Biomed Global

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TCBS / XLD Agar | Ready-to-use Media

a product by Biomed MDX

IVD

Intended Use:

TCBS Agar and XLD Agar (Biplate) medium are used for the selective isolation and presumptive identification of different bacterial organisms that are often implicated in gastrointestinal illnesses.

Principle of the Procedure:

TCBS Agar:

TCBS (Thiosulfate-Citrate-Bile Salts-Sucrose) agar is used to isolate and presumptively identify other enteropathogenic, particularly those that cause food poisoning. It contains selective agents (like bile salts and sodium cholate) that inhibit gram-positive bacteria and some gram-negative bacteria that are not members of the vibrio genus. Sucrose is fermented and produces acid that lowers the pH of the medium and turns to yellow colonies on agar for example *Vibrio alginolyticus*. Species such as *Vibrio parahaemolyticus*, do not ferment sucrose and produce blue-green colonies. Further microbiological identification tests are necessary to confirm and diagnose the presence of microorganisms.

XLD Agar:

XLD (Xylose Lysine Deoxycholate) agar is used to isolate and identify enteric gram-negative bacteria like *Salmonella spp.* and *Shigella spp*. It contains selective agents (like deoxycholate) that inhibit gram-positive bacteria, allowing gram-negative bacteria to grow. Xylose is fermented by most enteric bacteria, producing acid that lowers the pH of the medium. Lysine helps detect bacterial decarboxylation, which causes an alkaline reaction. The pH indicator phenol red turns yellow in acidic conditions (from xylose fermentation) and red in alkaline conditions (from lysine decarboxylation). The agar also contains iron salts that react with hydrogen sulfide (H_2S) from some bacteria (like *Salmonella spp*.), forming a black precipitate. Yeast extract provides nutrients to promote growth, and sodium chloride maintains osmotic balance.

Product Summary:

TCBS 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 general.

XLD Agar:

Xylose lysine deoxycholate (XLD) agar detects gastrointestinal pathogens, including *Salmonella spp*. and *Shigella spp*. by inhibiting gram-positive bacteria, allowing gram-negative bacteria to grow. Xylose is fermented by most enteric bacteria, producing acid that turns the medium yellow. Bacteria that decarboxylate lysine create an alkaline environment, resulting in red colonies. *Salmonella spp.* produces hydrogen sulfide (H_2S), forming black colonies when it reacts with iron salts in the medium. XLD agar is used mainly to isolate *Salmonella spp.* and *Shigella spp*. from clinical and environmental samples, including stool. Yellow colonies indicate xylose fermentation (e.g., *E. coli*), red colonies show lysine decarboxylation without xylose fermentation, and black colonies suggest H_2S production, typical of *Salmonella spp*. Further microbiological identification tests are necessary to confirm and diagnose the presence of microorganisms.

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Formulation* (PER LITER):

TCBS Agar		XLD Agar	
Yeast Extract	5.0g	Xylose	3.5g
Proteose Peptone No.3	10.0g	L-Lysine	5.0g
Sodium Citrate	10.0g	Lactose	7.5g
Oxgall	8.0g	Saccharose	7.5g
Saccharose	20.0g	Sodium Chloride	5.0g
Sodium Chloride	10.0g	Yeast Extract	3.0g
Ferric Ammonium Citrate	1.0g	Phenol Red	0.08g
Bromothymol Blue	0.04g	Sodium Desoxycholate	2.5g
Thymol Blue	0.04g	Ferric Ammonium Citrate	0.8g
Agar	15.0g	Sodium Thiosulfate	6.8g
		Agar	13.5g

pH 8.6 +/- 0.2 pH 7.4 +/- 0.2

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

Procedure

Materials Provided

90mm TCBS/XLD 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 on TCBS Agar, then the second sample on XLD 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.

TCBS Agar:

Strains	ATCC®	Growth
Vibrio alginolyticus	17749	Yellow colonies
Vibrio parahaemolyticus	17802	Blue green colonies
Escherichia coli	25922	No growth
Uninoculated plate	-	No growth

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XLD Agar:

Strains	ATCC®	Growth
Escherichia coli	25922	Yellow
Shigella flexneri	12022	Red
Salmonella choleraesuis subsp. choleraesuis serotype Typhimurium	14028	Red with black centers
Enterococcus faecalis	29212	No growth
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

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2. Newton, A., Kendall, M., Vugia, D. J., Henao, O. L., & Mahon, B. E. (2012). Increasing rates of vibriosis in the United States, 1996–2010: review of surveillance data from 2 systems. Clinical Infectious Diseases, 54(suppl_5), S391-S395.
3. Evans, T. J., & Riley, P. A. (2021). Principles of microscopy, culture and serology-based diagnostics. Medicine, 49(10), 648-653.
4. Hess, C., Drauch, V., Spergser, J., Kornschober, C., & Hess, M. (2023). Detection of atypical Salmonella Infantis phenotypes in broiler environmental samples. Microbiology Spectrum, 11(3), e00106-23.
5. Eslami, N., Anzabi, Y., & NourAzar, M. A. (2023). Comparison of the Effect of Temperature and Different Culture Media on the Possibility of Growth of Salmonella Typhimurium (ATCC: 14028). International Journal of Medical Laboratory.
6. Ali, N. S., Abdulkareem, R. A., & Ali, R. S. (2022, January). Study of diarrheagenic E. coli in Iraqi children. In AIP Conference Proceedings (Vol. 2386, No. 1). AIP Publishing.

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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:

For further information please contact your Biomed MDX representative.

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