

A & L GREAT LAKES LABORATORIES, INC.
Standard Operating Procedure

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TITLE: Microwave Digestion for Plant Tissues and Feeds

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PURPOSE OR SUMMARY: To assure that Plant Tissue and Feed material digestions are performed in a uniform and safe manner.

SCOPE / APPLICATION: This method is applicable for the digestion of Plant Tissues and Feeds in routine and non-routine samples in the Agricultural Division.

DISTRIBUTION: Quality Assurance Officer, Agriculture Department Manager, four (4) copies for Ag Division laboratory Manuals

APPROVAL		
Name	Job Title	Date
<i>Dorinda J. Singer</i>	President	12 10 9 , 2020
<i>[Signature]</i>	Quality Assurance Coordinator	12 10 9 , 2020
<i>Amanda Krey</i>	Quality Chemist	12 1 0 7 , 2020

HISTORY	
Supersedes	Reason for change
0	Addition of section: VIII.B

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I. DETECTION LIMIT

- A. N/A

II. APPLICABLE MATRIX OR MATRICES

- A. Plant Tissue and Feed materials

III. DEFINITIONS

- A. N/A

IV. INTERFERENCES

- A. N/A

V. SAFETY

- A. Always wear a face shield, gloves, and lab apron when working with nitric acid or hydrogen peroxide.
- B. Follow normal laboratory safety guidelines. Use caution when dealing with acids and bases. Consult MSDS/SDS sheets.
- C. The toxicity or carcinogenicity of each reagent used in this method has not been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably possible.
- D. The following chemicals have the potential to be highly toxic or hazardous, for detailed explanations consult the MSDS/SDS: Nitric acid and hydrogen peroxide.
- E. Use required protective equipment as indicated in the MSDS/SDS.

VI. EQUIPMENT AND SUPPLIES

- A. Milestone Ethos EZ Microwave Digestion Labstation
- B. Thermocouple and therma-well
- C. 50 place Microwave rotor (Milestone)
- D. CEM Mars Microwave Digestion Labstation
- E. 52 place Microwave rotor (CEM)
- F. Teflon caps with vent holes
- G. 50 mL Screw Cap Tubes conical base... SARSTEDT or equivalent...with caps
- H. 36 place wire racks
- I. Acid dispenser to deliver 2.0 mL Nitric Acid
- J. Repeating pipet to deliver 1.0 mL Hydrogen Peroxide
- K. 250 mL Griffin beaker
- L. 1000 mL Griffin beaker
- M. 3 decimal place analytical balance capable of data transfer to a computer
- N. Computer for weight data transfer

VII. REAGENTS AND STANDARDS

- A. De-ionized Water (DIW)
- B. Nitric Acid (conc.) **((CAUTION))**
- C. Hydrogen Peroxide 30%
 - 1. Store in refrigerator when not in use. **((CAUTION))**

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- D. A&L Feed Check
- E. "Alpha Resources" Certified Soybean Meal
- F. NIST Certified Peach leaves
- G. A&L Potato Petiole Check

VIII. SAMPLE COLLECTION, PRESERVATION, SHIPMENT AND STORAGE

- A. Samples are dried and stored in A&L Great Lakes Laboratories, Inc. Plants/Feeds and Manures Library.
- B. NEON ground samples in the analysis batch will be dried at 70°C for 2 hours, and stored in a desiccator until weighing. When NEON ground samples are placed in the 70°C oven, the caps on scintillation vials must be loosened or removed first.

IX. QUALITY CONTROL

- A. One A&L feed check, one blank and one soybean/peach NBS to be run with every 20 samples.

X. CALIBRATION AND STANDARDIZATION

- A. N/A

XI. PROCEDURE-WEIGHING

- A. With a marker, number each digestion tube with the last three digits of the lab number.
 - 1. Be sure to indicate the entire lab number on the first tube in every rack (every 36 samples).
 - 2. Mark each control tube with "REF"; this will be used as the temperature reference tube.
- B. For automatic weight transfer, use the weigh room balance connected to the computer.
 - 1. Weigh 0.195 to 0.205 g for each A&L standard, NIST (NBS) standard, and plant or feed sample.
 - 2. If not using the automatic weight transfer, weigh 0.200 g for all standards and samples.
 - a) **NOTE:** Transfer 0.200 g of the A&L Feed Check into each control tube and mark with **REF**.
 - b) These will be used as the temperature reference sample during the microwave digestion.

XII. PROCEDURE-DIGESTION WITH MILESTONE

- A. **MAKE SURE THE FUME HOOD IS ON.** After the first 100 samples (including, blanks and REF) have been transferred to the tubes, while working in the fume hood, clear the nitric acid dispenser of air pockets by dispensing several mLs of nitric acid into a waste beaker. Then dispense 2.0 mL of nitric acid into each tube.
 - 1. When not in use, always turn the acid dispenser away. **((CAUTION))**
- B. Place the **REF** sample (without a cap) into the appropriate spot in the first rotor.
 - 1. Then, place the tube in the next counter-clockwise spot (labeled "1") on the outside row of the rotor. **See NOTE below.**
 - a) Continue adding tubes until the first row is filled.
 - 2. Place the next tube in the second row in the spot labeled "**X**".
 - a) Add tubes in the next counter-clockwise spot.
 - b) Continue adding tubes until the second row is filled.
 - 3. Start the third row at the "**X**" and add tubes in the next counter-clockwise spot.

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a) Continue adding tubes until the third row is filled.

NOTE: If fewer sample tubes than a full row is being digested, spread the samples out as evenly as possible.

- C. Place a vented Teflon cap on each tube (no cap on the REF sample for Milestone).
- D. Turn on the scrubber pump and turn valve to spray.
- E. Turn on the microwave.
- F. Press **Select** to choose the correct program:
 - 1. For the first cycle - **newpt190**
 - 2. For the second cycle - **newpt2105**
- G. Place the wheel into the grooves in the microwave and insert the probe into the REF tube.
- H. Press **Start**
- I. Choose **USER** on the Log In screen and use **1 2 3** as the password. Press **"OK"**.
- J. Press **Sample** from the top menu.
- K. Place the rotor lid on the tubes so that the thermo-well port goes into the REF tube.
- L. Set the rotor on the open door of the microwave and place the thermo-well and thermocouple through the port into the **REF** sample.
 - 1. Make sure the thermocouple is completely inserted into the thermo-well and the thermo-well is in the nitric acid.
- M. Set the rotor in the microwave and gently turn the rotor until it settles down into the "D" shaped drive.
- N. The thermocouple should be in the back right corner of the microwave.
 - 1. If it is not, press the circular arrow on the **Sample** screen.
 - a) The rotor will turn while the circular arrow is pressed.
 - 2. Continue pressing the arrow until the thermocouple is in the back right corner of the microwave (2 o'clock position).
- O. Once the thermocouple is in the back right corner, attach the thermocouple to the port on the upper left front inside wall.
 - 1. Hold the metal sleeve of the thermocouple and line up the "D" shaped connectors.
 - 2. Push the connector completely into the port.
- P. Close the microwave door. Make sure the door latches shut.
- Q. Press **Method** on the top menu. Choose **"newpt190.mpr"**. Press **Start**.
- R. Press **Run** from the top menu to display the "real time" progress of the run.
 - 1. The temperature should ramp up to 90°C in 5 minutes as indicated by the red line.
 - 2. The black line is indicating the energy input from the microwave.
 - 3. The temperature should hold at 90°C for 90 seconds and then begin dropping.
- S. When the temperature is below 58°C, press **Stop**.
- T. After the rotor has stopped, open the door, grip the thermocouple connection by the metal sleeve, and pull the connector apart.
 - 1. Remove the rotor from the microwave and place it in the fume hood.

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- U. Move the rotor lid (along with the thermocouple) to the next rotor to go into the microwave, or remove the thermocouple and thermo-well, wipe off any acid, and place them on top of the microwave and set the rotor lid aside.
- V. Verify that the 1.0 mL dispenser is set at 1.0 mL. Prepare the 1.0 mL Hydrogen Peroxide repeating dispenser by disposing of the first couple of mLs in a waste beaker and then dispensing back into the bottle until any air pockets are cleared.
 - 1. Dispense 1.0 mL of the Hydrogen Peroxide into the **REF** sample and into each of the other tubes through the vent hole in each Teflon cap.
 - 2. Dispense in the same counter-clockwise direction used to place the tubes in the rotor...outside row first, middle row next, and inside row last. **((CAUTION))**

NOTE: The addition of the Hydrogen Peroxide should be done immediately after removing from the microwave. If more than a couple of minutes pass, the foam from the sample will stick to the sides of the tube and an incomplete digestion is possible.
- W. Follow **Steps J through O** to prepare for the final digestion step.
- X. Press **Method** on the top menu. Choose **newpt2105.mpr**. Press **Start**.
- Y. Press **Run** from the top menu to display the "real time" progress of the run.
 - 1. The temperature should ramp up to 105°C in 5 minutes as indicated by the red line.
 - 2. The black line is indicating the energy input from the microwave.
 - 3. The temperature should hold at 105°C for 10 minutes and then begin dropping.
- Z. When the temperature is below 58°C, press **Stop**.
- AA. After the rotor has stopped, open the door, grip the thermocouple connection by the metal sleeve, and pull the connecter apart.
 - 1. Remove the rotor from the microwave and place it in the fume hood.
- BB. Move the rotor lid (along with the thermocouple) to the next rotor to go into the microwave, or remove the thermocouple and thermo-well, wipe off any acid, and place them on top of the microwave. Wipe the rotor lid with a wet paper towel and place in holder.
- CC. Rinse the Teflon cap into the tube (place rinsed caps in the 1000 mL beaker) and add DIW to a final volume of 25 mL, making sure to rinse the sides of the tube as the DIW is added.
 - 1. Cap securely and mix.
 - 2. Place the tubes in a left to right, front to back order, 36 per rack.
 - a) **NOTE:** If a different final volume is used (i.e., if the 25 mL mark is overshoot, take the volume to 27.5 mL), immediately mark the cap with the different volume.
 - b) Let the ICP technician know that a different volume is in the rack.
- DD. When racks are full, take the racks to the ICP operator.

XIII. PROCEDURE-DIGESTION WITH CEM

- C. **MAKE SURE THE FUME HOOD IS ON.** After the first 100 samples (including, blanks and **REF**) have been transferred to the tubes, while working in the fume hood, clear the nitric acid dispenser of air pockets by dispensing several mLs of nitric acid into a waste beaker. Then dispense 2.0 mL of nitric acid into each tube.
 - 1. When not in use, always turn the acid dispenser away. **((CAUTION))**
- D. Place the **REF** sample (without a cap) into the appropriate spot in the first rotor.
 - 1. Then, place the tube in the next counter-clockwise spot (labeled "1") on the outside row of the rotor. See **NOTE** below.

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- a) Continue adding tubes until the first row is filled.
2. Place the next tube in the second row in the spot labeled "X".
 - a) Add tubes in the next counter-clockwise spot.
 - b) Continue adding tubes until the second row is filled.
3. Start the third row at the "X" and add tubes in the next counter-clockwise spot.
 - a) Continue adding tubes until the third row is filled.

NOTE: If fewer sample tubes than a full row is being digested, spread the samples out as evenly as possible.

- E. Place a vented Teflon cap on each tube.
- F. Turn on the scrubber pump and turn valve to spray.
- G. Turn on the microwaves.
- H. Select **Load Method** from the Method Menu.
- I. Select **User Directory**.
- J. Choose **NewPt190-HTV** and press the **Select** button.
- K. Set the rotor in the microwave and gently turn the rotor until it settles down into the "D" shaped drive.
- L. Insert the probe into the **REF** tube and connect to the top of the instrument cavity.
- M. Press the green **Start** button.
 1. The temperature should ramp up to 90°C in 5 minutes.
 2. The temperature should hold at 90°C for 90 seconds.
 3. The temperature will ramp down for 5 minutes, but can be stopped and opened when the temperature has reached 58°C.
- N. Press the red stop button when temp reads 58°C or less if it hasn't already.
- O. Open the door and remove the temperature sensor by pulling on the black sleeve at the port connection. **((CAUTION))** The probe is flexible and subject to breakage. DO NOT bend or pull on the green cable when removing from the port connection.
- P. Verify that the 1.0 mL dispenser is set at 1.0 mL for the Hydrogen Peroxide. Rinse the dispenser by disposing of a couple mLs into a waste beaker.
 1. Dispense 1.0 mL of the Hydrogen Peroxide into the **REF** tube and into the other tubes through the vent hole in the Teflon cap.
 2. Dispense in the same counter-clockwise direction used to place the tubes in the rotor.
- Q. Return the rotor to the microwave and connect temperature sensor probe again.
- R. From the Method Menu page select **NewPt2105-HTV** program and push the green start button.
 1. The temperature should ramp up to 105°C for 5 minutes.
 2. The temperature will hold at 105°C for 10 minutes.
 3. The temperature will ramp down for 5 minutes, but can be stopped and opened when the temperature has reached 58°C.
- S. When the temperature is at or below 58°C the program can be stopped and the rotor taken out.
- T. Press the red stop button when temperature has reached 58°C or less if it hasn't already.
- U. Open the door and remove the temperature sensor by pulling on the black sleeve.
- V. Remove the probe from the **REF** sample and wipe any excess acid off with a paper towel. Place the

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probe on top of the microwave.

- W. Rinse the Teflon cap into the tube (place rinsed caps in the 1000 mL beaker) and add DIW to a final volume of 25 mL, making sure to rinse the sides of the tube as the DIW is added.
- i. Cap securely and mix.
 - ii. Place the tubes in a left to right, front to back order, 36 per rack.
 1. NOTE: If a different final volume is used (i.e., if the sample was low and a smaller dilution was used). Immediately mark the cap with the different volume (15 mL or 20 mL).
 2. Let the ICP operator know that a different volume is in the rack.
- V. When racks are full take them to the ICP operator.

XIV. SHUT DOWN and CLEAN UP:

1. Log off of the microwave (Milestone).
2. Turn Microwaves off.
3. Turn off scrubber pump spray.
4. Remove repeating pipet tube from the hydrogen peroxide bottle and dispense hydrogen peroxide back into the bottle until the tubing is empty.
 - a) Place tubing in a beaker of DIW and rinse the dispenser (or attach to DIW faucet and flush the tubing and pipet).
 - b) Remove the tubing from the DIW and clear the dispenser of DIW. **CAUTION**
5. Immediately wash the Teflon caps in the beaker with DIW at least 3 to 4 times, if needed.
 - a) If caps are not needed immediately, cover with DIW and let them soak until needed.
6. Wipe down the inside floor of the microwave.
7. Wipe down the inside of the fume hood and the microwave counter.

XVI. DATA ANALYSIS AND CALCULATIONS

A. N/A

XVII. METHOD PERFORMANCE

A. N/A

XVIII. POLLUTION PREVENTION AND WASTE MANAGEMENT

- A. Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The USEPA has established a preferred hierarchy of environmental techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the USEPA recommends recycling as the next best option.
- B. The quantity of chemicals purchased should be based on expected usage during their shelf life and disposal cost of unused material. Actual reagent preparation volumes should reflect anticipated usage and reagent stability.
- C. It is the laboratory's responsibility to comply with all federal, state and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal

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restrictions, and to protect the air, water and land by minimizing and controlling all releases from fume hoods and bench operation. Compliance with all sewage discharge permits is also required.

XIX. DATA ASSESSMENT AND ACCEPTANCE CRITERIA FOR QUALITY CONTROL MEASURES

- A. Flex Control Acceptance Criteria is within 10%.
- B. NBS (soy) Acceptance Criteria – reference the Plant Analysis Quality Control sheet.
- C. A&L Feed Check Acceptance Criteria – reference the Plant Analysis Quality Control sheet.

XX. CORRECTIVE ACTIONS FOR OUT-OF-CONTROL DATA

- A. If the Flex Control or Quality Control Check(s) data is out-of-control the ICP will be recalibrated and the samples will be re-run. Samples may require being re-digested.

XXI. CONTINGENCIES FOR HANDLING OUT-OF-CONTROL OR UNACCEPTABLE DATA

- A. Reference A&L Great Lakes Laboratories, Inc. Quality Assurance Manual.

XXII. REFERENCES

- A. Peters, John. (Eds.). (1987). WISCONSIN PROCEDURES for Soil Testing, Plant Analysis and Feed & Forage Analysis. Madison, Wisconsin: Department of Soil Science College of Agricultural and Life Sciences University of Wisconsin-Extension-Madison. pp 34-35.
- B. Kalra, Y.P.; Maynard, D.G.; Radford, F.G. 1991. Microwave digestion for Ca, Mg, K, Na, Mn, Fe, Al, P, and S. Methods manual for forest soil and plant analysis. pp 97-100.
- C. CEM Microwave Owners Manual
- D. MILESTONE Microwave Owners Manual

XXIII. TABLES, DIAGRAMS, FLOWCHARTS AND VALIDATION DATA

- A. N/A