

Rapid Carbon Assessment Field Laboratory Instructions

A Step-by-Step Guide for Preparing, Scanning, and Shipping Samples

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Rapid Carbon Assessment Project

For questions/clarifications contact:

Cynthia Stiles

402-437-4007

cynthia.stiles@lin.usda.gov

Ellis Benham

402-437-5132

ellis.benham@lin.usda.gov

Rich Ferguson

402-437-5332

rich.ferguson@lin.usda.gov

Skye Wills

402-314-5659

skye.wills@lin.usda.gov

For shipping related questions contact:

Larry Arnold

402-437-5358

larry.arnold@lin.usda.gov

Linda Kruger

402-437-5016

Linda.kruger@lin.usda.gov

National Soil Survey Center
USDA-NRCS
100 Centennial Mall N., Rm. 152
Lincoln, NE 68508

Table of Contents

1. Sample Processing and Scanning Checklist	3
2. Weighing, Scanning and Processing Samples	4
2.1. Weights for Field Moisture Content	4
2.2. VNIR Scan the Field-Moist Sample (OPTIONAL)	4
2.3. Air-Dry the Sample	4
2.3.1. Slow method	4
2.3.2. Faster method	4
2.4. Sieving and Coarse Fragments	5
2.5. Oven Drying for Air-Dry Moisture Content	5
2.6. VNIR Scan Air-Dry Samples	6
2.7. File Management	6
3. Operating Instructions for the LabSpec 2500 Visible Near Infrared Spectrometer (VNIR)	7
3.1. Background	7
3.2. General Instructions	7
3.2.1. Lens and Window Cleaning Instructions	7
3.2.2. Fiber Optic Cables for Muglight and Contact Probe	9
3.3. Preparing the LabSpec 2500 Spectrometer	10
3.3.1. Component warm-up	10
3.3.2. Wavelength verification	11
3.3.3. Reference scans	11
3.4. High/Low QC Sample Check	12
3.4.1. General Instructions for QC Scanning	13
3.4.2. Scanning Low and High QC	14
3.5. Scanning Soil Samples	15
3.5.1. Scanning Field Moist Samples (OPTIONAL)	15
3.5.2. Scanning Air-Dried Prepared Samples	18
3.5.3. File Management	21
3.6. VNIR Instrument Operation Technical Support	21
4. Sending Samples to the Soil Survey Laboratory	21
4.1. Types of Samples	22
4.1.1. Central Pedon for Archive (CPA) samples	22

4.1.2.	Quality Control (QC3) samples.....	22
4.1.3.	O horizon (OHZ) samples	22
4.1.4.	How to identify Quality Control (QC3) pedons	22
4.2.	Procedure for Labeling SSL (CPA, QC3 and OHZ) Samples.....	23
4.2.1.	Labeling and storage	23
4.2.2.	Labeling convention for shipped samples	23
4.2.3.	Attaching labels to bags	24
4.3.	Instructions for Shipping SSL Samples	24
4.3.1.	Preparing samples for shipping.....	24
4.3.2.	Handling OHZ and QC3 Samples	24
4.3.3.	CPA: samples to be archived only	25

This document is a guide to that is designed to “walk through” the steps of Rapid Carbon Assessment sample processing and analysis in order for any individual sample. Instructions for operating the VNIR have been previously released as a separate document, but they are also provided within the step by step framework to provide additional reference for field offices.

Note 1: For all weights taken in the procedure, record to at least one decimal place; two places is preferred.

Note 2: In this document, spreadsheet data element names are indicated by angle brackets (<>) around the name.

1. Sample Processing and Scanning Checklist

Note: Instructional steps in this document follow the item in parentheses

- A. Weigh Field Moist Samples (Step 2.1)
- B. Setup VNIR (Step 3.3)
 - 1. Warm up (approximately 3 hours; Step 3.3.1)
 - 2. Wavelength verification (Step 3.3.2)
 - 3. Baseline scans (Steps 3.3.3-3.4.2)
- C. Scan Field-Moist Samples (Step 3.5.1)
- D. Air-dry Sample (Step 2.3)
- E. Sieve and Weigh Coarse Fragments (Step 2.4)
- F. Oven-dry Subsamples (Step 2.5)
- G. Scan Air-dry Samples (Step 3.5.2)
- H. Save and Backup ALL Files (Step 3.5.3)

2. Weighing, Scanning and Processing Samples

2.1. Weights for Field Moisture Content

- 2.1.1. Open the 'BD SSOL' worksheet of the **Rapid Carbon Assessment Workbook.xlsx** obtained from the Rapid Carbon SharePoint site.
- 2.1.2. Tare the balance with an empty sample bag on the balance.
- 2.1.3. Weigh the field moist sample, in the bag, and record weight <Moist Wt>.

2.2. VNIR Scan the Field-Moist Sample

NOTE: An accessory goal within the context of the Rapid Carbon Assessment Project is to build a library of VNIR spectra of unprocessed field moist samples to assess the feasibility of developing additional models for proximal (field) sensing such as for determining texture and mineralogy. In consideration of the workload to meet the main RaCA goals, the scanning of field moist samples is now OPTIONAL as of February 2, 2011, and is no longer mandatory. If you elect to not scan field moist samples, please safely store the Contact Probe and 1.5 m fiber optic cable in the Pelican Case.

Refer to Step 3.5.1 (Reminder – the instrument requires 3 hours to warm up)

2.3. Air-Dry the Sample

2.3.1. Slow method

- 2.3.1.1. Stand bag(s) upright using whatever means necessary to prevent the bags from falling over.
- 2.3.1.2. Open bag top such that it remains open, using a toothpick if necessary to prevent the bag from closing.
- 2.3.1.3. Cover bag(s) loosely with newspaper sheets or cloth.
- 2.3.1.4. Let bags stand until ready to finish drying (fast) and processing (once completely dry)

2.3.2. Faster method

- 2.3.2.1. Remove sample from bag. Place on brown paper on a bench (if moist or drier) or on a tray covered with plastic film (if wet).

2.3.2.2. Place the bag on the paper or tray, and put some of the soil on it to hold it down. For wet samples, turn the bag inside out before placing it on the paper or tray.

2.3.2.3. Crumble the soil if not too plastic, or spread wetter material to speed drying. If soil tends to remain clumped together, use a butter knife to gently separate the soil mass into smaller aggregates to speed up the drying process.

2.3.2.4. A fan can be used to blow air across the samples to speed drying. Light bulbs above the samples are not recommended to speed drying. Do not use heat lamps, and keep ordinary bulbs 18" or more from the sample surface.

2.3.2.5. The time required to reach air-dry state depends on the drying temperature, air circulation, and relative humidity.

2.3.2.5.1. For forced air circulation at 30-35 degrees C (86 – 95 degrees F, minimal relative humidity), 3-7 days is needed.

2.3.2.5.2. For still air at 21 degrees C (70 degrees F) with higher humidity, up to 30 days may be required.

2.3.2.5.3. Observation (color, hardness, lack of plasticity, dust produced by rubbing peds together) is used to decide when the soil is "air" dry.

2.4. Sieving and Coarse Fragments

2.4.1. Weigh the entire air-dry sample, record **<Air Dry Wt>**.

2.4.2. Crush the sample with a rolling pin, rubber or wood wheel, or large pipe roller. Unless you are positive the sample contains no coarse fragments, do not use a mortar and pestle or mechanical crusher.

2.4.3. Sieve the sample through a 2 mm square hole sieve. Transfer the sample that has passed through the sieve back to its original sample bag.

2.4.4. Weigh any coarse fragments retained on the sieve, record **<C frag wt>**, and dispose of coarse fragments.

2.5. Oven Drying for Air-Dry Moisture Content

2.5.1. Record an aluminum pan number for each sample in **<Pan No>** column.

- 2.5.1.1. Prior to the first use of the pans emboss a unique identifier (number) on each pan.
- 2.5.1.2. Embossing is done by placing the tab of the pan on a soft surface (such as a magazine) and using a sharp writing instrument/stylus (a fine-tip sharpie or ball point pen works well).
- 2.5.2. Tare the numbered aluminum weighing pan on a balance.
- 2.5.3. Stir the < 2mm sample in the bag so that larger and smaller particles are mixed. This can be done by stirring with a scoop or spatula.
- 2.5.4. After mixing is done, transfer ~30-40 g of sample to the weighing pan, weigh, and record **<Subsample, Air dry wt>**. If the sample has high organic matter, fill the pan level for maximum mass.
- 2.5.5. Save the rest of the <2 mm sample in the original sample bag.
- 2.5.6. Place the weighing pan(s) in a 110+/- 5°C oven for 12-16 hours.
- 2.5.7. Tare balance with an empty aluminum sample pan.
- 2.5.8. Weigh the numbered weighing pan(s) and sample(s) as soon as they have cooled, and record **<Subsample, Oven dry wt>**, approximately 10 min after removing from the drying oven.

Note: Do not allow to stand at room temperature for longer than that, to prevent the samples from "rewetting" or reabsorbing moisture in ambient air.
- 2.5.9. Discard the sample(s) in the pan(s). Remove clinging particles with brush or forced air. If necessary, soak the pans in soapy water until any adhering soil is loosened, clean as necessary, rinse thoroughly with distilled water, and allow to dry completely before reuse.

2.6. VNIR Scan Air-Dry Samples

Refer to Step 3.5.2

2.7. File Management

- 2.7.1. Frequent backup of all project files (RaCA workbooks and VNIR files) should be made to a CD, thumb drive, or other external media and kept in a separate physical location. See the "Data Backup" document on the SharePoint site for more information.

2.7.2. Project data should be sent to the SharePoint site, ideally at the end of each session: <https://nrsc.sc.egov.usda.gov/ssra/nssc/rapid/default.aspx>

3. Operating Instructions for the LabSpec 2500 Visible Near Infrared Spectrometer (VNIR)

3.1. Background

Visible Near Infrared (VNIR) spectra are obtained from a soil sample by exposing it to a light source and recording the reflected radiation. The spectra are used to build a library. These library spectra will then be used to develop or improve a model that can be used to predict the amount of an analyte in a soil. The two light sources principally used in this spectra-gathering are ASD's Contact Probe and Muglight. The analyzing instrument is a LabSpec 2500 Spectrometer, also made by ASD Inc, employing Indico Pro software (ASD proprietary) that interfaces the spectrometer with a computer to capture and store these spectra. The process of capturing the spectral information, regardless of the light source, is called a *scan*.

Samples for scanning will be either field-moist whole soil or air-dried <2 mm samples. The field moist samples are scanned at the same time as the first moist weights are determined. The air-dried samples are scanned after drying and processing these same moist samples to <2 mm size. **(Note: As of 02/02/2011, the scanning of field moist samples is now optional; scanning of the air-dried, < 2 mm material continues to be mandatory.)**

Reminder: Instrument requires at least 3 hours warm-up time prior to scanning

3.2. General Instructions

3.2.1. Lens and Window Cleaning Instructions

- 3.2.1.1. For minimal dust coatings on lenses and windows, use a clean dry microfiber cloth to clean the Muglight lens, inner and outer Muglight sample holder windows, the Contact Probe lens, and the ASD White Reference and ASD Wave Cal Puck windows. Do not use microfiber cloth on exposed white reference material that is not covered by a lens. Pictures below show examples of these items.
- 3.2.1.2. If smudges remain on lenses or windows, slightly dampen microfiber cloth with distilled water and wipe lens or window and then thoroughly dry with a clean dry microfiber cloth
- 3.2.1.3. If smudges still remain, use an isopropyl alcohol wipe and thoroughly dry with a clean dry microfiber cloth.



ASD White Reference & ASD Wave Cal Pucks

Double-sided Puck – White reference, Side #1

Double-sided Puck – Wave Cal, Side #2



Sample Holder



Exposed Muglight white reference insert



Muglight white reference (insert in holder)



Exposed Contact Probe white reference



Exposed additional white reference material



Muglight lens



Contact Probe lens

3.2.2. Fiber Optic Cables for Muglight and Contact Probe

3.2.2.1. The cables provided with the instrument are extremely expensive and easily compromised, so be mindful. To avoid damage to the fiber optic cables, be sure to follow these rules:

- 3.2.2.1.1. Do not bend or coil cables into a circle with less than 5" diameter.
- 3.2.2.1.2. Do not hit, strike, twist, or pull the cables.

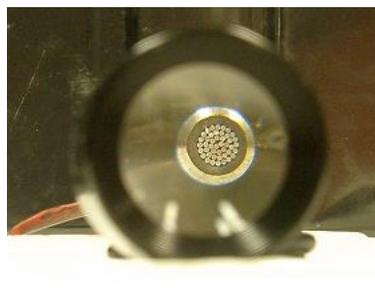
- 3.2.2.1.3. Do not set objects on the cables.
- 3.2.2.1.4. Do not leave the ends of the cables uncovered at any time unless connecting them to the spectrometer or a light source.
- 3.2.2.2. As a once-daily procedure before scanning samples, the magnifier (hand lens) should be used to examine the fiber optic cables to determine if any are broken (i.e., one or more are 'out' and no light can be transmitted through them). To do this:
 - 3.2.2.2.1. Plug the fiberoptic cable into the Muglight or Contact Probe.
 - 3.2.2.2.2. Examine the opposite end with the magnifier.
 - 3.2.2.2.3. When 5 or more fibers are 'out', the cable needs to be replaced.

Note: If the fiber optic cable has not been removed since last scanning run, checking the cable may not be necessary.

EXERCISE GREAT CARE WHEN HANDLING A FIBER OPTIC CABLE. Keep the ends covered (capped) at all times, except when removing or attaching them to devices.



Magnifier (only black loupe is needed)



Fiber optic strands through loupe magnifier

3.3. Preparing the LabSpec 2500 Spectrometer

3.3.1. Component warm-up

Before scanning samples, the components of the VNIR instrument need to be warmed up and ready to use.

- 3.3.1.1. The Muglight or Contact Probe needs to be on for 3 hours to allow for stabilization. It is optimal to leave the Muglight, Contact Probe, and Spectrometer on 24/7 during times of use. (Bulb life is rated at 2000 hours.)

3.3.1.2. The Spectrometer needs to be on for 20 minutes before scanning samples.

3.3.1.3. Although it is not advisable, scans can be taken before the devices are fully warmed up; however, a white reference will need to be run (scanned) immediately prior to scanning every sample until full warm-up time is reached (3 hours).

3.3.2. Wavelength verification

Once each day, before scanning samples, run the Wavelength Verification program as follows:

3.3.2.1. Go to Start → Wavelength Verification (should show Test type: USDA) → Next → Next (it may take several seconds for connection to be made and prompt “Next”).

3.3.2.2. If program is not here, look under Program Files → ASD → Wavelength Verification → WA.exe.

3.3.3. Reference scans

3.3.3.1. Check window on ASD White Reference Puck (#1) and lens on Muglight or Contact Probe to make sure they are clean. Clean if necessary (see Step 3.2.1).

3.3.3.2. Load ASD White Reference Puck (#1) onto Muglight or Contact Probe.

3.3.3.2.1. If using the Muglight, push the puck downward and inward to make sure it is in place over the Muglight lens

3.3.3.2.2. If using the Contact Probe, be sure it is in direct contact with the puck.

3.3.3.3. In some kits, pucks are dual-sided, with the ASD White Reference (#1) on one side and the ASD Wave Cal (#2) on the other; the side with “#1” must be placed face down onto the Muglight at this time. Then click Run. Wait until scan is complete (~30 seconds).

Note: Whatever is presented to a light source, whether an ASD White Reference Puck, ASD Wave Cal Puck, white reference, or an actual soil sample, it must be scanned IMMEDIATELY. The longer the material is exposed to the

light source and the sample warms up, the more significantly the IR signature will change.

3.3.3.4. Remove ASD White Reference Puck (#1) from Muglight or Contact Probe.

3.3.3.5. Check window on ASD Wave Cal Puck (#2) from Muglight or Contact Probe to make sure they are clean. Clean if necessary (see Step 3.2.1).

3.3.3.6. Load ASD Wave Cal Puck (#2) onto Muglight or Contact Probe.

3.3.3.6.1. If using the Muglight, push the puck downward and inward to make sure it is in place over the Muglight lens.

3.3.3.6.2. If using the Contact Probe, be sure it is in direct contact with the puck.

3.3.3.7. For dual-sided pucks, place side “#2” face down on the Muglight. Click Run. Wait until scan is complete (~15 seconds).

3.3.3.8. Does a green “Pass” show up in top right hand corner of dialogue box?

3.3.3.8.1. If Yes, do not click Next but go to Step 3.3.3.9.

3.3.3.8.2. If a red “Fail” appears, check the fiber optic cable so ensure there is a connection between the appropriate, warmed up light source (Muglight or Contact Probe) and Spectrometer, and also that the light source and Spectrometer are energized and have been properly warmed up

3.3.3.8.3. Repeat steps 3.3.3.1 through 3.3.3.7 above. If “Fail” appears again, do NOT proceed – contact Ellis Benham or Rich Ferguson.

3.3.3.9. Click Details. Do a Screen Capture (Print Screen) of the results, import to a Word File, save as proof of daily verification and print a hard copy for logbook.

3.3.3.10. Remove ASD Wave Cal Puck (#2) from Muglight or Contact Probe and store.

3.4. High/Low QC Sample Check

This should be performed when using the Muglight to scan air-dried samples only. If the plan of the day is to scan field moist samples using the contact probe, skip the High/Low QC Sample Check procedure, and use the wavelength verification puck as the sole validation

measurement. When scanning air-dried samples, use both wavelength verification puck AND the High/Low QC Sample Check QC samples as preferred validation measurements.

3.4.1. General Instructions for QC Scanning

- 3.4.1.1. Make sure the Muglight is connected to the Spectrometer via the fiber optic cable and that both the Muglight and Spectrometer are energized and have been properly warmed up (Step 3.3.1.)
- 3.4.1.2. Go to Start → ASD Programs → Indico Pro (or click Indico Pro icon on the desktop).
- 3.4.1.3. On the toolbar, click OPEN; under Project Name select QC Project → OK. If QC Project does not exist, close the dialogue box. On the toolbar, click NEW; under Project Name enter "QC Project". All future access to QC Project can be made by opening Indico Pro, clicking Open and selecting this project.
- 3.4.1.4. On the toolbar, click Spectrum → Sample Count/Average; under Instrument sample count, set number to "100"; under Averaging, select None; under Scan Type, select AB Even → OK. This step should only be necessary when the QC project is initially set up.
- 3.4.1.5. Check Muglight sample holder window and the Muglight lens to ensure that they are clean. Clean if necessary (see Step 3.2.1).
- 3.4.1.6. Place Muglight white reference insert into sample holder. Load this white reference holder onto the Muglight. It is recommended that one sample holder be dedicated for housing the white reference insert as it will be used continuously during the scanning process.

Note: the Muglight white reference (small letters) is NOT the same as the ASD White Reference Puck used for the Wavelength Analyzer program.

- 3.4.1.7. On the toolbar, click Baseline. Be sure Optimize First is checked → Yes. Wait until scan is complete (~30 seconds). The result should be a straight line at 1.00 on the graph (viewed as Reflectance on the y-axis).

Note: During scanning, and assuming that the spectrometer and source are warmed up, a baseline should be taken every 15 minutes, regardless of how many or how few samples have been scanned, to avoid spectrometer drift. A stopwatch is recommended to ensure this vital step is not missed.

3.4.1.8. On the toolbar, click log [1/R]. To put view in absorbance mode. The result should be a straight line at 0.00.

3.4.1.9. Remove white reference from Muglight. Clean if necessary (Step 3.2.1) and place in clean plastic bag or set on clean cloth and cover until next use.

3.4.2. Scanning Low and High QC

3.4.2.1. Load the QC sample labeled “Low” into a Muglight sample holder. (See Step 3.5.2.1 for instructions on the preparation of this sample.)

3.4.2.2. Examine outside window of sample holder and also the Muglight lens to be sure they are clean. Clean if necessary (Step 3.2.1). Place sample holder with the Low QC sample onto Muglight.

3.4.2.3. On the toolbar, click Scan. Wait until scan is complete (~15 seconds).

3.4.2.4. On the tool bar, click Chemometrics → Setup. Under Project Predictors, select Galactic PLS Plus/IQ; under Selected Predictor, select Galactic PLS/IQ; click the Add button; a dialogue box will appear; browse for and select the QC-IC.cal file located in C:\Program Files\ASD, click Open; within the Chemometrics dialogue box should now appear the file QC-IC.cal. Click OK.

3.4.2.5. On the toolbar, click Predict to view the calcium carbonate equivalent for the Low QC sample in the Prediction Result dialogue box; record this value and the M-distance in the Excel spreadsheet QC Log that will be provided (contact Rich Ferguson if you have not received this file).

3.4.2.5.1. If the predicted value or M-distance is outside of acceptable ranges specified in QC Log spreadsheet, check the fiber optic cable to make sure it is attached between the *Muglight* and spectrometer, and also that the Muglight and Spectrometer are energized and are warmed up; then repeat steps 3.4.5 – 3.4.11.

3.4.2.5.2. Should the predicted value or M-distance still be outside of acceptable range, do NOT proceed – contact Ellis Benham or Rich Ferguson.

3.4.2.6. If the Low QC sample passes, on the toolbar, click Save and enter the letter “L” (for “low”) followed by the File name field with the date (mm/dd/yy; e.g., L092710). The Prediction Result dialogue box should remain open for the next scan. Do not discard the Low QC sample, but return it to its original container for future use.

- 3.4.2.7. Load the QC sample labeled “High” into a Muglight sample holder (See Step 3.5.2.1 for instructions on the preparation of this sample.)
- 3.4.2.8. Examine outside window of sample holder and also the Muglight lens to be sure they are clean. Clean if necessary (Step 3.2.1). Place sample holder with the High QC sample onto Muglight.
- 3.4.2.9. On the toolbar, click Scan. Wait until scan is complete (~15 seconds).
- 3.4.2.10. The calcium carbonate equivalent for the High QC sample will display in the Prediction Result dialogue box; record this value and the M-distance in the Excel spreadsheet “QC Log”. If the predicted value or M-distance is outside of acceptable ranges specified in the QC Log spreadsheet, do NOT proceed – contact Ellis Benham or Rich Ferguson.
- 3.4.2.11. If the High QC sample passes, on the toolbar, click Save and enter the letter “H” (for “high”) followed by the File name field with the date (mm/dd/yy; e.g., H092710). Do not discard the High QC sample, but return it to its original container for future use.
- 3.4.2.12. On the toolbar, click File → Save Project; click File again → Exit.

3.5. Scanning Soil Samples

3.5.1. Scanning Field Moist Samples **(Note: As of 02/02/2011, the scanning of field moist samples is now optional)**

Field moist samples are to be scanned at the time they are weighed and then set out for air drying.

- 3.5.1.1. Make sure the Contact Probe is connected to the Spectrometer via the fiber optic cable and that both the Contact Probe and Spectrometer are energized and have been properly warmed up.
- 3.5.1.2. Go to Start → ASD Programs → Indico PRO (or click Indico Pro icon on the desktop). If the project to be scanned is a new one, go to Step 3.5.1.3. If the project to be scanned is an existing one, select OPEN, click on the appropriate project name → OK, go to Step 3.5.1.4.
- 3.5.1.3. On the toolbar, click NEW, and enter the Project Name as the RCA Site ID (contact Skye Wills if there are questions about this naming step) → OK. Enter Project Description as you wish → OK. All spectra

associated with a given sampling location will be stored under this project name.

Note: For every RCA sampling location, a new Project must be created. The Project serves as a folder into which all corresponding scan files should be stored, moist-scanned and air-dried-scanned.

3.5.1.4. On the toolbar, click Spectrum → Sample Count/Average; under Instrument sample count, set number to “100”; under Averaging, select Subset; under Subset, set the ‘Number of subsets to collect’ to “3”; **make sure the instruction “Prompt to accept each spectrum” is unchecked**; under Scan Type, select AB Even → OK. These parameters may already be set if the last samples scanned for this project were field-moist ones.

3.5.1.5. Make sure the rubber ring is removed from the end of the Contact Probe as its use would make cleaning the Contact Probe lens more difficult. Check the lens on the Contact Probe to make sure it is clean. Clean if necessary (see Step 3.2.1). Check Contact Probe white reference to make sure it is clean. If it is dirty, clean it or use another clean white reference.

3.5.1.6. Place the clean Contact Probe onto the clean white reference.

3.5.1.7. On the toolbar, click Baseline. Be sure Optimize First is checked → Yes. Wait until scan is complete (~30 seconds). The result should be a straight line at 1.00 on the graph viewed in reflectance mode or 0.00 if viewed in absorbance mode.

Note: During scanning, and assuming that the spectrometer and source are warmed up, a baseline should be taken every 15 minutes, regardless of how many or how few samples have been scanned, to avoid spectrometer drift. A stopwatch is recommended to ensure this vital step is not missed.

3.5.1.8. Remove Contact Probe from white reference. Place white reference in clean plastic bag.

3.5.1.9. Examine Contact Probe lens to be sure it is clean. Clean if necessary (see Step 3.2.1).

3.5.1.10. Each field moist sample will be scanned three times, with the average of these three scans then being stored. Judgment must be exercised in determining 3 spots on the sample that, combined, would represent a good ‘average’ for the sample as a whole.

- 3.5.1.10.1. Firmly press Contact Probe onto a representative location in the soil and hold steady. Click on Scan. Wait until scan is complete (~15 seconds).
- 3.5.1.10.2. Remove the Contact Probe from the soil, wipe the lens clean and firmly press onto another sample location and hold steady. Scan a second time.
- 3.5.1.10.3. Remove the Contact Probe from the soil, wipe the lens clean and firmly press onto another sample location and hold steady. Scan a third time.
- 3.5.1.10.4. A dialogue box will appear asking, "Accept average subset?" Click Yes. This is the average of the 3 scans just taken. Clean the lens for next use (Step 3.2.1).
- 3.5.1.10.5. Reminder: If any of the analyzed soils are APHIS regulated, applicable sterilization protocols need to be followed.
- 3.5.1.11. On the toolbar, click Save. The File name field is filled by copying the appropriate (cf) <VNIR File Name> from the 'VNIR' worksheet in the Rapid Carbon Assessment Workbook Excel spreadsheet, and pasting the sample identifier into the save dialog box → OK.

Note: In the Excel spreadsheet, the VNIR File Name field is unprotected. The user should make backup copies of this spreadsheet in the event that these unprotected file names are lost or accidentally changed. If this happens and no backup has been made, the user will have to call Skye Wills to get instructions on reconstructing the file name.

- 3.5.1.12. Files are named following a convention based on the site identification assignment, the horizon depth and the method used to obtain VNIR data. The convention is:

CxxyyLzzadddv.asd

Where:

C = Denotes Carbon

xx = MO number

yy = statistical group # for MO

L = land use

zz = Plot # within the group/land use combination

a = Pedon # within plot

ddd = upper depth of horizon (use 000 for surface, 010 if top is 10 cm below surface, and so on)

vv = VNIR method, as follows:

“cf” = contact probe / field moist soil

“md” = muglight / air-dried soil

e.g. C0803F092023cf.asd

3.5.2. Scanning Air-Dried Prepared Samples

Prepared samples are those that have been air-dried and processed to < 2 mm.

(Note: The scanning of air-dried, < 2 mm material is mandatory.)

3.5.2.1. Loading <2mm Air-Dried Sample into Muglight Sample Holder

3.5.2.1.1. Examine inner window of Muglight sample holder. Clean if necessary (see Step 3.2.1).

3.5.2.1.2. Heap prepared sample into sample holder to slight overflow capacity.

3.5.2.1.3. Level the sample to the top of sample holder by striking off excess sample with a straight edge.

3.5.2.1.4. Before packing, support underside of sample holder window with the wide end of a #7 rubber stopper; this will prevent sample holder window from being pushed out or broken. Align foot of the penetrometer press so it does not catch the sides as it presses into the well of the filled sample holder. Brush away excess soil from top and sides of sample holder while the sample is being packed, in order to help keep soil from spilling onto the Muglight lens (the next step in the procedure). The penetrometer is set to apply ~46 lb of pressure. Press sample for ~10 sec.

Note: As of date of release of this document, the penetrometer press to be used for packing samples was not yet available. Until this press becomes available, pack the samples by supporting the underside of the sample holder with the wide end of a #7 rubber stopper and using a #6 rubber stopper to pack the sample by applying moderate pressure.

3.5.2.1.5. After packing the sample, wipe the bottom of the sample holder free of extraneous dirt. Place the sample holder on a clean towel. This filled sample holder is ready to be loaded onto the Muglight for scanning.

3.5.2.1.6. During the compaction process, some soil particles will cling to the surface of the piston (stopper). The face of the piston (stopper) should be wiped clean before packing the next sample.

3.5.2.2. Scanning Samples

3.5.2.2.1. Make sure the Muglight is connected to the Spectrometer via the fiber optic cable and that both the Muglight and Spectrometer are energized and have been properly warmed up.

3.5.2.2.2. Go to Start → ASD Programs → Indico Pro (or click Indico Pro icon on the desktop).

3.5.2.2.3. On the toolbar, select Open, find the appropriate project. A project should already have been created when the field moist samples were scanned. If not, click Cancel on the Open Project dialogue box; on the toolbar, click NEW, and enter the Project Name as the RCA Site ID (contact Skye Wills if there are questions about this naming step) → OK. Enter Project Description as you wish → OK. All spectra associated with a given site will be stored under this project name.

Note: For every RCA sampling location, a new Project must be created. The Project serves as a folder into which all corresponding scan files should be stored, moist-scanned and air-dried-scanned.

3.5.2.2.4. On the toolbar, click Spectrum → Sample Count/Average; under Instrument sample count, set number to “100”; under Averaging, select → None; under Scan Type, select AB Even → OK.

3.5.2.2.5. Check outer window on white reference and lens on Muglight to be sure they are clean. Clean if necessary (see Step 3.2.1). Both surfaces should be wiped just prior to scanning. Load white reference onto Muglight.

3.5.2.2.6. On the toolbar, click Baseline. Be sure Optimize First is checked → Yes. Wait until scan is complete (~30 seconds). The result should be a straight line at 1.00 on the graph (viewed as Reflectance).

- 3.5.2.2.7. Remove white reference from Muglight. Clean if necessary (Step 3.2.1) and place in clean plastic bag or set on clean cloth and cover until next use.
- 3.5.2.2.8. Examine outside window of filled sample holder and lens on Muglight to be sure they are clean. Clean if necessary (Step 3.2.1). Load filled sample holder onto Muglight.

Note: During scanning, and assuming that the spectrometer and source are warmed up, a baseline should be taken every 15 minutes, regardless of how many or how few samples have been scanned, to avoid spectrometer drift. A stopwatch is recommended to ensure this vital step is not missed.

- 3.5.2.2.9. On the toolbar, click Scan. Wait until scan is complete (~15 seconds).
- 3.5.2.2.10. On the toolbar, click Save. The File name field is filled by copying the appropriate (md) <VNIR File Name> from the 'VNIR' worksheet in the Rapid Carbon Assessment Workbook Excel spreadsheet and pasting the sample identifier into the save dialog box → OK.

Note: In the Excel spreadsheet, the VNIR File Name field is unprotected. The user should make backup copies of this spreadsheet in the event that these unprotected file names are lost or accidentally changed. If this happens and no backup has been made, the user will have to call Skye Wills to get instructions on reconstructing the file name.

- 3.5.2.2.11. Files are named following the convention previously described in 3.5.1.12:

CxxyyLzzadddvv.asd

Where:

C = Denotes Carbon

xx = MO number

yy = statistical group # for MO

L = land use

zz = Plot # within the group/land use combination

a = Pedon # within plot

ddd = upper depth of horizon (use 000 for surface, 010 if top is 10 cm below surface, and so on)

vv = VNIR method, as follows:

“cf” = contact probe / field moist soil

“md” = muglight / air-dried soil

e.g. C0803F092023md.asd

3.5.2.2.12. To clean sample holder after scanning:

3.5.2.2.12.1. Working over a waste container and in front of dust collector, turn sample holder upside down and tap gently with wooden dowel or knife handle until compacted soil falls out.

3.5.2.2.12.2. Use compressed air from an oil-less air compressor to blow out remaining residue from inside of sample holder. Make sure that the compressor output regulator is set at ≤ 25 psi; higher pressures could cause pitting of windows should sand be present.

3.5.2.2.12.3. To more thoroughly clean inner window prior to loading the next sample, see Step 3.2.1.

3.5.2.2.12.4. Reminder: If any of the analyzed soils are APHIS regulated, applicable sterilization regulations need to be observed.

3.5.3. File Management

3.5.3.1. Before exiting Indico Pro, go to File menu → Save Project.

3.5.3.2. Frequent backup of all project files should be made to a CD, thumb drive, or other external media and kept in a separate physical location.

3.5.3.3. Project data should be sent to the SharePoint site:

<https://nrsc.sc.egov.usda.gov/ssra/nssc/rapidc/default.aspx>

3.6. VNIR Instrument Operation Technical Support

Ellis Benham – ellis.benham@lin.usda.gov ; 402-437-5132

Richard Ferguson – rich.ferguson@lin.usda.gov ; 402-437-5332

Deb Harms – deb.harms@lin.usda.gov ; 402-437-5324

Mike Pearson – mike.pearson@lin.usda.gov ; 402-437-5373

Skye Wills – skye.wills@lin.usda.gov ; 402-437-5310

4. Sending Samples to the Soil Survey Laboratory

4.1. Types of Samples

There are three types of samples that must be sent to the Soil Survey Laboratory (SSL) in Lincoln. They will be either stored as archive samples or analyzed because they are quality control samples or the carbon content is too high to be accurately modeled from VNIR scans. The following three categories of samples will be sent:

4.1.1. Central Pedon for Archive (CPA) samples

All samples from all central pedons will be archived.

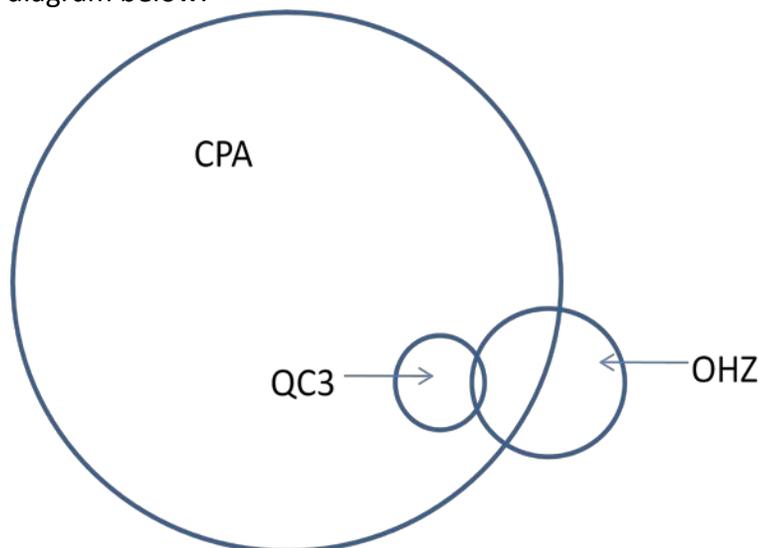
4.1.2. Quality Control (QC3) samples

Of the CPA pedons, 3% are randomly selected to be analyzed by conventional laboratory and spectral methods at the SSL. There are approximately 12 of these per MO.

4.1.3. O horizon (OHZ) samples

All O horizon samples will be analyzed by conventional laboratory and spectral methods at the SSL.

The relationship of the sample types to be sent to the SSL is illustrated in the diagram below:



Note: All QC3 samples are CPA samples. Some OHZ samples are CPA samples. Some OHZ samples are CPA and QC3 samples. All OHZ samples from outer pedons are neither CPA nor QC3 samples.

4.1.4. How to identify Quality Control (QC3) pedons

To assure that the data we are acquiring is accurate, QC3 samples collected in the field are evaluated at the SSL. The QC3 pedons are selected by using

functions in spreadsheets provided through the RaCA Share Point. All samples from the selected pedon are QC3 samples.

4.1.4.1. Go to the spreadsheet “MO#_group_prop.xlsx”, where # is the MO region (1-18).

4.1.4.2. In the worksheet “Sites_Assigned_list”, locate column A: “Pedons_for_lab”. In this column there is a number, x = 1, 2 or 3, representing the number of sites from that group / land use combination that must be identified as QC3 sites.

4.1.4.3. In order of site_ID number, identify the first (if x=1), first two (if x=2), or first three (if x=3) site(s) sampled within that group-land use combination as QC3 sites.

4.1.4.4. The central pedon(s) from the site(s) identified above will provide the QC3 samples.

4.2. Procedure for Labeling SSL (CPA, QC3 and OHZ) Samples

4.2.1. Labeling and storage

4.2.1.1. Air-dried, sieved samples should be stored in plastic Ziploc-style bags with complete label clearly and indelibly written on the bag.

4.2.1.2. After scanning the air-dried sample, fill out an additional provided standard label prior to shipping.

4.2.2. Labeling convention for shipped samples

Include the following information on labels for samples that are shipped to the SSL:

4.2.2.1. RCA_site_ID: for example CxxyyLzz

4.2.2.1.1. C is a constant

4.2.2.1.2. x = MO number [two digits]

4.2.2.1.3. yy = statistical group # for MO [two digits]

4.2.2.1.4. L = Land use code [one letter from CFXPRW]

4.2.2.1.5. zz = Plot # within the group or land use [two digits]

4.2.2.2. VNIR file name associated with the scan of the air-dried, processed sample using the Muglight

4.2.2.3. Sample type identifier(s): CPA, QC3 and/or OHZ. Circle one, two, or all three identifiers, as appropriate

4.2.3. Attaching labels to bags

4.2.3.1. Be certain that the label is legible, written in indelible ink and accurately matches the sample to which it is attached.

4.2.3.2. Attach the label with a heavy duty stapler in the upper right hand corner of the sample bag (above the seal) using two staples. Do not pierce bag with staples.

4.2.3.3. Consider laminating or otherwise protecting (clear shipping tape) the label to avoid smearing or destruction during storage and shipping.

4.2.3.4. Seal bag by running strong packaging-type tape along the top of the bag, half of the width of the tape. Cut tape to edge of bag and fold over to seal.

4.3. Instructions for Shipping SSL Samples

4.3.1. Preparing samples for shipping

4.3.1.1. Only air-dried, <2mm soil sample material will be shipped to the SSL.

4.3.1.2. From ½ - 1 pint (~ 400g of mineral soils) of sample will be archived, so very full bags or multiple bag samples should mixed well and a portion bagged and labeled before shipment.

4.3.1.3. Separate SSL samples that are to be analyzed (any sample identified as QC3 and OHZ) from those to be archived only (identified as CPA only).

4.3.2. Handling OHZ and QC3 Samples

4.3.2.1. If necessary, large samples should be reduced to ~ 1 pint

4.3.2.2. Seal sample bags securely (use a new bag if the old one is compromised).

4.3.2.3. Check that bag label and attached SSL label match.

4.3.2.4. Collect sample bags in a suitable box until ready for shipping.

4.3.2.5. Complete separate **'RaCA Shipping Log'** for each box

- 4.3.2.6. Before shipping, fill any open space in the box with paper or other shipping material
- 4.3.2.7. Please write "RaCA" in large bold letters on the outside of the box on at least two sides.
- 4.3.2.8. Plan to ship these samples on a regular basis, consider monthly or after 1 to 2 pedons have been processed.
- 4.3.2.9. Keep the total shipment size under 150 lb.
- 4.3.2.10. Arrange shipment with FedEx Ground or similar service by emailing or calling:

Linda Kruger
National Soil Survey Center
Federal Building, MS-41
100 Centennial Mall North
Lincoln, NE 68508
(402)437- 5016
Linda.kruger@lin.usda.gov

Linda will email you a FedEx shipping label. If FedEx is not a viable option at your location, let us know and we will make arrangements for shipment by an alternant company.

4.3.3. CPA: samples to be archived only

- 4.3.3.1. If necessary, large samples should be reduced to ~ 1 pint
- 4.3.3.2. Seal sample bags securely (use a new bag if the old one is compromised).
- 4.3.3.3. Check that bag label and attached SSL label match.
- 4.3.3.4. Collect sample bags in suitable boxes until full or 75 lbs. (an individual box must be < 75 lb for shipping).

Note: Unlike the OHZ and QC3 sample, there is no limit on the total shipment weight for CPA samples to SSL. More than one 75 lb box can be shipped at a time and larger shipments are preferred.

- 4.3.3.5. Complete separate '**RaCA Shipping Log**' for each box
- 4.3.3.6. Store filled boxes in a dry location.

4.3.3.7. Before shipping, please write “RaCA” in large bold letters on the outside of the box on at least two sides.

4.3.3.8. Plan to ship all CPA samples at once at the end of the project; if this is not possible (due to space constraints) arrangements will be made on an MO by MO basis (contact Ellis Benham or Skye Wills).

4.3.3.9. Arrange shipment with freight or similar service by contacting:

Larry Arnold, Supervisor, Processing Section
Soil Survey Research and Laboratory
National Soil Survey Center
Federal Building, MS-51
100 Centennial Mall North
Lincoln, NE 68508
(402)437-5358
larry.arnold@lin.usda.gov