

# 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:

Peptones and Lab Lemco powder are present to supply the materials required for the growth of bacteria and agar in the solidifying agent. Lactose provides a carbohydrate source. Bromothymol blue is a pH indicator which differentiates lactose fermenters (yellow) from non-fermenters. Cystine enhances the growth of cystine-dependent coliforms. Electrolytes are reduced in order to restrict the swarming of *Proteus spp.*

### Product Summary:

#### TSA with 5% Sheep Blood Agar:

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:

Urinary tract infections, often referred to as UTIs, are common infections that occur when bacteria invade the urinary tract by entering via the urethra. These infections can impact different parts of the urinary tract, but the most prevalent is cystitis (a bladder infection). The symptoms of cystitis can include frequent urination, or needing to urinate whilst having an empty bladder, a burning sensation or pain whilst urinating, blood in the urine and pressure or cramps in the lower abdominal area. The symptoms of kidney infections are very different. These can include chills, a fever, nausea and vomiting and pain in the lower back<sup>1</sup>. There are many species of bacteria that cause UTIs, but most common are *Escherichia coli*, *Staphylococcus aureus* and *Proteus mirabilis*<sup>2,3</sup>. Due to the severity of UTIs in a vast number of patients, it is very important to be able to isolate and identify common UTI-causing microorganisms from urine samples and wound swabs. Early diagnosis is vital in the prevention and reduction of urinary tract infections.

**Formulation\* (PER LITER):**

TSA with 5% Sheep Blood Agar		CLED Agar	
Tryptone	15.0g	Peptone	17.0g
Soya peptone	5.0g	'Lab-Lemco' powder	3.0g
Sheep Blood	50.0g	Tryptone	10.0g
Sodium chloride	5.0g	Lactose	1.5g
Agar	15.0g	L-Cystine	5.0g
		Bromothymol Blue	1.0mg
		Agar	13.5g

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 urine<sup>7</sup>

### 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.













**Limitations 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 <sup>8-11</sup>

## Reference

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**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:**

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