Bacitracin/SXT Sensitivity

**Intended Use:**
Bacitracin differential disks are used to presumptively identify Group A, beta-hemolytic streptococci from other beta-hemolytic streptococci. The combination of SXT sensitivity increases the accuracy of the results.

**Principle:**
Bacitracin is an antibiotic isolated from Bacillus subtilis. It inhibits cell wall synthesis mainly through inhibiting the biosynthesis of peptidoglycan. SXT inhibits folate metabolism which interferes with bacterial DNA synthesis. Group A, beta-hemolytic streptococci are more sensitive to bacitracin than other beta-hemolytic streptococci.

**Test Procedure:**
The standard protocol has been modified for our lab.
1. Using a loop, select 3-4 well isolated colonies, ideally from an 18-24 hour culture. Transfer to a small amount of sterile water.
   - Adjust the turbidity to 0.5 McFarland standard.
2. Use the procedure outlined in antimicrobial susceptibility testing to swab the entire plate to obtain confluent growth.
3. Visually divide the plate in thirds, place a bacitracin and SXT in their section of the plate. Using sterile forceps or a swab, lightly but firmly press the disks to the agar surface to adhere them.
   - Save the other section for the optochin disk.
4. Invert the plates and incubate them for 18-24 hours at 35°C in 5-10% CO₂.
5. Incubate another 24 hours if the results are negative.

**Results:**
- Any zone of inhibition around the disk is considered sensitive (S).
- No zone of inhibition with growth up to the disk is considered resistance (R).

<table>
<thead>
<tr>
<th>Bacitracin</th>
<th>SXT</th>
<th>Presumptive ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>R</td>
<td>Group A b-streptococci</td>
</tr>
<tr>
<td>R</td>
<td>R</td>
<td>Group B b-streptococci</td>
</tr>
<tr>
<td>R</td>
<td>S</td>
<td>Not Group A or B b-streptococci</td>
</tr>
<tr>
<td>S</td>
<td>S</td>
<td>Rule out Group A or B with serologic tests</td>
</tr>
</tbody>
</table>

This table is from MacFaddin, Biochemical Tests for Identification of Medical Bacteria.

**Limitations:**
- Only beta-hemolytic streptococci should be tested.
- While this test is accurate it is not highly specific. Other biochemical or serological tests are required for accurate identification.
- The growth should be confluent. Too light of a growth could cause some non-group A streptococci to appear susceptible to bacitracin.