LEM'S IMMUNOGENICITY ANALYSIS NOW – USER GUIDE

Updated 8/23/19

1. Introduction

1.1 Purpose

This document is meant to serve as a reference for all users in order to enlighten and gain an understanding of how to explore all avenues of functionality within the tool. It has been developed and deployed with the help and interests of the following groups in mind:

- *Scientific Implementation Group (SIG)*
- *Clinical Advisor Group (CAG)*
- *Clinical Laboratory Operations (CLO)*

This tool is meant to automate several data analytics processes for various vendor cumulative files, study reports, and other files that fit the standardized format for processing (Section 2). Logical checks are also built into the tool to flag discrepancies in data entry or identify inconsistent testing between tiered testing results.

1.2 Scope

The application includes generalized calculations for samples and subjects across all visits in each dataset. Standard summary statistics built within this tool include: number of samples tested and detected in each tier, putative positive rate, confirmed positive rate, number of evaluable subjects, baseline positive rate, treatment emergent rate, treatment-induced and treatment-boosted rates, and number of subjects who are unevaluable. Currently, the tool handles cumulative files from the following vendors: BAL, PBI, PPD, and Wuxi.

1.3 System Organization

The R programming language and Shiny package are the primary software tools used to run this application.

R version 3.6.0 (2019-04-26), nicknamed “Planting of a Tree”

Other installed packages and their purpose throughout the script:
dplyr_0.8.1 (filtering and transforming data)
DT_0.6 (provides R interface for data tables on web pages)
ggplot2_3.1.1 (creates aesthetic plots)
readxl_1.3.1 (loads tabular data from Excel spreadsheets)
reshape2_1.4.3 (implemented when creating pivot tables)
shiny_1.3.2 (builds interactive web apps with R)
shinyjs_1.0 (JavaScript-like operations to enhance user-experience)
shinythemes_1.1.2 (includes several Bootstrap themes for styling)

shinyWidgets_0.4.8 (custom input controls and user interface components)
xlsx_0.6.1 (provides R functions to read/write/format Excel 2007)

2. Preprocessing Data Files

2.1 Trimming files

The R Shiny app will load any dataset with columns, whether it be .csv, .txt, or .xlsx. However, the application itself is only searching for a few specific columns to conduct analyses and build calculations. Thus, it is recommended to delete unnecessary columns. This will not only reduce processing power and time to load data, but also boost user experience and allow for a much simpler interface on the page.

2.2 Modifying columns to the standardized format

R is case-sensitive when processing columns by name. In a nutshell, correct capitalization, spacing, and spelling are essential to making this Shiny app execute properly. The order of the columns is not critical to how the application performs. At a minimum, the following five columns (and their exact syntax) are required for the tool to run properly:

Subject
Visit
Tier1
Tier2
Tier3

Larger datasets with multi-tier testing can also processed with the correct column names:

Tier2b
Tier2c
Tier2d
Tier4
Tier4b
Tier4c
Tier4d

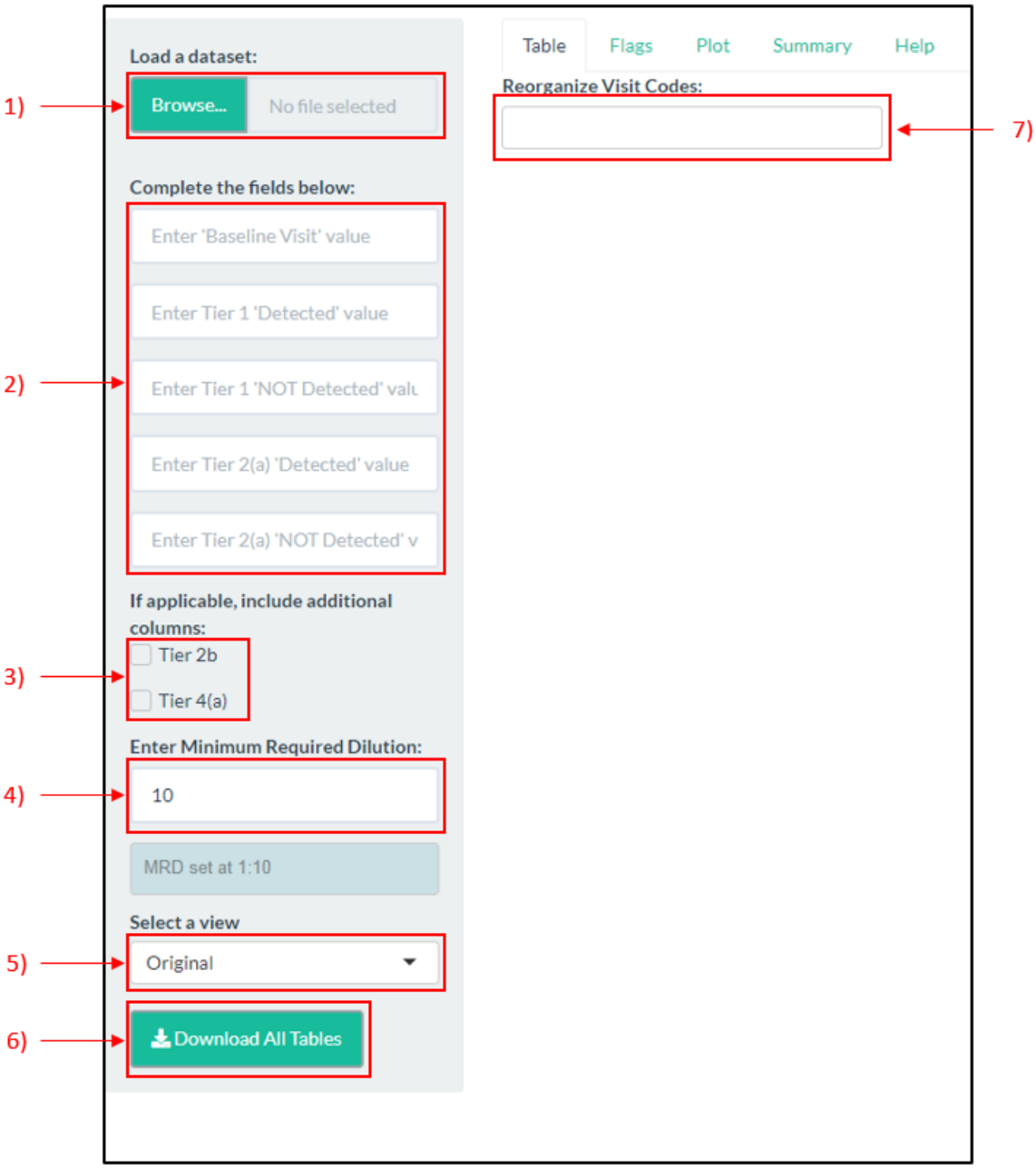
1	Subject	Visit	Tier1	Tier2	Tier3	Tier4
2	100-00102	BL/V2	NOTDETEC			
3	100-00102	V3	NOTDETEC			
4	100-00102	V4	NOTDETEC			
5	100-00102	V6	NOTDETEC			
6	100-00102	V7	NOTDETEC			
7	100-00102	EV1	NOTDETEC			
8	100-00102	EV2	DNR	DETECTED	1:10	NOTDETEC
9	100-00102	EV3	DNR	DETECTED	1:10	NOTDETEC
10	100-00102	EV6	NOTDETEC			
11	100-00103	BL/V2	NOTDETEC			
12	100-00103	V3	NOTDETEC			

3. Running the Application

3.1 Understanding the user interface

**Users must first have access to the Shiny Server – requires Lilly username and password.*
***This application lacks some functionality in Internet Explorer – Google Chrome is highly recommended.*

Once the web page has loaded, this is the landing page (Table tab) that will appear.

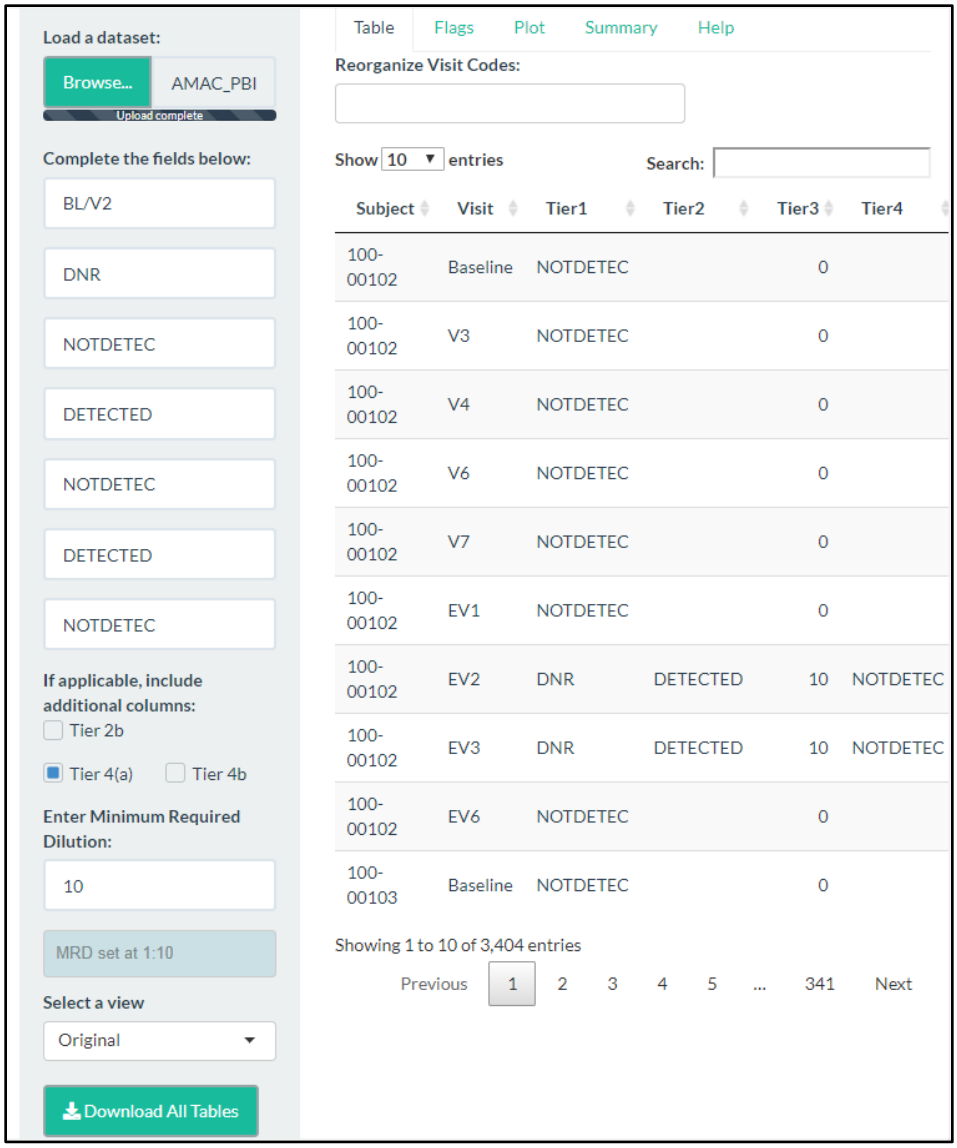


Elements of the primary interface include the following:

1) **File input field.** When clicked, the Browse button will open File Explorer.
2) **Text input field**. The user is required to complete all entry boxes with the corresponding, case-sensitive values for a loaded dataset.
3) **Checkboxes.** When selected, these will show or hide text input fields for additional tier columns.
4) **Numeric input field.** Sets the MRD for this particular dataset and is used for building many tables and summary statistics. Can be altered by the user to reactively update the tables and statistics. Default is set to 10.
5) **Dropdown list.** Shows more tables, many of which are reactively built once the "Baseline Visit" value has been entered. Others will require more user input.
6) **Download button.** Creates an export file that includes the original dataset along with all additional tables created in the app.
7) **Selection input field for visit codes.** This field loads all unique visit codes from the dataset, plus columns for Subject and highest titer per subject. It allows the user to rearrange the pivot tables in the best chronological order that they see fit.

3.2 First tab: Table

As data is being loaded into the page, the main panel will reactively display the table.



When the table is loaded on the page, the user should begin completing the text fields on the side panel. These include values for baseline visit, and detected and not detected values for Tier 1 and Tier 2. In the example above, the dataset contains an additional column for Tier 4. The corresponding checkbox for that tier is selected, revealing additional text fields for detected and not detected values in Tier 4.

Text fields are case-sensitive – capitalization, spacing, and spelling must match the values in the table. Copy and pasting values from the table to the text fields works fine, but double check for uneccessary white spaces in the text input field.

The "Select a view" dropdown shows seven tables that are available for viewing. Most will become accessible after the text input fields are completed:

1) **Original:** The initial table that was loaded into the tool. The titer column is processed automatically, populating rows with "0" if the value is non-numeric or blank, and trimming off every instance of the string "1:" if identified.
2) **Baselines:** A subset of the **Original** table, only displaying rows that match the user-entered baseline visit value for this dataset.
3) **Baseline Positives:** A subset of the **Original** table, only displaying rows that a) match the user-entered baseline visit value and b) match the user-entered tier 2 detected value for this dataset.
4) **Unevaluated Subjects:** Displays subjects who either a) are missing the baseline visit value and/or b) have a baseline visit without any follow-up visits. If the dataset does not logically identify any rows for either of these checks, the table is populated with an "EMPTY" premise. These subjects are not considered in the calculations for treatment emergence (Section 3.5).
5) **Subject Pivot Table:** Displays each unique subject and their Tier3 values for each visit. The highest post-baseline titer for each subject is also appended to this table (maxTiter). Any value of "0" indicates that the visit occurred for that Subject but no titer was reported. Any value of "–" is a placeholder, indicating that a visit for that subject has not occurred.
6) **Treatment Emergent Pivot Table:** A subset of the **Subject Pivot Table**, only displaying the following rows:
    a. Subjects who are negative at baseline, but have a reported titer that is at least two times greater than the MRD at any post-baseline visit (treatment-induced subjects).
    b. Subjects who are positive at baseline, but have a reported titer that is at least four times greater than the MRD at any post-baseline visit (treatment-boosted subjects).
7) **Titer Pivot Table:** Shows each unique baseline titer and the counts of each maximum post-baseline titer. Any value of "0" indicates a titer was not detected. Grand totals of each row and column are included.

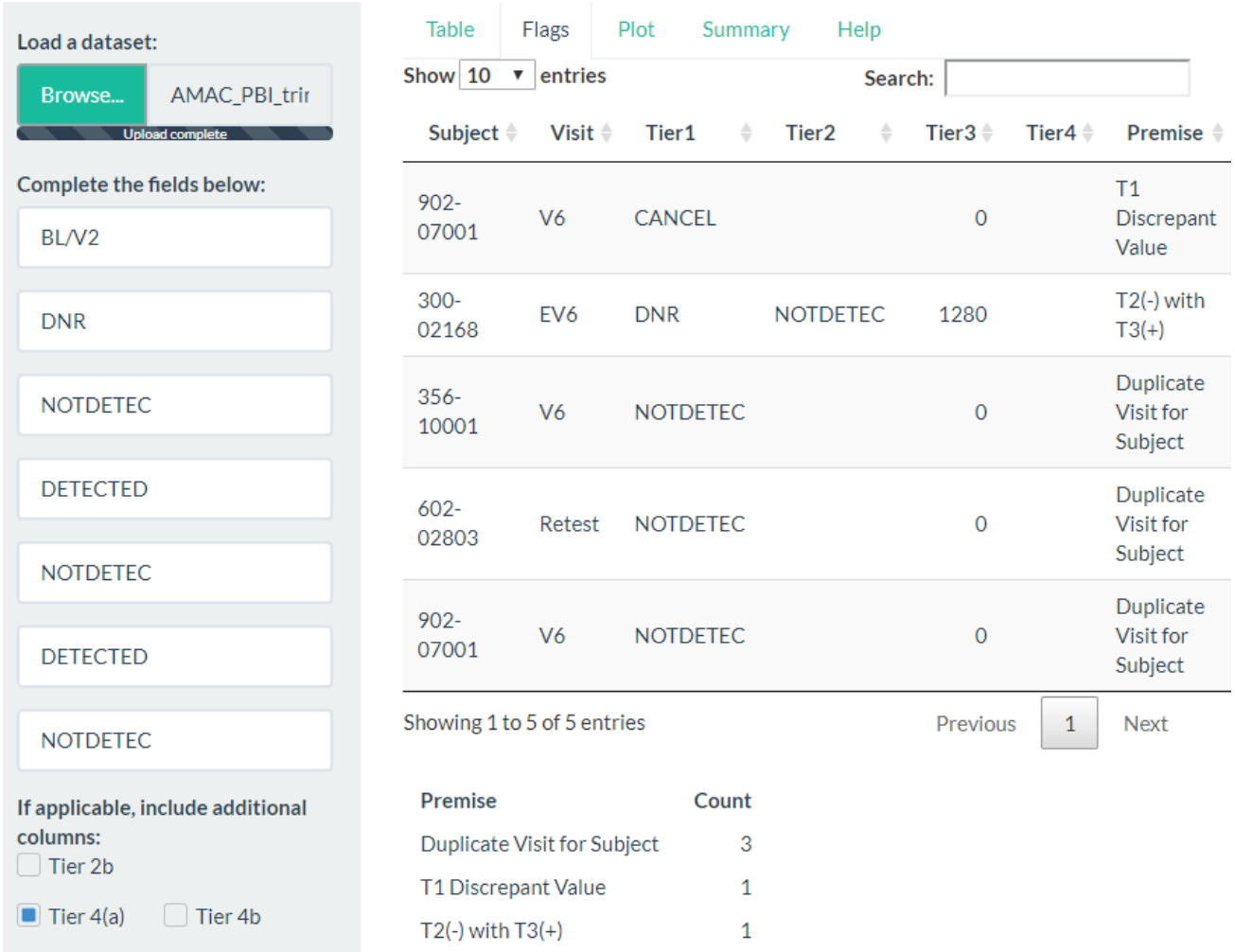
*The input field "Reorganize Visit Codes" only applies to the **Subject Pivot Table** and the **Treatment Emergent Pivot Table**.*

It is possible to have data that yields no results for baseline positives and/or treatment emergent subjects. These tables are handled in a similar way to the Unevaluated Subjects table, populating the tables with feedback that indicates no subjects were identified.

The Download All Tables button provides a quick and simple export to an Excel workbook. All seven tables in the tool are separated into separate Excel sheets. An eighth sheet is also included for any search results that a user completes in the search bar. In order for search results to appear in the Excel workbook, the search must be done on the **Original** table view.

3.3 Second tab: Flags

This QC table updates with each text input entry provided by the user. Logical statements are built into the tool to identify various types of QC checks from Tier 1 through Tier 4.



The reasoning behind each row being flagged in the dataset is included in the Premise column. A frequency table showing the sum of each Premise in the QC table is also included.

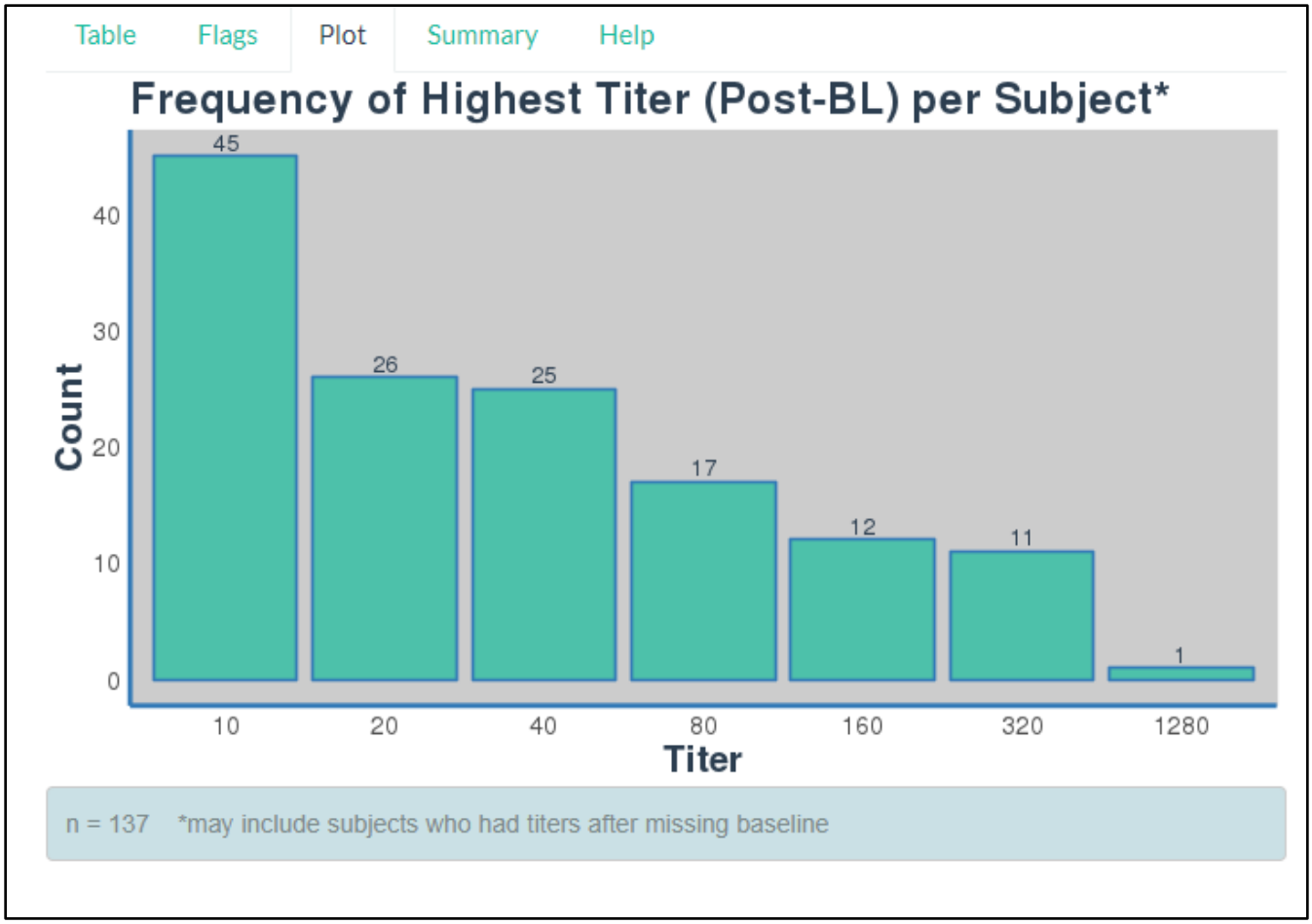
The QC checks can be categorized in the following ways:

- **Discrepant value flags:** instances where any of the values in the tier columns do not match the user-entered values for detected and not detected. In Tier 3, values that are not a multiple of the MRD or are non-numeric are also flagged.
- **Between tier flags:** scenarios where, logically, test results are inconsistent or unnecessary from one tier to the next.
- **Duplicate visit flags:** shows subjects who have multiple records of the same visit code (it is possible that these are retests).

An exhaustive list of all possible QC checks can be found on the Help tab.

3.4 Third tab: Plot

*On load, the titer histogram reactively charts the frequency of the highest titer in the **Subject Pivot Table**. It automatically excludes a bin for titers that are 0, or not detected.*



The total count of the histogram is shown beneath the table. It is worth noting that instances where subjects missed baseline but later had a reported titer in the **Subject Pivot Table** are also reported in this plot, but not considered for treatment emergence.

3.5 Fourth tab: Summary

A few tables reactively update and display statistics once the user begins filling out the text input fields. The second and third tables will be built upon the user entering a value for baseline visit.

Table | Flags | Plot | Summary | Help

	SamplesTested	Detected	PositiveRate
Tier 1	3403	1324	38.91%
Tier 2	1324	886	66.92%
Tier 4	886	532	60.05%

	Count	Rate
Subjects Evaluable for TE ADA	247	100%
Evaluable Subs with ADA Present at Baseline	38	15.38%
Subjects TE ADA	75	30.36%
Treatment-Induced	59	23.89%
Treatment-Boosted	16	6.48%

	Sort
Total Unique Subjects	253
Unevaluated Subjects	6
Highest Post-Baseline Titer	1280

The first table displays statistics for samples in each tier. This table automatically identifies unique tier columns in the dataset and creates a row for each tier.

1) **SamplesTested** is the sum of the user-entered detected and not detected values in each tier.
2) **Detected** is the sum of the user-entered detected values in each tier.
3) **PositiveRate** is number of samples **Detected** / number of **SamplesTested** in each tier. Putative positive rate and confirmed positive rate correspond to Tier 1 and Tier 2 respectively.

The second table displays treatment emergent statistics.

1) **Evaluable Subjects** are those that have a baseline visit and at least one follow-up visit.
2) **Evaluable Subs with ADA Present at Baseline** are considered Baseline Positive.
3) **Subjects TE ADA** are those that are treatment emergent and are either:
    a. **Treatment-Induced**: subject has an unreported titer at baseline but reaches a titer that is two times the MRD at a later visit.
    b. **Treatment-Boosted**: subject has a reported titer at baseline and reaches a titer that is four times their baseline value at a later visit.

The third table displays a few more general statistics about the dataset.

1) **Total Unique Subjects** is the sum of **Evaluable Subjects** and **Unevaluated Subjects**. It is the number of distinct patients in the dataset.
2) **Unevaluated Subjects** are those that either:
    a. Missed baseline visit
    b. Have a baseline visit without any follow-up visits
3) **Highest-Post Baseline Titer** in the dataset is reported from the **Treatment Emergent Pivot Table**.

3.6 Fifth tab: Help

Users can consult this page to get a synopsis on a few main ideas, such as how to preprocess a file correctly, get a detailed list of all logical QC checks built into the tool, and a recap of how the summary statistics tables are created.

Extended documentation to come soon