TOTAL PHOSPHOROUS DETERMINATION
(Feeds, Fecal and Manure)
Updated September 2013

I. Reference:

1963. P in feeds analysis. Alkalimetric ammonium molybdophosphate method using spectrophotometer. Journal of A.O.A.C.

Bremer, V.R., C.D. Buckner, G.E. Erickson, T.J. Klopfenstein. 2008. Total and Water Soluble Phosphorus Content of Feedlot Cattle Feces and Manure. MP 91: 62-63.

II. Personal Protective Equipment:

A. Lab coat
B. Safety glasses/goggles
C. Appropriate lab specified acid handling gloves

III. Reagents:

A. 3N Hydrochloric acid (HCl)
   1. Add 274.5 mL concentrated HCl to ~200 mL ddH_2O
   2. q.s. to 1 L with ddH_2O

B. Molybdovanadate Reagent
   1. Ammonium Molybdate
      a. Add 5.0 g Ammonium Molybdate ($(NH_4)_6Mo_7O_{24}*4H_2O$) to ~80 mL ddH_2O, stir to dissolve.
   2. Ammonium Metavanadate-Perchloric Solution
      Must handle in designated fume hood (Carcinogenic)
      a. Add 0.250 g Ammonium Metavanadate (NH_4VO_3) to ~120 mL ddH_2O in 250 mL volumetric flask, heat and stir to dissolve and cool
      b. Add 31.25 mL of concentrated (70%) Perchloric acid ($HClO_4$) to solution under **designated perchloric hood (highly caustic)**
   3. Gradually combine the two mixtures (~230 mL)
      Note: Once the solutions are mixed, they are stable for 1 day
   4. q.s. to 250 mL with ddH_2O

IV. Quality Control

A. SSRM
   Combine 50:50 DM ratio of Domestic Sludge standard reference material (SRM) with powdered silica sand. Mix well.

B. Blank
   Empty crucible run through the procedure with samples.

V. Standards:

A. Phosphorus Standard Stock Solution (10,000 μg/mL)
   1. Pipette into 25 mL volumetric flasks the reagent indicated in column 2 below:

1	2	3
Standard No.	Standard Solution (μL)	Phosphorus (μg/mL)
1	0.0	0
2	50.0	20
3	150.0	60
4	250.0	100
5	350.0	140

B. Blank Solution
   1. Pipette 20 mL 3N Hydrochloric acid (Hal) into a 200 mL volumetric flask
   2. q.s. to 200 mL with ddH_2O
   3. Use the Blank Solution to q.s. the standards flasks to 25 mL

VI. Procedure:

A. In duplicate, perform lab dry matter and ash analysis (Using the medium crucibles) using 1.0 g samples. Run 1 SSRM (0.250 g) and 1 blank QC per 10 sample crucibles (5 samples run duplicate = 10 crucibles).

B. Obtain ash residue in crucible and carefully add 10 mL of 3N HCl to each crucible.

C. Place on large hot plate under hood, cover crucible with a watch glass, and heat to a boil

D. Digest for 5 minutes

E. Remove from hotplate, cool, and pour crucible into 100 mL volumetric flask

F. Rinse crucible thoroughly with ddH_2O

G. q.s. to 100 mL volume with ddH_2O

H. Filter aliquot (~15 mL) into a capped vial using Whatman 42 filter paper (1:100 dilution) and store at 4-8°C until analysis.

I. Spectrophotometry:
   a. Pipette 50 μL of Blank Solution, standards, and samples into wells of a microtiter plate.
   b. Pipette 200 μL of Molybdovanadate Reagent to all wells
   c. Incubate in plate reader (SpectraMax 250) for 20 minutes at 25°C
   d. Read absorbance at 400nm
   e. Calculation:

$$\%P = 100 * \frac{\text{concentration (μg/mL) * dilution factor}}{(10^6)(\text{Sample wt, g})}$$

Notes: This preparation may also be analyzed for Ca and Mg; however, an adjustment to the initial dilution/sample size may be necessary.

There appears to be a matrix affect on the analysis of Phosphorus in samples. Keeping the matrix constant between and within a run is important for correct results.