TSI AGAR

INTENDED USE

Triple Sugar Iron Agar (TSI Agar) is used for the differentiation of gram-negative enteric bacilli based on carbohydrate fermentation and the production of hydrogen sulfide.

SUMMARY AND EXPLANATION

TSI Agar is used for the determination of carbohydrate fermentation and hydrogen sulfide production in the identification of gram-negative bacilli.[1,2]

Hajna developed the formulation for TSI Agar by adding sucrose to the double sugar (dextrose and lactose) formulation of Kligler Iron Agar.[3] The addition of sucrose increased the sensitivity of the medium by facilitating the detection of sucrose-fermenting bacilli, as well as lactose and/or dextrose fermenters.

Carbohydrate fermentation is detected by the presence of gas and a visible color change (from red to yellow) of the pH indicator, phenol red. The production of hydrogen sulfide is indicated by the presence of a precipitate that blackens the medium in the butt of the tube.

PRINCIPLE

TSI Agar contains three sugars (dextrose, lactose and sucrose), phenol red for detecting carbohydrate fermentation and ferrous ammonium sulfate for detection of hydrogen sulfide production (indicated by blackening in the butt of the tube).

Carbohydrate fermentation is indicated by the production of gas and a change in the color of the pH indicator from red to yellow. To facilitate the detection of organisms that only ferment dextrose, the dextrose concentration is one-tenth the concentration of lactose or sucrose. The small amount of acid produced in the slant of the tube during dextrose fermentation oxidizes rapidly, causing the medium to remain red or revert to an alkaline pH. In contrast, the acid reaction (yellow) is maintained in the butt of the tube because it is under lower oxygen tension.

After depletion of the limited dextrose, organisms able to do so will begin to utilize the lactose or sucrose.[2]

To enhance the alkaline condition of the slant, free exchange of air must be permitted by closing the tube cap loosely. If the tube is tightly closed, an acid reaction (caused solely by dextrose fermentation) will also involve the slant.

REAGENTS (FORMULA)

Beef Extract	3.0	g
Yeast Extract	3.0	g
Pancreatic Digest of Casein	15.0	g
Proteose Peptone No. 3	5.0	g
Dextrose	1.0	g
Lactose	10.0	g
Sucrose	10.0	g
Ferrous Sulfate	0.2	g
Sodium Chloride	5.0	g
Sodium Thiosulfate	0.3	g
Phenol Red	24.0	mg
Agar	12.0	g
Deionized Water	1000.0	ml

PROCEDURE

To inoculate, carefully touch only the center of an isolated colony on an enteric plated medium with a cool, sterile needle, stab into the medium in the butt of the tube, and then streak back and forth along the surface of the slant. Several colonies from each primary plate should be studied separately, since mixed infections may occur.

Incubate with caps loosened at 35°C and examine after 18-24 hours for carbohydrate fermentation, gas production and hydrogen sulfide production. Any combination of these reactions may be observed. Do not incubate longer than 24 hours because the acid reaction in the slant of lactose and sucrose fermenters may revert to an alkaline reaction.

EXPECTED RESULTS

Compare reactions produced by the unknown isolate with those produced by the known control organisms.

Carbohydrate fermentation is indicated by a yellow coloration of the medium. If the medium in the butt of the tube becomes yellow (acidic), but the medium in the slant becomes red (alkaline), the organism being tested only ferments dextrose (glucose).

A yellow (acidic) color in the slant and butt indicates that the organism being tested ferments dextrose, lactose and/or sucrose.

A red (alkaline) color in the slant and butt indicates that the organism being tested is a nonfermenter.

Hydrogen sulfide production results in a black precipitate in the butt of the tube.

Gas production is indicated by splitting and cracking of the medium. For final identification, perform biochemical tests and other identification procedures with a pure culture of the organism. Consult appropriate references for further information.[4-6]

QUALITY CONTROL

All lot numbers have been tested and have been found to be acceptable. Customers can test products using the following quality control organisms. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, sample results should not be reported.

Organisms	Incubation	Results
Escherichia coli ATCC 25922	35 ± 2°C for 18-24 hours	Growth, Slant (A), Butt (A), Gas (+), H_2S (-)
Shigella flexneri ATCC 12022	35 ± 2°C for 18-24 hours	Growth, Slant (K), Butt (A), Gas (-), H_2S (-)

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