West Nile Surveillance Spreadsheet Protocol 2023

Weekly File Prep Work

\*This can be done before samples are given\*

* Create a new folder within the sharepoint file for “WNV-S” with the correct week number
* There should be two files labeled “Data from VDCI and TA” and “Protocols and CSU Data” along with 1 word document, and 2 excel files
  + “Data from VDCI and TA” holds the data emailed from both companies containing the traps they collected mosquitoes from and the amount of samples they collected also- this can be added to the sharepoint immediately after receiving the data via email
  + “Protocols and CSU Data” holds:
    - the sample plates you created before running the plates (FC\_LV RNA Plate 20XX WXX) which was taken from the emailed data from the companies
    - The results taken from the quantstudio for each plate (Initials-Date Ran and Plate Number)
    - When taking data from flycycler-> download data in eds form by dragging it onto the flashdrive
      * Plug flashdrive into beta ebel quantstudio and reanalyze the eds file
      * Then export results like normal
    - The general q-RTPCR WNV-s Protocol to have just for reference
    - WNV-s Extraction protocol that is adjusted each week to prepare the correct amount of master mix based on the amount of samples collected
  + FC\_LV Document Reporting 20XX Week XX is the official report that all data analyzed will be put into to send out
  + FC\_LV Full Report will also be sent out and contains the graphs and analyzed data
  + FC\_LV q-RTPCR Plate 20XX Week XX is a depiction of the plates with the positives highlighted with their CT scores along with the CT values of the controls and standards
    - This needs to be updated weekly by changing the samples from the older plate to match the new plates and new samples from the current week
* Duplicate/Open “FC\_LV Full Report 20xx Week xx” from previous week
  + Delete the four macro-generated sheets, “InfRateCI”, “InfRateZO”, “InfRateZone”, and “InfRateTotal”.

Uploading Results from Quant Studio

* Get flash drive and plug into computer
* Open quantstudio program and select desired plate results from the “Landon” folder (for Beta) or the “WNV Surveillance” folder from the Flycycler
* Once plate has been open go to “results” tab and then select “export” tab at the top
* Re-enter the title of the plate “Initials-dateP#” and select the flash drive to export the data
* Only have the box checked “results” before selecting export onto the flash drive
* Close the program and open the flash drive file to make sure that it was uploaded correctly
* Eject flashdrive from computer
* Plug in flashdrive into desktop computer and open the file
* The file should look like an excel spreadsheet
* Upload the file into the “Protocols and CSU Data” folder of the desired week

Results Analysis for Plates

* Go through the excel file and highlight all the wells containing the standards blue
* Enter the CT value of the standards into the “FC\_LV q-RTPCR Plate 20XX Week XX” file
* Highlight all the wells containing the controls green
* Enter the CT value of the controls into the “FC\_LV q-RTPCR Plate 20XX Week XX” file
* Highlight all the wells with a number in the L column (Quantity) above 50 a yellow color
* Enter the CT value of the positive samples into the “FC\_LV q-RTPCR Plate 20XX Week XX” file
* This document should have matching samples with the plate ran through the Quant Studio as the RNA plate listed to the side
* Record the efficiency of each plate from the results file into the “FC\_LV q-RTPCR Plate 20XX Week XX” file at the top of each plate
  + Want the value to be between 90-120
* Record the R^2 value of each plate from the results file into the “FC\_LV q-RTPCR Plate 20XX Week XX” file at the top of each plate
  + Want the value to be as close to 1 as possible
* Save and repeat the process for every plate ran through quant studio

Sheets/Tables Overview

* Within the Full Report excel spreadsheet, there are a number of unique sheets. Most of these will persist from week to week and you will need to replace the data and redo certain calculations, while others will be deleted and generated anew each week.
* The Weekly Data Input sheet contains the data for all pools tested. This sheet will be used to calculate infection rates based on species and location of each pool.
* The Weekly 009 Input sheet contains the data for all traps set, and all Culex mosquitoes caught. Not all of the mosquitoes trapped by VDCI/TA are submitted for testing, so this sheet will not reflect the pools tested or positivity. Instead, it is used to calculate mosquito abundance based on species and trap location.
* The four macro-generated sheets (listed below) are generated each week by using the PooledInfRate macro for excel, which is based on an infection rate calculation devised by the CDC. These sheets must be deleted and regenerated each week and will yield infection rate data used to calculate WNV+ proportions and vector index.
* The Total Number of Ind sheet contains a Pivot Table that displays the overall values for Cx. tarsalis and Cx. pipiens caught in each trapping zone/city each week. This data is pulled from the Weekly 009 Input sheet.
* Total Number of Ind Examined reflects the actual number of mosquitoes that are tested each week, and the data here originates in the Weekly Data Input sheet.
* Total Number of Pools Examined shows the grouping of the total mosquitoes tested into pools. The maximum number of mosquitoes in one pool is 50, as any more leads to too thick a homogenized solution. This data also comes from the Weekly Data Input sheet, and the total number of pools equals the total number of samples tested.
* Total Number of WNV+ Pools reflects the positive sample data based on sample origin (zone/city) and species and pulls from the Weekly Data Input sheet.
* The CITYINFRATE and ZONEINFRATE sheets are simply locations to enter macro-generated infection rates based on city/zone so that the final tables in the Graphs sheet have somewhere to pull from. These sheets are necessary as the macro-generated sheets will be deleted/renewed each week.
* The Graphs sheet is the result of the data analysis and calculations, and contains 6 charts, which show vector index, vector abundance, and number of mosquitoes per one thousand that are WNV+. Each table has an A chart with the current week’s data, as well as a B chart with the season’s data for all previous weeks.

Data Analysis

* “Weekly Data Input” sheet
  + Clear all data from previous week.
  + Copy data in from “LC Week xx WNV 20xx CSU Datasheet” document from VDCI. Always paste values
  + Copy Boulder County data from Timberline Aquatics data sheet.
  + Fill in CSU IDA pool #’s (final IDA # of previous week +1 or CSU ID# +9606)
  + Fill in test code (negative=0, positive=1) & test result (positive/negative), highlight positive rows
  + In unoccupied space to the side, copy/paste “ =Left(G2,2)&"-"&Left(L2,3) ”, where G2 is city and L2 is species. Pull down for all rows, then copy/paste values into PIR-City column. This column must have values only to ensure proper formatting of Pivot tables.
  + In the same manner, copy/paste “ =LEFT(I2,2)&"-"&LEFT(L2,3) ”, where I2 is zone. Pull down for all rows and copy/paste values into PIR-Zone column. This column must have values only to ensure proper formatting of Pivot tables.
* “Weekly 009 Input” sheet
  + Clear all data from previous week up to “Trap Number”.
  + Copy data in from “LC Weekxx\_20xx\_Culex” document from VDCI.
  + Copy data from Timberline Aquatics “Culex” document.
  + Sort by trap type and delete all gravid traps, as well as any malfunctioned/omitted traps if specified by VDCI or TA.
  + Go to “Total Number of Ind” sheet, click within the Pivot table, click “PivotTable Analyze”, “Change Data Source”, and expand/reduce selection to current week’s data, including all headers. Refresh pivot table if nothing changes.
  + Go to “Graphs” sheet and update “Number CDC light trap nights” column under 2A, if anything changed from previous week. Standard light trap nights by zone/city are as follows:

NW- 9

NE- 10

SE- 15

SW- 9

FC- 43

LV- 37

BE- 5

BC- 6

\*VDCI/TA will indicate changes to the usual trap night numbers, if necessary.

* Macros!
  + Go to the main WNV-s file in the T-drive and activate macros by clicking on “PooledInfRate” file (this part requires a windows computer). It seems to also only work if you download the Full Report spreadsheet instead of working out of SharePoint.
  + Generate four new sheets using the following steps and criteria:
    - Click “Add-ins” while in Weekly Data Input sheet, select “Pooled Infection Rate” & “One Sample” options.
    - Select the following columns for each Group name:

Name Group Column

InfRateCI PIR-City

InfRateZO PIR-Zone

InfRateZone Zone

InfRateTotal Account

* + - For Pool Size, click the Total column.
    - For # Positive, click the Test Code column.
    - Leave Number of Pools blank.
    - Select “Ok”
    - Change Group selection and associated name before hitting apply for each new sheet, the result should be four unique sheets with the above names. When selecting columns for Groups, click the top of the column to select all data.
  + Copy/paste the first two columns of “InfRateZO” into “ZONEINFRATE” sheet by first pasting into unoccupied space to the side, then pasting values into the labeled columns. Directly copying/pasting will disrupt the formatting of the graphs. ZONEINFRATE is Fort Collins only, so delete all rows with non-FC data before copy/pasting.
  + Repeat the same process for the first two columns of “InfRateCI” into “CITYINFRATE” sheet.
* Update Remaining Pivot Tables
  + Go to the “Total Number Ind Examined”, “Total Number of Pools Examined”, and “Total Number of WNV+ Pools” sheets and update the Pivot tables. Following the same process from earlier, select “PivotTable Analyze”, then “Change Data Source”, and select all data rows up to the Test Result column, including headers. Refresh pivot table if nothing changes.
* Update Graphs & Word Document
  + Check “Graphs” sheet for any reference errors or discrepancies.
  + Take data from “InfRateZone” into 3A graph (four zones, BC, BE, LV), and from “InfRateTotal” into 3A graph (FC-citywide).
  + Take right-most “All Culex” colums from each A graph (1A, 2A, & 3A) into the current week of the corresponding B graph.
  + Any city that did not submit pools for testing should receive “N/A” for every graph section.
  + Update the week numbers in the top left of each A graph.
  + Double check cells for correct formatting and consistent decimal places.
  + Copy all graph data into the corresponding tables in the “FC\_LV Document Reporting 20xx Week xx” word document.

\*\*Troubleshooting\*\*

* When generating macro-sheets, sometimes multiple entries for a single city/zone will appear, or multiple columns for the same species (i.e. “Tarsalis”, “ Tarsalis”, or “tarsalis”). If this happens, go to “Weekly Data Input” sheet and make sure all species names are exactly the same (i.e. “Tarsalis” and “Pipiens”). You may need to cmd F for extra spaces or capitalization discrepancies. If the macro program detects inconsistencies, it will create new entries that break apart data that should otherwise be considered together.
* If the macro sheets do not generate due to issues with data/group selection, make sure correct data including headers is selected. If an error saying “Runtime error ‘9’ subscript out of range” appears, sort the data sheet by the column you are using for the group.
* When updating Pivot tables, make sure to include headers in the data selection, and refresh the table if it does not automatically do so. Similar to the macro-generated sheets, inconsistent spelling/capitalization of data entries will disrupt Pivot table formatting and may cause multiple/repeated entries.
* Sometimes the right-hand table in each pivot table sheet will have reference errors, if this happens, simply change the affected cell’s formula to equal the corresponding cell of the left-hand pivot table. The right-hand tables feed into the Graphs sheet, so any graph reference errors may stem from this initial error. This typically only occurs at the beginning/end of the season when some zones/cities do not submit both *Cx. pipiens* and *Cx. tarsalis* for testing.