I. Reference:


II. Personal Protective Equipment:
A. Lab coat
B. Safety glasses/goggles
C. Designated peroxide handling gloves

III. Reagents:
A. 0.3 N Barium Dihydroxide \( \text{Ba(OH)}_2 \)
   1. Add 47.3235 g \( \text{Ba(OH)}_2 \) per liter of solution
   2. Some of the \( \text{Ba(OH)}_2 \) may settle out after being put into solution
   3. DO NOT agitate prior to use
B. 5% (w/v) Zinc Sulfate \( \text{ZnSO}_4 \)
   1. 50 grams \( \text{ZnSO}_4 \cdot 7\text{H}_2\text{O} \) per liter of solution
   2. 10% (w/v) Barium Dichloride \( \text{BaCl}_2 \)
      a. 100 grams \( \text{BaCl}_2 \) per liter of solution
   3. 30% (w/v) trichloroacetic acid (TCA) \( (\text{C}_2\text{HCl}_3\text{O}_2) \) + 5.0% (w/v) \( \text{BaCl}_2 \)
      a. 300 grams TCA + 50 grams \( \text{BaCl}_2 \) per liter of solution

IV. Procedure:
A. Centrifuge abomasal or rumen samples for 10 minutes at 600-800 x g to remove particulate matter.
B. To a clean test tube add:
   1. 2 ml supernatant from step A
   2. 1 ml 0.3 N \( \text{Ba(OH)}_2 \)
   3. 1 ml 5% \( \text{ZnSO}_4 \)
   4. .5 ml 10% \( \text{BaCl}_2 \)
   5. 5.5 ml dd\( \text{H}_2\text{O} \)
C. Mix well and let stand for 5 minutes
D. Centrifuge for 10 minutes at 600-800 x g
E. To a clean test tube add:
   1. 1 ml supernatant from step D
   2. 4 ml dd\( \text{H}_2\text{O} \)
   3. 5 ml 30% TCA + 5.0% \( \text{BaCl}_2 \)
   4. Mix well – read at 525nm at exactly 20 minutes on SpectraMax

NOTE: Be sure to all used reagents go into a waste jar at the end of the procedure.
V. Standards:

3000, 2250, 1500, 750, 375, 187.5 µg/ml

**IMPORTANT**: Do not use the same standard solutions for more than a week.

VI. Calculations:

Calculate regression line of standards vs. O.D. and fit samples to regression line.