Optochin Disk Test

Intended Use:
Optochin sensitivity is specifically intended to differentiate *Streptococcus pneumoniae* from other α-hemolytic Streptococcus species.

Principle:
Optochin disks contain a low concentration (5 ug/disk) of optochin. Optochin causes changes in the surface tension of *S. pneumoniae* cell membranes increasing their fragility. A zone of inhibition is produced by contact with the optochin. Other organisms are also sensitive to optochin but at a 10 fold increased concentration.

Test Procedure:
Standard procedure has been slightly modified for our lab. You will use the same plate for the Bacitracin/SXT test.
1. Select 4-6 well-isolated colonies and suspend in a small amount of water to match a 0.5 McFarland standard. Swab a blood agar plate to obtain confluent growth. See the antimicrobial sensitivity lab for the inoculating procedure.
2. Visually divide the plate into three sections. Using sterile forceps or a sterile applicator, place the optochin disk in the middle of one section.
    - Do not place near the edge of the plate. Gently press it onto the surface of the agar with a fresh sterile swab or forceps.
3. Invert and incubate in the CO_2 incubator at 37°C for 18-24 hours.

Interpretation of Results:
- A sensitive (S) response is indicated by a ≥14 mm clear zone of inhibition with alpha-hemolysis measured from the edge of the disk. This is a presumptive identification of *S. pneumoniae*.
- Organisms with questionable diameters should only be considered pneumococcus if they are also bile soluble.
- Report your data as sensitive or resistance, not positive or negative. A negative report would indicate that the organism did not grow.

Limitations:
- An enhanced CO_2 environment is critical for *S. pneumoniae* growth.
- *S. pneumoniae* grown in less than 5% CO_2 have smaller zones of inhibition.
- Other strains of alpha-hemolytic streptococci may show slight inhibition.
- Optochin is a presumptive test. Further biochemical testing, such as bile solubility, should be done to confirm identification.