# AVP 48.01

# User Requirement Specifications (URS) for:

# QACAR (qPCR Auto Calc And Report)

Prepared by Ross Fu and James Frost

## TABLE OF CONTENTS

Table of Contents ..... 2
I. Executive Summary ..... 3
II. Definitions ..... 3
III. Responsibility ..... 4
IV. References ..... 4
V. User Requirement Specifications ..... 4
A. General Requirements ..... 4
B. Program Requirements ..... 5
C. Data Validation ..... 13
D. Conditional Formatting ..... 18
E. Formulas ..... 18
F. Reports, Printouts & Charts ..... 19
VI. Acceptance Criteria ..... 24
VII. Validation Summary Report ..... 24
VIII. Data Flow Diagram ..... 25
IX. Approvals ..... 26

## I. EXECUTIVE SUMMARY

The analysis of Quantitative Polymerase Chain Reaction (qPCR) data has several issues stemming from the inherently large volumes of data that are typically generated for a biodistribution study. The data analysis is currently time consuming and prone to anomalous behavior in Excel due to human error. A Python program designed to automatically perform qPCR data analyses could solve many of these challenges by quickly and consistently processing qPCR data. Analysts could use this program to produce accurate calculations and reports instead of doing them manually.

However, using a Python program to analyze qPCR data poses its own risks. If the program is not designed correctly, the output will not be correct either. Also, due to the variation in qPCR data files and the nature of how this program will be used (consecutive runs on the same data folder), the program has to be able to recognize and handle many different scenarios. However, once the program design addresses these issues, it will deliver accurate outputs consistently and quickly.

Avance will benefit from having an automated solution for qPCR data analysis. This program supports the endeavors of the quality team as well as the analysis team. The program works by opening the data file from each qPCR run, extracting information from the data, performing calculations (see section C for list of calculations), and writing both the data and calculations into a QACAR Excel spreadsheet, which can send the results to SCS 19. The program is able to analyze large volumes of qPCR data, calculate and report the results in a matter of seconds. QACAR is designed to integrate its analytic capabilities seamlessly into the existing workflow for SCS 19.

## II. DEFINITIONS

- **Python** – A programming language that will be used to create this program. Python is an interpreted high-level general-purpose programming language. Its design philosophy emphasizes code readability with its use of significant indentation. Its language constructs as well as its object-oriented approach aim to help programmers write clear, logical code for small and large-scale projects.

- **qPCR** – Quantitative Polymerase Chain Reaction. A PCR method used to quantify the number of copies or relative number of copies of a target sequence.

- **SCS19** – A controlled Excel workbook used to manage data during the course of a biodistribution project.

- **QACAR** – (qPCR Auto Calc and Report) the spreadsheet containing the VBA and Python code that analyzes, reports, and transfers qPCR data to SCS 19.

- **AutoForm10** – is a Python script designed to process qPCR instrument output files in text (.txt) or comma-delimited (.csv) format. Once the data is processed, it generates reports and provides basic analysis of the results.

- **QACAR Controls** – a sheet within QACAR, designed to control the QACAR program.
- **Root Path Settings** – a sheet within QACAR, designed to store/change root path settings
- **TeraCopy** – an application used to copy critical data file transfers with an automatic SHA-256 hash verification.

## III. RESPONSIBILITY

- It is the responsibility of the software developer to develop controlled software that meets the requirements spelled out in this URS.
- It is the responsibility of the employee conducting the validation to follow the validation plan, document deviations and prepare the summary report.

## IV. REFERENCES

- 21 CFR Part 11 Electronic Records; Electronic Signatures
- 21 CFR Part 58
- SOP LO QPCR 08 QPCR Absolute Quantification for a Biodistribution Study
- SCS 19 Biodistribution Study

## V. USER REQUIREMENT SPECIFICATIONS

### A. General Requirements

a. Excel version XX or above.

b. Windows 10 OS

c. Python version 3.9.6

d. Pandas version 1.3.1

e. TeraCopy 3.9

f. Datetime version 4.3.

g. Openpyxl version 3.0.7

h. SCS 19 needs to be stored in an Excel trusted location.

i. QACAR needs to be stored in an Excel trusted location.

j. The VBA code will be password protected and locked from viewing.

k. Parameters in Root Path Settings are password protected.

l. If a required parameter is not filled in prior to clicking a button, the user is prompted to enter the missing input.

m. Data validation is employed to ensure parameter values that have been inputted and are within acceptable ranges.

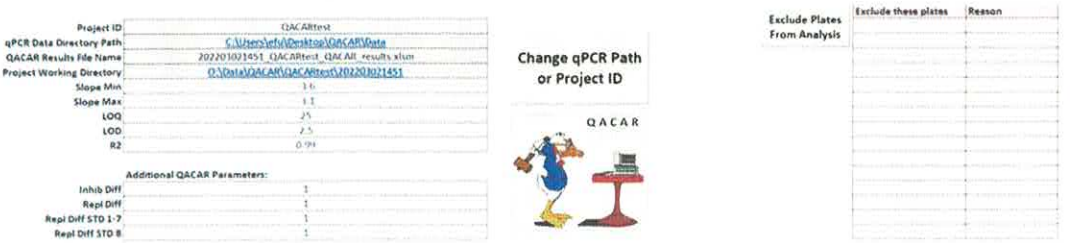
n. If a parameter value doesn't meet data validation requirements, a pop-up box will prompt them to do so.

o. DATA VALIDATION ALSO ENSURES TEXT-BASED PARAMETERS CONTAIN SPECIFIC SUBSTRINGS.

p. FILE AND FOLDER DIALOGS ARE EMPLOYED TO ENSURE VALID FILES AND FOLDERS ARE SELECTED.

## B. PROGRAM REQUIREMENTS

a. QACAR CONTROLS TAB




(1) *Change qPCR Path or Project ID Button*

This function is designed to allow the user to modify the Project ID, qPCR data directory path, QACAR results file name and Project working directory parameters. These parameters dictate how the program operates.

a. *After clicking the button, an input box comes up for the user to enter the Project ID.*

b. *The input is recorded in QACAR controls tab cell B2.*

c. *A select folder dialog box opens, titled 'Please select the qPCR Data Directory'*

d. *Once a folder is selected, the absolute path of the selected folder is recorded in QACAR controls tab cell B3.*

e. *QACAR controls tab cell B4, which corresponds to the resulting file name, is updated in this convention: {YYYYMMDDHHmm}_{ProjectID}_QACAR_results.xlsm*

f. *QACAR controls tab cell B5, which corresponds to the output folder path, is updated in this convention: \\birch\Operations\Data\QACAR\{ProjectID}\{YYYYMMDDHHmm}*

(2) *QACAR Button*

qPCR Auto Calculate and Report (QACAR) is a software pipeline designed to analyze qPCR data.

a. *QACAR VBA Code*
   i. *The program requires the user to input 13 parameters (Project_ID, qPCR data directory path, Excel QACAR Results File Name, Project Working Directory, Slope Min, Slope Max, LOQ, LOD, R2) from QACAR Controls cells B2:B10, (Inhib Diff, Repl Diff, Repl Diff STD 1-7, Repl Diff STD 8) from QACAR Controls cells B13:B16*
   ii. *Once the user clicks the QACAR (or Run AutoForm10) button the VBA code will: calculate a timestamp and update the QACAR results file name parameter and the Project Working Directory parameter to reflect the newly calculated timestamp.*
   iii. *Then it creates a temporary directory to store all the output files: \\birch\operations\QACAR\{Project_ID}\{YYYYMMDDHHMM} (hereby referred to as the Temp Share Directory)*
   iv. *Creates a VBS script file in the Temp Share Directory.*
   v. *Saves and closes the QACAR spreadsheet.*
   vi. *Finally, it executes the VBS script file, which runs a Python program to analyze the qPCR data.*

b. *QACAR Python Code*
   i. *The program makes the Temp Share Directory if it doesn't already exist.*
   ii. *The program creates a run log in the Temp Share Directory, which is named: {Project_ID}_{Date}_{Time}_AF10_run_log.txt*
      a. *The program log will record the username and computer ID that executed the program as well as timestamp each log entry executed.*
      b. *At the end of the run, the program log will also be copied to the Audit_Logs write once directory as an audit trail.*
   iii. *The program deletes the VBS script from the Temp Share Directory.*
   iv. *The program validates that the user input parameters are valid and that program requirements from section IV.A.c-g are met*
      a. *If the parameters are invalid or the requirements are not met, the program stops and reports that information via the Python window and in the run log.*
   v. *The program creates three folders for modified data if they don't already exist: 'Modified_7900_data_files', 'Modified_Quant_data_files', and 'Modified_7500_data_files' folders*
   vi. *The program creates two *.txt files if they don't already exist.*
      a. *Plate_information.txt: preserves info about each plate/file*
      b. *Re_Run_Sample_Info.txt: preserves info about which samples failed and if they were rerun.*

vii. *The program determines which files to exclude from analysis from columns I & J of the QACAR Controls tab of the spreadsheet. Then the program reports which files it will exclude via the Python window, Plate_Information.txt and Re_Run_Sample_Info.txt.*

viii. *The program creates a list of data files to analyze by filtering the files in the qPCR_Data_Directory_Path:*

a. *To include files ending with '.txt', '.csv' and '.xlsx'.*

b. *To exclude files that are listed in the Plate_Information.txt file.*

c. *To exclude run logs.*

d. *To exclude modified/re-encoded files.*

e. *To exclude data files that have records in the Plate_information.txt file.*

f. *To exclude files that are in the exclude list.*

g. *To exclude the Plate_Information.txt and Re_Run_Samples_Info.txt files.*

h. *To exclude files that do not match the expected format of the 7900, 7500 or QuantStudio instruments.*

ix. *The program checks if the QACAR spreadsheet is open and if open, pauses to ask the user to close the file before continuing.*

x. *The program will create the QACAR spreadsheet that will store the QACAR results, if it doesn't already exist.*

xi. *The program begins processing every data file in the list generated in Step viii.*

a. *The program identifies which instrument created the data file.*

b. *The program extracts the raw data from the data file.*

c. *For the 7900 instruments an intermediate file is made: (MODIFIED_7900_FILE_filename).*

d. *For the ABI 7500 Real-Time PCR instrument, the process results in the generation of two intermediate files: (RE_ENCODED_7500_FILE_filename, and MODIFIED_7500_FILE_filename).*

e. *For the QuantStudio 12K Flex qPCR instrument, the process results in the generation of an intermediate file: (MODIFIED_QUANTSTUDIO_FILE_filename).*

f. *The data files are normalized through further processing:*

a) *clear N/A samples*

b) *remove commas from all numbers*

c) *standardize column names*

d) *standardize SPK Control name*

e) *standardize Assay Control name*

*f) clear white space from sample names*

*g. The program uses the normalized data to calculate: Replicate Quantity Mean, Replicate Ct differences, SPK average, +SPK sample Ct difference from SPK average, Inhibition (True/False). Then the program pastes the calculations as a table for each file within the QACAR spreadsheet's Form10_Raw Data tab.*

*h. The program writes the plate failure status next to the raw data for that plate in the Form10_Raw Data tab.*

*i. The program lists any healthy samples from a plate next to the raw data for that plate on Form10_Raw Data*

*xii. The program lists all healthy samples from the entire run in Form10_Raw Data columns AF & AG. These batches of healthy samples are positioned in such a way that they will fall into SCS 19's Form 10 tables when transferred.*

*xiii. The program generates four new tabs:*

*a. Form12_Plate Report: summarizes whether qPCR plates/samples failed and why. Passing plates are additionally indicated by a green status indicator.*

*b. Form13_Re Run Samples Report: summarizes all of the qPCR samples that failed, the reason and identifies if previously failed samples were rerun.*

*c. Form14_AC Report: produces calculations on the assay control (AC) values of passing plates.*

*d. Form15_Y Intercept Report: summarizes whether STD values and Y intercept values have shifted throughout the course of the project.*

*xiv. The program will save the Plate_Information.txt and Re_Run_Sample_Info.txt files, the QACAR spreadsheet, and its backup file to the Temp Share Directory.*

*xv. If the run was successful, these additional actions are performed:*

*a. The Plate_Information.txt and Re_Run_Sample_Info.txt files are updated so that the plates within those files are marked as successfully processed.*

*b. The run logs are updated and archived.*

*c. The program calls TeraCopy to copy the entire Temp Share Directory into the write once Final Share Path. TeraCopy logs the copy details in a MySQL database and SHA-256 hash verifies the file copies.*

d. *The program checks if the Temp Share Directory and Final Share Path have the same number of files, if they do, the program deletes the Temp Share Directory. If they don't, the program reports the TeraCopy operation was unsuccessful.*

xvi. *In the event the run was unsuccessful, these additional actions are performed:*

a. *The Python window stays open until the user closes it.*

b. *The Plate_Information.txt and Re_Run_Sample_Info.txt files are updated. The unsuccessfully processed plates are removed from the files.*

c. *The run logs are updated and archived to the Data Directory Path in the Audit_Logs subdirectory.*

xvii. *Whether the program is successful or has an error, the updated run log files should contain the following information:*

a. *User name and computer ID of person who ran the program*

b. *Whether the user's system has validated software versions*

c. *Time and date the program was run*

d. *A list of all files processed*

e. *A list of all errors encountered during the run*

f. *Confirmation that the run completed successfully or not.*

(3) *Exclude Plate Button*

This function is designed to exclude files from the QACAR program's analysis/reports.

a. *After clicking the button, a file dialog box opens.*

b. *Once the user selects a file, the user is prompted to enter a reason for the exclusion.*

c. *The function handles invalid inputs:*

- If the selected file is invalid then the user is notified with a message box and no file is added to the exclusion list.
- If an exclusion reason is not provided then the user is notified with a message box and the excluded file is removed from the list.

d. *The excluded file and its reason are written in QACAR Controls tab columns I & J.*

b. FORM10_RAW DATA TAB

(1) *Move Data to SC19 Button*

This function is designed to transfer the Form10_Raw Data outputs from the QACAR spreadsheet into the specified SCS19's Form10_Raw Data tab.

a. *If the user tries to use this button before populating Form10_Raw Data with QACAR's outputs, the function reports that the QACAR program must be run first.*
b. *If the QACAR results are in the Form10_Raw Data tab, a select file dialog box opens.*
c. *Once the user selects the file, the function checks cell T11 of the selected file for the words "Plate Fail Reason" and if they exist, then transfers QACAR's Form10_Raw Data contents (cells B1:AM20000) into the same cells in the same tab in the selected file.*
d. *If cell T11 doesn't have the specified text, the function reports that the transfer target file didn't have the correct format and exits.*
e. *The function then activates the Form10_Raw Data tab to display the transferred data.*
f. *Finally, the function saves a copy of the original QACAR results file (with transferred data) under a new name: {date}{time}_QACAR_Results_ (selected filename).xlsm. This file gets saved to the same directory as the file selected.*

c. ROOT PATH SETTINGS TAB

| | | |
|---|---|---|
| Assign QACAR Template Path | **QACAR Template Path** | O:\5 GlobalShare\Python Scripts\AutoForm10\QACAR\QACAR_Master_Template.xlsm |
| Assign qPCR Data Root Path | **qPCR Root Path** | C:\Users\efu\Desktop\QACAR |
| Assign Project Output Path | **Project Output Root Path** | O:\Data\QACAR |

(1) *Assign QACAR Template Path Button*

This function is designed to input a file path for the QACAR template.

a. *Once the user clicks the button, the function asks for a password.*
b. *If an incorrect password is given, the function brings up a message box to inform the user of the incorrect password and exits.*
c. *Once the correct password is given, a select file dialog box opens.*
d. *After the user selects a file, the path to that file is written to the QACAR Template Path in cell F4 of the Root Path Settings tab.*

(2) *Assign qPCR Data Root Path Button*

This function is designed to set the root directory that the "Change qPCR Path or Project ID" function of QACAR Controls will begin in when opening a folder select dialog box for setting the qPCR Data Directory Path.

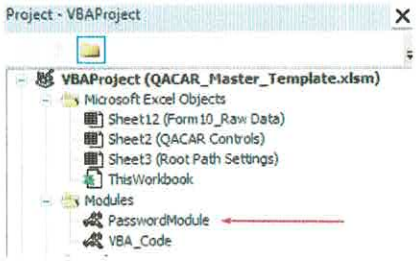
a. *Once the user clicks the button, the function asks for a password.*
b. *If an incorrect password is given, the function brings up a message box to inform the user of the incorrect password and exits.*
c. *Once the correct password is given, a select file dialog box opens.*
d. *After the user selects a file, the path to that file is written to the Root Path Settings cell F5.*

(3) *Assign Project Output Path Button*

This function is designed to input a root directory for the Project Output Path.

a. *Once the user clicks the button, the function asks for a password.*
b. *If an incorrect password is given, the function brings up a message box to inform the user of the incorrect password and exits.*
c. *Once the correct password is given, a select file dialog box opens.*
d. *After the user selects a file, the path to that file is printed in Root Path Settings cell F6.*

d. PASSWORDMODULE



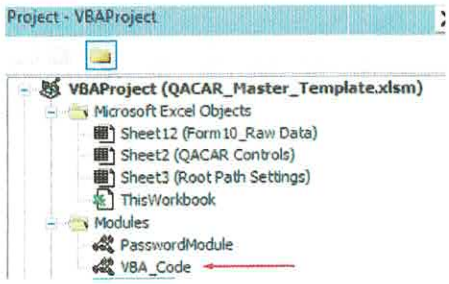

(1) *Function NewProc*

A function used to create the password masking for the InputBoxDK function.

(2) *Function InputBoxDK*

Function used for creating an input box for entering passwords without being able to view the password entered.

e. VBA_CODE MODULE



(1) *Sub AssignParams*

Called when the program opens or when the QACAR Controls tab Change qPCR Path or Project ID button is clicked. Used to assign the Project ID, qPCR Data Directory Path, QACAR Results File Name and Project Working Directory.

(2) *Sub ReassignParams*

Clears the cells B2:B5 on the QACAR Controls tab and then calls AssignParams.

(3) *Sub ExcludePlates*

Called when the "Exclude Plates from Analysis" button is clicked on the QACAR Controls tab. Allows the user to exclude qPCR runs from the QACAR analysis.

(4) *Sub WriteVBScriptForAutoForm10*

Called when the QACAR Controls tab QACAR button is clicked. Used to run the QACAR VBA, VBS, and Python programs. Processes all qPCR files in the path specified by the user.

(5) *Function ShellRun*

Function used to execute the VBS script that starts the Python QACAR programs.

(6) *Function SelectFolder*

Function used to bring up a folder selection dialog.

(7) *Function SelectFile*

Function used to bring up a file selection dialog.

(8) *Function CheckFolderExists*

Function used to determine if a folder exists.

(9) *Function CreateDirectory*

Function used to create a file directory.

(10) *Sub GetQPCRRootPath*

Called when the Assign qPCR Data Root Path button is clicked on the Root Path Settings tab.

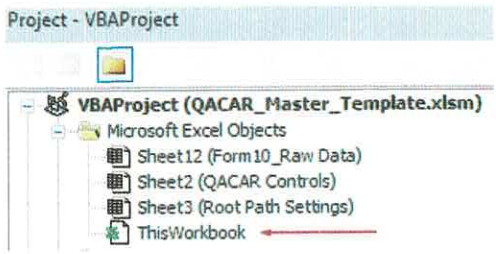
(11) *Sub GetTemplatePath*

Called when the Assign SCS19 Template Path button is clicked on the Root Path Settings tab.

(12) *Sub PutInfoInSCS19*

Called when the Assign Project Output Path button is clicked on the Root Path Settings tab.

f. THISWORKBOOK




(1) *Sub Workbook_Open*

Called whenever the spreadsheet is opened. Selects the QACAR Controls tab and calls the sub AssignParams.

## C. DATA VALIDATION

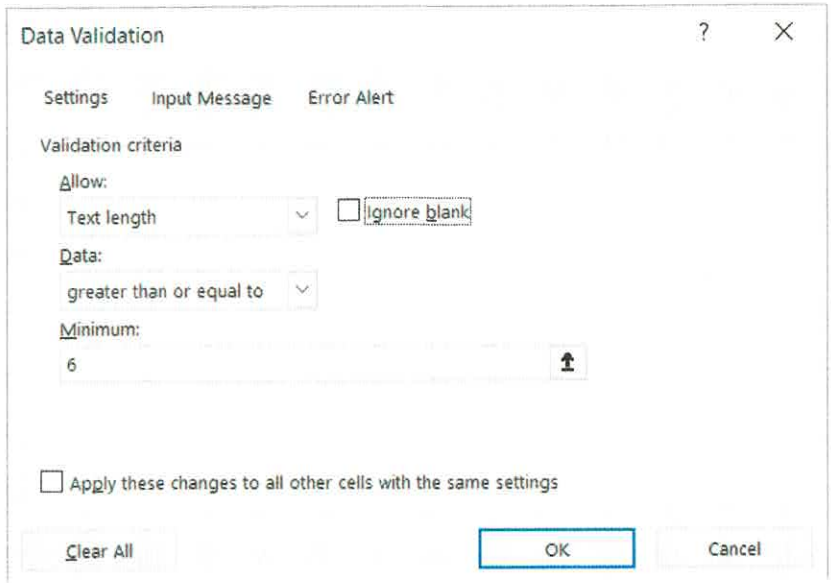
a. QACAR CONTROLS TAB

Yellow cells have data validation

| | |
|---|---|
| Project ID | |
| qPCR Data Directory Path | |
| SCS1900 Results File Name | |
| Project Working Directory | |
| Slope Min | -3.6 |
| Slope Max | -3.1 |
| LOQ | 25 |
| LOD | 2.5 |
| R2 | 0.99 |

Additional QACAR Parameters:

| | |
|---|---|
| Inhib Diff | 1 |
| Repl Diff | 1 |
| Repl Diff STD 1-7 | 1 |
| Repl Diff STD 8 | 1 |

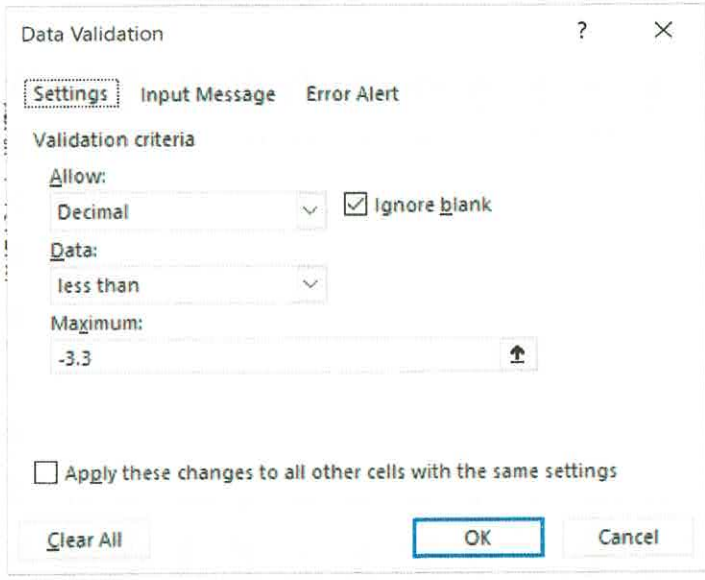



(1) *Cell B2 –*

(2) *Cell B3 – Uses "=(FIND("\",B3)<>0)" to ensure a path is entered*

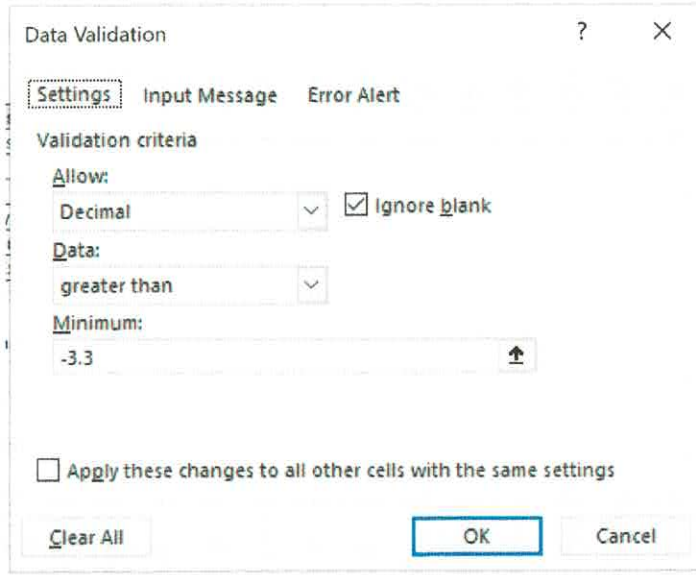
(3) *Cell B4 – Uses "s=(FIND(".xlsm",B4)<>0)" to ensure an Excel VBA enabled workbook was entered*

(4) *Cell B5 – Uses "=(FIND("\",B5)<>0)" to ensure a path was entered*

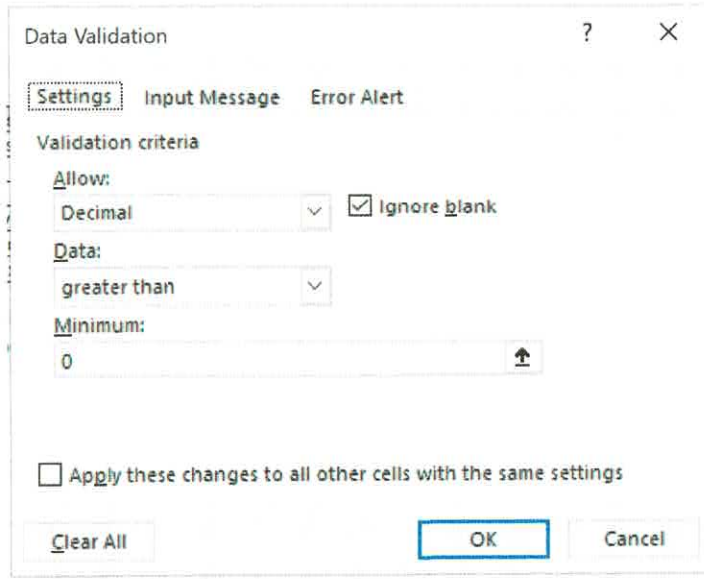



(5) *Cell B6 -*

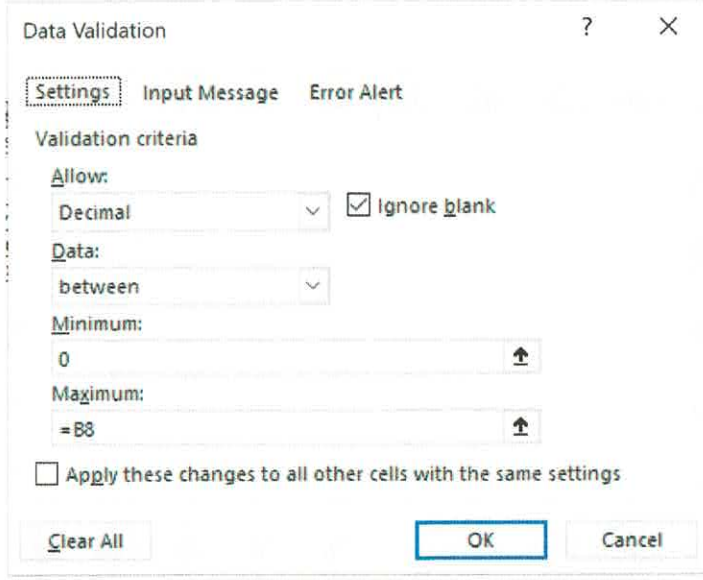
(6) *Cell B7 -*



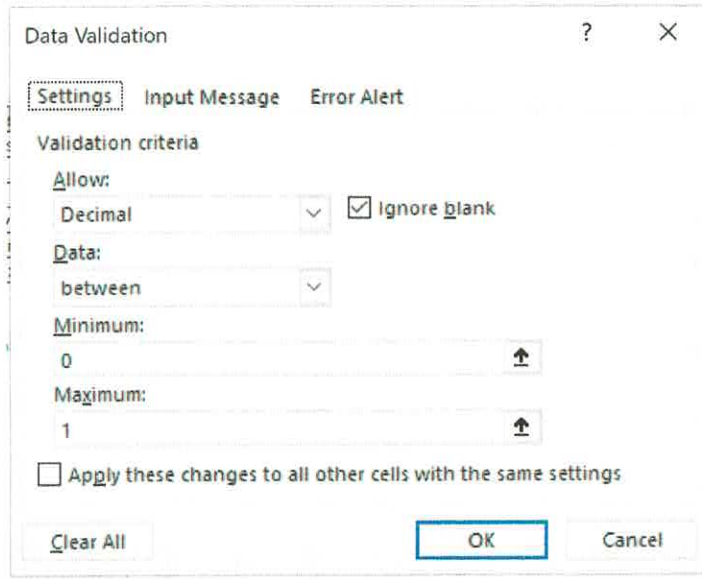

(7) *Cell B8 -*

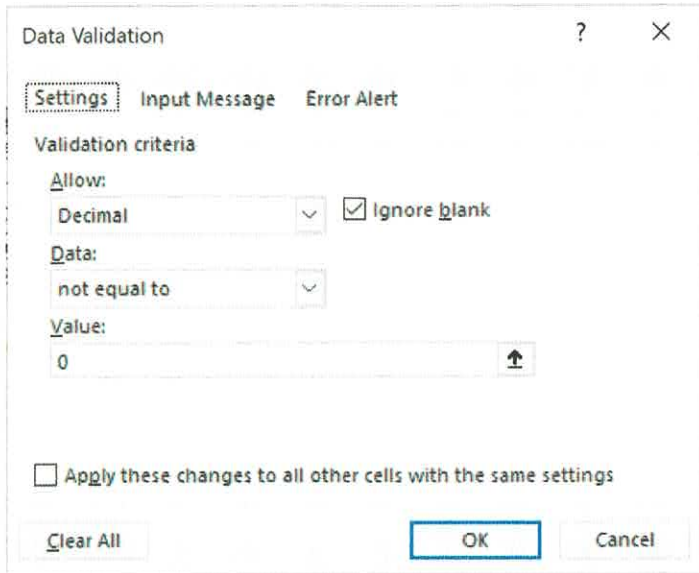


(8) *Cell B9 -*




(9) *Cell B10 -*






(10) *Cell B13:B16 -*

b. FORM10_RAW DATA TAB – N/A

c. ROOT PATH SETTINGS TAB – N/A

d. MODIFIED_7900_DATA_FILES TAB (AFTER RUNNING QACAR BUTTON) – N/A

e. MODIFIED_QUANT_DATA_FILES TAB (AFTER RUNNING QACAR BUTTON) – N/A

f. MODIFIED_7500_DATA_FILES TAB (AFTER RUNNING QACAR BUTTON) – N/A

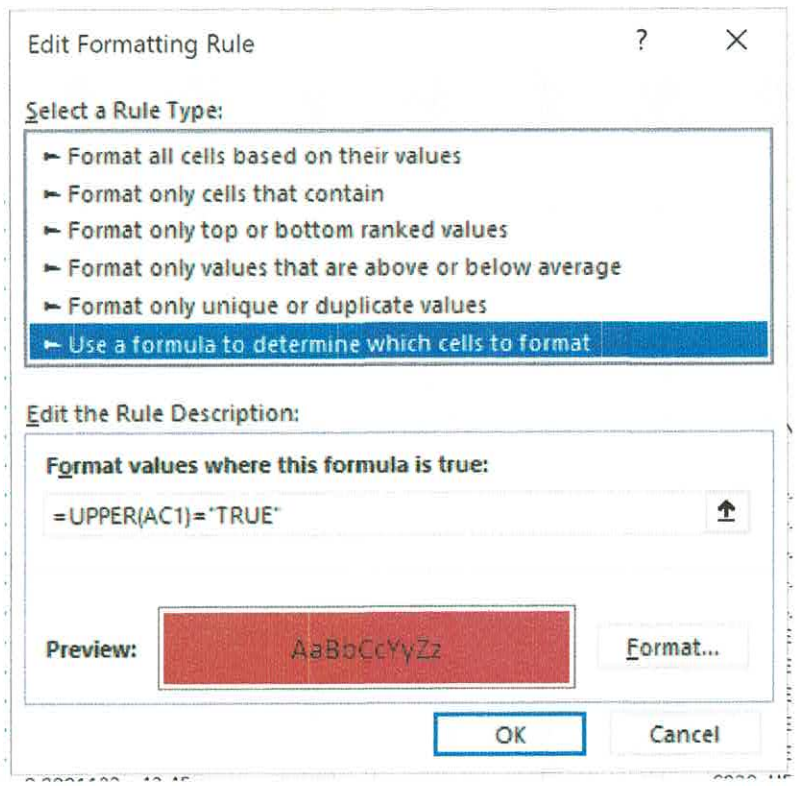
## D. Conditional Formatting

a. QACAR Controls Tab – N/A

b. Form10_Raw Data Tab

(1) *Column A:A*

(2) *Column AC:AC*




c. Root Path Settings Tab – N/A

d. Modified_7900_data_files Tab (after running QACAR button) – N/A

e. Modified_Quant_data_files Tab (after running QACAR button) – N/A

f. Modified_7500_data_files Tab (after running QACAR button) – N/A

## E. Formulas

a. QACAR CONTROLS TAB – N/A

b. FORM10_RAW DATA TAB – N/A

c. ROOT PATH SETTINGS TAB – N/A

d. MODIFIED_7900_DATA_FILES TAB (AFTER RUNNING QACAR BUTTON) – N/A

e. MODIFIED_QUANT_DATA_FILES TAB (AFTER RUNNING QACAR BUTTON) – N/A

f. MODIFIED_7500_DATA_FILES TAB (AFTER RUNNING QACAR BUTTON) – N/A

## F. REPORTS, PRINTOUTS & CHARTS

a. QACAR CONTROLS TAB – N/A

b. FORM10_RAW DATA TAB

This is a summary of the qPCR data along with calculations on the data such as: Quantity Mean, Replicate Difference, +SPK Difference and Inhibition True/False.

(1) *Raw qPCR Data*

Quant Studio Results

For File:
Q091021P1EC_QuantStudio 12K Flex_export

Sample Information

| Well | Sample Name | Target Na | Task | Reporter | Ct | Quantity | Qty Mean | Y-Intercept | R^2 | Slope |
|---|---|---|---|---|---|---|---|---|---|---|
| 72 | AC | OMYX | UNKNOWN | FAM | 21.157 | 148413.92 | 149774 | 38.37 | 1 | -3.3 |
| 60 | AC | OMYX | UNKNOWN | FAM | 21.13 | 151134.22 | 149774 | 38.37 | 1 | -3.3 |
| 3 | C102_TU_A2221.10DIL | OMYX | UNKNOWN | FAM | 15.6 | 6931551 | 6567224 | 38.37 | 1 | -3.3 |
| 4 | C102_TU_A2221.10DIL | OMYX | UNKNOWN | FAM | 15.761 | 6202897.5 | 6567224 | 38.37 | 1 | -3.3 |
| 5 | C102_TU_A2221.10DIL+ | OMYX | UNKNOWN | FAM | 15.681 | 6556057.5 | 6556058 | 38.37 | 1 | -3.3 |
| 15 | C114_TU_A2261.10DIL | OMYX | UNKNOWN | FAM | 16.944 | 2735931.8 | 2728148 | 38.37 | 1 | -3.3 |
| 16 | C114_TU_A2261.10DIL | OMYX | UNKNOWN | FAM | 16.952 | 2720363.5 | 2728148 | 38.37 | 1 | -3.3 |
| 17 | C114_TU_A2261.10DIL+S | OMYX | UNKNOWN | FAM | 16.921 | 2780053 | 2780053 | 38.37 | 1 | -3.3 |
| 36 | NEG | OMYX | UNKNOWN | FAM | Undetermined | | | 38.37 | 1 | -3.3 |
| 48 | NEG | OMYX | UNKNOWN | FAM | Undetermined | | | 38.37 | 1 | -3.3 |
| 12 | NTC | OMYX | NTC | FAM | Undetermined | | | 38.37 | 1 | -3.3 |
| 24 | NTC | OMYX | NTC | FAM | Undetermined | | | 38.37 | 1 | -3.3 |
| 84 | SPK CONTROL | OMYX | UNKNOWN | FAM | 21.751 | 98374.641 | 99244.6 | 38.37 | 1 | -3.3 |
| 96 | SPK CONTROL | OMYX | UNKNOWN | FAM | 21.726 | 100114.47 | 99244.6 | 38.37 | 1 | -3.3 |
| 2 | STD1 | OMYX | STANDAR | FAM | 14.99 | 10000000 | | 38.37 | 1 | -3.3 |
| 1 | STD1 | OMYX | STANDAR | FAM | 15.04 | 10000000 | | 38.37 | 1 | -3.3 |
| 14 | STD2 | OMYX | STANDAR | FAM | 18.417 | 1000000 | | 38.37 | 1 | -3.3 |
| 13 | STD2 | OMYX | STANDAR | FAM | 18.332 | 1000000 | | 38.37 | 1 | -3.3 |
| 25 | STD3 | OMYX | STANDAR | FAM | 21.745 | 100000 | | 38.37 | 1 | -3.3 |
| 26 | STD3 | OMYX | STANDAR | FAM | 21.874 | 100000 | | 38.37 | 1 | -3.3 |
| 37 | STD4 | OMYX | STANDAR | FAM | 25.051 | 10000 | | 38.37 | 1 | -3.3 |
| 38 | STD4 | OMYX | STANDAR | FAM | 25.15 | 10000 | | 38.37 | 1 | -3.3 |
| 49 | STD5 | OMYX | STANDAR | FAM | 28.39 | 1000 | | 38.37 | 1 | -3.3 |
| 50 | STD5 | OMYX | STANDAR | FAM | 28.45 | 1000 | | 38.37 | 1 | -3.3 |
| 61 | STD6 | OMYX | STANDAR | FAM | 29.458 | 500 | | 38.37 | 1 | -3.3 |
| 62 | STD6 | OMYX | STANDAR | FAM | 29.274 | 500 | | 38.37 | 1 | -3.3 |
| 73 | STD7 | OMYX | STANDAR | FAM | 32.847 | 50 | | 38.37 | 1 | -3.3 |
| 74 | STD7 | OMYX | STANDAR | FAM | 32.462 | 50 | | 38.37 | 1 | -3.3 |
| 86 | STD8 | OMYX | STANDAR | FAM | 33.47 | 25 | | 38.37 | 1 | -3.3 |
| 85 | STD8 | OMYX | STANDAR | FAM | 33.964 | 25 | | 38.37 | 1 | -3.3 |

## (2) *qPCR Calculation Table*

| Sample Name | Qty Mean | Replicate Difference | (+) SPK Value | SPK Difference | Inhibition? |
|---|---|---|---|---|---|
| AC | 3012214 | 0.152965 | | | |
| C1027_KI_A884_A1 | 0 | Undetermined | 23.192764 | 0.923992 | False |
| C1028_KI_A884_B1 | 0 | Undetermined | 22.9554 | 0.686628 | False |
| C1029_KI_A884_C1 | 0 | Undetermined | 22.93113 | 0.662358 | False |
| C1030_KI_A884_D1 | 0 | Undetermined | 23.623713 | 1.354941 | True |
| C1031_KI_A884_E1 | 0 | Undetermined | 23.445175 | 1.176403 | True |
| C1032_KI_A884_F1 | 0 | Undetermined | 22.605192 | 0.33642 | False |
| C1033_KI_A884_G1 | 0 | Undetermined | 23.021933 | 0.753161 | False |
| C1034_KI_A884_H1 | 0 | Undetermined | 22.960161 | 0.691389 | False |
| C1035_LU_A884_A2 | 0 | Undetermined | 22.97151 | 0.702738 | False |
| C1036_LU_A884_B2 | 0 | Undetermined | 22.989397 | 0.720625 | False |
| C1061_LU_A884_C2 | 0 | Undetermined | 23.146753 | 0.877981 | False |
| C1062_LU_A884_D2 | 0 | Undetermined | 23.225681 | 0.956909 | False |
| C1063_LU_A884_E2 | 0 | Undetermined | 22.96156 | 0.692788 | False |
| C1064_LU_A884_F2 | 0 | Undetermined | 23.070309 | 0.801537 | False |
| C1065_LI_A884_G2 | 0 | Undetermined | 22.761429 | 0.492657 | False |
| C1066_LI_A884_H2 | 11.94065 | 0.493067 | 23.06485 | 0.796078 | False |
| C1067_LI_A884_A3 | 0 | Undetermined | 22.949793 | 0.681021 | False |
| C1068_LI_A884_B3 | 0 | Undetermined | 23.040094 | 0.771322 | False |
| C1069_LI_A884_C3 | 0 | Undetermined | 23.077658 | 0.808886 | False |
| C1070_LI_A884_D3 | 0 | Undetermined | 23.125202 | 0.85643 | False |
| C1071_LI_A884_E3 | 0 | Undetermined | 23.021221 | 0.752449 | False |
| C1072_LI_A884_F3 | 0 | Undetermined | 22.939548 | 0.670776 | False |
| C1073_LI_A884_G3 | 0 | Undetermined | 22.903294 | 0.634522 | False |
| C1074_LI_A884_H3 | 0 | Undetermined | 22.988874 | 0.720102 | False |
| NEG | | Undetermined | | | |
| NTC | | Undetermined | | | |
| SPK CONTROL | | -0.040692 | **22.268772** | | |
| STD1 | | -0.11729 | | | |
| STD2 | | -0.214933 | | | |
| STD3 | | -0.148747 | | | |
| STD4 | | -0.213128 | | | |
| STD5 | | -0.239007 | | | |
| STD6 | | -0.13603 | | | |
| STD7 | | -0.334194 | | | |
| STD8 | | -0.39072 | | | |

## (3) *Healthy Plate Samples*

| Healthy Samples | Qty Mean |
|---|---|
| C898_BR_A880_B1 | 0 |
| C901_BR_A880_C1 | 0 |
| C908_BR_A880_G1 | 0 |
| C913_TU_A880_C2 | 0 |
| C914_TU_A880_D2 | 0 |
| C915_TU_A880_E2 | 0 |
| C916_TU_A880_F2 | 0 |
| C917_TU_A880_G2 | 0 |
| C918_TU_A880_H2 | 0 |
| C919_TU_A880_A3 | 0 |
| C920_TU_A880_B3 | 0 |
| C921_TU_A880_C3 | 0 |
| C922_TU_A880_D3 | 0 |
| C923_TU_A880_E3 | 0 |
| C924_TU_A880_F3 | 0 |

## (4) *Plate Fail Reason*

Plate Fail Reason

Slope Requirement

(5) *Form 11 transfer samples*

Form11 Samples

| | |
|---|---|
| C898_BR_A880_B1 | 0 |
| C901_BR_A880_C1 | 0 |
| C908_BR_A880_G1 | 0 |
| C913_TU_A880_C2 | 0 |
| C914_TU_A880_D2 | 0 |
| C915_TU_A880_E2 | 0 |
| C916_TU_A880_F2 | 0 |
| C917_TU_A880_G2 | 0 |
| C918_TU_A880_H2 | 0 |
| C919_TU_A880_A3 | 0 |
| C920_TU_A880_B3 | 0 |
| C921_TU_A880_C3 | 0 |
| C922_TU_A880_D3 | 0 |
| C923_TU_A880_E3 | 0 |
| C924_TU_A880_F3 | 0 |
| C10_CO_A3 | 607.391 |
| C11_IR/CB_A4 | 168.0365 |
| C13_CO_A5 | 649.6135 |
| C14_IR/CB_A6 | 8168.665 |
| C16_CO_A7 | 8.384105 |
| C17_IR/CB_A8 | 90.82605 |
| C19_CO_A9 | 28.8194 |
| C20_IR/CB_A10 | 147.818 |
| C22_CO_A11 | 246.72 |

c. ROOT PATH SETTINGS TAB – N/A

d. FORM12_PLATE REPORT TAB

This is a summary of each plate and its samples are compiled into Form 12

(1) *Plate Rerun Status*

(2) *Samples Failed*

(3) *Reason Failed*

(4) *qPCR Calculation Table*

(5) *Slope, R2, NTC, LOD, LOQ*

| Plate 1 | Re Run Status | Samples | Reason |
|---|---|---|---|
| 01202221P4 | [illegible] | | |

Table

| Sample Name | Qty Mean | Replicate Q | Difference (+) | SPK Value | SPK Q | Difference (-) | Inhibition? |
|---|---|---|---|---|---|---|---|
| C101_A372 | 14733 | 0.028 | 22.160 | | | 0.1395 | False |
| C101_A373 | 24322.1 | 0.112 | 22.169 | | | 0.3105 | False |
| C101_A374 | 10370.2 | 0.013 | 22.410 | | | 0.0495 | False |
| C87_A375 | 3130.4 | 0.094 | 22.541 | | | 0.0815 | False |
| C87_A376 | 3292.94 | 0.1 | 22.454 | | | 0.0255 | False |
| C87_A377 | 4250.47 | -0.043 | 22.432 | | | 0.0475 | False |
| C92_A378 | 12060 | 0.015 | 22.457 | | | 0.0225 | False |
| C92_A379 | 12454.1 | -0.067 | 22.440 | | | 0.0395 | False |
| C92_A380 | 11503.1 | 0.078 | 22.489 | | | 0.0065 | False |
| NEG | Undetermined | | | | | | |
| NTC | Undetermined | | | | | | |
| SPK CONTROL | | | -0.055 | 22.4795 | | | |
| STD1 | | | -0.107 | | | | |
| STD2 | | | -0.133 | | | | |
| STD3 | | | -0.02 | | | | |
| STD4 | | | 0.099 | | | | |
| STD5 | | | 0.024 | | | | |
| STD6 | | | 0.023 | | | | |
| STD7 | | | 0.048 | | | | |
| STD8 | | | -0.445 | | | | |

| Slope | R^2 | NTC | LOD | LOQ |
|---|---|---|---|---|
| 3.242 (+/-) | 0.998 | Undetermined | 35 | |

e. FORM13_RE RUN SAMPLES TAB (AFTER RUNNING QACAR BUTTON)

This is a summary to keep track of samples that needed to be rerun: which plate is the sample from, why does it need a rerun, which plate contains the Rerun sample, what's the Rerun sample's name.

| Need_Re_Run_Plate | Need_Re_Run_Samples | Reason_Re_Run | Re_Run_Plate | Re_Run_Samples |
|---|---|---|---|---|
| Q072721P1 | C893_BR_A880_A1 | Inhibited | | |
| Q072721P1 | C902_BR_A880_D1 | Inhibited | | |
| Q072721P1 | C903_BR_A880_E1 | Inhibited | | |
| Q072721P1 | C906_BR_A880_F1 | Inhibited | | |
| Q072721P1 | C909_BR_A880_H1 | Inhibited | | |
| Q072721P1 | C911_BR_A880_A2 | Inhibited | | |
| Q072721P1 | C912_BR_A880_B2 | Inhibited | | |
| Q072721P1 | C925_BL_A880_G3 | Inhibited | | |
| Q072721P1 | C926_BL_A880_H3 | Inhibited | | |
| Q072721P2 | C932_BL_A881_D1 | Inhibited | | |
| Q072721P2 | C944_HE_A881_H2 | Inhibited | | |

(1) *Plate Need Rerun*

(2) *Samples Need Rerun*

(3) *Reason Need Rerun*

(4) *Rerun Plate*

(5) *Rerun Sample*

f. FORM14_AC REPORT (AFTER RUNNING QACAR BUTTON)

This is a summary of technical calculations performed on the passing plates that contained assay control values.

AC raw data value:

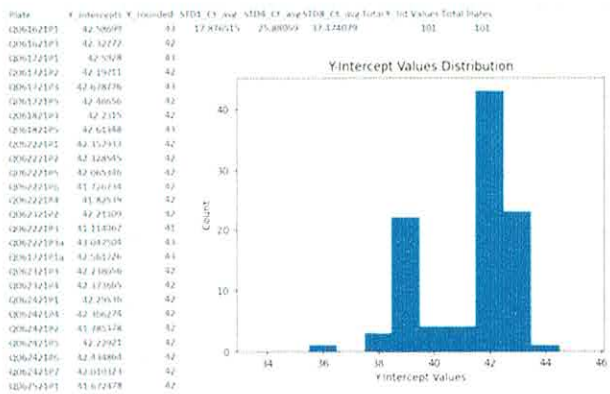
| Plate | Sample Name | Ct | Qty | Qty Mean |
|---|---|---|---|---|
| Q061621P1 | AC | 24.704838 | 71764.21 | 67044.72 |
| | AC | 24.930372 | 62325.23 | |
| Q061621P3 | AC | 24.453995 | 78688.15 | 68840.225 |
| | AC | 24.910755 | 58992.3 | |
| Q061721P2 | AC | 24.594654 | 74181.08 | 67562.155 |
| | AC | 24.903194 | 60943.23 | |
| Q061721P5 | AC | 24.3209 | 87374.13 | 73600.185 |
| | AC | 24.924942 | 59826.24 | |
| Q061821P3 | AC | 24.49999 | 75636.97 | 71297.18 |
| | AC | 24.692383 | 66957.39 | |

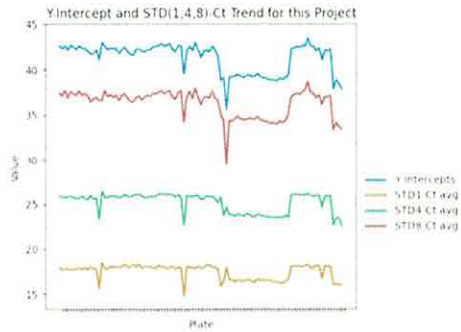
Calculated data:

| Std Dev of Qty Mean | Avg of Qty Mean | Percent CV | # Passing AC Plates | # Passing No AC Plates | # of Failed Plates | # Total Plates |
|---|---|---|---|---|---|---|
| 74173.67199 | 117509.7703 | 0.6312128 | 87 | 28 | 19 | 134 |

(1) *Plate*
(2) *Sample Name*
(3) *CT*
(4) *Quantity*
(5) *Quantity Mean*
(6) *Standard Deviation of Quantity Mean*
(7) *Average of Quantity Mean*
(8) *Percent CV*
(9) *Number of Passing Plates with AC values*
(10) *Number of Passing Plates with no AC values*
(11) *Number of Failed Plates*
(12) *Total number of Plates*

g. FORM15_Y INTERCEPT REPORT TAB (AFTER RUNNING QACAR BUTTON)




This is a graph showing the distribution of Y intercept values.

(1) *Plate*
(2) *Y Intercepts*
(3) *Rounded Y Intercepts*
(4) *STD1 Ct avg*
(5) *STD4 Ct avg*
(6) *STD8 Ct avg*
(7) *Total Y Intercept values*
(8) *Total Number of Plates*

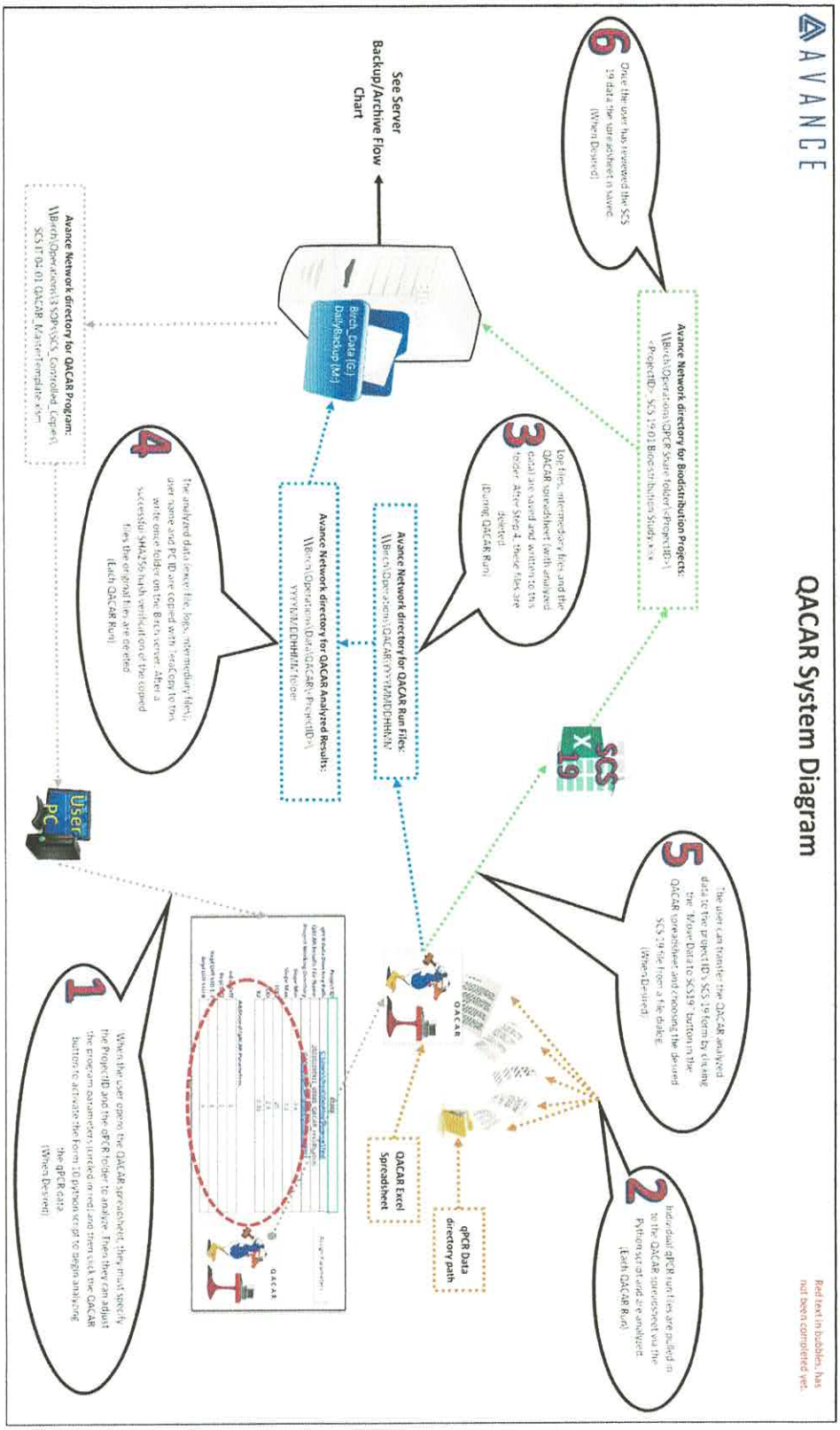
## VI. ACCEPTANCE CRITERIA

- For the validation, code will be tested to ensure all data is moved correctly:
  - When files are moved SHA-256 hashes will be used to ensure data integrity and verified using SCS IT 02.
  - When data is moved, it will be verified against the original data.
- Pass/Fail criteria will be established to test all required functions listed in this URS.

## VII. VALIDATION SUMMARY REPORT

A summary report will be prepared to include the conclusions of the software validation tests, any required screen shots or printouts specified in the validation plan, and any planned and/or unplanned deviations.

## VIII. DATA FLOW DIAGRAM



## IX. APPROVALS

08Mar 2022

IT/Engineering (signature/date)

08Mar2022

Management (signature/date)

08 Mar 2022

QA (signature/date)