

**College of  
Agriculture and Life Sciences**  
**Nutrient Management Spear Program**

# **Laboratory Manual**

## **Soil, Plant and Water Analysis**

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Cornell University



### **Cornell Nutrient Management Spear Program**

A collaboration among the Department of Animal Science, Cornell Cooperative Extension and PRODAIRY.

<http://nmssp.cals.cornell.edu>

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## **1. Nutrient Management Spear program: Purpose**

Updated date: 4/12/2017

The Laboratory of the Nutrient Management Spear Program (NMSP) at Cornell University is an agricultural and environmental research facility. The main objectives of this laboratory are to provide soil, plant, and water-testing services for research samples and conduct applied laboratory-based research that leads to the development of soil, plant and water analyses that aid farmers and farm advisors in management of nutrients in agricultural operations. Once interpretations for new tests are developed as part of our research program, we work with commercial laboratories with an interest in offering the tests for their clients to ensure proper implementation and continued quality control of new methodology. We also guide undergraduate and graduate students with analytical aspects of their projects.

The procedures described in this manual reflect the current methodologies used for soil, plant, and water testing. The NMSP laboratory uses the Morgan extraction procedure for extracting soil samples for  $\text{NO}_3^- + \text{NO}_2^-$ -N test, 2 M KCl extraction for both  $\text{NH}_4^+$ -N and  $\text{NO}_3^- + \text{NO}_2^-$ -N tests, and 0.01 M  $\text{SrCl}_2$  extraction for K, Ca, Mg, and S tests.

This manual was prepared and updated by Sanjay Gami and reviewed by Quirine Ketterings. Some of the protocols were updated or reviewed by others and their names are included in the individual chapters.

## **2. Sample Submission**

Updated date: 4/12/2017

A sample submission form needs to be filled out entirely for commercial samples and submitted for testing. The form that corresponds to the tests and testing options can be downloaded and printed from the links on the NMSP website (<http://nmsp.cals.cornell.edu>). Other information can also be assessed by visiting the NMSP website.

### **3. Soil Sample Preparation – Drying and Grinding**

Updated date: 2/2009

#### **Introduction:**

For routine soil testing, it is suggested to dry the samples before analysis. Analysis of undried samples is not recommended because of the effect of the moisture content on soil to extractant ratio and the need for determination of moisture content.

#### **Apparatus:**

1. Drying oven
2. Crusher, mill, grinder, mortar and pestle.
3. Plate sieve, mesh sieve

#### **Procedure:**

##### Soils:

1. Once samples have been collected, they must be processed promptly to prevent any changes that might affect analysis.
2. Break up large chunks of soil and spread out to air dry where the sample will not be contaminated. Remove stones and large objects by hand picking. The sample may also be placed in a forced air oven set at 50°C.
3. Soil should be ground in a soil grinder to pass through a 2-mm sieve (or 10 to 20-mesh screen) (Jackson, 1958) for routine soil test. However, analysis of carbon, nitrogen, and total elements (arsenic, cadmium, copper, chromium, iron, lead, manganese, molybdenum, nickel, phosphorus, selenium, zinc) requires that the sample be ground to a fine powder (to pass through a 60-mesh screen).
4. Mix each soil sample to homogenize before weighing or scooping the subsample.

##### Plants:

1. After plant samples have been received in the lab, fresh samples should be placed into paper bags or trays and dried in a forced air oven at 60°C. In general, adequate drying time is approximately 12 to 48 hours or until the material breaks easily.

2. Most plant and feed samples should be ground to pass through a screen according to test requirements. For corn stalk nitrate test, samples should be ground to pass through a 2-mm screen. See agronomy factsheet 31 for sampling procedure and sample submission protocol for corn stalk samples.

**References:**

1. Jackson, M.L. 1958. Soil Chemical Analysis. Prentice-Hall, Inc. Englewood Cliffs, N.J.

## 4. Determination of Moisture Content in Soil

Updated date: 2/2009

### Introduction:

Soil moisture influences crop growth by affecting nutrient availability, nutrient transformations and soil biological behavior<sup>1</sup>. The method is based on removing soil moisture by oven drying a soil sample until the weight remains constant. The moisture content (%) is calculated from the sample weight before and after drying.

### Apparatus:

1. Oven – capable of maintaining a temperature of 105°C
2. Crucibles - 10-mL, porcelain
3. Crucible racks
4. Balance - minimum capacity of 50 g,  $\pm 0.001$  g
5. Scoop – 1 cm<sup>3</sup> capacity

### Procedure:

1. Dry the samples in a forced air oven set at 50°C.
2. Prepare the soil sample for weighing by removing any stones, twigs, and plant matter; grind in a soil grinder to pass through a 2-mm sieve.
3. Take the weight of empty crucibles ( $W_c$ ).
4. Accurately weigh 2 g of the soil sample into the crucible, and record the weight of the crucible and soil sample ( $W_{cs}$ ).
5. Place the crucibles containing sample into the oven, set at 105°C and dry for one hour (after temperature reaches 105°C).
6. Remove the crucible containing sample from the oven and allow to cool in a dry atmosphere for 20 minutes.

7. Weigh the crucibles with the dried sample ( $W_{os}$ ) and determine the moisture content.

Note: Record all weights to  $\pm 0.001$  g.

**Calculations:**

Calculate the moisture content of the soil as a percentage of the dry soil weight.

$$\text{Moisture (\%)} = \frac{W_{cs} - W_{os}}{W_{cs} - W_c} \times 100$$

Where,

$W_c$  = Weight of empty crucible

$W_{cs}$  = Weight of crucible plus soil before drying at  $105^\circ\text{C}$

$W_{os}$  = Weight of crucible plus soil after drying at  $105^\circ\text{C}$

**References:**

1. Analytical Laboratories, Department of Agronomy, Cornell University, procedure no. S1810, issued: 03/12/2087, revised: 01/06/2004.
2. <sup>1</sup>Soil and Plant Analysis Laboratory Manual available at [www.icarda.org/Publications/Lab\\_Manual/read.htm](http://www.icarda.org/Publications/Lab_Manual/read.htm).

## 5. Determination of pH

Updated date: 8/18/2009

### **Introduction:**

Soil pH is a representation of hydrogen ion activity in a liquid. It is the negative logarithm of the amount of hydrogen ions (in moles) per liter of liquid. The pH found in soils ranges from 3 to 9. It reflects whether a soil is acidic, neutral, basic or alkaline. Soil pH is one of the most indicative measurements of the chemical properties of a soil (McLean, 1982). The availability of most plant-essential elements depends on soil pH. It is one of the single most informative measurements that can be made to determine soil characteristics (Thomas, 1996). A suspension, one part water to one part soil (McLean, 1982; Thomas, 1996), is prepared and allowed to stand at room temperature for one hour. The pH of this suspension is determined using a standard pH meter and electrode.

### **Apparatus:**

1. pH Meter equipped with glass and reference electrode or combination electrode
2. 1.25 or 3 oz plastic cups or 50-mL beakers
3. Dispenser calibrated to deliver 20 mL deionized (DI) water
4. Weighing balance
5. Stirring rod

### **Reagents:**

1. Standard buffers: pH 4.0, pH 7.0, and pH 10.0
2. Deionized water

### **Procedure:**

#### pH in soil samples:

1. Weigh out or scoop 20 g of soil in a 3 oz disposable plastic cup or 50 mL beaker.
2. Add 20 mL of DI water to the soil in the cup/beaker.
3. Stir each sample several times until homogeneous slurry is achieved. Rinse the stirring rod with DI water after stirring each sample.



4. Allow to stand for one hour.
5. Refer to the instrument manual for specific instructions supplied with the specific meter for the operation of the instrument. Standardize the instrument using the pH buffers.

Note: Allow the pH buffers to come to room temperature before attempting to calibrate the instrument. Two pH standards should be used to calibrate the pH meter. They must be chosen in accordance with the pH range expected for the samples analyzed (pH 4.0 and 7.0 or pH 7.0 and 10.0).

6. Determine the pH of all samples.
7. Between pH readings, rinse the electrodes with DI water.
8. To check the meter during analysis, measure the pH of a buffer solution after every 10 samples and at the end of the analysis. The pH should be within 0.1 pH units of the buffer value.
9. When finished, be sure the electrode is immersed in pH 4.0 buffer for storage.

#### pH in aqueous sample:

This method is applicable to drinking water, surface, ground, and saline waters, and other liquid samples such as acid whey, manure samples etc.

Note: Water and other liquid samples for pH determination are to be preserved by refrigeration at 4°C. Allow the samples to come to room temperature prior to measuring the pH.

1. Pour approximately 25 mL of each sample into individual cups or beakers. You can use 1.25 or 3 oz plastic cups or 50 mL beakers for the samples.
2. Follow steps 5-9 as described above for pH in soil samples.

#### **Calibration of pH Meter:**

1. Touch main screen.
2. Press 1.
3. Press pH.
4. Press Standard.
5. Press Clear.

6. Put electrode in pH 4.0 buffer; Press Standard and wait for it to stabilize.
7. Put electrode in pH 7.0 buffer; Press Standard 2 times.
8. If the slope shown on the instrument screen is 90% or above, continue to take readings for samples.

**Clean-Up:**

1. Pour off the supernatant liquid, then dispose of the cups and soil in the waste soil containers.

**Maintenance:**

1. Always keep the electrode in water or pH 4.0 buffer when not in use.
2. Always keep the filling hole of the electrode covered when not in use.
3. For further details on maintenance of the instrument and the electrode, refer to the instrument standard operating procedure (SOP) and related hardware manuals.

**References:**

1. EPA Method 150.1 (4-79-020).
2. Hendershot, W.H., H. Lalonde, and M. Duquette. 1993. Soil reaction and exchangeable acidity. In: M.R. Carter Soil Sampling and Methods of Analysis. Lewis Publishers, pp 41-145.
3. Mclean, E.O. 1982. Soil pH and lime requirement. p. 199-224. In A.L. Page et al. (ed.) Methods of soil analysis. Part 2. 2nd ed. Agron Monogr. 9. ASA and SSSA, Madison, WI.
4. Thomas, G.W. 1996. Soil pH and soil acidity. p. 475-490. In D.L. Sparks et al. (ed.) Methods of soil analysis. Part 3. Book series no. 5. SSSA and ASA, Madison, WI.

## 6. Determination of Electrical Conductivity

Updated date: 7/2009

### Introduction:

The conductivity is manually determined using a conductivity meter. Samples are preferably analyzed at 25°C. If the meter does not provide automatic temperature compensation, readings should be corrected to 25°C based on measured temperature of the samples and known compensation factors (Janzen, 1993). This method is used for determining the electrical conductivity (EC) in milliSiemens per meter (mS/m) in the samples. It is often called “Soluble Salts”. Soluble salts refer to the inorganic soil constituents that are soluble in water (EPA Method 120.1). Electrical conductivity estimates the amount of total dissolved salts or the total amount of dissolved ions in the water and in the soil-water suspension. It is a function of quantity and specific types of cations and anions in the soil-water suspension (Rhoades, 1982).

Soluble salts are readily accumulated in arid regions where leaching is limited. Salts may be released by weathering of parent material or they may be transported to a soil by wind or water. The accumulation of soluble salts in the soil profile limits plant growth by increasing the osmotic potential of the soil solution and inducing specific ion toxicities or nutrient imbalance (Janzen, 1993).

### Apparatus:

1. Conductivity meter
2. Conductivity cell, with constant K-1.0
3. 1.25 or 3 oz plastic cups or 50-mL beakers
4. Dispenser, capable of delivering 20 mL
5. Soil Scoop, 20 cm<sup>3</sup>
6. Stirring rod

### Reagents:

1. 0.01 M KCl standard solution: Dissolve 0.7456 g potassium chloride (KCl) in deionized (DI) water. Make up to 1 L with DI water and mix at 25°C. This solution is used as a standard and reference solution.

## **Procedure:**

### Electrical conductivity in soil samples:

1. Weigh out or scoop 20 g of soil in a 3 oz plastic cup or 50 mL beaker.
2. Set the dispenser capable to deliver 20 mL, and add 20 mL of DI water to the soil in the cup/beaker.
3. Stir each sample several times, until homogeneous slurry is achieved. Rinse the stirring rod with DI water after stirring each sample.
4. Allow to stand for one hour.
5. Calibrate conductivity meter using the standard KCl solution following manufacturer's instructions.
6. Rinse the conductivity cell with DI water and insert the cell into the soil-water suspension making sure that the tip of the cell is completely immersed. Allow the EC reading to become stable. Read the electrical conductivity of the suspension. To check the meter during analysis, measure EC of the standard after every 10 samples and at the end of the analysis.
7. Between EC readings, rinse the cell with DI water.

### Electrical conductivity in aqueous samples:

1. If analysis is not completed within 24 hours of sample collection, sample should be filtered through a 0.45 micron filter and stored at 4°C.
2. Filter and apparatus must be washed with DI water and pre-rinsed with sample before use.
3. Pour approximately 25 mL sample into a 1.25 or 3 oz plastic cup or 50 mL beaker.
4. Rinse the cell with DI water and place the cell in the water samples. Allow the EC reading to become stable. Record the EC in the Data Notebook.
5. Between EC readings, rinse the cell with DI water.
6. To check the meter during analysis, measure EC of the standard after every 10 samples and at the end of the analysis.

**Calculations:**

The laboratory reports all electrical conductivity measurements in deciSiemens per meter (dS/m), which is equal to the reading in mS/m divided by 100. The relationship between electrical conductivity and salt concentration are approximate and can be calculated as given below:

$$\text{Soluble salts in ppm (mg/liter)} = \text{EC in dS/m} \times 640$$

$$\text{Total Cations or Anions (meq/liter)} = \text{EC in dS/m} \times 10$$

**References:**

1. EPA Method 120.1 (4-79-020)
2. [http://www.epa.gov/region6/6pd/qa/qadevtools/mod5\\_sopsfield\\_Measurements/29palms\\_field\\_conductivity.pdf](http://www.epa.gov/region6/6pd/qa/qadevtools/mod5_sopsfield_Measurements/29palms_field_conductivity.pdf)
3. Janzen, H.H. 1993. Soluble salts. Soil reaction and exchangeable acidity. In: M.R. Carter Soil Sampling and Methods of Analysis. Lewis Publishers, pp 161-166.
4. Rhoades, J. D. 1982. Soluble salts. p. 167-179. *In*: A. L. Page et al. (ed.) Methods of soil analysis: Part 2: Chemical and microbiological properties. Monograph Number 9 (Second Edition). ASA, Madison, WI.

## 7. Soil Organic Matter by Loss on Ignition

Updated date: 6/18/2009

### Introduction:

We follow the same protocol as revised by Analytical Laboratories, Cornell University in 2004. Loss-on-ignition (LOI) is an inexpensive and reliable technique that has been used for decades for the determination of soil organic carbon (SOC) and soil organic matter (SOM). The LOI method is generally used to determine the OM content of soils by weighing the sample before and after an ashing (Storer, 1984; Bisutti et al., 2004). The method is based on the principle that at the chosen temperature, all OC is converted to CO<sub>2</sub>. To obtain the OC value from the weight difference, it is necessary to develop individual conversion factors or equations. These regression equations and conversion factors vary with the nature of OM and are different for specific areas, soils and soil horizons (Bisutti et al., 2004). Loss in weight gives an indication of the content of the organic matter in the sample. Soil organic matter is oxidized at a moderate to high temperature, with the weight loss being proportional to the amount of SOM in the sample (Konen et al., 2002). A known weight of soil is placed in a tared crucible. The sample is dried at 105°C and moisture content is determined. The sample is then ashed for two hours at 500°C. The percent weight loss of the soil ignition is calculated.

### Apparatus:

1. Oven - capable of maintaining a temperature of 105°C
2. Muffle furnace or oven - capable of maintaining a temperature of 500°C
3. Crucibles, 10-mL, porcelain
4. Balance - minimum capacity of 50 g, ±0.001 g
5. Scoop - 1 cm<sup>3</sup> capacity

### Procedure:

The following procedure is written for use by the Nutrient Management Spear Program Laboratory, where 50 soil samples, including a quality control soil and a replicate are prepared for organic matter analysis by loss on ignition:

1. Take the weight of empty crucibles (W<sub>c</sub>).
2. Accurately weigh 2 g of the soil sample into the crucible, and record the weight of the crucible and soil sample (W<sub>cs</sub>). Remove the crucible from the balance.

3. Dry the soil in the crucible in an oven set at 105°C for one hour.
4. Remove the crucibles and allow cooling for 15 minutes.
5. Weigh the crucibles with the dried sample ( $W_{os}$ ).
6. When all the crucibles containing a dried soil sample have been weighed, place into a muffle furnace, which equilibrated at 500°C, for two hours.
7. Remove the crucibles and allow cooling for one hour.
8. Place the crucible and the ashed sample on the balance and record the weight ( $W_{ca}$ ). Remove the crucible from the balance.

Note: Record all weights to  $\pm 0.001$  g.

#### Calculations:

1. Moisture (%):

$$\text{Moisture (\%)} = \frac{W_{cs} - W_{os}}{W_{cs} - W_c} \times 100$$

2. Loss on ignition (%):

$$\text{LOI (\%)} = \frac{W_{os} - W_{ca}}{W_{os} - W_c} \times 100$$

Where,

LOI = Percent weight loss on ignition

$W_c$  = Weight of empty crucible

$W_{cs}$  = Weight of crucible plus soil before drying at 105°C

$W_{os}$  = Weight of crucible plus soil after drying at 105°C

$W_{ca}$  = Weight of crucible plus ashed soil

3. Organic matter (%):

$$\text{OM (\%)} = (0.7 \times \text{LOI}) - 0.23$$

**References:**

1. Analytical Laboratories, Department of Agronomy, Cornell University, procedure no. S1810, issued: 03/12/2087, revised: 01/06/2004
2. Bisutti, I., I. Hike, and M. Raessler. 2004. Determination of total organic carbon- an overview of current methods. *Trends in Analytical Chemistry* 23: 716-726.
3. Konen, M.E., P.M. Jacobs, C. Lee Burras, B.J. Talaga, and J.A. Mason. 2002. Equations for predicting soil organic carbon using loss-on-ignition for North Central U.S. soils. *Soil Science Society of America Journal* 66: 1878-1881.
4. Storer, D.A. 1984. A simple high sample volume ashing procedure for determination of soil organic matter. *Communications in Soil Science and Plant Analysis* 15 759-772.



## **8. Haney-Brinton Solvita Test for Soil Biomass and C-N Mineralization**

Updated date: 4/2/2012

### **Introduction:**

The procedure and detail information are taken from Solvita booklet. The Solvita soil test is a tool which easily and accurately measures soil biological CO<sub>2</sub> respiration. This is a measure of microbial activity in the soil and is highly related to the fertility of the soil. Dried and weighed samples of soils are moistened at the time of testing, and run for 24 hours to obtain the CO<sub>2</sub>-burst potential, which is proportional to biomass. The results are read with the Digital Color Reader (DCR).

### **Apparatus:**

1. Digital color reader
2. Plastic beaker
3. Fiber filter
4. Glass jar with lid
5. Weighing balance
6. Solvita paddle (probe)
7. Dispenser calibrated to deliver 25 mL deionized (DI) water

### **Procedure:**

1. Place fiber filter in the bottom of the small plastic beaker.
2. Weigh 40 g ( $\pm 0.02$  g) of the pre-dried and 2-mm sieved soil into each plastic beaker.
3. Place the plastic beaker into the glass jar.
4. Add 25 mL of DI water into the glass jar (do not get soil wet).
5. Using plastic tweezers, place the Solvita paddle (probe) next to plastic beaker in glass jar.

6. Put the paddle with gel facing out next to the clear side of the jar.
7. Screw the lid on jar tightly.
8. Record the time.
9. Store jars at room temperature (25°C) for 24 hours.
10. After 24 hours ( $\pm 30$  minutes), remove paddle from glass jar.
11. Insert paddle (gel up) into the DCR.
12. Press the Low CO<sub>2</sub> button.
13. Press the READ button.
14. The DCR reports the color chart number on the first line and the ppm CO<sub>2</sub>-C on the second line. Record both.
15. Use the appropriate interpretation tables from the Solvita booklet for test results.

**References:**

1. Solvita 2011. Official Solvita guidelines soil respiration test. Booklet with digital color reader. Also information found at [www.solvita.com](http://www.solvita.com).

## 9. Determination of Permanganate Oxidizable Carbon

Updated date: 3/3/2017

### Introduction:

Weil et al. (2003) originally developed this method and Culman et al. revised this protocol in 2012. We follow exactly the same protocol as revised by Culman et al. (2012). We just copied their protocol here. However, some of the points and steps are modified/adjusted for our equipment and facilities. This procedure estimates the permanganate oxidizable carbon. In short, this is called POXC (Culman, 2012). In this method, slightly diluted alkaline  $\text{KMnO}_4$  reacts with the soil readily oxidizable (active) forms of soil C that converts Mn(VII) to Mn(II) and proportionally lowering absorbance of 550 nm light (Weil et al., 2003). Calcium chloride (0.1 M) addition to permanganate helps in settling of the soil after shaking.

### Apparatus:

1. Spectrophotometer
2. pH meter
3. Weighing balance, resolution  $\pm 0.01$  g
4. Dispensers calibrated to 2 mL, 18 mL and 49.5 mL
5. Horizontal mechanical shaker
6. Magnetic stirrers
7. Adjustable 1 and 10 mL pipettor and tips
8. 50 mL centrifuge tubes with lids
9. Timer
10. Cuvettes

## Reagents:

### Preparation of $\text{KMnO}_4$ stock solution 0.2 M:

1. 0.1 N NaOH solution (need for pH adjustment): Completely dissolve 4 g of NaOH in 1 L of deionized (DI) water.
2. Dissolve 147 g of  $\text{CaCl}_2$  in about 600 mL of DI water. Dilute to 1 L DI water and mix thoroughly.
3. Weigh 31.60 g of  $\text{KMnO}_4$  into a 1 L beaker/volumetric flask and add approximately 900 mL of the  $\text{CaCl}_2$  solution. Place on the magnetic stir plate with gentle heat and stir until dissolved completely. Dissolution may be very slow and check the solution for undissolved  $\text{KMnO}_4$ .
4. Once dissolution is complete, bring the volume to 1 L with the  $\text{CaCl}_2$  solution and mix well. Begin by adjusting the pH to 7.2 by adding a few drops of 0.1 N NaOH. Once the pH unit is close to approach 7.2, add 1 drop at a time. Allow the pH reading to stabilize between additions of NaOH. Once the pH is adjusted, transfer to a brown glass bottle and store in a dark place. If stored as indicated, the stability of this solution will be 3-6 months.

## Standards:

Four standard concentrations (0.005, 0.01, 0.015 and 0.02 M) will be prepared from the  $\text{KMnO}_4$  stock solution. The standard preparation involves first making a standard stock solution and then diluting each standard stock solution to a final working standard.

Part 1 Standard stock solutions: Use the table below to prepare standard stock solutions. These stock solutions can be prepared in 50 mL volumetric flask or centrifuge tubes and used for three days (in glass and in the dark) to prepare working standards.

Concentration	Volume of $\text{KMnO}_4$ stock solution	Volume of DI water
0.005 M	0.25 mL	9.75 mL
0.01 M	0.5 mL	9.5 mL
0.015 M	0.75 mL	9.25 mL
0.02 M	1.0 mL	9.0 mL

Part 2 Dilution step: Dilute each standard stock solution to a working standard by adding 0.5 mL of each stock solution to 49.5 mL of DI water in 50 mL volumetric flask or centrifuge tubes. These flask now contain the working standards (0.00005, 0.0001, 0.00015, and 0.0002 M  $\text{KMnO}_4$ ) and should be prepared fresh daily.

## **Procedure:**

Procedure for sample preparation involves two-parts; a sample reaction and sample dilution, as detailed below:

### Part 1 Sample reaction:

1. Label two 50 mL centrifuge tubes for each sample. Weigh 2.50 g ( $\pm$  0.05 g) of air-dried soil (sieved to 2-mm) into one of the 50 mL centrifuge tubes.
2. Use two quality control samples, one with soil (soil standard) and one without soil (solution standard) in each batch. For soil standard, 2.50 g of known pulverized soil should be used. Place the other set of tubes aside.
3. Add 18.0 mL of deionized water to each of the centrifuge tubes. Using the 1.0-10.0 mL pipettor or 1.0-5.0 mL dispenser, add 2.0 mL of 0.2 M  $\text{KMnO}_4$  stock solution to each tube.
4. Working quickly, cap tubes tightly and hand-shake each tube vigorously for 2 seconds to assure soil dispersion within the solution.
5. Place tubes on shaker and shake on high speed 240 oscillations per minute for 2 minutes.
6. After 2 minutes, remove samples from shaker and swirl or shake the tube vigorously to ensure that there is no soil clinging to the sides or cap of the tube. At this point, remove caps to avoid further disturbance of soil after settling. Place the samples in a dark area and allow soil to settle for 10 minutes. Settling time is a critical step.

Note: It is important that the timing of each step be consistent, particularly the shaking and settling times. The permanganate will continue to react with soil as long as it remains in contact. Hence, working quickly with small batches of 10 samples or less is advised.

### Part 2 Sample dilution:

1. While samples are settling, add 49.5 mL of DI water to the second set of centrifuge tubes.
2. Once the 10-minute settling period has passed, quickly transfer 0.5 mL of supernatant (avoiding any particulate matter) to the second tube containing 49.5 mL of DI water. This step should be performed as quickly as possible as the permanganate will continue to react with soil as long as it remains in contact.
3. Cap second set of tubes and invert to mix. These are the final sample solutions for analysis. They are stable for up to 24 hours if stored in the dark.

### Reading Samples on Spectrophotometer:

1. Measure and record the absorbance of standards and samples at 550 nm using AquaMate spectrophotometer single cuvette reader.

### Calculating Mass of POXC for Unknown Soil Samples:

1. The amount of carbon oxidized is a function of the quantity of permanganate reduced. Consequently, the higher the POXC values the lower the absorbance (intensity of the color of the solution).
2. Use the following equation to determine POXC, Weil et al. (2003):

$$\text{POXC (mg kg}^{-1}\text{)} = \frac{0.02 \text{ mol/L} - (a + b \times \text{Abs})}{0.02 \text{ L solution/Wt}} \times (9000 \text{ mg C/mol}) \times (0.02 \text{ L solution/Wt})$$

Where,

0.02 mol/L = initial solution concentration

a = intercept of the standard curve

b = slope of the standard curve

Abs = absorbance of unknown

9000 = milligrams of C oxidized by 1 mole of  $\text{MnO}_4$  changing from  $\text{Mn}^{7+}$  to  $\text{Mn}^{2+}$

0.02 L = volume of stock solution reacted

Wt = weight of air-dried soil sample in kg

### Example for Calculation:

Construct standard curve with the following values:

Y-axis (Molarity of stock $\text{KMnO}_4$ standards)*	0.005	0.01	0.015	0.02
X-axis (Abs values from spectrophotometer)	0.1000	0.1984	0.3034	0.3966

\* Note: The standard curve should use the molarity of the stock standards, and not the working standards, since the stock standards represent the actual concentration (0.02 M  $\text{KMnO}_4$ ) used to react with the soil.

This produces the regression line:  $y = 0.0502x - 0.00004$ ;  $R^2 = 0.999$

Unknown sample absorbance: 0.3087; unknown sample soil weight: 2.48 g

$$\text{POXC (mg kg}^{-1} \text{ soil)} = [0.02 M - (-0.00004 + (0.0502 \times 0.3087))] \times (9000 \text{ mg C/ mol}) \times (0.02 \text{ L solution/ } 0.00248 \text{ kg}) = 329.75 \text{ mg POXC kg}^{-1} \text{ soil}$$

### **Clean-up and Disposal:**

Leaving the centrifuge tubes capped but on the bench top for a week or more will allow the permanganate to completely react with the soil and lose all purple pigmentation. Liquid can then be safely disposed of down the sink and tubes with soil thrown out or cleaned and reused. The second dilution of samples and standards contains very little  $\text{KMnO}_4$  and may be safely flushed down the drain with copious amounts of water. However, check environmental health and safety rules for proper disposal.

### **Equipment Operation and Calibration Procedure:**

Turn instrument “ON” and warm up instrument for about three minutes.

Select “GENERAL TEST” → Select “QUANT” → Select “WAVELENGTH” (change according to methodology) → “ENTER STANDARDS” → Enter the concentration of the standards. To enter Standard concentration, use the “UP” and “DOWN” arrow keys to move through the list of standards. Use the numeric keys to enter the concentration of the standard and press “ENTER” when finished. The instrument returns to the Standards Entry Page with the highlight on the next Standard on the list. When all the Standards have been entered press the “ACCEPT” function key to return to the QUANT Page with the new list of Standards.

Press “ZEROBASE” to zero the instrument and press “CALIBRATE”. The Quant Calibration graph will be displaced and the instrument will prompt for each standard (and replicate) in turn. As the measurements of the standards proceed, the data points are marked on the graph. When all the standards have been measured, the system calculates the equation, rescales the graph then draws and displays the line of best fit. Now we can take sample measurement and then switch to the Quant Results screen. Press “ZEROBASE” to zero again before taking sample measurement.

### **References:**

1. AquaMate User Manual, AM Issue 3b (07/2001).
2. Culman, Steve, M. Freeman, and S. Snapp. 2012. Procedure for determination of permanganate oxidizable carbon. Kellogg Biological Station, Michigan State University, Hickory Corners, MI 49060. Found at <http://lter.kbs.msu.edu/protocols/133>.
3. Weil, R.R., K.R. Islam, M.A. Stine, J.B. Gruver, and S.E. Samson-Liebig. 2003. Estimating active carbon for soil quality assessment: A simplified method for laboratory and field use. American Journal of Alternative Agriculture. 18: 3-17.

## 10. Illinois Soil Nitrogen Test

Updated date: 3/25/2008 and 6/2009

### Introduction:

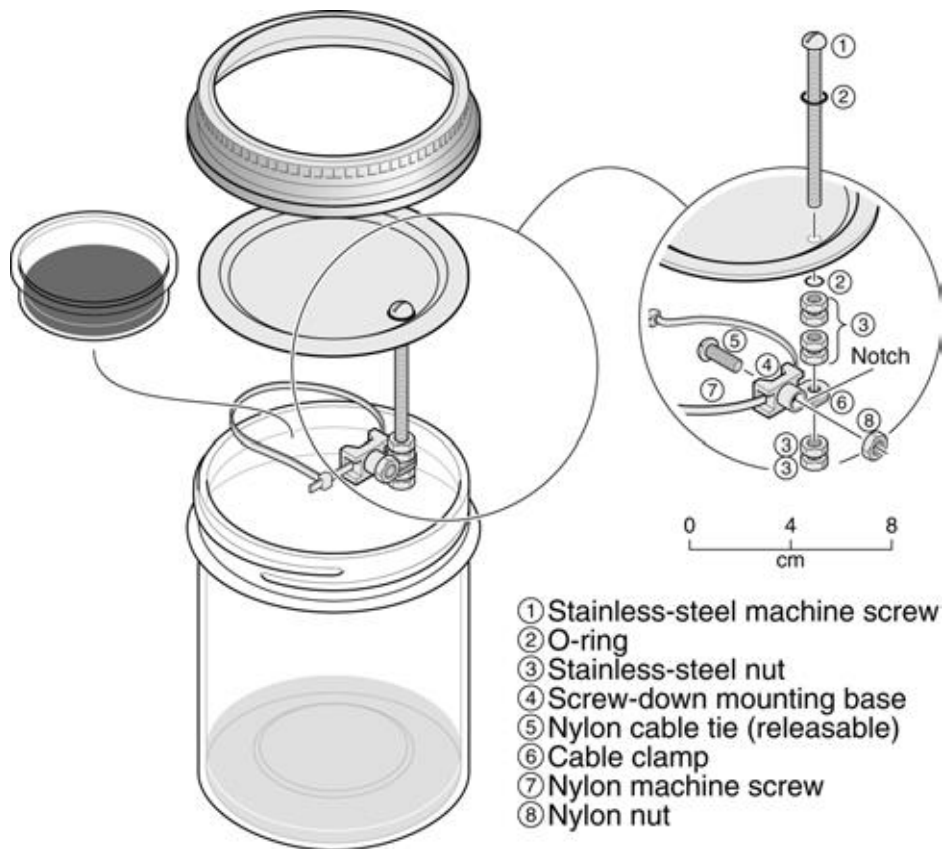
The protocol for the Illinois soil nitrogen test (ISNT) was originally developed by Nathalie Galens and Jon Klapwijk and updated on 3/25/2008 by Kevin Dietzel. Sanjay Gami updated this protocol in 6/2009. The ISNT is a simple laboratory analysis that can be used as a tool to predict if additional nitrogen (N) application for corn (*Zea mays* L.) will result in a yield increase. Initial testing of the method at Cornell University showed a high degree of day-to-day and within-griddle soil test N variability. At the University of Illinois, similar findings led to modification of the method to include jar rotations 1.5 and 3 hours after initiation of the 5-hour incubation period used in the test. To determine if variability could be reduced without additional labor by enclosing the griddles using plywood boxes, experiments were designed to compare soil test variability using both the open-bench and enclosed-griddle methods. Enclosing the griddles decreased the coefficient of variance ( $p=0.013$ ) to 2.5% from 3.8% when analyses were done using the open-bench method (using 10 exterior positions of the griddle for each method). Enclosing griddles did not impact recovery of added glucosamine-N but resulted in lower N test values when set at the same temperature as the open-bench griddles. A comparison study of 14 soils analyzed at the University of Illinois using the standard open-bench procedure with jar rotation and at Cornell University using the enclosed-griddle method without rotation showed sample means to be comparable when the enclosed griddles were set at 50.6°C (Klapwyk and Ketterings, 2005). These results imply that the modified method could be adopted for use beyond well-controlled laboratory environments but that griddle temperature setting is extremely important. Field work indicated the test reflects past manure and compost N applications (Klapwyk et al., 2006) and was accurate in predicting corn responsiveness in New York (Klapwyk and Ketterings, 2006; Lawrence et al, 2009).

### Apparatus:

1. Weighing balance: An analytical balance with at least 0.1 mg accuracy is used to weigh out soil for analysis.
2. Quality control soil: Two samples of a quality control soil are used on each griddle to monitor the accuracy of the values determined from that griddle. This soil (a mix of various soils, thoroughly mixed and ground to pass 250  $\mu\text{m}$ ) was run more than 100 times with an average value of 230 ppm, maximum 255 ppm and minimum 205 ppm.
3. Mason jars: Pint sized glass canning jars (cat. No. 14400-66000, Alltrista, Muncie, IN).



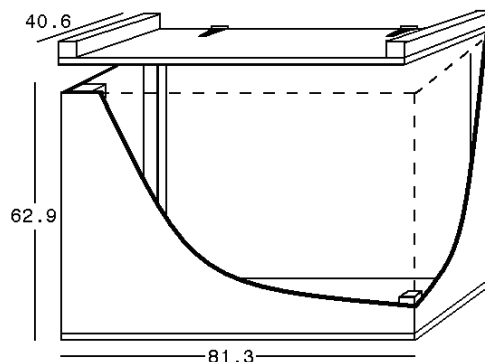
4. Lid: Attached onto the jar lids is a screw with a turnable apparatus where the Petri dishes can be mounted. See diagram. Supplies list for lid:
- i. Machine screws (Part No. 91783A161, McMaster Carr, Aurora, OH).
  - ii. Stainless steel nuts (Part No. 91841A007, McMaster Carr).
  - iii. Nylon screw (Part No. 94609A150, McMaster Carr).
  - iv. Nylon nut (Part No. 94812A133, McMaster Carr).
  - v. Viton O-ring (Part No. 9464K11, McMaster Carr).
  - vi. Nylon strap (Part No. 8876T14, McMaster Carr).
  - vii. Releasable cable tie (Part No. 7134K3, McMaster Carr).
  - viii. Panduit Cable tie mounts (Part No. TM2S6-C, available through Horizon Solutions Corp., Holyoke, MA).



5. Petri dishes: 60 mm diam. by 15 mm deep Pyrex Petri Dishes (C-3160-60B0, Labglass, Buena, NJ).
6. HOBO temperature sensor: HOBO (Model H08-006-04, Onset Computer Corp., Bourne, MA) four-channel external data loggers with water/soil temperature sensors (Part No. TMC6-HD, Onset Computer Corp.) are used to record temperatures within griddles.
7. Timer: A Big-Digit timer (Cat No. S407992, Fisher Scientific) is used to time the 5 h incubation and the 7 min loading and unloading times.

8. Lamp timers: Lamp timers (Model No. TN111, Intermatic Inc., Spring Grove, IL) are used to preheat griddles at least 1 hr 15 min. before start time.

9. Griddles: Electric Griddles with the original temperature controller (Model 76220, West Bend, West Bend, WI).



10. Plywood boxes: Plywood boxes are 12.7 mm thick for the walls of the box, and 19 mm thick for the bottom and lid. Outside dimensions of the boxes are 81.3 cm in length, 40.6 cm in width, and 62.9 cm in height (see diagram). A 19 by 25 mm hole was cut into the side of the box for electrical plug of the griddle. Inside corners and sides of the top of the lid were secured with 38 by 38 mm spruce lumber. The bottom of the lid was lined with 32 by 5 mm weather stripping (Prod. No. V447, Thermwell Products Co., Mahwah, NJ).

11. Titrator: An automatic titrator (719 S Titrimo, Metrohm Ltd., available through Brinkmann Instruments, Inc., Westbury, NY) with propeller titration stand (Metrohm 727 Ti Stand) performs a pH titration using 0.01 M H<sub>2</sub>SO<sub>4</sub> of H<sub>3</sub>BO<sub>3</sub> and DI water in Petri dishes after 5 h incubation.

12. Printer: An Epson LX-300+ 9-pin dot matrix printer (Seiko Epson Corp., Nagano, Japan) is connected to the titrator for automatic printing of outputs.

13. Acid Bath: Acid bath is made by adding 160 mL of Concentrated H<sub>2</sub>SO<sub>4</sub> to 8 L of DI water.

14. Drying Oven: Any model drying oven with temperature set to 50°C.

**Reagents:**

1. 2.00 M NaOH (Cat. No. VW6720-7, VWR International, Bridgeport, NJ)
2. 4% H<sub>3</sub>BO<sub>3</sub> with pH indicator (Cat. No. VW3251-7, VWR International, West Chester, PA)
3. 0.01 M H<sub>2</sub>SO<sub>4</sub> (SA226-20, Fisher Scientific, Fair Lawn, NJ)

**Procedure:**Sample preparation:

1. Dry samples in a 50°C oven and crush to pass through a 2-mm sieve.
2. Weigh out two samples of 1 g (0.9975-1.005 g) each from each sample to be tested.
3. Place in glass jar and cover with lid marked with griddle # and position.
4. Designate the placement of each sample in a randomized fashion so that the two duplicate samples are assigned to different positions on different griddles.
5. Positions 6 and 7 are always quality control samples (ground to 250 μm).

Data loggers:

HOBO 4-Channel External data loggers from Onset Computer Corporation are used to monitor temperature. They are used with HOBO Water/Soil Temperature sensors (part Number TMC6-HD). Data is saved using BoxCar Pro 4.3.

Launch HOBOS:

1. Open BoxCar Pro 4.3 from the desktop.
2. Plug in data logger.
3. Click Launch Data Logger button from the toolbar (or hit Ctrl + L).
4. In Launch window, change date in Description field to current date.
5. Click Start.
6. A reminder will pop up that launching will erase previous data. Click Yes to continue.
7. When Disconnect Logger window opens, unplug logger and then press OK.

8. Check for flashing light on logger.

#### Readout HOBOS:

1. Open BoxCar Pro 4.3 from the desktop.
2. Plug in data logger.
3. Click Readout Data Logger button from the toolbar (or hit Ctrl + R).
4. When Disconnect Logger window opens, unplug logger then press OK.
5. In Save As window, confirm that file name is ok and click Save.
6. In Select Series to Show in View window, uncheck all °F boxes and check all °C ones.
7. Click OK.
8. A graph of the temperature data will appear. Check for anything unusual.
9. Click Export Excel Spreadsheet button from the toolbar.
10. In Export Window, click Export.
11. In Save As window, confirm that file name and location to save are correct and click Save.
12. Currently, temperature data is saved in the Shared → ISNT Results → Temperature → 2017 folder.
13. An Export window with the message “Writing series Info...” will remain open until the export is complete, this may take awhile.
14. If the data logger batteries run low, replacements (3 V Maxell CR203 microlithium cells) are in Morrison Hall Room no. 306 in a drawer beneath the reagent dispensing bottles.

#### Loading griddles:

##### Preheat:

1. Fill water jars with 120 mL DI water the night before.
2. Set timers to preheat griddles at least 1 h 15 min. before start time (lids closed).

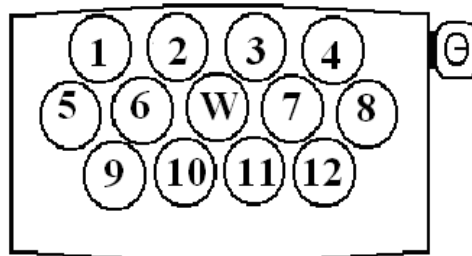
3. For Monday runs: fill water jars with 150 mL DI water on Friday. Set timers to run for 3 h in the morning. On Monday, be sure to reset timers before loading griddles so they run for the full 5 h period.
4. Launch HOBO's.
5. Shake/swirl then prime reagent bottles; check volume setting of dispensers with graduated cylinder.

Loading (repeat for each griddle):

1. Plug in HOBO to first temperature sensor.
2. Open box lid.
3. Start timer to count down from five hours.

For each jar:

1. Swirl dry soil in jar.
2. Add 10 mL 2 M NaOH (clear) around walls of jar to wash down any soil; gently swirl jar.
3. Add 5 mL H<sub>3</sub>BO<sub>3</sub> (pink) to Petri dish; insert dish into jar.
4. Hold jar tilted towards screw and screw rings on tightly.
5. Place jar in correct position (see diagram on right) on griddle with screw facing the front.
6. Load each jar within 30 seconds of NaOH addition; load with all 12 jars in ~7 minutes.
7. Close and latch box lid after griddle is loaded.



### Removing jars:

1. Open box lid at 5 h and remove jars in same order as loading (jars 1-12).
2. Tilt jar towards screw.
3. Level the Petri dish
4. Allow condensed water to run down screw and not drip into Petri dish.
5. Remove Petri dish and place on tray.
6. Make sure to not drip water from gloves/hands into Petri dish.
7. Unplug HOBO and griddle power after each box is done.
8. Add 5.0 mL DI water to each Petri dish.
9. Keep entire griddle (12 samples) at the same time and in the same order as they were unloaded.
10. Make sure electrode is in solution and the propeller is under the acid line.

### **Setup for Titration:**

#### Check standards:

1. Turn titrator ON (red switch on back left side), keep printer OFF.
2. Spin propeller/electrode/acid line (P/E/A) in DI water for 2+ minutes.
3. Wash/dry P/E/A. Spin again in DI water.

#### Recalibration:

1. Press mode = CAL. Enter. Start. 25°C: Enter.
2. pH 7: Place electrode in “old” pH 7 buffer; wash/dry electrode.
3. Place electrode in new pH 7 buffer and press START. Watch for final mV value displayed and record this value (it will no longer be displayed once calibration is complete); wash/dry electrode.
4. pH 4: Place electrode in “old” pH 4 buffer; wash/dry electrode.

5. Place electrode in new pH 4 buffer and press START. Watch for final mV value displayed and record this value, as well as the pH and slope.
6. Press STOP.
7. Wash/dry electrode.

Measure pH of 3 blank samples:

1. Add 5.0 mL boric acid (pink) and 5.0 mL DI water to 3 clean/dry Petri dishes.
2. Mode = MEAS. Enter. Start.
3. Write down pH of each of the three samples. Wash/dry after each sample.
4. Press STOP.

Set titrator to average of last 2 samples:

1. Mode = SET. Press ENTER. Press ENTER.
2. Press USER METH. Recall: ENTER. Dif1: ENTER.
3. Press PARAM. Set1: ENTER. Input pH average of last 2 blank samples from above. ENTER. Dynamics: Press STOP.
4. Press USER METH twice until get to 'store method': Press ENTER. Dif1: ENTER. Overwrite: ENTER.
5. Now the titrator is set and can be used; but first: Press and hold DOS to remove air bubbles from acid line. Wash/dry. Press STOP.
6. Make sure printer is turned off.
7. Do one practice titration by adding a bit of "old" pH 7 buffer to one of the blank samples (see below for titration method).
8. Turn printer on (this will remove the error message).

Samples titration:

1. Titrate in same order as load/remove (griddle 1: 1-12, griddle 2: 1-12...).
2. Place Petri dish on platform, lower P/E/A, press START.

3. Wait for titration to print, making sure the printer did not jam. Remove Petri dish, wash/dry electrode.
4. If washing jars at the same time, make sure to not drip from gloves into Petri dish.

**Printer:**

To adjust printer paper:

1. Press LF/FF to advance enough to tear off.
2. Press Load/Eject.
3. Press LF/FF.
4. Check to make sure pause light is off (if on, press pause button to take printer off pause).

**Shutdown:**

1. Place electrode in pH 4 buffer.
2. Turn off titrator and printer.

**Cleaning:**

1. Always wear safety goggles, lab coat and large gloves when using acid bath.

Jars:

1. Tap water rinse 3x (remove all soil; no soil should get into the acid bath), scrub with brush.
2. Put in 0.1 M H<sub>2</sub>SO<sub>4</sub> acid bath for at least one minute.
3. Put jars in tub of water; scrub each jar.
4. Rinse outside of jar under DI water 1x, inside 2x; put into the drying oven.

Lids:

1. Let lids soak/rinse in tub of warm tap water for a couple of minutes (this makes inserting Petri dishes easier). They should not stay in water overnight or they will rust.



### Petri dishes:

1. Place in tub of water and rinse.
2. Put in 0.1 M H<sub>2</sub>SO<sub>4</sub> bath for 30+ seconds.
3. Put into tub of tap water; scrub with brush.
4. DI water rinse outside 1x, inside 2x.
5. Fit into lids/cable ties while still wet, place upside down to air dry with 36 lids/dishes per tray on two wooden trays (72 total).

### **Data Entry:**

The outputs from the titration are automatically printed in the order that the samples are titrated. From this printout, enter the number following the label “N DTD” into an Excel spreadsheet. This number is displayed in units of  $\mu\text{g}$  (H<sub>2</sub>SO<sub>4</sub> added). The values can then be matched to their sample ID's. From the duplicate values of each sample, calculate the average, standard deviation, and the coefficient of variance (% CV). Re-run samples with CV>10 % (%CV = standard deviation/average \* 100).

1. Quality control samples should be between 205 and 255 ppm.
2. Average griddle temperature should be  $50.6 \pm 0.5^\circ\text{C}$ . Calculate the average beginning with the values at the time the sensor was plugged into the HOBO, as temperatures begin to be recorded as soon as loggers are launched and end when loggers are read out.

### **References:**

1. Klapwyk, J.H., and Q.M. Ketterings (2006). Soil nitrogen tests for predicting if corn will respond to nitrogen fertilizer in New York. *Agronomy Journal* 98: 675-681.
2. Klapwyk, J.H., Q.M. Ketterings, G.S. Godwin, and M. Wang (2006). Response of the Illinois Soil Nitrogen Test to liquid and composted dairy manure application in a corn agroecosystem. *Canadian Journal of Soil Science* 86: 655-663.
3. Klapwyk, J.H., and Q.M. Ketterings (2005). Reducing laboratory variability of the Illinois soil N test with enclosed griddles. *Soil Science Society of America Journal* 69: 1129-1134.
4. Lawrence, J.R., Q.M. Ketterings, M.G. Goler, J.H. Cherney, W.J. Cox, and K.J. Czymmek (2009). Accuracy of the Illinois Soil Nitrogen Test (ISNT) in predicting N responsiveness of corn in rotation. *Soil Science Society of America Journal* 73: 303-311.

## 11. Soil Nitrate+Nitrite-N Test Extracted with Morgan's Solution

Update date: 4/27/2013

### Introduction:

For the determination of nitrate-N, soils are extracted with Morgan's solution (diluted sodium acetate/acetic acid solution, buffered at pH 4.8). Activated carbon is added to the extraction to help in the removal of organic matter and decolorize the extraction solution. After shaking, the extract is filtered and the concentration of available nutrients is measured in the clear filtered extract.

The automated procedure for the determination of nitrate utilizes the reaction whereby nitrate is reduced to nitrite by a proprietary Chemical R1. The reduced nitrate is then treated with sulfanilamide and N-1-napthylethylenediamine dihydrochloride under acidic conditions to form a soluble dye which is measured colorimetrically at 546 nm. The final product measured represents the nitrite ion originally present plus that formed from the nitrate.

### Apparatus:

1. EasyChem analyzer
2. Computer for data collection
3. Printer
4. Stainless steel scoop
5. Reciprocating or rotary shaker
6. 100 mL Erlenmeyer flasks or plastic bottles
7. Test tubes
8. Balance with sensitivity of  $\pm 0.01$  g
9. Automatic pipette or dispenser capable of dispensing 50 mL
10. Funnels
11. Funnel rack
12. Whatman 2V, Size: 150 mm (Cat No. 1202-150) filter paper or its equivalent

**Reagents:**

1. Morgan's Solution: Weigh 400 g of sodium acetate trihydrate ( $\text{CH}_3\text{COONa}_3\text{H}_2\text{O}$ ) and add deionized (DI) water in a 4 L volumetric flask. Add 116 mL glacial acetic acid, and mix until the sodium acetate is dissolved. Dilute to the 4 L mark, and mix well. The pH of this solution should be  $4.80 \pm 0.05$ . If not, adjust a lower pH with sodium acetate or a higher pH with acetic acid.
2. Darco G-6 activated carbon
3. R1 – Systea (1-Reagent) nitrate solution (proprietary); R1 – 1 L size, part number E/R1-10 and R1 – 0.5 liter size, part number E/R1-05; available for purchase from Chinchilla Scientific, 900 Jorie Blvd., Suite 35, Oak Brook, IL 60523; Phone: 630-645-0600; Fax: 630-645-0601; Email: info@easy.chem.com
4. Potassium nitrate ( $\text{KNO}_3$ )

**Standards:**

1. Stock Standard (1000 mg/L  $\text{NO}_3^-$ -N): Dissolve 7.218 g of potassium nitrate in approximately 600 mL of DI water. Mix thoroughly and dilute to 1 L with DI water.
2. Working Standard (100 mg/L): Dilute 10 mL of 1000 mg/L stock standard in a 100 mL volumetric flask with DI water.

**Procedure:**

1. Weigh 10 g of dried and sieved (<2mm) soil into a 100 mL Erlenmeyer flask or plastic bottle. See chapter for details on sample preparation - drying and grinding.
2. Add approximately 0.15 g of activated carbon to each sample.
3. Add 50 mL of Morgan's solution to each flask, place the flask horizontally in the reciprocal shaker and shake for 15 min on low speed (180 oscillation per minute).
4. Filter the extracts through filter paper (Whatman 2V or equivalent) into the test tubes.
5. Use a quality control soil sample in each batch.
6. Remove the funnel racks, dispose of the filter papers, and place the test tubes containing the extract in the 50-place test tube rack.
7. The extracts are now ready for analysis.

8. Enter cup IDs into computer and pour samples into the cup and keep in tray in order.
9. Analyze the sample for nitrate+nitrite-N test with discrete analyzer.
10. Follow directions for EasyChem Discrete Analyzer start-up and shut down procedures. Also, refer to the instrument manual for specific instructions for the operation of the instrument.
11. Wash the plastic bottle and funnels thoroughly with hot water, then rinse several times with DI water and allow them to dry.
12. After all analyses have been completed, rinse the test tubes thoroughly with hot water, then clean with DI water, and dry.

**Calculations:**

Report mg/kg of  $\text{NO}_3^- + \text{NO}_2^- - \text{N}$  in sample:

mg/kg of  $\text{NO}_3^- + \text{NO}_2^- - \text{N}$  =  $\text{NO}_3^- + \text{NO}_2^- - \text{N}$  in extract mg/Lx50/sample weight

**References:**

1. Analytical Laboratories, Department of Agronomy, Cornell University. Procedure No. S1100-1, issued on 2/27/89.
2. EasyChem Methodology. 2009. Nitrate by discrete analysis, Syssta easy (1-reagent) nitrate method (colorimetric, automated, 1 reagent). Syssta Scientific, LLC, 900 Jorie Blvd., suite 35, Oak Brook, IL 60523.
3. Morgan, M.F. (1941), Chemical Soil Diagnosis by the Universal Soil Testing System., Conn. Agr. Exp. Sta., Bulletin 450.

## 12. Soil Ammonium-N and Nitrate+Nitrite-N Tests Extracted with Potassium Chloride

Updated date: 4/27/2013

This 2 M KCl effectively extracts both exchangeable  $\text{NH}_4^+$ -N and  $\text{NO}_3^- + \text{NO}_2^-$ -N from soils. The aqueous sample containing ammonia reacts hypochlorite to form monochloramine. In the presence of excess hypochlorite, monochloramine reacts with phenol to give indophenol blue in an amount that is proportional to the ammonia concentration. The blue color is intensified with sodium nitroferriyanide. The absorbance is measured at 670 nm.

### Apparatus:

1. EasyChem analyzer
2. Computer for data collection
3. Printer
4. Stainless steel scoop
5. Reciprocating or rotary shaker
6. 100 mL Erlenmeyer flasks or plastic bottles
7. Test tubes
8. Balance with sensitivity of  $\pm 0.01$  g
9. Automatic pipette or dispenser capable of dispensing 50 mL
10. Funnels
11. Funnel rack
12. Whatman 2V, Size:150 mm (Cat No. 1202-150) filter paper or its equivalent

### Reagents:

1. Extractant: 2 M KCl: Dissolve 596.4 g analytical grade KCl (Potassium chloride) in deionized water (DI) bring to 4 L.
2. Darco G-6 activated carbon

3. Ammonium Sulfate,  $(\text{NH}_4)_2\text{SO}_4$
4. Sodium Hydroxide, NaOH
5. Sodium Nitroferricyanide,  $\text{Na}_2\text{Fe}(\text{CN})_5\text{NO}\cdot 2\text{H}_2\text{O}$  Stock Solution: dissolve 0.2 g of sodium nitroferricyanide in 75 mL DI water, mix thoroughly, and dilute to 100 mL with DI water. Transfer to a light-resistant container. Stable for at least one month at room temperature.
6. Liquefied Phenol (88%),  $\text{C}_6\text{H}_5\text{OH}$  Stock Solution: Dissolve 3.2 g of sodium hydroxide in 75 mL DI water, mix thoroughly, and cool. Slowly add 9.4 mL of liquefied phenol and dilute to 100 mL with DI water. Transfer to a light-resistant container. Stable for at least one month at room temperature.
7. Ethylenediamine Tetraacetic Acid, Disodium Salt Dehydrate; EDTA,  $\text{C}_{10}\text{H}_{14}\text{N}_2\text{Na}_2\text{O}_8\cdot 2\text{H}_2\text{O}$  Stock Solution: Add 0.1 g of sodium hydroxide to 75 mL DI water and cool, dissolve 5 g of EDTA, mix thoroughly, and dilute to 100 mL with DI water. Transfer to a light-resistant container. Stable for at least one month at room temperature.
8. Phenol/nitroferricyanide/EDTA Solution: Add 20 mL EDTA stock solution, 5 mL nitroferricyanide stock solution, and 20 mL phenol stock solution and mix well. Transfer to an EasyChem reagent bottle. Stable for one single day of analysis.
9. Sodium Hypochlorite ( $\text{NaOCl}$ ) Solution: Add 20 mL sodium hypochlorite to 30 mL DI water and mix well. Transfer to an EasyChem reagent bottle. Best if prepared daily.

### Standards:

1. Stock Standard (1000 mg/L): Dissolve 4.7168 g of ammonium sulfate, dried at  $110^\circ\text{C}$ , in about 600 mL of DI water. Dilute to 1 L with DI water and mix thoroughly.
2. Working Standard (100 mg/L): Dilute 10 mL of 1000 mg/L stock standard in a 100 mL volumetric flask with DI water.

### Procedure:

1. Weigh 5 g of dried and sieved ( $<2\text{mm}$ ) soil into a 100 mL Erlenmeyer flask or plastic bottle. See chapter for details on sample preparation - drying and grinding.
2. Add approx. 0.15 g of activated carbon to each sample.
3. Add 50 mL of 2 M of KCl solution to each flask.

4. Place the flask horizontally in the reciprocal shaker and shake for 1 hour on low speed (180 oscillations per minute).
5. Filter the extracts through filter paper (Whatman 2V or equivalent) into the tubes.
6. Use a quality control soil sample in each batch.
7. Remove the funnel racks, dispose of the filter papers, and place the test tubes containing the extract in the 50-place test tube rack.
8. The extracts are now ready for analysis.
9. Enter cup IDs into computer and pour samples into the cup and keep in tray in order.
10. Analyze the sample for exchangeable  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_3^-+\text{NO}_2^-\text{-N}$  analysis with discrete analyzer at NMSP lab.
10. Follow directions for EasyChem Discrete Analyzer start-up and shut-down procedures. Also, refer to the instrument manual for specific instructions for the operation of the instrument.
11. Wash the plastic bottle and funnels thoroughly with hot water, then rinse several times with DI water and allow to dry.
12. After all analyses have been completed, rinse the test tubes thoroughly with hot water, then clean with DI water, and dry.

### Calculations:

Report mg/kg of  $\text{NH}_4^+\text{-N}$  or of  $\text{NO}_3^-+\text{NO}_2^-\text{-N}$  in sample:

$\text{mg/kg of } \text{NH}_4^+\text{-N or } \text{NO}_3^-+\text{NO}_2^-\text{-N} = \text{NH}_4^+\text{-N or } \text{NO}_3^-+\text{NO}_2^-\text{-N in extract mg/L} \times 50/\text{sample weight}$

### References:

1. EasyChem Methodology. 2009. Ammonia, USEPA by discrete analysis, Systea easy ammonia method 350.1-02 (colorimetric, automated). Systea Scientific, LLC, 900 Jorie Blvd., suite 35, Oak Brook, IL 60523.
2. Recommended Soil Testing Procedures for the North Eastern United States. 2<sup>nd</sup> edition, Northeast Regional Publication, December 1995, Bulletin # 493. Agricultural Experiment Station, University of Delaware, Newark, DE 19717-1303.

### 13. Potassium, Calcium, Magnesium, and Sulfur Tests Using 0.01 M Strontium Chloride

Updated date: 11/26/2014

#### Apparatus:

1. 50-mL centrifuge tubes with lid
2. Weighing balance, resolution  $\pm 0.001$  g
3. Dispensers calibrated to 20 mL
4. Horizontal/reciprocal shaker
5. Whatman 2V, Size: 150 mm (Cat No. 1202-150) filter paper or its equivalent
6. Timer
7. Test tubes
8. Funnels
9. Funnel rack

#### Reagents:

1. 0.01 M Strontium Chloride,  $\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$ : Dissolve 2.6662 g of  $\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$  and make up to 1 L with deionized water (DI) water.

#### Procedure:

1. Place 2 g of dried and sieved (<2 mm) soil into a 50 mL centrifuge tube.
2. Add 20 ml of 0.01 M  $\text{SrCl}_2$  in each centrifuge tube, place the tube horizontally in the reciprocal shaker and shake for five minutes on low speed (180 oscillation per minute).
3. Filter the extracts through filter paper (Whatman 2V or equivalent) into the tubes.
4. Filter 2 blank (0.01 M  $\text{SrCl}_2$  extracting solution) samples through filter paper in each batch. Subtract the mean value of K, Ca, Mg and S of blank samples from each sample.



5. Submit the sample to laboratory for K, Ca, Mg and S analysis with ICP.

**Calculations for K, Ca, Mg, and S in Mg/Kg:**

mg/kg of K or Ca or Mg or S = K or Ca or Mg or S in extract mg/Lx20/sample weight

**Equation to Adjust Soil S:**

$$Y = 0.9685X$$

Report the soil S data after adjusting with this equation.

**Calculations for CEC:**

Total CEC is calculated by summation of the three major nonacid cations (K, Ca, and Mg).

$$\text{Total CEC (cmol/kg)} = \text{K(mg/kg)/390} + \text{Ca (mg/kg)/200} + \text{Mg (mg/kg)/120}$$

**Equation to Adjust CEC:**

$$Y = 1.0453x - 0.2419$$

Report the CEC data after adjusting with this equation.

**Calculations for Base Saturation:**

$$\text{Ca (\%)} = \text{Ca (mg/kg)/200/CECx100}$$

$$\text{Mg (\%)} = \text{Mg (mg/kg)/120/CECx100}$$

$$\text{K (\%)} = \text{K (mg/kg)/390/CECx100}$$

**Note:** Our data showed that a single 0.01 M SrCl<sub>2</sub> extraction is a simple, rapid, and inexpensive method of estimating effective cation exchange capacity (ECEC), cation saturation ratios and sulfur. We also concluded that three major cations (Ca, Mg and K) are enough to calculate ECEC (Ketterings et al., 2014).

**References:**

1. Ketterings, Q.M., S. Gami, R.R. Mathur, and M. Woods (2014). A simple method for estimating effective cation exchange capacity, cation saturation ratios and sulfur across a wide range of soils. *Soil Science* 179:230-236.

## 14. Determination of Water Stable Aggregate

Updated date: 8/2016

### Introduction:

Sarah Hetrick and Sanjay Gami prepared the protocol for the determination of water stable aggregate in August 2016. The wet aggregate stability is determined by the principle that unstable aggregates will break down more easily than stable aggregates when immersed into water.

### Apparatus:

1. Eijkelkamp wet sieving apparatus
2. 0.25 mm sieves
3. 150+ mL beakers
4. Cans
5. Rubber policeman

### Procedure:

1. Soil should be air dried prior to fraction separation. Use 0.25-2 mm fraction aggregates to measure small aggregate and use 2-4 mm fraction to measure large aggregate. Use eight 0.25 mm sieves.
2. Label and weigh 2 sets of 150+ mL beakers: one set for stable aggregate (label as A) and another one for sand/large residue (label as S). Record the weights of all beakers with labels.
3. Gently mix soil sample and weigh  $4.00 \pm 0.02$  g of soil into sieve for small aggregate and weigh  $2.00 \pm 0.01$  g of soil into sieve for large aggregate.
4. Label and fill cans with 100 mL deionized (DI) water.
5. Lower rack holding sieves so that aggregates are just submerged. Soak for 3-5 minutes.
6. Raise rack to middle prong and run apparatus for three minutes. Lift the sieves to the top prong, drain for about 30 seconds, then gently submerge and run once again for three minutes. Raise rack and allow sieves to drain for 3-5 minutes.

7. Discard contents of can and rinse cans with tap and DI water thoroughly.
8. Replace can under proper sieve and add 100 mL of 2 g/L NaOH to each can as a solvent.
9. Lower sieves into cans and operate apparatus for three minutes. Lift sieves and stir contents of each gently and thoroughly with rubber policeman for about 10 seconds, break up remaining aggregates and unclogging the sieves. Be sure to not transfer residue across sieves by rinsing with DI water above the sieves between samples so that all residue is returned to the sieve. Lower sieves and operate another three minutes.
10. Raise sieves and let drain for 3-5 minutes. Transfer entire contents of can (aggregate residue) into the properly labeled A beaker with a wash bottle and set aside.
11. Using a wash bottle, transfer remaining contents of sieves into the S beaker; Again, be sure to capture all residue in the beaker.
12. Now place both beakers for each soil size/number in an oven at 110°C until completely dry.
13. Weigh beaker + dry aggregate residue and record weight. Weigh second beaker + sand/large residue and record weight. Subtract initial weights for corresponding beakers to find the relative amounts of water-stable aggregate residue, and sand/large residue. Use these to infer the amount of water unstable aggregate residue. Turn these to percentages of the initial ~2.00 or ~4.00 g.
14. Ensure that all materials have been thoroughly cleaned of residue for next trials.
15. If replicates have a relative difference > 10%, repeat one additional replicate sample run until any two replicates agree within 10%.

**References:**

1. Operating instructions, 08.13 Wet Sieving Apparatus. Found at [www.eijkelkamp.com](http://www.eijkelkamp.com).
2. Water stable aggregate procedure (protocol from University of Maine).

## 15. Corn Stalk Nitrate Test

Updated date: 8/2007, 8/20/2013, and 2/2/2016

### Introduction:

Sanjay Gami prepared the protocol and Renuka Rao reviewed this protocol for the corn stalk nitrate test (CSNT) in August 2007. Sanjay Gami updated the protocol on 8/20/2013. A third update was prepared by Sanjay Gami and Sheryl Swink on 2/15/2016. The end-of-season corn stalk nitrate test analysis provides a method of assessing the N available to the corn (*Zea mays* L.) crop during the latter part of the season (Wilhelm et al. 2005). The method semiquantifies the concentration of nitrate-nitrogen ( $\text{NO}_3\text{-N}$ ) in plant tissue by extraction with an aluminum sulfate solution [ $\text{Al}_2(\text{NH}_4)_2\text{SO}_4$ ] without filtration and subsequent determination by nitrate electrode (Baker and Smith, 1969; Milham et al., 1970; Carlson and Keeney, 1971; Mills, 1980; Baker and Thompson, 1992 as cited by Miller 1998). In this method, the need to filter the media is eliminated, because the electrode is placed directly into the agitating extraction media and electrometer readings are observed (Wilhelm et al. 2000).

### Apparatus:

1. pH/ISE meter
2. Nitrate electrode
3. Weighing balance, resolution  $\pm 0.001$  g
4. Dispenser, calibrated to 50 mL  $\pm 0.2$  mL
5. 50 mL centrifuge tubes with lid
6. Horizontal mechanical shaker
7. Magnetic stirrer and 1" stir bar

### Reagents:

1. Ionic Strength Adjustor (ISA): add 264.28 g of ammonium sulfate [ $(\text{NH}_4)_2\text{SO}_4$ ] to 1 L volumetric flask and fill to volume with deionized (DI) water to make 2 M  $(\text{NH}_4)_2\text{SO}_4$  ionic strength adjustor (ISA).
2. Aluminum sulfate [ $\text{Al}_2(\text{SO}_4)_3$ ] extracting solution: add 320 grams of  $\text{Al}_2(\text{SO}_4)_3$  (16 g per L DI water) to 20 liter carboy. This solution is also used to make standards.

3. Place 7.218 g of potassium nitrate ( $\text{KNO}_3$ ) in 1 L DI water to make a 1000 ppm N solution.

**Standards:**

1. 1 ppm: add 0.5 mL of  $\text{KNO}_3$  solution in 500 mL volumetric flask and filled with  $\text{Al}_2(\text{SO}_4)_3$  extracting solution.
2. 4 ppm: add 2 mL of  $\text{KNO}_3$  solution in 500 mL volumetric flask and filled with  $\text{Al}_2(\text{SO}_4)_3$  extracting solution. .
3. 10 ppm: add 5 mL of  $\text{KNO}_3$  solution in 500 mL volumetric flask and filled with  $\text{Al}_2(\text{SO}_4)_3$  extracting solution.
4. 40 ppm: add 20 mL of  $\text{KNO}_3$  solution in 500 mL volumetric flask and filled with  $\text{Al}_2(\text{SO}_4)_3$  extracting solution.
5. 100 ppm: add 50 mL of  $\text{KNO}_3$  solution in 500 mL volumetric flask and filled with  $\text{Al}_2(\text{SO}_4)_3$  extracting solution.

**Calibration:**

Calibration and measurement of concentration:

1. It is good idea to remove standard from refrigerator and leave them out overnight to reach room temperature.
2. Fill the combination electrode with the filling solution (Catalog No. 13-620-807, Scientific Fisher) shipped with the electrode to a level just below the fill hole. Connect the electrode to the proper terminal of the meter as recommended by the meter manufacturer. The system is now ready for use.
3. Store the electrode in distilled water; place it in 100 ppm standard for at least 1 h before running standards and samples.
4. Do the calibration and measurement of the instrument according to AR 50 Accumet Meter Instruction Manual.
5. Set the meter in MV mode.

6. Measure 50 mL of each standard (1, 4, 10, 40, and 100 ppm) into individual cups and add 1 mL of ISA to each cup.
7. Drop a stir bar into each cup of standard to be measured.
8. Place the standard on a magnetic stirrer at medium (moderate) setting and lower the electrode into the standard solution.
9. Record the MV reading.
10. Repeat to get to obtain readings for each of the standards.
11. Calculate the difference between readings for 1 and 10 ppm standards, 4 and 40 ppm standards, and 10 and 100 ppm standards. If the differences in readings for each pair of standards are between 54 and 60, then continue running samples. Sometimes the difference between the 1 and 10 ppm standards may not be in this range; if that is the only one out of range, you can still continue the work.

Calibration of instrument:

1. Set the meter in Ion mode.
2. Use the 1, 10, and 100 ppm standards just used for MV mode.
3. Place the first standard on the magnetic stirrer (on medium speed as before).
4. Touch “Std” on the instrument screen to access standardize mode; Press “Std” again.
5. Place the electrode in each standard (1, 10, and 100 ppm) and enter the correct concentration of std. into the key pad of instrument screen.
6. If the slope shown on the instrument screen is 70% or above, continue on to take readings for previously prepared CSNT samples (see next).

**Procedure:**

1. Dry the CSNT stalk samples at about 60°C and grind with a Wiley mill to pass a 2-mm screen (Gami and Ketterings, 2010). For sampling protocol see the Factsheet 31 at NMSP website (<http://nmisp.cals.cornell.edu>).

2. Weigh 0.5 g of each corn stalk sample into individual centrifuge tubes and record weight of tared sample to two decimal points (can be done prior to day of analysis).
3. Add 50 mL of aluminum sulfate extracting solution to each tube of sample, shake to suspend and wet all of sample, and cap tubes.
4. Organize the sample tubes securely on a shaker set on low speed; shake for 15 minutes.
5. While first set of tubes are shaking, calibrate the instrument with standard solutions as described in calibration section.
6. When samples are done shaking, hand swirl or shake each tube to fully suspend CSNT sample, especially material settled into bottom of tube. Then pour into numbered plastic cups and arrange in trays.
7. Add 1 mL of ISA to each cup - one tray row (10 samples) at a time in case you need to stop or take a break. All samples with added ISA need to be analyzed before stopping for a break.
8. Drop a stir bar into the plastic cup of sample; place on magnetic stirrer set to medium speed. Lower the electrode into the sample and record the stabilized reading of each sample on data sheet. Do not submerge the electrode above the sensing module (located ~1.5 inches above tip of electrode).
9. Clean electrodes with DI water and wipe with a Chem Wipe after each sample.
10. Rinse stirring bar with DI water and dry with Chem Wipe before placing in new samples.
11. After every 10 samples, take a reading of the standard that is closest to midrange of your CSNT sample values (usually the 10 ppm standard, but if your values are high, use a higher standard) to make sure the electrode is working properly. If the value of the standard is 10% or more above or below its ppm value, repeat above calibration steps before running any more samples.
12. After taking the reading of all samples, place the nitrate electrode in a 100 ppm nitrate solution.
13. Keep all the standards and ISA solution in the refrigerator.

14. When through with CSNT analyses for the day, throw out the set of standards used that day and do Cleanup.

**Cleanup:**

1. Drain the extracting solution from cups into sink and throw the cups and residue in garbage.
2. Soak all flask and glassware in hot water. Rinse all centrifuge tubes, flask, and glassware thoroughly with hot water 3-4 times (use a brush inside the centrifuge tubes the first time to release any CSNT sample residue), then give a final rinse with DI water 3-4 times.

**Calculations:**

Report mg/kg of NO<sub>3</sub>-N in sample:

$$\text{mg/kg} = \text{NO}_3\text{-N in extract mg/L} \times 50 / \text{sample weight}$$

**Notes:**

1. Routinely check electrode calibration every 10<sup>th</sup> sample using a mid-range standard.
2. Clean electrodes with DI water and wipe with a Chem Wipe after each sample.
3. Rinse stirring bar with DI water and dry with Chem Wipe before placing in new samples.
4. Change in sampling protocol for stalk length (August 2013): It has the option for stalks taken between 2-8 inches or 6-14 inches. This should be noted for every sample we receive as the results need to be adjusted. For sample taken between 2-8 inches, divide the lab results by 1.5 and report that new number in our reporting system. The conversion only applies when samples are taken between 2 and 8 inches off the ground.
5. Sorghum stalk nitrate test (SSNT): the exact same reagents, standards, and protocol were used for sorghum stalk nitrate test for the first time in January 2017. This test will be tested and verified again in the coming 2017 fall season.



## References:

1. Gami, S.K., and Q.M. Ketterings, 2010. Comparison of Nitrate Concentrations of corn stalk samples ground with Wiley Mill using 1 and 2-mm Screen Size. Unpublished Report. Nutrient Management Spear Program, Department of animal Science, Cornell University.
2. Miller, R.O. 1998. Extractable nitrate in plant tissue: ion selective electrode method. P 85-88. In Y.P. Kalra (ed.) Handbook of Reference Methods for plant Analysis. CRC Press LLC, Boca Raton, FL.
3. Stalk Analysis. Methodology prepared by Soil Nutrient Analysis Laboratory, University of Connecticut.
4. Wilhelm, W.W., S.L. Arnold, and J.S. Schepers. 2000. Using nitrate specific ion electrode to determine stalk nitrate-nitrogen concentration. *Agronomy Journal* 92:186-189.
5. Wilhelm, W.W., G.E. Varvel, and J.S. Schepers. 2005. Corn stalk nitrate concentration profile. *Agronomy Journal* 97:1502-1507.

## 16. Corn Stalk Potassium Test Using Potassium Electrode

Updated date: 5/2015

### Introduction:

The potassium electrode is used to quickly, simply, accurately and economically measure potassium ions.

### Apparatus:

1. Potassium electrode; other apparatus are similar to corn stalk nitrate test.

### Reagents:

1. Aluminum sulfate [ $\text{Al}_2(\text{SO}_4)_3$ ] extracting solution: add 320 grams of  $\text{Al}_2(\text{SO}_4)_3$  (16 grams per liter distilled water) to 20 liter carboy; this solution is also used to make standards.
2. Ionic Strength Adjustor (ISA), 5 M NaCl
3. Potassium standard, 10,000 ppm K
4. Filling solution, 0.1 M NaCl

### Standards:

1. 40 ppm: add 2 mL of 10,000 ppm K solution in 500 mL volumetric flask and filled with  $\text{Al}_2(\text{SO}_4)_3$ .
2. 400 ppm: add 20 mL of 10,000 ppm K solution in 500 mL volumetric flask and filled with  $\text{Al}_2(\text{SO}_4)_3$ .

### Procedure:

Use the same protocols as described for the corn stalk nitrate test, except use a 5 M NaCl solution as the ISA, a 0.1 M NaCl solution for the filling solution, and 10,000 ppm K solution for preparing potassium standards.