TRIPLE SUGAR IRON AGAR

Triple sugar iron agar tests for 3 things---sugar fermentation (glucose/lactose/sucrose), CO₂, and H₂S. It is a good medium for some bacteria, although you can run all of these tests in other ways, such as running individual sugar discs, SIM for H₂S, and phenol red glucose for CO₂.

Gases are easy to spot. The H₂S is identified the same way as in SIM, but be sure that the black is INSIDE of the medium. The colorless H₂S gas reacts with an iron compound to become black inside of the agar. Carbon dioxide is identified by cracks and bubbles inside of the medium, sometimes a few bubbles and sometimes enough to push the slant up to the top.

There are 3 possibilities of sugar reactions and different reactions in different areas (butt vs. slant) of the medium, so the physiology behind it is pretty complex. The outcome of sugar use is always acid, so the pH indicator phenol red will turn yellow---reported as A. No use of the sugar or alkaline by-products (which is NO sugar use) from the other non-sugar nutrients in the medium will cause the indicator to stay the same color red/orange or maybe even change it to a red (if peptones/proteins are used as an energy source, producing alkaline products)---reported as a K.

The reactions in TSIA are reported as slant (A or K), butt (A or K), a circle around the butt for CO₂, and + for H₂S. For example K/ A + H₂S = red slant, yellow butt, with both CO₂, and H₂S. The glucose (=dextrose) is 1/10 in concentration as the other 2 sugars. The fermentation of the sugars causes the anaerobic butt to turn yellow and stay yellow. However, if only glucose is used, even though the slant turns yellow only after a few hours it will revert to red because the protein in the medium is broken down to alkaline products when the small amount of glucose is used up. If lactose and/or sucrose are used, the large amount of fermentation products neutralizes the basic products and the slant stays yellow.

Therefore,

A/A = glucose and lactose and/or sucrose are used
K/A = glucose alone
K/K = no sugars used

There is no way to get a A/K reaction when using a G-rod on this medium. IF YOU do, it means that 1) you did not inoculate correctly with a stab and streak or 2) you inoculated something other than a G-rod.

NOTE: This is a stab and streak inoculation.

THIS MEDIUM IS RUN ON GRAM - RODS ONLY!
SOME COMMON SUGAR REACTIONS IN TSIA

<table>
<thead>
<tr>
<th>Type</th>
<th>Organisms</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/A</td>
<td><em>E. coli, Yersinia, Aeromonas, Vibrio</em></td>
<td></td>
</tr>
<tr>
<td>K/A</td>
<td><em>Salmonella, Edwardsiella, Shigella</em></td>
<td></td>
</tr>
<tr>
<td>K/K</td>
<td>nonfermenters such as <em>Pseudomonas</em> and others</td>
<td></td>
</tr>
<tr>
<td>A/K</td>
<td>erroneously inoculated a Gram + or some other weird thing</td>
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</tbody>
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OBJECTIVES:

Identify the various sugar reactions in TSIA.
Identify the presence of carbon dioxide and hydrogen sulfide gases.

MATERIALS NEEDED:

1 TSIA per unknown

THE PROCEDURE:

1. Inoculate the medium using an inoculating NEEDLE. Stab the inoculum down through the butt, then pull the needle out and streak up the slant (Do NOT take another inoculum to do the slant).
2. Incubate at 30 or 37 degrees C.

There will be some demo bacteria so that you can see some different reactions using this medium.

INTERPRETATION:

A/A = yellow throughout
K/A = red slant, yellow butt
K/K = red or red/orange throughout

carbon dioxide = bubbles or breaks in medium
black precipitate = hydrogen sulfide

QUESTIONS:

1. What would the medium look like if the bacterium is KA +H₂S +CO₂.
2. If the organism uses glucose only, without lactose or sucrose, the medium will be ____.

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