

QUALITY ASSESSMENT OF IN-HOUSE DIAGNOSTIC REAL-TIME PCR WITH SEMI-AUTOMATED DATA MONITORING BY MULTI-QC SOFTWARE

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Background: in-house diagnostic assays require careful quality control measures and quality data recording which is often done with Excel® and manual keyboarding. This takes time and may be associated with errors during data transfer.

Aim: Adapt real-time PCR data files for automated import and statistical analysis by Multi-QC to monitor quality of real-time PCR assays.

Permanent record of “quality values”

Figure 1 illustrates with cytomegalovirus (CMV) the various items that technicians should consider for quality assessment. Ct and DeltaRn values of standards as well as PCR efficiency are good indicators of the quality and reproducibility of a PCR diagnostic assay. These values must be traced to monitor PCR drift/shift underlying defective reagents or lab anomalies. Negative controls must also be traced to monitor contaminations.

To avoid technician time being wasted on data transfer, the use of software to automate this task appeared valuable. Since the format of the Taqman results file (highlighted in yellow) is predictable, one can automate data import in Multi-QC for immediate results validation and long term data storage.

Multi-QC (<http://www.mutiqc.com/>)

Multi-QC is a software developed for clinical chemistry. The program can automatically import values from pertinent fields within results file exported from analytical instruments, and can perform immediate statistics that are valuable for quality assessment (Figure 2).

I programmed a VBA application in Excel® to transform Taqman results .txt files into .mqc files adapted for Multi-QC (Figure 3). Multi-QC reads them through a file link importer that has been customized to match their structure (Table 1).

T090601A.txt					T090601A.mqc							
Well	Sample Na	Detector N	Reporter	Task	Ct	Well	Sample N	Detector	Reporter	Task	Ct	
49	S-CMV3	M-CMV	FAM	Standard	27.58559	49	S-CMV3	M-CMV	FAM	Standard	27.58559	
50	S-CMV2	M-CMV	FAM	Standard	30.82049	50	S-CMV2	M-CMV	FAM	Standard	30.82049	
51	S-CMV1	M-CMV	FAM	Standard	33.993484	51	S-CMV1	M-CMV	FAM	Standard	33.993484	
52	TEM92252	M-CMV	FAM	Unknown	Undetermined	52	TE	M-CMV	FAM	Unknown	55	
53	TEM92311	M-CMV	FAM	Unknown	Undetermined	53	TE	M-CMV	FAM	Unknown	55	
Slope					-3.203947 cycles/log decade	400 Slope					M-CMV	-3.203947
Y-Intercept					37.20775	400 R^2					M-CMV	0.9999689
R^2					0.9999689	400 DeltaRn					M-CMV	1.6

Figure 3. ABI 7900 export results file (.txt) and its .mqc counterpart after VBA processing.

The position of the Slope and R^2 fields are moved by one column and the DeltaRn entry is created together with updating the Detector field of the corresponding rows in the .mqc file. The values of the original fields are moved in the Ct column while TEx values are changed to TE, “undetermined” Ct values are changed to 55 and DeltaRn is set to 99. This value will need to be updated by the technician within the .mqc file.

Multi-QC checks periodically for .mqc files in a specified folder on the network. Once a new file is found, it reads it and imports automatically the values specified in the file link importer in a matter of seconds, and updates the Multi-QC analyte files (Figure 2).

Table 1. Parameters of the Multi-QC file link importer.

[Interface]
Name = File-link A
Default section = Routine
[Fields]
Section =
Analyte = 3
Date = 2, 5
Time = 2, 5
QC material = 2
Value = 6
Flag =
[Format]
Delimiter = #9
Date = MM/DD/YYYY hh:mm:ss:tt
Time = MM/DD/YYYY hh:mm:ss:tt
[Sections]
[Tests]
M-CMV = CMV / S-CMV3, S-CMV2, S-CMV1, TE, Slope, DeltaRn

The [Fields] item indicates where in the txt file the values are recorded (column, row).
Delimiter indicates how the fields are separated in the txt file (here #9 corresponds to “carriage return”).
Date and Time indicates the format of these items in the export txt file.
For sake of simplicity only the CMV analyte is shown under [Tests], among the > 60 in use in our Molecular Diagnostic Laboratory

Conclusions

- Multi-QC allows for global statistical evaluation as well as data recording and tracing of events pertinent to each molecular diagnostic test. This helps preventing errors and facilitates biomedical validation of daily results.
- File import is amply facilitated by VBA programming. Several export files can be processed consecutively in an unattended mode to further reduce the time needed to import data from retrospective Taqman analyses.
- Together with the Taqman file setup that could be generated by our diagnostic institutional database and with our already automated analytic platform, we hope to fully automate real-time PCR diagnosis with our in house PCR assays.

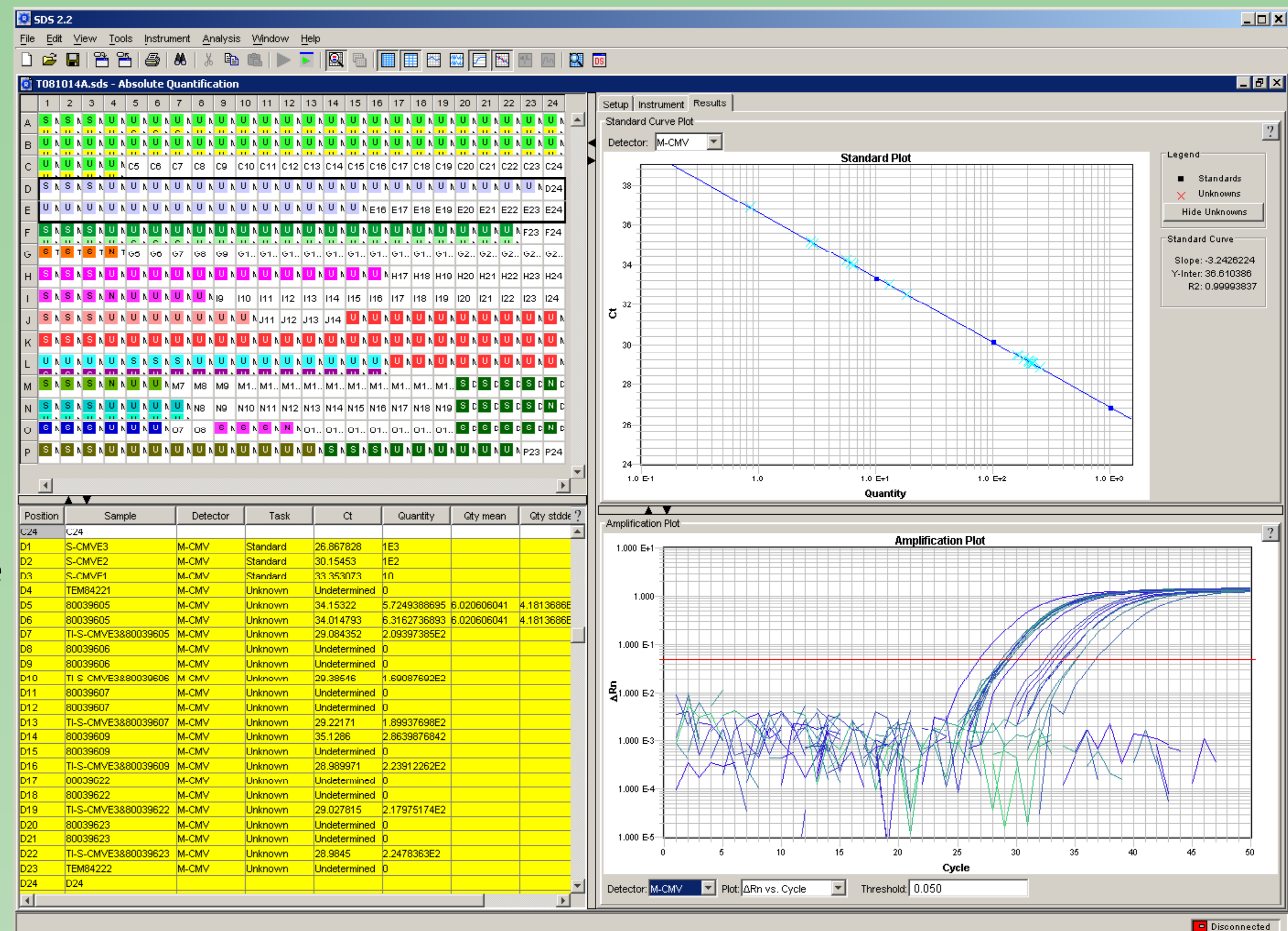


Figure 1. ABI 7900 results display of a cytomegalovirus quantitative assay.

Top: 384 well plate scheme (left) and standard curve (right); bottom: results table (left) and amplification plot (right). Note that such plates can be quite complex (> 20 different analytes) and necessitate at least 30 minutes for manual data transfer in Excel®.

The standards (S-CMVEx) are evaluated for their Ct consistency and reproducibility and the standard curve for efficiency of amplification (slope) and sensitivity (minimal viral load). The DeltaRn value is a measure of the quality of the probe. The TEx samples are negative controls that are used to monitor contamination and Ti-S-x are clinical samples spiked with standards to monitor PCR inhibitors.

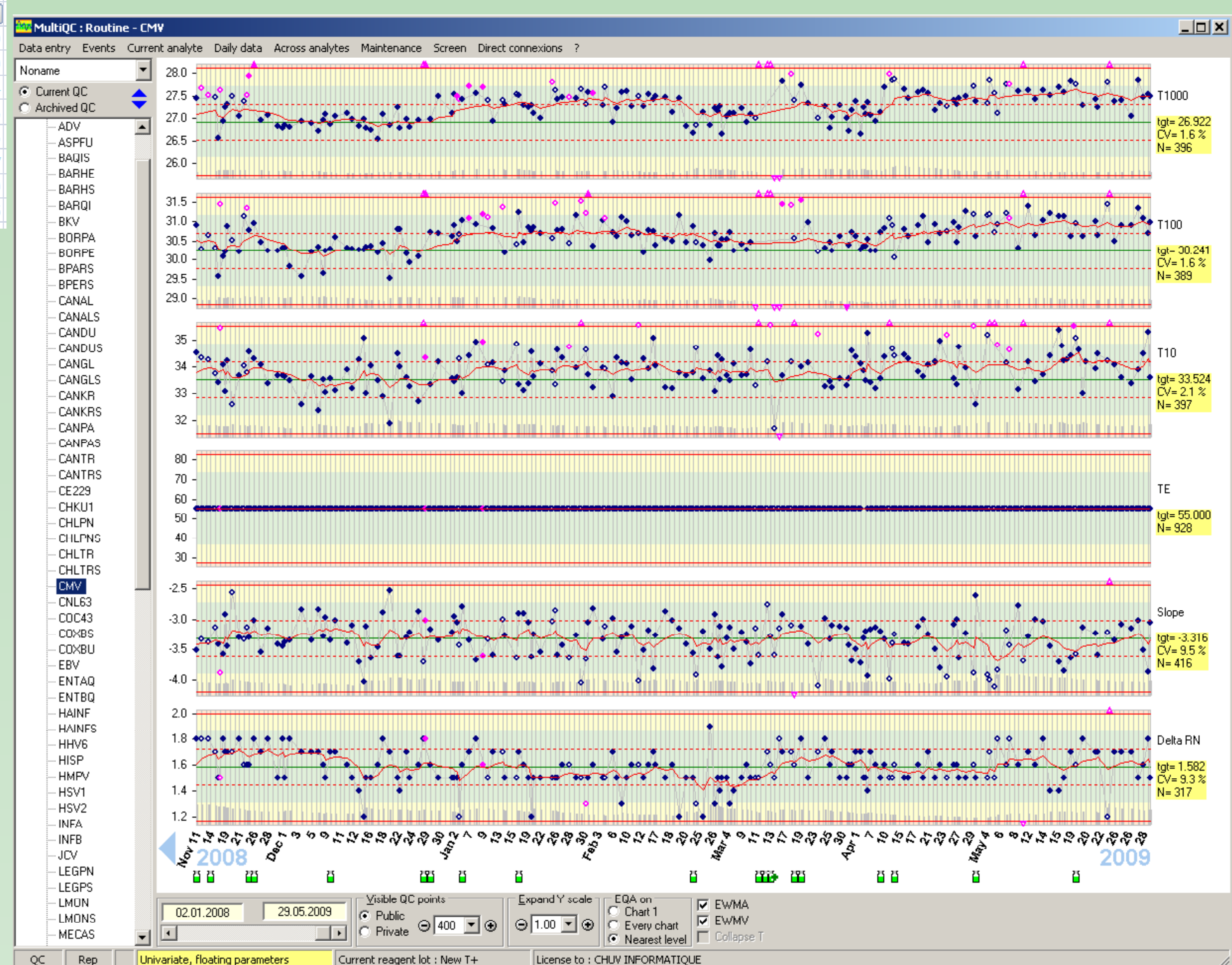


Figure 2. MULTI-QC representation of CMV data over 6 months.

Ct of standards (Tx) and TE, slope and DeltaRn values (y axis) are plotted against their date of acquisition (x axis). The green lines correspond to the mean, dotted red lines are the mean plus/minus 1 standard deviation, the straight red line is the mean plus/minus 3 standard deviations of each item. The continuous red line is the mobile mean value exponentially pondered with the last 10 measures. The mean and standard deviations can be established either continuously or with the values acquired during the assay setup period (validation).

Note the absence of contamination (Ct of TE arbitrarily defined as 55, five cycles above the maximum number of cycles used in our PCR) and the drift of the positive control values towards higher Ct that suggests alteration of positive control DNA.

Filled blue symbols are representative of accepted data (within two standard deviations, green background). Open symbols are associated with comments, and pink symbols, as well as the mobile mean value crossing the dotted red lines, warn for possible anomalies. Bottles at the bottom of the screen indicate the date of introduction of new lots of reagents.

Acknowledgements

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