Fully automated Extraction, Amplification and Real-time Detection of CMV-DNA from different Specimens with the BD MAX™ Instrument

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INTRODUCTION

The BD MAX™ is a fully automated instrument for molecular diagnostics. The nucleic acid extraction and subsequent polymerase chain reaction (PCR) is done by adding the appropriate reagent cartridges and test tubes. Further intervention is not necessary (walk-away system)

1-24 samples can be processed and various tests can be performed in one run. The test duration varies between 2 and 3.5 hours depending on the number of samples and test format.

The BD MAX™ is an open test platform. Self developed tests can be implemented besides the BD IVD tests.

A variety of test formats are available.

Sample processing occurs in cartridges. Depending on the test format appropriate reagents are added. Supplied BD reagents are lyophilized

The aim of the study was to evaluate a fully automated quantitative CMV-PCR on the BD MAX™ instrument.

Evaluation was performed according to the criteria described by Rabenau et al. (J Lab Med 2007; 31 (2): 41-47).

Based on the results of preliminary tests, extraction reagents, master-mix buffer and IC were supplied by BD. Primer and probes were developed and provided by altona diagnostics (formerly astra diagnostics), Hamburg, Germany

BD MAX™ 1st generation (used in the study)
Width. 94 cm, Depth 75 cm; Height 72 cm
Weight: 114 kg

2 Detection channels:
FAM (512-559 nm),
CalRed (612-647)

Computer on-board

BD reagent strip with tubes for master-mix and DNA extraction.

In house specific primer and probes (2-fold concentrated) can be pipetted by user into tube 3

Microfluidic cartridge with 12 individually controlled and wax sealed PCR reaction chambers

BD MAX™ 2nd generation

6 detection channels:
FAM, ROX, HEX, Cy5, Cy5.5
(6 detection channels with 5 active colors)
LIS-Connectivity
Computer on-board

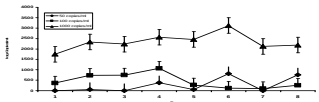
BD reagent strip with tubes for master-mix and DNA extraction.

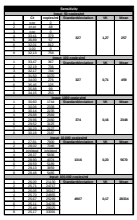
In house specific primer and probes (2-fold concentrated) can be pipetted by user into tube 3

Microfluidic cartridge with 24 individually controlled and wax sealed PCR reaction chambers

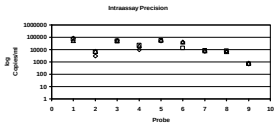
Analytical Sensitivity & Precision

Analytical sensitivity was determined with sample pools with indicated virus loads. Each pool was tested eight times. All eight samples from the 100 copies/ml pool and five samples with 50 copies/ml were tested positive.

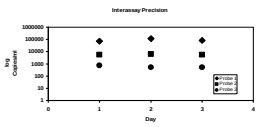




Intra- and Interassay-Precision



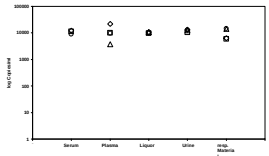
Dilutions from positive clinical urine samples were prepared to obtain different concentrations of CMV. Samples 7, 8 and 9 were a pool of negative samples. Sample volume was 700 µl.
9 positive and 3 negative probes were analyzed in triplicate. All negative results remain negative.



For interassay precision three positive (low, middle and high) and one negative sample from the first series of tests (intra-assay precision) were tested three times on two further days.

Specimens

Matrix CMV Urine/Plasma - Kit Input: 10.000 Copies/ml				
Serum	Copies/ml	Mean	Standarddeviation	VK
1	9207			
2	13347	10748	1396	0,13
3	10284			
4	8819			
Plasma				
1	22859			
2	10145	11413	6646	0,58
3	3742			
4	3712			
Liquor				
1	10353			
2	9709	10255	537	0,05
3	10876			
4	9736			
Urin				
1	13299			
2	10800			
3	12708	13158	1732	0,13
4	13355			
5	16023			
Resp. Material				
1	6458			
2	6056			
3	14463	10430	4176	0,40
4	14753			



Previous negative tested serum, plasma, liquor, urine and respiratory specimen-pools were spiked with standard CMV-DNA (concentration: 10.000 copies/ml). Each pool was tested four times in one run with the Urine/Plasma-Kit.

This Kit is very suitable for processing indicated specimens.

QCMD Proficiency Test

QCMD	QCMD copies/ml	BD Max copies/ml
CMV 10-1	1879	1114
CMV 10-2	230	391
CMV 10-3	275423	67272
CMV 10-4	5534	2532
CMV 10-5	0	0
CMV 10-6	2552701	470348
CMV 10-7	1799	2522
CMV 10-8	23988	11531
CMV 10-9	684	697
CMV 10-10	16904	7845

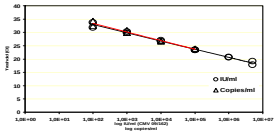
QCMD proficiency testpanel CMV 2010 was tested with the BD UrinePlasma-Kit.

The results have a good correlation to values indicated by QCMD.

Calibration with International CMV-Standard 09/162

Calibration with the International CMV-Standard was performed with dilutions of spiked urine samples. Starting with 5.000.000 IU/ml probes with 1.000.000 IU/ml, 100.000 IU/ml, 10.000 IU/ml, 1.000 IU/ml 100 IU/ml and 10IU/ml were tested in duplicate and compared with samples spiked with the *altona diagnostics* standards in the range from 100.000 – 10 copies/ml.

The results shows
1. a linear decrease of the Ct values of about 3 Cts/dilutionstep and
2. a very good correlation between the two dilution series (r^2 value: 0,99)



Summary & Conclusion

- ✓ **The BD MAX™ instrument is a true walk-away system and saves hand on-time in a routine molecular diagnostic laboratory.**
- ✓ **Different clinical specimens (urin, serum, plasma, liquor, respiratory materials) can be processed and analyzed in combination with the altona diagnostics CMV-DNA-Kit.**
- ✓ **Analytical sensitivity is 100 copies/ml.**
- ✓ **Calibration with the International CMV standard 09/162 showed a very good conformity to the altona diagnostics standards.**
- ✓ **The results showed a good Intra- and Interassay precision.**
- ✓ **Proficiency test results from QCMD were confirmed.**

The BD MAX™ instrument in combination with the altona diagnostics CMV-Kit is a suitable and reliable tool for analyzing CMV infections in different clinical specimens.