Rev: 0
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TSA with 5% Sheep Blood / Orientation Agar | Ready-to-use Media

a product by Biomed MDX

IVD

Intended Use:

TSA with 5% Sheep Blood and Orientation 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. Orientation 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.

Orientation:

The CHROMagar™ Orientation Medium is a differential chromogenic medium that enables the simultaneous isolation and presumptive identification of urinary tract pathogens (UTPs). This is achieved by incorporating multiple proprietary chromogenic substrates. Specific microbial enzymes produced by target organisms hydrolyze these substrates, releasing an insoluble, colored chromophore that accumulates within the colony structure. This enzymatic action results in species-specific colony colors, allowing for rapid differentiation and enumeration directly from urine specimens, followed by further confirmation tests.

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.

Orientation:

CHROMagar™ Orientation Medium is a differential chromogenic agar designed for the rapid isolation, enumeration, and presumptive identification of urinary tract pathogens (UTPs) directly from urine samples. By utilizing proprietary substrates, the medium generates species-specific colony colors (chromophores) via enzymatic cleavage, significantly accelerating the diagnosis of UTIs.

Formulation* (PER LITER):

TSA with 5% Sheep Blood Agar		Orientation Agar	
Pancreatic Digest of Casein	15.0g	Peptones and yeast extract	17.0g
Papaic Digest of Soybean	5.0g	Chromogenic mix	1.0g
Sheep Blood	50.0g	Agar	15.0g
Sodium Chloride	5.0g		
Agar	15.0g		

pH 7.3 +/- 0.2 pH 7.0 +/- 0.2

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

Procedure

Materials Provided

90mm TSA with 5% Sheep Blood / Orientation 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 Orientation 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[4]

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

Orientation Agar:

Strains	ATCC®	Growth
Enterococcus faecalis	29212	Turquoise blue
Streptococcus agalactiae	12386	Light blue
Staphylococcus aureus	25923	Golden, opaque, small
Escherichia coli	25922	Dark pink to reddish
Klebsiella aerogenes	13048	Metallic blue (+/- reddish halo)
Proteus vulgaris	8427	Blue with brown halo
Candida albicans	10231	Cream, pinpoint colonies
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. Lüthje, P., Pranada, A. B., Carruthers-Lay, D., Desjardins, M., Gaillot, O., Wareham, D., Ciesielczuk, H., & Özenci, V. (2017). Identification of microorganisms grown on chromogenic media by MALDI-TOF MS. *Journal of Microbiological Methods, 136*, 17–20. https://doi.org/10.1016/j.mimet.2017.03.001

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Packaging Symbol

Symbol	Definition
REF	Catalogue number
IVD	In Vitro Diagnostic Medical Device
LOT	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.

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