Our Optochin Disks are used to aid in the presumptive identification of *Streptococcus pneumoniae* from other alpha-hemolytic streptococci. *Streptococcus pneumoniae* is commonly found in the human respiratory tract along with other streptococci. When thought to be implicated in disease, *Streptococcus pneumoniae* can be differentiated from other alpha-hemolytic streptococci based on its susceptibility to optochin (ethylhydrocupreine hydrochloride). The growth of pneumococci is markedly inhibited by optochin, which results in the formation of zones of inhibition around disks impregnated with optochin.

**Recommended Procedure**

1. Obtain a fresh, overnight culture of the alpha-hemolytic test organism grown on blood agar. Ensure that it is from a pure culture and not a mixed culture plate.
2. Using a sterile swab, streak a sample of the organism onto a non-selective TSA blood agar plate in three directions to obtain a confluent lawn of bacteria.
3. Aseptically, place an optochin disk on the agar surface using sterilized forceps or tweezers. Tap gently to ensure that the disk is adhering to the agar surface.
4. Incubate at 35°C in an aerobic environment supplemented with 5% CO₂.
5. Examine the growth pattern around the disk after 24 hours of incubation. If zone of inhibition is present measure the diameter using a millimeter ruler or caliper.

**Interpretation of Results**

A presumptive identification of *Streptococcus pneumoniae* can be made if the alpha-hemolytic organism produces a zone of inhibition of 14-mm or greater around the disk. Alpha-hemolytic organisms producing smaller zone sizes (6 to 13-mm) should be tested for bile solubility; if they are bile soluble they can be presumptively identified as pneumococci until further tests are performed.

Other non-pneumococcal alpha-hemolytic streptococci will grow right up to the disk and show no zone of inhibition.

This is only a presumptive test, therefore additional biochemical and/or serological tests may need to be performed on isolated colonies from pure culture in order to complete identification.

- Occasional, rare strains of pneumococci not inhibited by optochin have been reported

- Some strains of non-pneumococcal alpha streptococci have been reported to form zones of inhibition approximately 10-12-mm in diameter when light inocula was used. Therefore ensure that you are getting a confluent lawn of bacteria when reading results

**Quality Control**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Expected Results</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Streptococcus pneumoniae</em></td>
<td>Susceptible Growth with zone of inhibition ≥ 14-mm</td>
</tr>
<tr>
<td>ATCC 6305</td>
<td></td>
</tr>
<tr>
<td><em>Streptococcus pyogenes</em></td>
<td>Resistant Growth up to the disk with no zone of inhibition</td>
</tr>
<tr>
<td>ATCC 12384</td>
<td></td>
</tr>
</tbody>
</table>

**Storage and Shelf Life**

Our Optochin Disks should be stored at -20°C and protected from light. Under these conditions they have a shelf life of 52 weeks from the date of manufacture.
References


Original: September 2008
Revised / Reviewed: May 2014