The cobas® CMV Test, with Workflow and Throughput Advantages, Shows Excellent Reproducibility and Clinical Concordance to the TaqMan CMV Test

Joseph Yao[1], Ann Butcher[2], Pari Hemyari[2], Paul Baum[2], Stephen Young[3], Alexandra Valsamakis[4]

[1]Mayo Clinic, Rochester, MN, USA; [2]Roche Molecular Diagnostics, Pleasanton, CA, USA; [3]Tricore Laboratories, Albuquerque, NM, USA; [4]Johns Hopkins University, Baltimore, MD, USA

1. Introduction and Purpose

Cytomegalovirus (CMV) viral load (VL) testing is a critical tool in managing the major viral threat to transplant recipients and other immunocompromised patients. Here, we study the clinical performance of a new CMV VL test, the **cobas**® CMV for use on the **cobas**® 6800/8800 Systems (**cobas**® CMV). These fully automated systems use a uniform assay design that permits mixed batching of CMV with other analytes, thereby improving laboratory workflow and time to result.

The clinical reproducibility of the **cobas**® CMV test was evaluated in three laboratories. To understand how results might be expected to compare with an existing FDA approved CMV assay, we conducted method comparison and clinical concordance studies comparing **cobas**® CMV with the COBAS® AmpliPrep/COBAS® TaqMan® CMV test (TaqMan CMV), using clinical samples from solid organ transplant and stem cell transplant patients.

2. Methods

The clinical reproducibility of the **cobas**® CMV test was assessed using a panel consisting of four members representing the linear range tested at 3 sites across multiple lots, operators and days.

The clinical concordance of the **cobas**® CMV test with the TaqMan CMV test was studied using de-identified longitudinal remnant plasma samples from solid organ transplant patients (SOT; n=1913 samples from 107 subjects) and hematopoietic stem cell transplant patients (HSCT; n=1532 samples from 257 subjects). The subjects were participants in CMV prophylaxis trials who underwent CMV viral load monitoring approximately weekly; some of these patients developed and were treated for CMV viremia. Concordance of all samples was analyzed at a representative viral load threshold (1800 IU/mL). We also conducted a concordance analysis for the decision to stop therapy at 14, 21, 28, 35 and 49 weeks after the onset of treatment. The stopping rule was defined as two sequential results <137 IU/mL, which is the lower limit of quantitation for the TaqMan test.

Finally, method comparison analysis was performed, using the SOT and HSCT samples, supplemented with 68 cross-sectional clinical samples and 219 contrived samples.

Table 1: Assays at a Glance

	COBAS® Ampliprep/ COBAS® TaqMan®	cobas® 6800/8800 System
System		
Throughput	Medium	Medium to High, with mixed batching
Gene Target	UL54 DNA polymerase	UL54 DNA polymerase
Sample type	Plasma	Plasma
95% LOD claim	56 IU/mL plasma (CE probit claim) 91 IU/mL plasma (US hit rate claim)	34.5 IU/mL plasma
LLOQ	137 IU/mL plasma	34.5 IU/mL plasma
Amplicon size	~350 bp	~150 bp
Quantitation standard	Competitive	Non-competitive
Mastermix	Z05 enzyme	Z05D enzyme and other improvements

3. Results

cobas® CMV was highly reproducible with a standard deviation of <0.114 $\log_{10}$ IU/mL and a detectable fold difference of <2.06. Most variability was in the low end of the range and due to within-run effects.

Table 2: Clinical reproducibility of cobas® CMV

CMV DNA Concentration ($\log_{10}$ IU/mL)		Number of Tests	Percent of Total Variance (Lognormal CV(%))						Total Precision	
Expected	Observed Mean		Lot	Site	Operator	Day	Run	Within-Run	SD	Log-normal CV (%)
2.01	**2.07**	**324**	1% (3.00)	5% (6.05)	2% (3.36)	0% (0.00)	2% (3.75)	90% (25.15)	0.114	26.61
3.26	**3.27**	**322**	10% (4.31)	11% (4.60)	3% (2.23)	2% (1.90)	0% (0.00)	74% (11.71)	0.059	13.64
3.86	**3.90**	**324**	23% (7.26)	0% (0.00)	0%(0.00)	0% (0.22)	0% (0.00)	77% (13.50)	0.066	15.36
6.70	**6.74**	**324**	15% (5.17)	3% (2.24)	0%(0.79)	1% (1.40)	0% (0.00)	81% (11.98)	0.058	13.35

Note: The table only includes results with detectable viral load. CMV = cytomegalovirus; CV(%) = percent coefficient of variation; SD = standard deviation.

In SOT patients the **cobas**® CMV and Taqman CMV were concordant at a 1800 IU/mL decision point, with 96.7% overall percentage agreement. The decision to stop antiviral therapy (based on two sequential results <137 IU/mL) would be 85% concordant, with the **cobas**® CMV usually resulting in a longer duration of therapy.

Table 3: Concordance of paired samples from SOT subjects at a representative 1800 IU/mL threshold

		TaqMan CMV Test			Row Agreement (95% Exact CI)[a] (95% Bootstrap CI)[b]
		< 3.3 $\log_{10}$ IU/mL (< 1800 IU/mL)	≥ 3.3 $\log_{10}$ IU/mL (≥ 1800 IU/mL)	Total	
cobas® CMV	**< 3.3 $\log_{10}$ IU/mL (< 1800 IU/mL)**	1693	1	1694	99.9% (99.7%, 100.0%) (99.8%, 100.0%)
	≥ 3.3 $\log_{10}$ IU/mL (≥ 1800 IU/mL)	62	142	204	69.6% (62.8%, 75.8%) (63.5%, 75.5%)
	Total	1755	143	1898	
Column Agreement (95% Exact CI)[a] (95% Bootstrap CI)[b]		96.5% (95.5%, 97.3%) (95.7%, 97.2%)	99.3% (96.2%, 100.0%) (97.4%, 100.0%)		
Overall Percent Agreement (95% Exact CI)[a] (95% Bootstrap CI)[b]		96.7% (95.8%, 97.4%) (95.9%, 97.4%)			
p-value[c]		<.0001			

[a]Assumed independence between all samples; [b]Adjusted correlation between samples from same subjects by the bootstrap method with 500 iterations; [c]Calculated using McNemar's Test.

Table 4: Concordance for the decision to stop therapy in SOT patients who developed CMV viremia

Time Point	Overall Percent Agreement between cobas® CMV and TaqMan CMV Test	95% Exact CI
Day 14	98.2% (54/55)	(90.3%, 100.0%)
Day 21	94.6% (53/56)	(85.1%, 98.9%)
Day 28	92.2% (47/51)	(81.1%, 97.8%)
Day 35	86.2% (50/58)	(74.6%, 93.9%)
Day 49	87.5% (42/48)	(74.8%, 95.3%)

In HSCT patients, the tests were concordant at a 1800 IU/mL decision point with 99.3% overall percent agreement. The decision to stop therapy would be 100% concordant although this was only evaluable in 26 subjects.

Table 5: Concordance of paired samples from HSCT subjects at a representative 1800 IU/mL threshold

		TaqMan CMV Test			Row Agreement (95% Exact CI)[a] (95% Bootstrap CI)[b]
		< 3.3 $\log_{10}$ IU/mL (< 1800 IU/mL)	≥ 3.3 $\log_{10}$ IU/mL (≥ 1800 IU/mL)	Total	
cobas® CMV	**< 3.3 $\log_{10}$ IU/mL (< 1800 IU/mL)**	1,320	2	1,322	99.8% (99.5%, 100.0%) (99.6%, 100.0%)
	≥ 3.3 $\log_{10}$ IU/mL (≥ 1800 IU/mL)	8	37	45	82.2% (67.9%, 92.0%) (71.8%, 91.3%)
	Total	1,328	39	1,367	
Column Agreement (95% Exact CI)[a] (95% Bootstrap CI)[b]		99.4% (98.8%,99.7%) (99.0%, 99.7%)	94.9% (82.7%, 99.4%) (87.8%, 100.0%)		
Overall Percent Agreement (95% Exact CI)[a] (95% Bootstrap CI)[b]		99.3% (98.7%, 99.6%) (98.8%, 99.6%)			
p-value[c]		0.1094			

[a]Assumed independence between all samples; [b]Adjusted correlation between samples from same subjects by the bootstrap method with 500 iterations; [c]Calculated using McNemar's Test.

Table 6: Concordance for the decision to stop therapy in HSCT patients who developed CMV viremia

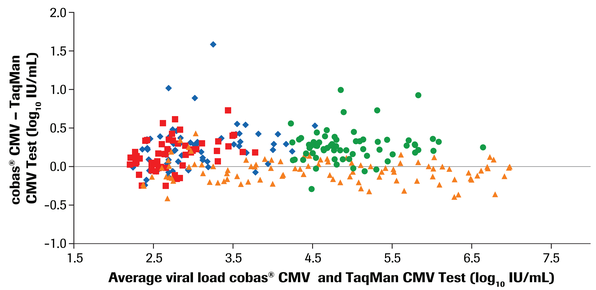
Time Point	Overall Percent Agreement between cobas CMV and TaqMan CMV Test
Day 14	100.0% (4/4)
Day 21	100.0% (7/7)
Day 28	100.0% (19/19)
Day 49	100.0% (2/2)

Due to insufficient patients, agreement at Day 35 was not assessed and no confidence intervals were calculated.

In a method comparison analysis, the two tests were highly concordant with no significant bias in contrived samples made from cultured virus. When baseline viremic clinical samples were tested, **cobas**® CMV yielded higher viral loads than Taqman® CMV with a bias of 0.16 $\log_{10}$ IU/mL in HSCT patients, and 0.25 $\log_{10}$ IU/mL in SOT patients.

Figure 1. Bland-Altman Bias Plot (cobas® CMV vs. TaqMan CMV Test)

Symbol	Sample Type	N	Mean Offset ($\log_{10}$ IU/mL)
◆	SOT	66	0.25 (0.19 to 0.32)
■	HSCT	53	0.16 (0.10 to 0.22)
●	Mixed clinical lab remnants	67	0.24 (0.19 to 0.29)
▲	Spiked (cultured virus)	99	-0.06 (-0.09 to -0.03)



4. Conclusions

- The new **cobas**® CMV test is highly reproducible and is calibrated to the WHO international standard.
- **cobas**® CMV provides workflow and throughput advantages over existing CMV testing platorms.
- **cobas**® CMV may result in higher quantitated values than the TaqMan CMV test in clinical samples, although both assays closely agree when testing samples spiked with cultured virus.
- This discrepancy is possibly due to limitations in the commutability of the WHO standard caused by template fragmentation in clinical samples which is not modeled by cultured virus samples.
- Both tests lead to concordant clinical decision making.

Disclosures: This study was funded by Roche Molecular Diagnostics, the manufacturer of both CMV tests described in this study. Ann Butcher, Pari Hemyari and Paul Baum are employees of Roche Molecular Diagnostics.

Note: cobas® CMV is not commercially available in all markets. Performance claims for the assay in the USA will be subject to FDA approval.

Roche

cobas®
Life needs answers