TRIPLE SUGAR IRON (TSI) AGAR

INTENDED USE
Remel Triple Sugar Iron (TSI) Agar is a solid medium recommended for use in qualitative procedures for differentiation of enteric gram-negative bacilli on the basis of carbohydrate fermentation and hydrogen sulfide (H_2S) production.

SUMMARY AND EXPLANATION
In 1911, Russell described a medium containing dextrose and lactose for differentiation of enteric gram-negative bacilli.[1] Krumwiede and Kohn modified Russell's double sugar medium by adding sucrose which allowed for detection of sucrose-fermenting gram-negative bacilli.[2] In 1940, Sulkin and Willet further modified the medium by adding ferrous sulfate, an indicator of H_2S production.[3] Hajna developed the recent formulation for Triple Sugar Iron Agar by adding sucrose to Kligler Iron Agar.[4]

PRINCIPLE
Casein and meat peptones provide nitrogenous compounds, amino acids, and peptides necessary for bacterial growth. Dextrose, lactose, and sucrose are fermentable carbohydrates. Phenol red is an indicator of carbohydrate fermentation. Fermentation reactions are read on the slant and in the butt, with a color change from red (alkaline) to yellow (acid). The dextrose concentration in TSI Agar is one-tenth the concentration of lactose and sucrose. This serves to distinguish dextrose-only fermenting organisms from those which ferment lactose and/or sucrose, as well. The small amount of acid produced in the slant during dextrose fermentation oxidizes rapidly, causing the slant revert to alkaline (red). The yellow acid reaction is maintained in the butt of the tube due to the absence of oxygen. If the lactose or sucrose is also fermented, sufficient acid is produced to retain the yellow color on the slant and in the butt. Ferric ammonium citrate is an indicator of H_2S production. If H_2S is produced from sodium thiosulfate, it reacts with ferric ammonium citrate to form a black precipitate (ferrous sulfate) in the butt of the tube. Gas production is indicated by bubbles, splitting of the agar, or displacement of the agar in the tube.

REAGENTS (CLASSICAL FORMULA)*

Casein Peptone	10.0	g	Dextrose	1.0	g
Lactose	10.0	g	Sodium Thiosulfate	0.2	g
Meat Peptone	10.0	g	Ferric Ammonium Citrate	0.2	g
Sucrose	10.0	g	Phenol Red	25.0	mg
Sodium Chloride	5.0	g	Agar	13.0	g
			Demineralized Water	1000.0	ml

pH 7.3 ± 0.2 @ 25°C

*Adjusted as required to meet performance standards.

PROCEDURE
1. The performance of this medium is dependent on proper inoculation.
2. Using an inoculating needle, inoculate TSI Agar with a single well-isolated colony from an 18-24 hour culture. Streak the slant back and forth across the entire surface and stab to the bottom of the agar butt.
3. Incubate the tube with cap loosened at 33-37°C for 18-24 hours in an aerobic atmosphere.
4. Examine for fermentation reaction, gas production, and H_2S production.

INTERPRETATION OF THE TEST
Carbohydrate Fermentation:

Positive Test for Slant Reaction - Yellow (acid = A)
Negative Test for Slant Reaction - Red (alkaline = ALK)

Positive Test for Butt Reaction - Yellow (acid = A)
Negative Test for Butt Reaction - Red (alkaline = ALK)

ALK/A - Dextrose only fermented
A/A - Dextrose and/or sucrose and/or lactose fermented
ALK/ALK - No sugar fermented

Hydrogen Sulfide Production:

Positive Test - Black color throughout the entire butt (may mask acidity), a black ring at the juncture of the slant and butt, or a black precipitate
Negative Test - No blackening of medium

Gas Production (CO_2 and H_2):

Positive Test - Bubbles in the medium, cracking and displacement of the medium, or separation of the medium from the side and bottom of the tube
Negative Test - No bubbles and no separation or displacement of the medium

(Continued on back)

QUALITY CONTROL

All lot numbers of Triple Sugar Iron (TSI) Agar have been tested using the following quality control organisms and have been found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

		RESULTS			
CONTROL	**INCUBATION**	**GAS**	**SLANT**	**BUTT**	**H_2S**
Escherichia coli ATCC® 25922	Aerobic, 18-24 h @ 33-37°C	+	A	A	-
Salmonella enterica serovar Typhimurium ATCC® 14028	Aerobic, 18-24 h @ 33-37°C	+	ALK	A	+
Pseudomonas aeruginosa ATCC® 27853	Aerobic, 18-24 h @ 33-37°C	-	ALK	ALK	-

LIMITATIONS

1. To enhance the alkaline condition in the slant, a free exchange of air must be permitted. If TSI tubes are tightly capped, an acid reaction caused solely by dextrose fermentation will also involve the slant. Therefore, tubes must have loosened caps during incubation.[5]
2. Before inoculation, a slight precipitate may be present on the slant. This will not effect the performance of the medium.[6]
3. Studies have demonstrated that sucrose utilization may suppress the enzyme mechanisms responsible for H_2S production.[7]
4. SIM (sulfide-indole-motility) agar has been reported to be more sensitive for the detection of H_2S than TSI.[5]
5. Some organisms, such as *Proteus* spp., may produce reactions similar to *Salmonella* and *Shigella*. Additional biochemical testing is required for definitive identification. Consult appropriate references for further instructions.[8,9]

BIBLIOGRAPHY

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Refer to the front of Remel *Technical Manual of Microbiological Media* for **General Information** regarding precautions, product storage and deterioration, specimen collection, storage and transportation, materials required, quality control, and limitations.

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