TITLE: Triple Sugar Iron (TSI)	Revision Date: 20-April-2018	Issue Date: 20-April-2016
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PRINCIPLE:

Triple Sugar Iron (TSI) agar contains casein and meat peptones, phenol red as the pH indicator and 3 sugars used for fermentation and oxidative metabolism: 0.1% glucose, 1% sucrose, 1% lactose. Ferric or ferrous ions and sodium thiosulfate are also present for the detection of hydrogen sulfide production which is visualized by the production of a **black** butt or a **black** ring at the junction of the butt and slant. Gas production from sugar fermentation is also be detected by the presence of bubbles, agar displacement or fracturing.

There is no oxygen penetration in the butt of the slant therefore no oxidative metabolism occurs. Fermentation occurs in the butt and the acid (A) reaction results in a yellow butt. Non-lactose fermenters will initially turn the slant yellow as it metabolizes the small amount of glucose. Once the glucose is utilized oxidative metabolism continues in the slant producing an alkaline (K) pH from the breakdown of peptone turning the slant **red**. Thus non-lactose fermenters have alkaline slants and yellow butts (K/A). Lactose fermenters (and sucrose fermenters) continue to produce large amounts of acid in the slant and in the butt so the reaction remains acidic (A/A) resulting in a yellow butt and a yellow slant. Non-fermenters do not ferment or metabolize any sugars so no acid production occurs thus leaving the slant and butt **red** (K/K).

SAMPLE INFORMATION:

Type	One well isolated colony

REAGENTS and/or MEDIA:

Source	Oxoid Cat#MT2052
Storage Requirements	2-8C

SUPPLIES:

- Inoculating needle
- MAC plate

SPECIAL SAFETY PRECAUTIONS:

Containment Level 2 facilities, equipment, and operational practices for work involving infectious or potentially infectious materials or cultures.

- Lab gown must be worn when performing activities with potential pathogens.
- Gloves must be worn when direct skin contact with infected materials is unavoidable.
- Eye protection must be used where there is a known or potential risk of exposure to splashes.
- All procedures that may produce aerosols, or involve high concentrations or large volumes should be conducted in a biological safety cabinet (BSC).
- The use of needles, syringes, and other sharp objects should be strictly limited.

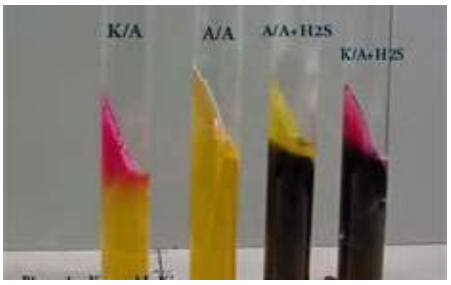
PROCEDURE INSTRUCTIONS:

Step	Action
Performing a Triple Sugar Iron Test	
1	In plate log – Order **^TSI**
2	Allow medium to come to room temperature. Look for cracks or signs of deterioration

3	Using a sterile inoculating loop, touch the centre of a well isolated colony
4	Stab the media until the needle reaches 3-5mm from the bottom of the tube
5	Remove the needle and streak along the entire surface of the agar slant
6	Loosely cap the tub
7	Incubate aerobically at 35-37°C for 18-24 hours
8	Examine the reaction in the slant and butt
9	Observe for gas and H2S production • Gas production can be seen as bubbles, agar splitting or agar displacement • H2S production is **blackening** of the butt
10	If readings are delayed – refrigerate tubes. Do not interpret sugar reactions after 24 hour incubation period.
11	Extended readings can be performed for H2S production only

INTERPRETATION OF RESULTS:

IF	THEN
Acid reactions	Yellow
Alkaline reactions	Red
No change	Pink
Black butt, black precipitate	**H2S present, acid reaction**
Bubbles/agar split or displaced	Gas present
Acid Slant, Acid Butt	A/A (glucose/sucrose and lactose fermented)
Alkaline Slant, Acid Butt	K/A (glucose fermented)
Alkaline Slant, Alkaline Butt	K/K (no carbohydrates fermented)



REFERENCES:

- Clinical Microbiology Procedures Handbook. (n.d.). Kligler's Iron Agar Test and Triple Sugar Iron Agar Test. p. 13.17.25.

REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	31Dec13	Initial Release	Darrach (A)
2.0	31Mar16	Update of “Special Safety Precautions” to reflect risk assessment recommendations.	C. Russell