

TSA with 5% Sheep Blood / CLED Agar

| Ready-to-use Media

a product by Biomed MDX



Intended Use:

TSA with 5% Sheep Blood and CLED Agar (Biplate) medium are used for urinary microbiology analysis. TSA with 5% sheep Blood Agar is a general-purpose medium for the isolation and cultivation of both non-fastidious and fastidious microorganisms from a variety of clinical and nonclinical materials. It also enables the observation of hemolytic reactions. CLED Agar is a medium for isolating, enumerating, and presumptively identifying microorganisms from urine.

Principle of the Procedure:

TSA with 5% Sheep Blood Agar:

TSA enriched with 5% sheep blood is a widely used culture medium in microbiology laboratories. Its formulation provides a nutrient-rich environment suitable for the cultivation of diverse bacterial species, encompassing both fastidious and non-fastidious organisms. The inclusion of sheep blood serves a dual purpose which is to enhance the cultivation of fastidious bacteria that may have complex nutritional requirements and allows for the differentiation of bacterial species based on their hemolytic properties.

CLED Agar:

The nutrients in CLED Agar are supplied by peptones, pancreatic digests of gelatin and casein, and beef extract. Lactose is included to provide an energy source for organisms capable of utilizing it by a fermentative mechanism. The cystine permits the growth of dwarf colony coliforms. Bromothymol blue is used as a pH indicator to differentiate lactose fermenters from lactose-no fermenters. Organisms that ferment lactose will lower the pH and change the color of the medium from green to yellow. Electrolyte sources are reduced to restrict the swarming of *Proteus* species.

Product Summary:

TSA with 5% Sheep Blood:

TSA is a widely used growth medium derived from a soybean-based formula outlined in the U.S. Pharmacopeia. The inclusion of blood in this medium enhances its ability to support the growth of fastidious bacteria, those with complex nutritional requirements. Furthermore, the presence of blood allows for the observation of hemolysis, the breakdown of red blood cells. This characteristic, particularly the type of hemolysis observed, is an important tool for differentiating various bacterial species, especially those belonging to the *Streptococcus* genus. The absence of carbohydrates in the medium ensures that hemolysis is accurately observed and not masked by other metabolic reactions.

CLED Agar:

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. Previous chemical methods used to inhibit swarming by *Proteus* included the addition of chloral hydrate, alcohol, sodium azide, surface-active agents, boric acid, and sulfonamides to the culture medium. This electrolyte-deficient medium of Sandys was modified by Mackey and Sandys for use in urine culture by substituting lactose and sucrose for the mannitol and increasing the concentrations of the bromothymol blue indicator and of the agar. These two investigators further modified the medium by the incorporation of cystine to enhance the growth of cystine-dependent "dwarf colony" coliforms and by deletion of sucrose. They designated the new medium as Cystine-Lactose-Electrolyte-Deficient (CLED) medium and reported it to be ideal for dip-inoculum techniques and for urinary bacteriology in general¹⁻³

Formulation* (PER LITER):

TSA with 5% Sheep Blood Agar		CLED Agar	
Pancreatic Digest of Casein	15.0g	Pancreatic Digest of Gelatin	4.0g
Papaic Digest of Soybean	5.0g	L-Cystine	128.0mg
Sheep Blood	50.0g	Pancreatic Digest of Casein	4.0g
Sodium Chloride	5.0g	Bromothymol Blue	0.02g
Agar	15.0g	Beef Extract	3.0g
		Lactose	10.0g
		Agar	15.0g

pH 7.3 +/- 0.2

pH 7.3 +/- 0.2

*Adjust and/or supplemental as required to meet performance criteria

Procedure

Materials Provided

90mm TSA with 5% Sheep Blood / CLED Agar.

Materials Required but Not Provided

Ancillary culture media, reagents, and laboratory equipment as required.

Test Procedure

1. Collect a sample of the undiluted, well-mixed urine using a calibrated loop (0.01 or 0.001 ml) for each of the two media of this biplate.
2. First, streak a sample of the urine on TSA with 5% Sheep Blood Agar., then the second sample on CLED Agar.
3. Incubate plates at 35°C ± 2°C for 18 to 24 hours.
4. Observe the result according to user requirements.
5. Dispose of all used reagents and contaminated materials as infectious waste. Laboratories must handle and dispose of all waste safely according to regulations.

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 urine⁴

Quality Control

Inoculate representative samples with the following strains. Incubate the inoculated plates at 35 ± 2°C for 18 to 24 hrs. to allow colonies to develop on the medium.

TSA with 5% Sheep Blood Agar:

Strains	ATCC®	Growth
<i>Escherichia coli</i>	25922	Growth at 24 hours, beta hemolysis
<i>Streptococcus pyogenes</i>	19615	Growth at 24 hours, beta hemolysis
<i>Streptococcus pneumoniae</i>	6305	Growth at 24 hours, alpha hemolysis
<i>Candida albicans</i>	60193	Growth at 24 hours, no hemolysis
<i>Enterococcus faecalis</i>	29212	Growth at 24 hours, gamma hemolysis
Uninoculated plate	-	No growth

CLED Agar:

Strains	ATCC®	Growth
<i>Proteus mirabilis</i>	12453	Growth; colonies blue, medium blue-green to blue
<i>Escherichia coli</i>	25922	Growth; colonies yellow, medium yellow
<i>Staphylococcus aureus</i>	25923	Growth; colonies small, yellow; medium yellow
<i>Proteus vulgaris</i>	8427	Growth; colonies colorless to blue; swarming inhibited; slight spreading acceptable
Uninoculated plate	-	No growth

Transportation:

Temperature fluctuations may occur during transportation. However, these fluctuations do not affect the performance, quality, or safety of the media.

Storage and Shelf Life:

Upon receipt, store plates at 2 to 8°C, in their original sleeve wrapping until just before 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.

Warning and Precautions:

For in vitro diagnostic use. For Professional Use Only. Do Not Reuse.

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

Limitation of the Procedure











This medium is for laboratory use only and is not intended for the diagnosis of disease or other conditions. Identifications are presumptive and colonies should be identified using appropriate methods ⁵⁻⁸

Reference

1. Mackey, J.P., and G.H. Sandys. 1965. Laboratory diagnosis of infection of the urinary tract in general practice by means of a dip-inoculum transport medium. *Br. Med. J.* 2:1286–1288.
2. Sandys, G.H. 1960. A new method of preventing swarming of *Proteus* sp. with a description of a new medium suitable for use in routine laboratory practice. *J. Med. Lab. Technol.* 17:224–233.
3. Mackey, J.P., and G.H. Sandys. 1966. Diagnosis of urinary infections. *Br. Med. J.* 1:1173.
4. Thomson, R.B., and J.M. Miller. 2003. Specimen collection, transport, and processing: bacteriology. In: Murray, P. R., E. J. Baron, J.H. Jorgensen, M. A. Pfaller, and R. H. Tenover (ed.). *Manual of clinical microbiology*, 8th ed. American Society for Microbiology, Washington, D.C.





5. Holt, J.G., N.R. Krieg, P.H.A. Sneath, J.T. Staley, and S.T. Williams (ed.). 1994. *Bergey's Manual™ of determinative bacteriology*, 9th ed. Williams & Wilkins, Baltimore.
6. MacFaddin, J.F. 2000. *Biochemical tests for identification of medical bacteria*, 3rd ed. Lippincott Williams & Wilkins, Baltimore.
7. Koneman, E.W., S.D. Allen, W.M. Janda, P.C. Schreckenberger, and W.C. Winn, Jr. 1997. *Color atlas and textbook of diagnostic microbiology*, 5th ed. Lippincott-Raven, Philadelphia.
8. Isenberg, H.D. (ed.). 2004. *Clinical microbiology procedures handbook*, vol. 1, 2 and 3, 2nd ed. American Society for Microbiology, Washington, D.C.

Packaging Symbol

Symbol	Definition
	Catalogue number
	In Vitro Diagnostic Medical Device
	Batch code
	Date of manufacture
	Temperature limit
	Use-by date
	Keep away from sunlight
	Do not re-use
	Fragile, handle with care
	Consult instructions for use or consult electronic instructions for use
	Do not use if packaging damaged and consult instructions for use
	Manufacturer

Further Information:

For further information please contact your Biomed MDX representative.

 Biomed MDX Sdn Bhd
8, Jalan IAN 3, Industri Angkasa Nuri,
76100 Durian Tunggal, Melaka, Malaysia
 +6063370191
 <https://biomedmdx.com/>
 info@biomedmdx.com