XLD AGAR

- For in vitro use only - Catalogue No. PX75

Our XLD Agar is a selective, differential medium used for the isolation and differentiation of gram-negative enteric pathogens, in particular *Shigella* and *Salmonella* species.

XLD Agar is a modification of Taylor's original work, whom first developed XL Agar as a non-selective medium for the isolation of enteric organisms. The addition of desoxycholate made the medium selective while the addition of ferric ammonium citrate and sodium thiosulfate made the medium more differential by allowing for the detection of hydrogen sulfide production.

The selectivity of the medium is due to the presence of sodium desoxycholate, a bile salt that is inhibitory to gram-positive organisms.

The presence of carbohydrates (lactose, sucrose, xylose), lysine, and the pH color indicator, phenol red, make the medium differential. The utilization of these substrates results in a pH change indicated by a color change in the color indicator. The three carbohydrate sources added are present at different concentrations, xylose is limited while both lactose and sucrose are considered inexhaustible during the prescribed incubation period. Degradation of xylose, lactose, and sucrose generate acid end products that change the indicator and the medium from red to yellow.

Organisms able to ferment only xylose, such as *Salmonella*, will exhaust the supply of xylose and begin to utilize lysine. The decarboxylation of lysine results in the release of the alkaline end product, cadaverine, and a reversion of the medium color back to red. Uninhibited organisms able to utilize either lactose and/or sucrose will not exhaust their carbohydrate source and generate acid continually resulting in yellow zones around those colonies. Organisms unable to utilize any of the carbohydrates, such as *Shigella*, will not produce any significant change and therefore the colonies and medium will remain red after incubation.

The other differential characteristic detectable on XLD Agar is the production of hydrogen sulfide; organisms capable of reducing sodium thiosulfate release hydrogen sulfide gas as a byproduct; ferric ammonium citrate reacts with H_2S producing a visible black precipitate under alkaline conditions. Therefore H_2S-positive organisms, such as *Salmonella*, develop colonies with black centers.

Formula per Litre of Medium

Yeast Extract	3.0 g
Lactose	7.5 g
Sucrose	7.5 g
L-Lysine	5.0 g
Xylose	3.5 g
Sodium Chloride	5.0 g
Sodium Desoxycholate	2.5 g
Sodium Thiosulfate	6.8 g
Ferric Ammonium Citrate	0.8 g
Phenol Red	0.08 g
Agar	15.0 g

pH 7.5 ± 0.2

Recommended Procedure

1. Allow medium to reach room temperature.
2. Using an inoculum from the specimen streak so as to obtain isolated colonies. If desired, feces and rectal swabs may be directly streaked onto the medium. Roll the swab several times over a small portion of the medium near the edge of the plate. Using a sterile loop, perform a four-quadrant streak starting where the swab was inoculated.
3. Incubate aerobically at 35°C.
4. Examine after 18-24 hours.
5. Incubate for a further 24 hours for complete color development.

Interpretation of Results

Shigella species do not ferment xylose, lactose or sucrose and therefore colonies are red. The colonies are actually colorless and transparent but appear red due to the color of the medium. *Providencia*, *Pseudomonas*, and H_2S-negative *Salmonella* may produce similar red colonies.

Salmonella species ferment only xylose and undergo alkaline reversion due to lysine decarboxylation. The alkaline pH results in colonies being red. Centers appear black due to a positive H_2S reaction under alkaline conditions. *Edwardsiella tarda* of the wild-type biogroup may produce *Salmonella*-like colonies.

Other uninhibited organisms, able to ferment lactose or sucrose will produce yellow colonies due to continual acid production and a resultant shift in the pH. These organisms include *Escherichia*, *Klebsiella*, *Serratia*, *Citrobacter koseri*, *Yersinia enterocolitica*, *Providencia rettgeri*, and *Morganella morganii*. Yellow colonies are also observed for those organisms that are lysine-negative, such as *Proteus* species. *Proteus mirabilis* and *Proteus vulgaris* are H_2S-postivie and may also produce colonies with black centers.

Additional biochemical and/or serological tests should be performed on isolated colonies from pure culture in order to complete identification.

- *Prolonged incubation beyond recommended times is not prescribed since false-positives may arise due to alkaline reversion of normally acidic colonies*
- *Some Shigella species (1%) ferment lactose and produce atypical yellow colonies*
- *Non-enteric organisms such as Pseudomonas and Providencia may also grow on XLD and produce red Shigella-like colonies*

Quality Control

After checking for correct pH, colour, depth, and sterility, the following organisms are used to determine the growth performance of the completed medium.

Organism	Expected Results
Salmonella typhimurium ATCC 14028	Growth, red colonies with black centers
Shigella flexneri ATCC 12022	Growth, red colonies
Escherichia coli ATCC 25922	Inhibition (partial) Yellow colonies
Enterococcus faecalis ATCC 29212	Inhibition (partial)

Storage and Shelf Life

Our XLD Agar should be stored away from direct light at 4°C to 8°C. The medium side should be uppermost to prevent excessive accumulation of moisture on the agar surface. Under these conditions this medium has a shelf life of 8 weeks from the date of manufacture.

References

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