Utilization of Azure PCR AccuCall Software to Improve Analysis of PCR Data

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Azure PCR

Background

Quantitative Polymerase Chain Reaction (PCR) assays have become an essential tool for laboratory diagnostics, especially in the field of molecular virology. Interpretation of PCR results is performed by experienced technologists, and can be both time-consuming and error prone. In addition, the volume of specimens being submitted to clinical laboratories for testing has increased drastically over the past decade and indicates the need for automated analysis software to reduce turnaround time, costs and result variability.

Objective

We evaluated Azure PCR AccuCall software on our laboratory developed CMV PCR assay to determine its ability to provide standardized quantitative results without the need for technologist manipulation or review. The software is available as either an internet-based service or can run from a local host computer.

Methods

Clinical CMV specimens were extracted on the Roche MagNA Pure 96 instrument and amplified by Real-Time PCR on the Applied Biosystems (ABI) StepOnePlus thermocycler.

- 2,252 samples from 48 runs spiked with internal control were amplified by RT-PCR and analyzed for CMV with both ABI and AccuCall software
  - 1,692 patients
    - 79 high positives (>1,000 IU/ml)
    - 343 low positives (6 to 1,000 IU/ml)
    - 1,270 negatives
  - 275 controls
  - 285 standard points

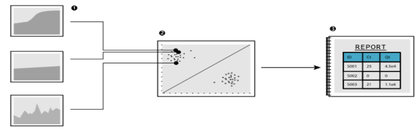
ABI Method:

1. Each target is analyzed and positive samples assigned a CT. Analysis settings are decided on by the lab. A technologist verifies all amplification plots.

2. Results are handwritten on a worksheet, which is checked for accuracy by a second technologist.

3. Results are transferred from worksheet into lab information system by a technologist, then released to practitioners.

Azure AccuCall Method:



❶ For each target, the fluorescence data is analysed and converted to a data point.

❷ By using the target label, the data point is correctly compared to the COR template data in order to classify the target.

❸ Quantification is calculated if relevant.

AccuCall does not require manipulation of analysis settings or verification of amplification plots by a technologist.

Results

Correlation

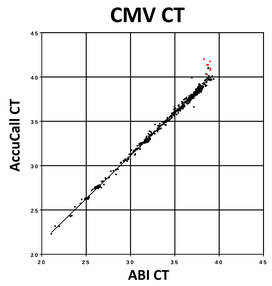


Figure 1: Linear regression analysis of all CMV cycle threshold results
y = 0.973x + 1.939
R^2=0.999

Red data points indicate discrepancies

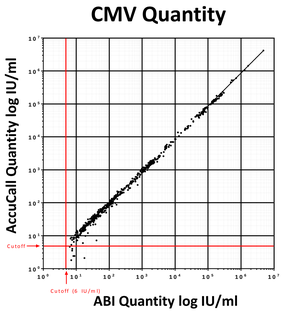
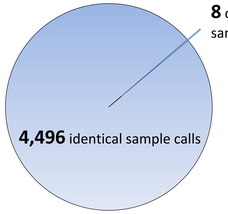


Figure 2: Linear regression analysis of all CMV quantitative results
y = 0.865x – 905
R^2=0.995

Discrepancies

99.8% identical qualitative sample calls



Out of 4,496 targets (CMV & internal control) interpreted by ABI and AccuCall, 8 results were found as discrepant. All discrepancies were below the 95% reproducibility cutoff of 20 IU/ml.

SAMPLE #	ABI Quantity (IU/ml)	AccuCall Quantity (IU/ml)
605	7	4
632	8	4
837	7	5
2254	9	2
2344	7	2
2350	8	2
2487	18	2
4342	6	5

Table 1: Discrepant results
8 results were below the positive cutoff of 6 IU/ml by the AccuCall method.

ABI False Amplification

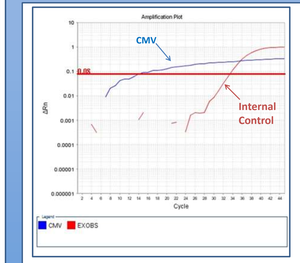


Figure 3: ABI amplification plot
False amplification of CMV target

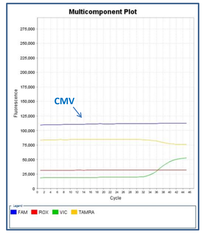


Figure 4: ABI multicomponent plot
No increase in CMV (FAM) fluorescence

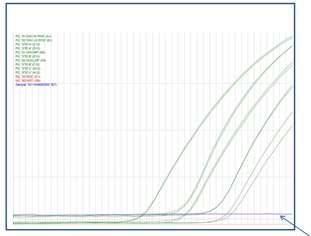
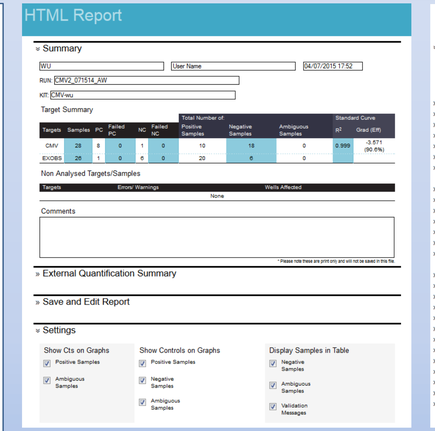
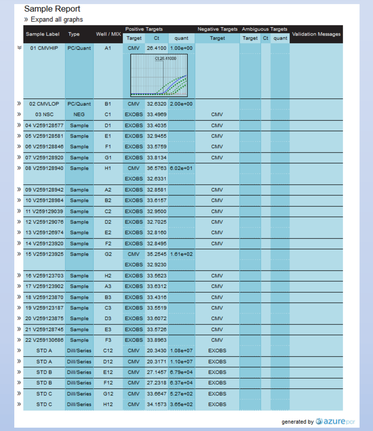


Figure 5: AccuCall amplification plot
No amplification of CMV target

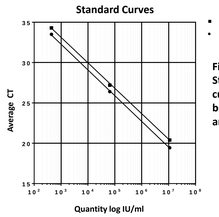
8 out of 4,496 targets analyzed by both methods showed false amplification with the ABI software. AccuCall interpreted all of these samples correctly.

AccuCall HTML Report





Standard Curve Comparison



- AccuCall curve
- ABI curve

Figure 6: Standard curves for both methods are linear

	ABI		AccuCall	
	Slope	R^2	Slope	R^2
Average	-3.25	1.00	-3.46	1.00
SD	0.14	0.00	0.14	0.00
% CV	4.25	0.24	4.01	0.26

Table 2: Standard curve variability
Standard curve data from 48 PCR runs was generated by ABI and AccuCall. The slope of the curve and coefficient of determination (R^2) are very similar between the two methods.

Conclusion

- Azure PCR's AccuCall software is a highly accurate and efficient method for quantifying CMV viral loads in a clinical lab.
- The automatic data analysis provided by AccuCall reduces errors and requires less hands-on technologist analysis time.

Contributors

Aron Cohen and Simon Bengen, of Azure PCR Ltd, UK