Our Salt Tolerance Broths are used to differentiate non-β-hemolytic strains of catalase-negative, gram-positive cocci (i.e. Enterococcus and Aerococcus) based on their ability to grow in a 6.5% sodium chloride broth. Hajna devised the first formulation of a salt tolerance medium. The high salt concentration inhibits a range of bacteria but allows salt-tolerant organisms such as enterococci to grow. The Quadri formulation includes the fermentable carbohydrate, dextrose, and the color indicator, bromcresol purple. Organisms capable of growing in the high salinity medium, utilize the sugar and release acid as a by product of their metabolism. The pH drop results in the indicator, bromcresol purple, to change from purple to yellow.

The mediums were designed to aid in the differentiation of enterococci from other streptococci. In the clinical laboratory, an accurate presumptive identification of a catalase negative, gram-positive cocci as a Enterococcus strain can be accomplished by demonstrating that the unknown organism is PYR and LAP positive and grows in salt tolerance broth (6.5% NaCl) at 45°C. Aerococcus species such as A. viridans and A. urinae can also grow in 6.5% NaCl, therefore salt tolerance broth can also be used to differentiate Aerococcus species from other similar organisms such as Stomatococcus and Helcococcus.

**Formula per Litre of Medium**

**TS27 Salt Tolerance Broth**
- Infusion from Beef Heart .................. 10.0 g
- Pancreatic Digest of Animal Tissue ......... 10.0 g
- Sodium Chloride .................................. 65.0 g

  pH 7.0 ± 0.2

**TS28 Salt Tolerance Broth with Indicator**
- Infusion from Beef Heart .................. 10.0 g
- Pancreatic Digest of Animal Tissue ........... 10.0 g
- Sodium Chloride .................................. 65.0 g
- Dextrose ........................................ 1.0 g
- Bromcresol Purple .............................. 0.016 g

  pH 7.0 ± 0.2

**Recommended Procedure**

1. Allow medium to reach room temperature.
2. Pick two or three colonies of the isolate and inoculate the broth.
3. Incubate tubes aerobically with loose caps at 35 to 37°C.
4. Examine tubes for turbidity after 24 hours and if negative again at 48 and 72 hours.

**Interpretation of Results**

For both mediums, a positive reaction is obvious growth or turbidity in the inoculated tube. For Salt Tolerance Broth with Indicator, turbidity is usually accompanied by a color change from purple to yellow, but it is not necessary for a positive result as growth in the broth itself is indicative of salt tolerance.

A negative result is indicated by no growth in the medium after 72 hours of incubation.

- Some strains of Pediococcus, Leuconostoc, and beta-hemolytic Streptococcus species may grow in Salt Tolerance Broth
- Turbidity alone is indicative of a positive test
- Enterococcus and Aerococcus species typically give a positive result after 24 hours of incubation.
Quality Control

After checking for correct pH, color, depth, and sterility, the following organisms are used to determine the growth performance of the completed medium.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Expected Result</th>
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</thead>
<tbody>
<tr>
<td><em>Enterococcus faecalis</em> ATCC 29212</td>
<td>Turbidity (yellow color change if an indicator is present)</td>
</tr>
<tr>
<td><em>Streptococcus gallolyticus</em> (Formerly <em>S. bovis</em>) ATCC 9809</td>
<td>Clear, no growth (no color change if an indicator is present)</td>
</tr>
</tbody>
</table>

Storage and Shelf Life

Our Salt Tolerance Broths should be stored in an upright position at 4 to 8°C and protected from light. Under these conditions this medium has a shelf life of 26 weeks from the date of manufacture.

References


Original: January 1998
Reviewed / Revised: December 2018