Appendix B:

Preparation of 0.5 McFarland standard

- A 0.5-ml aliquot of 0.048 mol/L BaCl₂ (1.175% w/v BaCl₂·2H₂O) was added to 99.5 ml of 0.18 mol/L H₂SO₄ (1% v/v) with constant stirring to maintain a suspension.

- The correct density of the turbidity standard was verified by using a spectrophotometer with a 1-cm light path and matched cuvette to determine the absorbance. The absorbance at 625 nm was 0.008 to 0.10 for the 0.5 McFarland standard.

- The Barium Sulfate suspension was transferred in 4 to 6 ml aliquots into screw-cap tubes of the same size as those used in growing or diluting the bacterial inoculum.

- These tubes were tightly sealed and stored in the dark at room temperature.

- The barium sulfate turbidity standard was vigorously agitated on a mechanical vortex mixer before each use and inspected for a uniformly turbid appearance. If large particles appeared, the standard was replaced.

- The barium sulfate standards were replaced or their densities verified monthly.