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Laboratory Evaluation of the Vela Diagnostics *Sentosa*® SA CMV Quantitative PCR Assay

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Amended Abstract

Introduction. PCR is considered the gold standard for detecting CMV in clinical samples. Vela Diagnostics (Vela DX) offers a new platform for CMV testing. It combines nucleic acid extraction, master mix preparation and PCR set-up in a Rotor-Gene 72-Disk on the *Sentosa* SX101 instrument followed by amplification on a Rotor-Gene Q instrument. The objectives of this study were: (a) to evaluate the Vela DX CMV assay for the detection and quantification of CMV, and (b) to compare the results to our standard of care test, the Simplexa™ Focus DX CMV ASR (Focus Diagnostics, USA).

Methods. Two hundred and twenty-nine plasma and 61 bronchial lavage (BAL) or bronchial wash (BW) samples were evaluated, along with 3 CAP survey samples. Vela DX CMV assay was compared to the Simplexa™ Focus DX CMV ASR. For the Focus assay, samples were extracted using the EasyMag extractor (bioMerieux, France). Nucleic acid was mixed with the Focus CMV primer set and master mix, followed by amplification on the 3M Integrated Cycler (Focus Diagnostics), using the 96 well universal disk. For the Vela DX CMV test, samples were loaded onto the *Sentosa* SX101 instrument, along with the reagents to prepare the CMV PCR. The instrument performed extraction of nucleic acids, master mix preparation and set-up the PCR assay in Rotor-Gene 72 well disks. Finally, the disks were transferred to a sealer, which applied a film to the disk wells, and then transferred to the Rotor-Gene Q for real-time PCR.

Results. For the two-hundred and twenty nine plasma samples, 34 were negative and 148 were positive. Forty samples were positive only on the Focus CMV assay and 7 were positive only on the Vela CMV assay. All Focus positive and Vela DX negative samples, as well as Vela DX positive and Focus negative samples had <500 copies/mL, most were <200 copies/ml. CAP survey samples were observed on both assays and correlated well. The correlation between quantitative values from both assays was 0.93, indicating very good overall agreement. Most variances were less than 0.2 log and all were less than 0.5 log for viral loads. For the 61 BAL/BW samples, 34 were positive and 21 were negative with both assays. Two samples were positive only on the Vela CMV assay while 4 were positive only on the Focus CMV assay. All Focus positive and Vela DX negative samples, as well as Vela DX positive and Focus negative samples had very low levels of virus (< 200 copies/mL), one was <500/ml.

Conclusions. The new Vela DX CMV PCR assay performed well in comparison to the Focus DX CMV PCR. The discrepant samples had low viral loads. Samples were frozen at -20°C in order to batch the samples for comparison. A separate look at the quality of freeze/thaw samples showed that low viral load samples were affected. This caused most of the low positives to repeat as negatives if there was a freeze/thaw cycle. Overall, the absolute quantitation by the two assays was highly concordant.

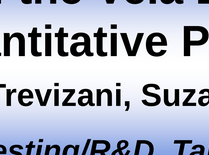
The Vela DX system includes the Sentosa SX101 and the Rotor-GeneQ instruments. The Sentosa SX101 instrument performs sample extraction, nucleic acid purification, set up of master mix, as well as addition of the nucleic acids and master mix in the PCR rotor-disk.

Methods

- A total of 290 clinical samples (229 plasmas and 61 BAL/BW) and 3 CAP survey samples were included in this study.
- Clinical samples were first tested by the standard of care procedure, the Simplexa™ Focus DX CMV ASR (Focus Diagnostics, USA), according to manufacturer's instructions.
- Samples were frozen after standard of care procedure and thawed right before the Vela DX CMV test was performed by the Vela DX System, as described below.
- Specificity Test was carried out by testing 15 different organisms using the Vela DX CMV assay at the Vela System.
- Applied Biosystems CMV standard controls were tested to compare the Vela DX CMV PCR and the Focus CMV assay.

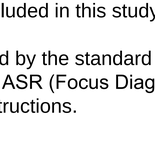
Vela DX System and CMV Quantitative Assay

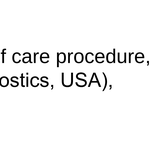
1. Transfer 230 µL of sample to a Safety-Lock tube.



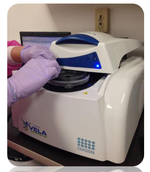
2. Load samples, reagents and consumables on the Sentosa SX101

3. Choose the correct assay definition parameters and load in sample identifiers. Start the run.





4. At the end of the run, apply seal to rotor-disc and place it in heat sealer.



5. Transfer sealed rotor-disc to the RGQ instrument and start run.

Results

Table 1. Comparative Evaluation of Plasma Samples

Focus DX \ Vela DX	Positive	Negative
Positive	148	40
Negative	7	34

*See conclusions for analysis of discrepant samples.

Table 2. Comparative Evaluation of BAL/BW Samples

Focus DX \ Vela DX	Positive	Negative
Positive	34	4
Negative	2	21

Figure 1. Correlation of the two assays for CMV Quantitation on plasma.

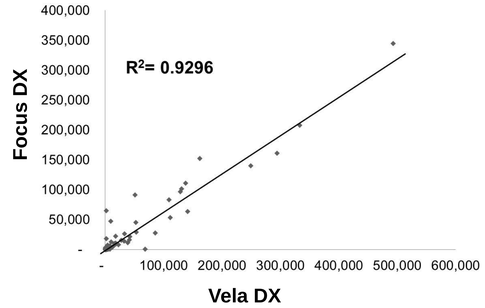


Table 3. Specificity Tests

Organism Tested	Vela CMV	Organism Tested	Vela CMV
Influenza A	negative	*Coronavirus*	negative
Adenovirus	negative	*RSV*	negative
hMPV	negative	*VZV*	negative
EBV	negative	*JC virus*	negative
Influenza B	negative	*Enterovirus*	negative
Parainfluenza 1-4	negative	*BK virus*	negative

Table 4. Applied Biosystems CMV Standard Control Results:

Dilution	Focus DX CMV PCR	Vela DX CMV PCR
3 x 10^5 IU/mL	Positive(3/3)	Positive(3/3)
3 x 10^4 IU/mL	Positive(3/3)	Positive(3/3)
3 x 10^3 IU/mL	Positive(3/3)	Positive(3/3)
3 x 10^2 IU/mL	Positive(2/3)(*)	Positive(3/3)

*Sample were tested in triplicate and one Sample was invalid

CONCLUSIONS

- The Vela DX CMV PCR demonstrated to have an excellent sensitivity and specificity for CMV patient samples.
- Out of 293 samples tested, 53 had different results when both assays were compared. All discrepants were <500c/mL while most were <200 c/mL.
- A comparative side study (data not published) showed that freezing and thawing the CMV samples for batch testing affected the results. Samples with low CMV viral load were shown to lose their positivity on both Focus DX instrument and Vela DX instrument.
- This would suggest that most of the discrepant results where Vela DX CMV PCR was negative from a positive sample is due to freezing and thawing the sample.
- The Vela DX Sentosa CMV is a rapid, sensitive and specific method for qualitative or quantitative detection of CMV in clinical samples.