LIMS QC Manager Data Upload, Verification and Sample Authorization Instructions for qPCR Samples

1. INTRODUCTION

1.1 This SOP provides instructions for uploading data after qPCR analysis of environmental samples. Prior to uploading data, the worksheet should be completed with the necessary test codes added (SOP PCR- 5.0). The raw data file is then exported as a text file from StepOne Plus software (SOP PCR-2.3) or the QuantStudio software (SOP PCR-2.6).

1.2 The export file is converted from a text file in QC Manager (QCM). Raw and review files are created after calculating the results, and these are verified before the samples and job are authorized. Quality Control (QC) failures for the assay are identified in the results.

2. RESULTS ENTRY AND CALCULATIONS

2.1 In the LIMS menu, select **Laboratory/QC/QC Manager** from the drop-down list.

2.2 Select the instrument where the qPCR assay was run: Biology: qPCR StepOne Plus 3 or Biology: qPCR QuantStudio3 1.

2.3 Select the **Convert Instrument File icon**  , and choose the file to upload from the Export Files folder. Click OK to save to LIMS after checking the file name. Enter the analyst's name who set up the run and the start date/time of the run. Use the printed Experiment Results report from the StepOnePlus or QuantStudio3 to find the start time. Click **Convert**. Sometimes crude samples might need purification due to inhibition. In this case, those samples can be removed from the crude worksheet and added to a newly created purified worksheet. If the samples have not been removed from the crude worksheet, they will be listed in both crude and purified worksheet. Select the correct worksheet when prompted by QCM at the file import step.

2.4 Data will show in the Sample Table. Check that three rows (test result, SPK and SUR recovery) are associated with each sample. If there is missing information, check the StepOnePlus or QuantStudio3 file for nomenclature errors, fix them, save the raw data and export the txt file again. In the sample table:

2.4.1 Check the "Prep Batch" column. It should correspond to the number specified in the worksheet. If the worksheet has not been completed, a "Prep Batch" number will not appear.

2.4.2 Check the "Dilution X" column. Dilutions of the samples are added to the prep worksheet. Verify sample dilutions are correct by comparing the plate preparation file to the prep. worksheet and QCM file.

2.4.3 Check the "Test ID" column. It should correspond with the test ID in the prep. worksheet.

2.4.4 Fill in the Sample Table by clicking on the column header and highlighting all rows (the control/sample rows). Select the "Analysis Batch" column and click "New".

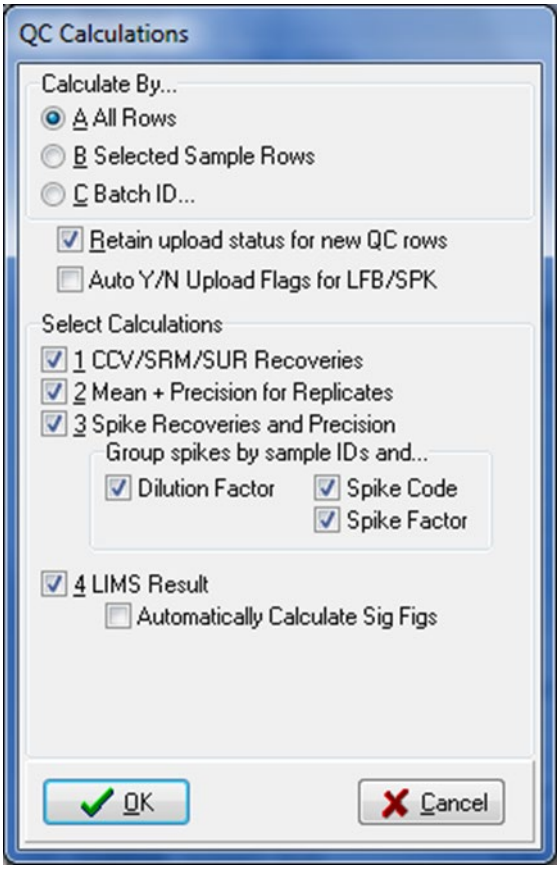
2.5.5. For crude analyses: The MBLK should have the Pre-Conc column entered as 83.333333 (filtration volume (0.05 L)/extraction volume (0.0006 L)), a factor used to calculate MDL/PQL.

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2.5.6 For purified samples, manually edit the Pre-Conc. factor for MBLK to "500" (0.05 L/ 0.0001 L =500). LIMS will calculate the Pre Conc-factor for the rest of the samples automatically using data from the prep worksheet manager.

2.5.7 The SUR rows should always have a "Pre Conc" of 1. The filtration volume will show up in the "Weight (g)" column, and the extraction volume will show up in the "Prep Volume (L)" column.

2.6 Select **Tools**/**Calculate** from the menu. Choose Calculate by "A All Rows", check everything except "Auto Y/N Upload Flags for LFB/SPK" (see picture below). Click "OK". Click "OK" in the Calculations complete pop-up window.



3. RESULTS ANALYSIS

3.1 Results will show in the Component Table. For each sample, check the SPK and SUR recovery in LIMS Result column. Rows corresponding to DUP samples are analyzed differently (see below 3.2).

3.1.1 If both are >50%, then change the Upload flag to "Y" for the sample line.

3.1.2 If the recovery is low and SPK or SUR are flagged "F", the sample is inhibited and needs to be purified or diluted. This should correspond with observations from data analysis of the instrument file. In the Component table, change the upload flag to "N" for the sample, SUR and SPK rows. In the Sample table, add "NO UPLOAD- Inhibited, need to purify" or "NO UPLOAD - Low control recoveries, need to re-run" to the comment field and change the Type

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to NONE for all three rows. Record the sample ID number of inhibited samples to either re-run as diluted or add the corresponding SOP PCR-4.2 test codes.

3.1.3 For purified samples, the extraction controls and sample surrogates will usually fail due to material loss during extraction. These samples can still be uploaded. A "J" qualifier and comment are automatically added at the job authorization step.

3.1.4 If the failure is for over-recovery, notify a supervisor to determine if the sample should be uploaded or rerun. If uploading a sample result with a failing surrogate or spike, a "J" qualifier and comment are automatically added at the job authorization step.

3.2 To upload the results for DUP samples, look at each sample result. (Refer to Section 4.6.7 for a description of qualifiers):

3.2.1 If both replicates are U-qualified, then select "Y" for the AVG upload flag. Remove the A-qualifier.

3.2.2 If one replicate is U-qualified and the other replicate is I-qualified, select "Y" for the upload flag of the I-qualified result. Make a comment of the lower result in the comments section of the sample table. "Duplicate was analyzed, and the result was x." Copy the result of the lower result from the component table into the comments to avoid transcription errors.

3.2.3 If both replicates, are I-qualified, select "Y" for the upload flag of the higher result. Make a comment of the lower result in the comments section of the sample table. "Duplicate was analyzed, and the result was x." Copy the result of the lower result from the component table into the comments.

3.2.4 If both replicates are above PQL, select "Y" for the AVG upload flag. If values are above PQL, "sample_RPD" may be uploaded instead of sample_SPRPD. Only one precision measure should be uploaded per analysis batch.

3.2.5 For qPCR runs where no duplicate samples were analyzed, all samples will need the comment "Precision data unavailable for x component.". If "SPRPD" and "sample_RPD" are flagged as F triggered by SPK and SUR control failures, no RPD will be uploaded and all the samples need the following comment, "Precision data unavailable due to inhibition in QC sample". If "SPRPD" is flagged as F but not related to DUP sample inhibition, the upload flag can be set to "Y". A "J" qualifier and comment will automatically be added at the job authorization step.

3.3 The analyst has the option to run a set of DUPs to serve as a precision measure if a sample was re-analyzed due to failing QC (SUR or SPK failure). DUP sample results are uploaded only with the original prep batch worksheet number. Set the upload flag to "N" for the DUP rows in the Component Table except for the SPRPD. In the Sample Table, edit the Type column to "NONE" and add the comment "NO UPLOAD - Only spike was replicated for precision, not sample." Give a new Prep Batch ID to this upload file.

3.4 Select the save icon . The name of the file will automatically show. Click OK to save. Print the results using the "PCR" template icon  and use the Run ID as the Report Title. Paperclip the instrument reports, copy of plate layout, and QCM printouts and give to second analyst to review.

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4. SAMPLE LEVEL UPLOADING AND AUTHORIZATION

4.1 Open the .REV file in QCM to review the results.

4.2 Check that the analysis date and time on the QCM file matches the Run Started time on the Experiment Results Report. Check that Analyst also matches.

4.3 Check the standard curve R^2 and efficiency from the printout. $R^2 \geq 0.99$ and Eff%: 85-110%. The samples need to be re-run if the $R^2 < .97$ or the Eff% < 70%.

4.4 On the plate layout printout:

4.4.1. Check if any standard curve replicates were omitted from the calculation. If multiple points were omitted, determine if standard curve is still usable or plate should be rerun.

4.4.2. Check for any flags that may be of concern. Blanks and undetected samples will have flags for no amplification. The flags can be identified on the QC Summary printout.

4.5 Check Prep Worksheet Manager to make sure it has been completed correctly:

4.5.1. Spikes entered for the correct assays.

4.5.2. Sample IDs have the correct naming conventions.

4.5.3. Filtration Dates and Volumes are entered. Check the volumes recorded on the labels for each sample.

4.5.4. Prep batch ID matches the QCM printout.

4.5.5. Extraction date, start time, end time, and analyst name entered.

4.5.6. PBS serial number entered in Reagent 1. PMA serial number entered in Reagent 2 for PMA treated samples.

4.5.7. Dilution factor entered for any extracts diluted in the plate.

4.5.8. Test codes added, if necessary.

4.6 Check the QCM file/printout:

4.6.1. Check that the prep batch, spike code, test ID, matrix, dilution x, and analysis batch are filled correctly.

4.6.2. Check the Pre Conc values.

4.6.3. Check the blank controls (MBLK, EXBLK, NTC) to make sure they are non-detect.

4.6.4 Check the spike and extraction controls (LFB_XXSPK#, LFB_XXLFB#, SUR_XXLFB#) to make sure they are passing.

4.6.5. Check each sample and control to see if it is flagged correctly. Samples with passing controls are set to “Y” for upload. Samples with failing third well spike or surrogates are set to “N” and will not usually be uploaded unless the sample is purified. Discuss with supervisor before uploading failing results

4.6.6. Check to make sure dups are uploaded correctly and appropriate comments are added if necessary (See section 3.2).

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4.6.7. Check all qualified data. Samples with no detection or detection below the MDL will have a "U" qualifier. Samples detected between the MDL and PQL will have an "I" qualifier. Samples being uploaded with failing controls (including SPRPD) will have a "J" qualifier added at the job authorization step.

4.6.8. Check the "Notes" column for any QC comments from the StepOnePlus or QuantStudio3 file.

4.7. Return the packet to the initial analyst to correct any mistakes. Double-check corrections before authorizing.

4.8. When all checks are done, select Tools/Upload/By Upload Flag from the menu.

4.8.1. If there are any errors that come up, check these and make corrections as needed.

4.9. Select Data/Upload from the menu. The samples will be uploaded to LIMS and the status will change from "V" to "C" at the sample level.

4.10. Select Data/Authorize Test from the menu. Click the "Select All" button, then click the "Authorize Selected Tests" button. This will authorize at the sample level and change the status of the samples from "C" to "A".

5. REFERENCES

5.1 SOP PCR-2.3 Use and Calibration of StepOnePlus Real-Time PCR System and QuantStudio3 Real-Time PCR System

5.2 SOP PCR-4.0 Preparation of samples for qPCR analysis

5.3 SOP PCR-4.1 Crude Lysate Extraction Method for qPCR samples

5.4 SOP PCR-4.2 Purification of Filtered Samples using the Qiagen DNeasy PowerSoil Pro Kit

5.5 SOP PCR-5.0 Sample Custody, Labels, and Worksheet Instructions for Molecular Biology Samples

Appendix of Significant Changes

01/10/2021 Added QCM names for PUR target assays to table 6.

12/15/2022 Added procedure for purified test codes in section 2.3. Restructured numbered sections in 2.4. Added data review process steps in sections 4.5 and 4.6. Clarified procedure for uploading replicates with different qualifiers in section 3.2.

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6. TABLES

Table 6.1. Naming conventions

Type	**QCM Type**	**QCM Name**	**Comments**
Sample, Target Assay	SAMP	SampleID_ SampleID_PUR PURXX	_PUR is added to purified samples. XX is the target assay name (HF, BR, D3, G2, GD, C2, H2)
Sample, Salmon Assay	SUR	SUR_SampleID_ SUR_SampleID_DUP_A_ SURPUR_SampleID_PUR SURPUR_SampleID_DUP_A_PUR	The salmon assay is considered a surrogate for QCM. QCM will calculate the % recovery of the surrogate.
Lab Duplicate, Target Assay	REPLA	SampleID_DUP_A_ SampleID_DUP_A_PUR	second dup is “B” _PUR for purified samples.
Method Blank	MBLK	MBLK_#	# is the prep batch ID
Extraction blank	MDL	EXBLK_#	# is the prep batch ID
No Template Control	MDL	NTC_#	Each NTC rep is shown (1, 2, 3)
NTC, Salmon assay	MDL	SURNTC_#	
Extraction Control, Target Assay	LFB	LFB_XXLFB# LFB_XXLFBP# (for PUR samples)	# is Standard Prep Tracker S# XX is spike code (BD, BR, etc)
Extraction Control, Salmon Assay	SUR	SUR_XXLFB# SURPUR_BDLFB#	# is Standard Prep Tracker S# XX is spike code (BR, BR, etc)
3rd well spike control	LFB	LFB_XXSPK#	# is Standard Prep Tracker S# XX is spike code (BR, BR, etc)
3rd well spike, samples	SPK	SampleID_SPK_XXSPK# SampleID_SPK_DUP_A_XXSPK# SampleID_SPK_PUR_XXSPK# SampleID_SPK_DUP_A_PUR_XXSPK#	# is Standard Prep Tracker S# XX is spike code (BR, BR, etc)

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