for the isolation and counting of many aerobically growing microorganisms, such as *Enterobacteriaceae*, *Pseudomonas* and other non-fermenting Gram negative rods, enterococci, staphylococci, *Candida* species, and many others from urine specimens.

Performance Results

In an internal evaluation, **BD CLED Agar (Bevis)** was tested with strains of *Enterobacteriaceae*, *Streptococcus agalactiae* and *Candida albiacans*.[8] Most strains were collection strains, but several clinical isolates were also included. **BD Columbia Agar with 5% Sheep Blood** was used as a growth reference medium. After 20 hours of incubation at 35 ± 2°C, all *Enterobacteriaceae* and *C. albicans* grew very well on the medium and produced the expected color reactions. Growth of *S. agalactiae* was acceptable, and the colonies were tiny to small. Detailed results were as follows:

Species	**Results on BD CLED Agar (Bevis)**
Candida albicans	Tiny to small whitish colonies on a blue medium
Citrobacter freundii	Red colonies on a red medium
Enterobacter cloacae	Red colonies on a red medium
Klebsiella pneumoniae	Red colonies on a red medium
Proteus mirabilis	Greyish colonies on a blue medium; swarming partially inhibited
Proteus vulgaris	Greyish colonies on a blue medium; swarming partially inhibited
Providencia stuartii	Pale blue colonies on a blue medium
Salmonella Typhimurium	Pale blue transparent colonies, on a blue medium
Serratia liquefaciens	Grey-white colonies on a blue medium
Shigella sonnei	Pale blue transparent colonies on a blue medium
Streptococcus agalactiae	Tiny to small orange colonies with rose to pink halos on a red medium

Limitations of the Procedure

Streptococci and other organisms requiring blood or serum for growth may only be insufficiently recovered on this medium or may need extended incubation. Therefore, the specimen should also be cultivated onto a blood agar plate if such organisms are expected.
Genitourinary pathogens such as *Neisseria gonorrhoeae, Gardnerella vaginalis, Chlamydia, Ureaplasma,* or other fastidious organisms do not grow on this medium. Consult the reference for the appropriate detection techniques of these organisms.[6]
Although a differentiation according to lactose fermentation and certain diagnostic tests may be performed directly on this medium, biochemical and, if indicated, immunological testing using pure cultures is necessary for complete identification.[9]
CLED Agar (Bevis) must not be incubated longer than 24 hours as this might cause wrong color reactions.

REFERENCES

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PACKAGING/AVAILABILITY

BD CLED Agar (Bevis)
Cat. No. 255529 Ready-to-use Plated Media, cpu 20

FURTHER INFORMATION

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