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

Thiemann, F.1; Laue, T.2.; Priscoglio, C. 3

1 MVZ Dr. Löer, Dr. Treder & Kollegen, Münster, Germany; 2 altona DIAGNOSTICS GmbH, Hamburg, Germany, Becton Dickinson; Heidelberg, Germany

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.

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

The aim of the study was to evaluate a fully automated quantitative CMV-PCR on the BD MAX™ instrument. Sample processing occurs in cartridges. Depending on the test format appropriate reagents are added. Supplied BD reagents are lyophilized and have to be reconstituted with provided reagent vials. probes (2-fold concentrated) can be pipetted by user into tube 3. 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 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.

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 in triplicate. Each negative result remains negative.

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 10 IU/ml were tested in duplicate and compared with samples spiked with 1,000 IU/ml.

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 750 µl. All negative samples were tested three times on two further days. For intraassay precision three positive (low, middle and high) and one negative (low) dilutions were tested three times. For interassay precision three positive (low, middle and high) dilutions were tested three times on two further days.

QCMD Proficiency Test

QCMD proficiency test panel CMV J018 was tested with BD Ultraflurox-AK. The results have a good correlation to values indicated by QCMD.

Summary & Conclusion

- The BD MAX™ instrument is a true walk-away system and saves hands on-time in a routine molecular diagnostics laboratory.
- Different clinical specimens (saliva, serum, plasma, liquor, respiratory materials) can be processed and analyzed in combination with the altona diagnostics CMV-DNA-Kit.
- Analytical sensitivity: 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 confimed.

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.