TSI AGAR

INTENDED USE

TSI (Triple Sugar Iron) Agar is used for the identification of enterobacteria by the rapid detection of the fermentation of lactose, glucose (with or without gas production) and of sucrose, as well as the production of hydrogen sulfide.

HISTORY

Hajna developed the formulation of this agar containing three sugars by adding sucrose to a two-sugar (lactose and glucose) medium of Kligler. The addition of sucrose increases the sensitivity of the medium by leading to the early detection of coliform bacteria which ferment lactose slowly and degrade sucrose more rapidly. The medium also favors the differentiation of certain Proteus strains (lactose-negative) which ferment sucrose within 24 hours of incubation.

PRINCIPLES

- Sugar fermentation results in an acidification which makes phenol red (pH indicator) turn yellow.
- The detection of bacteria fermenting only glucose is facilitated by decreasing the concentration of the sugar to 1/10 of that of lactose or sucrose, so that the small quantity of acid produced on the slant during fermentation is rapidly oxidized. This causes a rapid return to the red color or else a more pronounced re-alkalinization. The acid reaction (yellow color), on the contrary, is maintained in the depth of the agar, in the butt of the tube.
- Bacteria fermenting lactose or sucrose will make the slant of the tube turn yellow.
- Bacteria fermenting none of these sugars will not change the color of the medium.
- The production of H₂S is revealed in the butt by the appearance of black iron sulfide, due to the reduction of thiosulfate in the presence of ferric citrate.
- The production of gas (H₂, CO₂), resulting from sugar fermentation, is revealed by the appearance of bubbles or by the fragmentation of the agar.

PREPARATION

- Suspend 60.1 g of dehydrated medium (BK059) in 1 liter of distilled or deionized water.
- Slowly bring to boiling, stirring with constant agitation until complete dissolution.
- Dispense in tubes.
- Sterilize in an autoclave at 121°C for 15 minutes.
- Incline the tubes to obtain a butt 3 cm high and a slant.

NOTE:

When the medium will not be used within 8 days of its preparation, it is recommended to regenerate it in a boiling water bath and solidify it again in the correct position.
INSTRUCTIONS FOR USE

- Inoculate the butt by stabbing and the slant by streaking close together, using a suspected colony taken from a selective isolation medium.
- Pure cultures taken from the center of well isolated colonies must be used, since otherwise cross reactions will make identification impossible.
- Incubate at 37°C for 24 hours (caps loosened) to favor gas exchanges.

NOTE:

In the case of water analysis according to the NF ISO 19250 standard protocol, incubate at (36 ± 2)°C during (24 ± 3)°C.

RESULTS

The use of one of the sugars in the medium results in acidification (phenol red turns yellow). Alkalinization is shown by a dark red color. Hydrogen sulfide production from thiosulfate is detected by a black color due to the formation of iron sulfide in the presence of ferric citrate.

TSI Agar supplies four types of information:

(1) Glucose fermentation
   - Butt red : glucose not fermented
   - Butt yellow : glucose fermented

(2) Lactose and/or sucrose fermentation
   - Slant red : lactose and sucrose not fermented
   - Slant yellow : lactose and/or sucrose fermented

(3) Gas production
   - Production of gas bubbles in the butt

(4) Formation of H₂S
   - Formation of a black color between the butt and the slant or along the inoculation stab.
Typical reactions are in the following table:

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>SLANT</th>
<th>BUTT</th>
<th>H₂S</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lactose/Sucrose</td>
<td>Glucose</td>
<td>Gas</td>
</tr>
<tr>
<td><strong>Salmonella Typhi</strong>&lt;sup&gt;(2)&lt;/sup&gt;</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><strong>Salmonella Paratyphi A</strong>&lt;sup&gt;(2)&lt;/sup&gt;</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>Salmonella Choleraesuis</strong>&lt;sup&gt;(2)&lt;/sup&gt;</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>Salmonella Pullorum</strong>&lt;sup&gt;(2)&lt;/sup&gt;</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>Salmonella Paratyphi B</strong>&lt;sup&gt;(2)&lt;/sup&gt;</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>Salmonella Typhimurium</strong>&lt;sup&gt;(2)&lt;/sup&gt;</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>Salmonella Enteritidis</strong>&lt;sup&gt;(2)&lt;/sup&gt;</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>Salmonella Gallinarum</strong>&lt;sup&gt;(2)&lt;/sup&gt;</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><strong>Shigella dysenteriae</strong></td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><strong>Shigella flexneri</strong></td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><strong>Shigella sonnei</strong></td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><strong>Shigella boydii</strong></td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><strong>Proteus vulgaris</strong></td>
<td>+</td>
<td>+</td>
<td>[+</td>
</tr>
<tr>
<td><strong>Proteus mirabilis</strong></td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>Proteus morganii</strong></td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>Proteus rettgeri</strong></td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><strong>Serratia marcescens</strong></td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><strong>Enterobacter hafniae</strong></td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>Enterobacter aerogenes</strong></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>Enterobacter cloacae</strong></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>Escherichia coli</strong>&lt;sup&gt;(1)&lt;/sup&gt;</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>Citrobacter freundii</strong></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>Klebsiella pneumoniae</strong></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>Alcaligenes faecalis</strong></td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Pseudomonas aeruginosa</strong></td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Yersinia enterocolitica</strong></td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

<sup>(1)</sup> Some strains of *Escherichia coli* ferment lactose only very late in growth.

<sup>(2)</sup> In the case where interpretation may suggest the presence of salmonellae, it is possible to use TSI medium to detect β-galactosidase, urease and lysine decarboxylase.

**TYPICAL COMPOSITION**
(can be adjusted to obtain optimal performance)

For 1 liter of medium:
- Tryptone.................................................................14.0 g
- Yeast extract.........................................................3.0 g
- Meat extract ..........................................................3.0 g
- Glucose.........................................................................1.0 g
- Lactose ........................................................................10.0 g
- Sucrose .........................................................................10.0 g
- Sodium chloride.........................................................5.0 g
- Sodium thiosulfate.......................................................0.3 g
- Ferric ammonium citrate ..............................................0.3 g
- Phenol red.....................................................................24.0 mg
- Bacteriological agar....................................................13.5 g
pH of the ready-to-use medium at 25°C : 7.4 ± 0.2.

QUALITY CONTROL

- Dehydrated medium : pinkish powder, free-flowing and homogeneous.
- Prepared medium : orange red agar.
- Typical culture response after 24 hours of incubation at 37°C :

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Growth</th>
<th>Slant</th>
<th>Butt</th>
<th>H2S</th>
<th>Gas</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>ATCC® 25922</td>
<td>good, score 2</td>
<td>yellow</td>
<td>yellow</td>
<td>-</td>
</tr>
<tr>
<td><em>Citrobacter freundii</em></td>
<td>CIP 57.32T</td>
<td>good, score 2</td>
<td>yellow</td>
<td>yellow</td>
<td>+</td>
</tr>
<tr>
<td><em>Proteus vulgaris</em></td>
<td>ATCC 13315</td>
<td>good, score 2</td>
<td>yellow</td>
<td>yellow</td>
<td>[+]</td>
</tr>
<tr>
<td><em>Salmonella Enteritidis</em></td>
<td>CIP 82.97</td>
<td>good, score 2</td>
<td>red</td>
<td>yellow</td>
<td>+</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>CIP 82.118</td>
<td>good, score 2</td>
<td>red</td>
<td>red</td>
<td>-</td>
</tr>
</tbody>
</table>

STORAGE / SHELF LIFE

**Dehydrated medium** : 2-30°C.
- The expiration date is indicated on the label.

**Prepared medium** (benchmark value*) :
- Media in non-slanted tubes : 6 months at 2-8°C.
- Media in slanted tubes : 8 days at 2-8°C.

PACKAGING

**Dehydrated medium** :
- 500 g bottle

BIBLIOGRAPHY


PHOTO SUPPORT:

Product reference: BK059HA

Media used for: Identification of enterobacteria through fermentation reactions.

Diverse bacteria / reactions

Triple Sugar iron (T.S.I.) agar
Ref: BK059HA
Incubation: 24 hours / 37°C
Characteristics: Various biochemical reactions can be observed. See text above.

*Benchmark value refers to the expected shelf life when prepared under standard laboratory conditions following manufacturer's instructions. It is provided as a guide only and no warranty, implied or otherwise is associated with this information.
The information provided on the package take precedence over the formulations or instructions described in this document. The information and specifications contained in this technical data sheet date from 2010-11-02. They are susceptible to modification at any time, without warning. Code document : BK059/A/2000-09 : 8.