

NEON Tick Identification Instructions

2021 edition v.3

Contents

Processing a new shipment from NEON	2
Domain folders.....	2
Chain of Custody Emails.....	2
Receiving a shipment	2
Assigning RMLs.....	3
Domain Data Files	3
Print RMLs.....	3
Google Drive File.....	4
File Check	4
Created:.....	4
Modified:.....	4
Storing the Samples	4
Processing Ethanol Samples.....	5
Printing Vial ID and CEN/RML labels	7
Processing an identified and verified Domain	8
Creating Data Files for NEON reports	9
Domain Files.....	9
Files for Box.....	15
Creating Specify Files from NEON Box Files.....	15
Printing NEON labels.....	20
NEON Invoices.....	14
Preparing Samples for Shipment to UMass	21

Processing a new shipment from NEON

Domain folders

A template Domain folder is in the shared Google folder designed as “NEON_year”. The template folder contains four folders and two files. The folders are:

1. *Identified and Billed*: As material for that Domain has been identified and billed to NEON all of the files get saved into this folder.
2. *Manifests*: The shipment manifests attached to the Chain of Custody emails get saved here.
3. *Receipts*: The receipt files attached to the Chain of Custody emails get saved here.
4. *Site Upload*: Site upload files generated for ingest into the USNTC database are saved here.

The files are:

1. *_current*
2. *_RMLs for printing*

Both of these files should be renamed to contain “Domain #” for the domain the main folder is being created for. These files contain the correct formatting for those files.

Chain of Custody Emails

Upon shipment from a domain, a Chain of Custody email is created. This email contains both a manifest for the shipment and a receipt form. Download both of these files to the folder for that Domain.

Receiving a shipment

1. Shipments are sent to us by the NEON domains. Upon shipment, a Chain of Custody email is created which contains both a manifest and a receipt file.
2. As soon as possible, pick up the shipment from the front office and bring to the lab.
3. Compare the contents of the package to the Receipt file attached to the Chain of Custody email
4. Fill out the Receipt file using the information in Table 1 on page 2.
5. Save the Receipt file as a CSV. Do not change the file name.
6. Upload the Receipt file to the data portal* at: <http://data.neonscience.org/web/external-lab-ingest/>
7. Some packages will contain a return shipping label. After removing the samples, replace all packing material in the package, seal it back up, and tape the return shipping label to the outside of the container. Be sure to remove the previous shipping label!
8. Bring the package to Melissa so she can schedule a pickup.

*Note: You must have an account with NEON in order to access the data portal. As of August 2018, only Lorenza has account.

Table 1: Data Dictionary for Receipt files

Field	Format	Required?	Example	Remarks
ShipmentID	Alphanumeric	--	D0220180806121654937	This field is already populated by NEON
shipmentReceivedDate	YYYYMMDD	Y	20180813	
receivedBy	xxxxx@xxxx.com	Y	crevans@georgiasouthern.edu	Email address of the receiver
sampleID	Sitecode.YYYYMMDD	--	SERC_005.20180430	This field is already populated by NEON
sampleCode	Prepopulated	--	A00000001185	This field is already populated by NEON. Barcode number on sample vials.
sampleReceived	Y/N	Y	Y	
acceptedForAnalysis	Y/N	N	Y	REQUIRED if sampleReceived is 'Y', choice of 'Y' or 'N'. If sampleReceived is 'N', leave this field blank.
sampleCondition	Cold chain broken; damaged; sample incomplete; handling error; other	N	Damaged	REQUIRED if acceptedForAnalysis is 'N', leave this field blank otherwise. If a sample is not fit for analysis, please document the reason why in the sampleCondition field. If selecting 'other', include a description of the problem in the remarks column.
unknownSamples	Sitecode.YYYYMMDD	N	SERC_005.20180430	Add the sampleID of all samples found that do not correspond to any provided sampleID or sampleCode. Please also notify CLA_admin@battelleecology.org
Remarks	Text	N	---	usually optional, REQUIRED if sampleCondition is 'other'

Assigning RMLs

1. Open the manifest attached to the Chain of Custody email in Excel.
2. Open the current Google Drive file for RMLs*. This file is in the *RML, Jar Numbers, etc.* folder.
3. Scroll to the last used number in the document.
4. Copy the **vial IDs** and **sampleCodes** from the Excel file
5. Paste so the **vial IDs** are in the *vialID (NEON)* column and the **sampleCodes** are in the next column. (Right click on the cell that you are pasting into. Choose *Paste special*→*Paste values only.*)
6. The RMLs are now considered assigned.

Domain Data Files

1. In the folder for that Domain, open the *Domain #_current* file.
Note: If this is the first shipment for a Domain, this file may only contain column headings.
2. Copy the **vialIDs**, **sampleCodes** and **RMLs** from the Google Drive RML file into the *_current* file. Do not paste over existing data (if any).
 - a. **vialID** into **column B (sampleID)**
 - b. **sampleCodes** into **column C (sampleCode)**
 - c. **RMLs** into **column D (RML-temp, remove before sending to NEON)**
3. Save the file.
4. Save the file a second time using the following format:
 - a. Domain #_DDMonYYYY
(the date is the current date with numbers representing the day and year and the first three letters of the month)

Print RMLs

1. In the folder for that Domain, open the *Domain #_RMLs for printing* file.
Note: If this is the first shipment for a Domain, this file may be blank. The formatting is still there!
2. Copy the **sampleIDs** and **RMLs** from the *_current* file and paste them into *the _RMLs for printing file*.
 - a. Be sure to only copy/paste the new **sampleIDs** and **RMLs** and not previously printed ones.
 - b. When pasting, right click and choose the second paste option called *Paste Values*. This will adapt the data to the format in the RML file.
3. Copy and paste the same data two more times, so you have a total of three copies for each ID/RML.
4. Highlight the new data entered.
5. Select File→Print.
6. On the print screen, change the first setting to *Print Selection*.
7. Print the labels on the inkjet printer in M/P 1023. Make sure cotton paper is loaded into the printer before printing.
8. Cut apart the printed labels based on the different bags of samples in the shipment.
9. Add the cut labels to the appropriate bag.

Google Drive File

1. In the *Identification Files_2017 samples* folder in Google Drive, open the appropriate Domain file.
Note: If this is the first shipment for a Domain, there may only be column headings in this file.
2. Copy the **sampleIDs** and **RMLs** from the *_current* file and paste them into the Google Drive file.
3. Make sure the statement *All changes saved in Drive* appears next to the menu at the top.
4. Close the file.

File Check

After all of these steps, you should have created one new file and modified five others.

Created:

1. Shipment#_Domain #_DDMonYYYY.xlsx

Modified:

1. RML128XXX (Google Drive)
2. Shipment#_Domain #_current.xlsx
3. Shipment#_Domain #_RMLs for printing.xlsx
4. Shipment#_Domain #_identifications (Google Drive)
5. Shipment#_Receipt file

Storing the Samples

Once all data files are created/modified, store the samples in M/P 1017. There are pre-labeled trays for each Domain. Place the samples, separated by shipment, into the correct tray.

Processing and Identifying Samples

Processing Ethanol Samples

1. Log on to the laptop in 1017 using your GSU credentials.
 - a. You can use a personal laptop if desired, but keep in mind that there is a chance of chemical spills.
2. Using your preferred web browser, open and log in to Google Drive.
3. Navigate to the NEON files that have been shared with you.
4. The NEON samples are bagged in small batches, usually based on the original shipment. These bags are sorted into bins by Domain. This sample baggie should contain a set of labels for those vials. If the labels are missing or there are not enough, you can print new ones using the printer in 1023. (See *Printing Vial ID and RML Labels*)
5. Open a bag of samples and remove the vials and labels.
6. Cut the labels apart by row. There should be three to four copies of each Vial ID/RML set.
7. The sample vials are labeled using the Vial ID, which is assigned by NEON. Match the cut apart labels to the sample vials.
8. Select a sample to process.
9. Prepare archival vials for the samples. For each NEON sample, you will need up to four 1 mL shell vials and up to four 8ml vials (with black lid). The shell vials will eventually be placed into the 8ml vials.
10. Put a label set for that sample into each of the 8ml vials.
 - a. You may be short one label set at this point. This is okay. It is very unlikely you have up to four samples. If needed, you can print a fourth copy of the label set.
11. Dump the NEON sample into a petri dish. If there is a large amount of sample media, you can fish out the specimens using a large pipette or forceps.
12. Make sure all of the specimens made it into the dish. Double check the sample lid and the sidewalls of the vial.

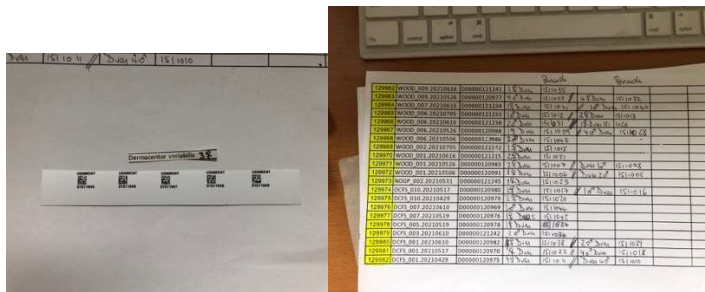
Keep in mind that larvae in particular are very small and sneaky!
13. Using a microscope, sort and count the tick specimens by life stage.
 - a. If you have been trained by Dr. Beati, you may also identify samples to Genus/species at this time.
14. In Google Drive, open the identification file for the Shipment#_Domain you are currently working on.
15. Record the counts for each life stage on the appropriate row for the sample.
 - a. For samples with multiple life stages, you will need to duplicate the row. Add a new row below the one for your sample and copy/paste the NEON identifier, Shipment#_, and the RML into this new row.
 - b. If you are identifying the samples as you go, you should also record the identification at this time. If there is more than one species present in a vial, you will need to duplicate the row. Every life stage/species combination should have its own row in the file.
16. Every life gender/stage/species combination should be placed into their own archival vial. Place the counted specimens into the shell vial (shell vial = internal vial) containing ethanol along with a label containing the life stage/gender. Always use a pencil or a Pigma pen to write the count onto the pre-prepared life stage/gender labels.

17. Counting larvae: if a vial appears to contain <200 specimens, the ticks in that vial have to be manually counted. If the number appears to be more important, ticks are placed in shall vials that have been calibrated for approx. number of larvae/volume. Although not perfect, this method allows in general for less than 2-5% error (as verified with counted large batches of ticks).
18. Top off the shell vial with 90% ethanol.
19. Place the shell vial into a 8ml vial (=external vial). The 8ml vial should already contain a set of vial ID and RML labels.



- 20.
21. At this point, you do not need to fill the 8ml “external” vial with ethanol. That will wait until after the samples have been verified by Dr. Beati.
22. Notify Dr. Beati via email when the sorting/counting for one shipment is complete.

Dr. Beati identifies the samples, records IDS on a paper spreadsheet, adds an identification (genus, species, gender/stage, amount) label in the shell vial and a barcode (USNMENT#) provided by the Smithsonian Institution in the 8ml vial. Dr. Beati, also enters the data into the Google identification spreadsheet (separated by shipment) and fills up the 8ml vial with ethanol.



23. Place completed samples back into their appropriate tray (separated by shipment).
24. Discard any unused ethanol from the original sample in the waste container after verifying that no ticks are left).
25. Once an entire Shipment# is complete, download the identification file from Google Drive and add the term “_complete” to the saved file name and the Google Drive file name.
26. Print the file and place it with the samples.
27. Move on to the next shipment.

QA Evaluations

On a yearly basis, once all ticks are counted and identified, and before nymphs are shipped to the testing laboratory, the quality of the work performed is tested as follows:

- 1) The “random” function in excel is used to assign random numbers to each database entry. These numbers are then sorted in ascending order and the first samples corresponding to 3% of the total number of entries are selected.
- 2) The selected samples are recovered, ticks are recounted and identifications verified. Discrepancies are recorded (if any) and the following equations are used to evaluate quality of work.

Equation 1, Percent Difference in Enumeration (PDE):

$$PDE = (|Count1-Count2|/|Count1+Count2|) \times 100$$

Equation 2, Percent Taxonomic Disagreement (PTD):

$$PTD = (1 - (agreements/N)) \times 100$$

Where: agreements is the number of consistent identifications (species and sex match; if species and sex are identical, but identification qualifier differs, that is still considered an agreement); N is the total number of individuals in the larger of the two counts.

Printing Vial ID and CEN/RML labels

1. Log on to the computer in M/P 1023 that is hooked up to the printer.
2. Make sure the printer is loaded with archival ethanol-resistant paper. There is additional paper in the desk drawers if it is empty.
3. Log in to Google Drive and navigate to the NEON folder that has been shared with you.
4. Open the folder for the year you are currently working on.
5. Open the folder for the Domain you are currently working on.
6. Download the *Domain #_RMLs for printing* file.
7. Open the downloaded file in Excel.
8. Once opened in Excel, highlight the label(s) you need to print.
9. Open the **File** menu and select **Print**
10. BEFORE printing, change the first drop down menu under **Settings** to **Print Selection**.
11. Verify that the print preview on the right shows the label(s) you need to print.

12. Verify that the print job is going to the HP Envy printer.
13. Click "Print"

Processing an identified and verified shipment.

All identifications must either be performed or verified by Dr. Beati. She will write her ids directly onto the paper spreadsheets printed by the students when they complete the initial processing of a shipment. The identifications will then need to be entered into the spreadsheets (Dr. Beati has been doing this since 2019, but students will start entering identification data again in 2022). These files must be processed in a timely fashion.

1. Open the *Shipment #_identifications_complete* file for the shipment you are entering. You may need to download this from Google Drive.
2. Using the handwritten notes, transcribe the species names into the file in the **Genus** and **species** columns. Make sure the RML and barcode numbers match.
Note: since the spreadsheet was printed from this file, the records should be in the same order. You may need to rearrange the pages.
3. If more than one species was identified out of a batch, you will need to duplicate the row. Insert a new blank row and copy/paste the vialID and RML. Enter all other data, including the barcode Dr. Beati assigned.
4. Update the date and identifier fields for all records identified by Dr. Beati.
5. Once the file is complete, save it as an Excel file using the following naming format:
Domain #_identifications_complete_LB
6. Use this file to create the data files.

Data and Invoice Files for NEON

Creating Data Files for NEON reports (sent to NEON within 3 months from shipment receipt).

Domain Files

1. Open the *Domain #_identifications_complete_LB* file and the *Domain #_current* file in MS Excel.
2. Split screen the files.
 - a. On a PC, you can drag the window to the center of either side of the screen to “snap” the windows into place.
3. Sort both files by RML.
 - a. Click the **Data** tab
 - b. Click the **Filter** button
 - c. Click the arrow next to the **RML** heading.
 - d. Select the **Sort A to Z** option.
 - e. Do this for both files.
4. In the *_complete_LB* file, select **column B (CEN/RML)** and set it to highlight all duplicate values.
 - a. On the Home tab, select Conditional Formatting→Highlight Cells Rules→Duplicate Values
 - b. In the window that appears, click okay.
 - c. All duplicate RMLs should now be highlighted in red.
5. The *_current* file has each vialID/RML combination listed once. Some of these will need to be duplicated to match the *_complete_LB* file. Insert a blank row below each record that needs to be duplicated. Insert more than one if needed. The number of rows per record should match the *_complete_LB* file.
6. Copy and paste the data from **column B** in the *_complete_LB* file into **column D** in the *_current* file, so you can verify you inserted the new rows correctly. Do not copy and paste the header.
7. Fix any mistakes you notice.
8. Copy/paste the **sampleID** and **RML** down into each blank row. Do not drag data down, as it will increase any numbers by 1.
9. Set the **RML column** in *_current* to highlight duplicate values. Verify that the blocks of highlighted cells match the data you copy/pasted in from the *_complete_LB* file.
10. Clear Rules in *_current*
 - a. Home→Conditional Formatting→Clear Rules→Clear Rules from entire sheet
11. Delete the data out of **column D** in the *_current* file. Do not delete the header.
12. Copy and paste all columns from the *_complete_LB* file into the *_current* file. Make sure you are pasting data into the correct places. See Table 2.

Table 2: Origin in *_complete_LB*, Destination in *_current*

Column in <i>_complete_LB</i>	Data contained	Column in <i>_current</i>
C	Genus	K
D	Species	M
E	Life stage/sex	P
F	Counts	Q
G	Identified Date	A

H	Identified by	AA
I	Barcode Number	T
J	Remarks	AB

13. Double check that the data in *_current* matches the data in *_complete_LB* and that nothing is scrambled. Data checks are performed when data are converted to the final format (Tick IDs and Archival files).
14. If everything is okay, close out of *_complete_LB*.
15. Fill out the remaining columns in *_current*. Note: many will be left blank as it is data we do not report. See Table 3.

Table 3: Data for Remaining Columns in *_current*

Column Letter	Column Heading	Actions
E	Target Taxa Present	Fill Y all the way down, unless there was something barcoded that is not a tick. In that case, use N.
F	Species Name	<ol style="list-style-type: none"> 1. Sort file by Genus. 2. Fill in the following formula: =J#&" "&L# 3. Drag down for all records with an ID 4. Copy and paste values on top of itself (this will delete the formula)
G	Family	Fill in Ixodidae for anything with an identification.
R	Sample condition	OK or Damaged
S	Archive Medium	90% ethanol or RNA stabilization buffer
T	Archive facility ID	Needs to be in the following format: USNMENT##### Correct if needed
V	Subsample ID	<ol style="list-style-type: none"> 1. Fill in the following formula: =B#&"."&left(J#, 3)&left(L#, 3)&"."&left(O#, 1) 2. Drag down for all records. 3. Copy and paste values* on top of itself (this will delete the formula) 4. In column V fill in the following formula: =upper(U#) 5. Drag down for all records. 6. Copy and paste values from column V into column U. 7. Delete all data in column V. Do not delete the header. 8. Highlight column U 9. Open the Find/Replace tool (cntrl F) 10. In the replace tab, set "Find what" to "." and "Replace with" to "." 11. Click Replace All. 12. Close Find/Replace

W	Subsample fate	archived
X and Y	Scientific Name Authorship and Identification References	<ol style="list-style-type: none"> 1. Highlight the data and open the sort menu. 2. Set "Sort by" to Genus 3. Add a second parameter by clicking "Add Level" 4. Set "Then by" to specific epithet 5. Click OK 6. Hide all columns in between the Genus/Species and the current columns. 7. Open the <i>Species Authors and Resources</i> file 8. Copy and paste the authorship and references information. 9. Be sure to Copy/Paste for each record, do not drag down as that will increase numbers by 1. 10. Close the <i>Species Authors and Resources</i> file.
Z	Identification Protocol Version	Always: GSU_tickIdentification_SOP_2016-11-01
AA	Identified by	<ol style="list-style-type: none"> 1. Open Find/Replace tool (cntrl F) 2. Click on the Replace tab 3. Set "Find What" to the name you need to expand 4. Set "Replace With" to the full name of that person 5. Click Replace All 6. Repeat for each person listed in the Identified by column. 7. Close Find/Replace
AB	Remarks	For anything not being invoiced, put "Over invoice limit, will not be billed."

*To Paste Values:

1. Copy something to your clipboard (Do not Cut)
2. Right click where you want to paste.
3. Under "Paste Options" select the icon for Paste Values.

16. Sort the file by RML.

17. Save the file.

18. Save a second time, replacing *_current* with the day's date in DDMonYYYY format.

Table 4: Data Dictionary for NEON Files

Field Name	Required	Format	Example	Notes
identifiedDate	N	YYYY-MM-DD	2013-09-12	
sampleID	Y	SITE_###.YYYYMMDD	HARV_003.20130712	sampleID reported by Field Operations
sampleCode	N	[A/B]#####	A00000001347	Barcode assigned to sample vials before shipment. May start with either the letters A or B followed by a string of numbers
targetTaxaPresent	Y	Y/N	Y	
scientificName	N	Genus species	Ixodes scapularis	
family	N		Ixodidae	
subfamily	N		Ixodinae	Ignore, Leave blank
tribe	N		Ticktribe	Ignore, Leave blank
subtribe	N		Ticksubtribe	Ignore, Leave blank
genus	N	Genus	Ixodes	
subgenus	N		Ticksubgenus	Ignore, Leave blank
specificEpithet	N	species	scapularis	
infraspecificEpithet	N	sub species	forma Beati	
identificationQualifier	N		aff. species	
instar	if targetTaxaPresent = Y, Y	Male/Female/Nymph/Larvae	Male	
individualCount	N	#	1	integer >= 0; leave blank if uncounted
sampleCondition	Y	OK/Damaged, analysis affected/Handling error/Other	Damaged, analysis affected	
archiveMedium	Y	RNA stabilization buffer/95% ethanol/Other/90% ethanol	RNA stabilization buffer	

archiveFacilityID	N	USNMENT#####	USNMENT00853485	Equivalent to internalLabID1
deprecatedVialID	N	SITE_###.YYYYMMDD.#.Genus species	HARV_003.20130712.1.3.Ixodes scapularis	What is on the tube label only if it does not match the sampleID; Usually blank
subsampleID	Y	SITE_###.YYYYMMDD.GEN SPE.I	HARV_003.20130712.IXOSCA.M	
subsampleFate	Y	archived/active/lost	archived	Most samples will be "archived", those sent for testing will be "active" - consult NEON Science for other situations
scientificNameAuthorship	N	Name, YYYY	Say, 1821	See <i>Species Authors and Resources</i> file
identificationReferences	N	[citation for key used]	[citation for key used]	See <i>Species Authors and Resources</i> file
identificationProtocolVersion	Y	GSU_tickIdentification_SOP_YYYY-MM-DD	GSU_tickIdentification_SOP_2016-11-01	Currently always: GSU_tickIdentification_SOP_2016-11-01
identifiedBy	N	First Last	Lorenza Beati	

NEON Invoices

Invoices are prepared at the beginning of the month for the previous month's work. Identifications must be completed or verified by Lorenza Beati in order to be billed. We charge per identification for adults and nymphs. Larvae are charged as one for each batch that is counted. We do not identify the larvae.

Billing has been done by Dr. Beati since 2019. She will keep doing this in future.

1. Open the file that needs to be billed in Microsoft Excel. This will be the Shipment's *_current* file.
2. Click on the **Data** tab.
3. Select **Filter**. This will make it possible to sort the file quickly.
4. Click the arrow next to the **Instar** heading and select **Sort A to Z**.
5. Click the arrow next to the **Instar** heading again and uncheck the term "larvae" from the list. Click **OK**.
6. Open the *NEON Billing Template*.
7. Copy and paste the contents of **column V (subsampleID)** in the identification file into **column C (Ticks in vials)** in the billing file. Because the larvae are filtered out they will not be pasted over at this time.
8. Copy and paste the contents of **column Q (individualCount)** from the identification file to **column F (Quantity)** in the Billing file.
9. Copy and paste the contents of **column A (Identification Date)** into **column B (Identification Date)**.
10. Click the arrow next to the **Instar** heading again. Recheck the term "larvae" from the list and uncheck all other terms. Click **OK**.
11. Copy and paste the contents of **column V (subsampleID)** in the identification file into **column C (Ticks in vials)** in the billing file. Because the adults and nymphs are filtered out they will not be pasted over at this time.
12. Enter "1" as the Quantity for all larvae.
13. Copy and paste the contents of **column A (Identification Date)** into **column B (Identification Date)**.
14. Click on the arrow next to the **Instar** heading and select **Clear filter from instar**.
15. Save the file three different ways:
 - a. Save as "Shipment #_DDmonYYYY", where the date is the current day's date
 - b. Save in the "Identified and Billed" folder for that domain with the file name "Domain #_DDmonYYYY_invoiced"
 - c. Delete the contents of the file, leaving only the column headings and save it as "Domain #_current"
16. If there are other Domains to invoice for this cycle, repeat steps 1-15. Otherwise move on.
17. **Column A** of the Billing file has two statements: "Identification to species with long-term storage and curation" and "Larval count with long-term storage and curation". All of the adults and nymphs should have column A populated with the first statement. All larvae should have column A populated with the second.
18. In **column D (Location Collected)**, drag the first populated cell down to copy the formula to all cells. The column should populate with the first four letters of the vial code in column C.
19. In **Column E (Individual cost)**, populate the entire column with "amount applicable for that year". Do not drag this down as it will increase by 1 each time.

20. In **Column G (Total Cost)**, drag the first populated cell down to copy the formula to all cells. This formula should multiply **column E (Individual Cost)** by **column F (Quantity)**.
21. Scroll to the very bottom of the data.
22. In the first empty cell for **column F (Quantity)**, get the sum of the column. Do the same for **column G (Total Cost)**
23. Double check the math for the sum of the total cost by multiplying the sum of the quantity by 15.
24. In **column H (Date Invoiced)**, enter the date you are preparing the invoice.
25. Save the entire file using the following naming scheme: NEON_billing_DDMonYYYY (the day and year are both digits, the month is the first three letters of that month's names).

Send the completed invoice file to Jennifer Dippolito (jdippolito@georgiasouthern.edu), accountant (in Research Accounting), and Krista Brinson (kbrinson@georgiasouthern.edu), Associate Director for Financial Accounting and Sponsored Programs, Office of Research Services and Sponsored Programs and CC Melissa Gast Goodman, the ICPS administrative coordinator (mgoodman@georgiasouthern.edu).

Files for Upload

When an invoice is sent to NEON a matching data file needs to be uploaded the NEON data portal. Report to NEON all counts and ids, including those that are not billed.

1. Open a blank data template file.
2. Open the *_DDmonYYYY_invoiced* file for each Domain included in the invoice.
3. Copy and paste records from the *_DDmonYYYY_invoiced* file into the blank template file.
4. Repeat for all Domains that were invoiced.
5. Remove column C (RML)
6. Save as a CSV file with the file name: tck_identification_in_GSU_YYYYMMDD (the date is the date of the invoice)
7. Upload the file to the NEON data portal*

*Note: You must have an account with NEON in order to access the data portal. As of August 2018, only Lorenza has account.

Creating USNTC FileMaker Files from NEON Files

Once files are uploaded to the Box, the file needs to be rearranged and reformatted for upload into the FileMaker-USNTC database.

Some of the fields will be discarded during the import process and can be ignored. Do not delete these fields. Additional fields will need to be added. See the table on the following page for the list of fields to be discarded and new fields to be added.

Table 5: Specify Workbench File

Field Name	Required	Format	Example	Notes	Keep/Discard/Add	Specify field
identifiedDate	N	YYYYMMDD	2013-09-12	Convert to MM/DD/YYYY format	Keep	Determination: Determined Date 1
sampleID	Y	SITE_###.YYY YMMDD	HARV_003.201307 12	sampleID reported by Field Operations	Discard	--
RML	N	#####	125555	For GSU use only. Not reported to NEON	Keep	Collecting Information: RML/CEN
targetTaxaPresent	Y	Y/N	Y		Discard	--
scientificName	N	Genus species	Ixodes scapularis		Discard	--
family	N		Ixodidae		Discard	--
subfamily	N		Ixodinae	Ignore, Leave blank	Discard	--
tribe	N		Ticktribe	Ignore, Leave blank	Discard	--
subtribe	N		Ticksbtribe	Ignore, Leave blank	Discard	--
genus	N	Genus	Ixodes		Keep	Determination: Genus 1
subgenus	N		Ticksbgenus	Ignore, Leave blank	Discard	--
specificEpithet	N	species	scapularis		Keep	Determination: Species 1
infraspecificEpithet	N	sub species	forma Beati		Keep	Determination: Subspecies 1

identificationQualifier	N		aff. species		Keep	Determination: Qualifier 1
instar	if targetTaxaPresent = Y, Y	Male/Female/Nymph/Larvae	Male	For Specify, rearrange into columns	Keep	Collection Object Attributes: #males; #females; #nymphs; #larvae
individualCount	N	#	1	integer >= 0; leave blank if uncouncted	Keep*	Insert counts into appropriate previous columns
sampleCondition	Y	OK/Damaged, analysis affected/Handling error/Other	Damaged, analysis affected		Discard	--
archiveMedium	Y	RNA stabilization buffer/95% ethanol/Other/90% ethanol	RNA stabilization buffer		Keep	Preparations: Prep Type
archiveFacilityID	N	USNMENT## #####	USNMENT0085348 5	Equivalent to internalLabID1	Keep	Collection Object: Catalog Number
deprecatedVialID	N	SITE_###.YYY YMMDD.#.Genus species	HARV_003.201307 12.1.3.Ixodes scapularis	What is on the tube label only if it does not match the sampleID; Usually blank	Discard	--

subsampleID	Y	SITE_###.YYY YMMDD.GEN SPE.I	HARV_003.201307 12.IXOSCA.M		Keep	Collection Object: Alt Cat Number
subsampleFate	Y	archived/active/lost	archived	Most samples will be "archived", those sent for testing will be "active" - consult NEON Science for other situations	Keep	Collection Object Attribute: Deposition
scientificNameAuthorship	N	Name, YYYY	Say, 1821	See <i>Species Authors and Resources</i> file	Discard	--
identificationReferences	N	[citation for key used]	[citation for key used]	See <i>Species Authors and Resources</i> file	Keep	Determination: nameaccordingto
identifiedBy	N	First Last	Lorenza Beati	For Specify separate into three columns	Keep	Determination: Determiner first name 1; Determiner last name 1; Determiner middle 1
Remarks	N				Keep	Collection Object Attribute: Remarks
Cataloger first	N				Add	Collection Object: Cataloger first name
Cataloger last	N				Add	Collection Object: Cataloger Last Name
Cataloger middle	N				Add	Collection Object: Cataloger Middle Initial

Cataloged date	N	MM/DD/YYYY Y	09/12/2013		Add	Collection Object: Cataloged Date
Count 1	Y				Add	Preparation: Count 1

Archiving Samples

Printing NEON labels

1. Log into Specify.
2. Click the **Reports** icon.
3. Under the **Labels** heading on the left, select the appropriate label. There are three to choose from for NEON:
 - a. *NEON labels*
 - b. *NEON locality only*
 - c. *NEON ID only*
4. In the window that appears, use the fields listed to create the labels you need. You will use either the **barcode number** (*labels and ID only*) or the **RML** (*locality only*)
 - a. Use the drop-down menu of operators as needed. The most useful is 'Between' which allows you to set ranges.
5. Click **OK** to run your Report. It will take a moment to load.
6. Zoom in on the labels to make sure they populated properly and that none of the text is truncated or overlapping.
7. Select the **Printer** icon at the top of the workspace to print your labels.
 - a. Alternately, you can choose to print to PDF at this point and save the labels, so they can be printed later or from a different computer.
8. Print the labels in 1023 using the computer that is hooked up to the ink jet printer. The printer should be loaded with cotton paper, not standard paper.
9. Carefully cut apart the labels. If you printed both label types at once, be careful not to separate the two labels.
10. Insert the labels into the sample vial. The locality label should face out against the glass. The identification label nests behind the locality label with the text facing into the vial.

Arranging Samples into Archival Jars

Samples are grouped into archival jars by site, not just domain. Up to five scintillation vials can fit into one jar. As jars are created they should be labeled "SITE YYYY Jar #". Write the labelling on the top of the lid using pencil. Once all jars for a Domain are completed, print the jar names using the label maker.

Arrange the jars in the drawers by Domain and year. As drawers are filled, label the outside of the drawer with the range of Domains for that year.

Subsampling for Disease Testing

Preparing Samples for Shipment to UMass

Only nymphs are shipped to University of Massachusetts for disease testing. Instructions for which samples to send will be received from NEON.

1. Consult the second page of the file *ticksForPathogenTesting2017_counts added.xlsx* or the printed list of samples.
2. Select a sample for preparation.
3. Choose specimens for archiving (retained). Try to select ones that are fully intact with mouthparts and all legs still attached. Place these back into the archival vial.
4. Place each individual specimen in separate 2ml sterile Eppendorf vials with NO ETHANOL. Verify simultaneously that counts are correct.
5. Place batches of ticks corresponding to single NEON collection events in small sealable plastic bags. Place the sampleID label and nymph count into the shipment plastic bag. If more than a species is contained in a batch, create separate and add the name of the species to the label.
6. Bags from same site and same species are placed in larger sealable bags.
7. Place the “# nymphs sent to UMass...” label into the archival vial.
8. Top off the archival vials with sample media (90% ethanol).
9. Repeat steps 2-8 for each sample to be prepared.
10. Return archival vials to their original location.
11. Group the samples for shipment by Domain.
12. Pack bagged samples into a cardboard shipping box.
13. Add enough air bags to cushion the samples while in transit.
14. Send Lorenza any corrections to the nymph counts, if needed.
15. Once all samples are prepped, contact Lorenza so she can ship the samples.