Optochin Disk Procedure

**Principle:**
The optochin disc is used to assist in differentiating between *Streptococcus pneumoniae* and other alpha-hemolytic streptococci. This procedure uses the chemical optochin (ethylhydrocupreine hydrochloride) to test the fragility of an organism’s cell membrane. *Streptococcus pneumoniae* is sensitive to optochin, but other alpha-hemolytic streptococci are resistant at a concentration of 5.0 ug.

**Specimen Collection and Preparation**
Testing should be performed on well isolated aerobic, catalase-negative, alpha hemolytic gram-positive cocci. All colonies should be taken from a blood agar plate and growth must be less than 24 hours old, 15-18 hours being optimal.

**Reagents**
- 5% Sheep’s blood agar plate
- Optochin disc (P disk)
- Inoculating loop
- Incinerator

**Storage**
1. 5% Sheep’s blood agar: Store at 2-8°C and bring to room temperature before use.
2. Optochin disc: Store upon receipt at -20 to +8°C. After use, store vial or cartridge at 2-8°C to protect product integrity. The expiration date applies to unopened containers stored as directed. Do not open until ready to use.

**Quality Control**
Quality control should be performed per lot/shipment date.
- **Positive** control - *Streptococcus pneumoniae* ATCC 6305
- **Negative** control - *Streptococcus sanguis* ATCC 15506

**Expected Results:**
1. *Streptococcus pneumoniae* should exhibit a zone of inhibition >14 mm in diameter.
2. *Streptococcus sanguis* should show no zone of inhibition (<6mm).

**Procedure**
1. Allow containers to come to room temperature before opening.
2. Streak a sheep blood agar plate with the organism(s) to be tested to obtain confluent growth on 1/3 of plate surface, then streak for isolation.
3. Aseptically, with forceps, remove an optochin disc, and apply it to the center of the streaked area. Do not place the disc too near the edge of the plate. Press gently on the disc to make it adhere to the medium.
4. Invert the plate, and incubate it at 35°C in a CO2 incubator for 18-24 hours.
5. Return unused discs to the refrigerator.

**Interpretation of Test**
1. Sensitive
   a. Inhibition of growth around the disc will result in a >14 mm zone with a distinct margin.
   b. Optochin sensitive alpha-hemolytic streptococci (with characteristic morphology) are *S. pneumoniae*.
2. Resistant
   a. Growth will not be inhibited around the disc (<6 mm).

**References:**
2. BBL Taxo P Disc package insert, Sparks, MD.