Rapid Detection of VZV - DNA with the BD MAX™-Instrument in the Routine Laboratory

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Introduction

Varizella zoster virus (VZV) is an α-herpesvirus with a double stranded DNA genome. Primary infection with VZV causes chickenpox, while reactivation of the virus is responsible for varicella zoster, or shingles.

In immunocompromised persons (AIDS, transplant recipients, cancer patients) a generalized zoster infection with life-threatening pneumonia and encephalitis may develop.

Diagnosis depends on the ability to detect VZV DNA in a rapid and sensitive fashion.

We developed a real-time PCR assay (altona diagnostics, Hamburg, Germany) for rapid and sensitive detection of VZV DNA using the BD MAX™ Instrument (Becton Dickinson).

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.

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

Microfludic 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

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

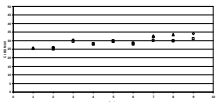
Material & Methods

Swabs
Previously positive tested swabs from clinical samples were washed out in Tris-EDTA buffer, pH 8.0. Buffer solutions were pooled and diluted to obtain samples with defined concentrations of VZV-DNA or buffer was spiked with appropriate amounts of standard VZV-DNA (altona diagnostics, Hamburg, Germany).

DNA Preparation
Reagents for DNA preparation as well as Master Mix-buffer and Internal Control were provided by Becton Dickinson, Heidelberg, Germany. Reagents were delivered lyophilized in appropriate cartouches (Swab/Whole Blood-Kit).

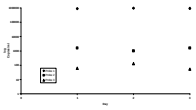
Master-Mix & PCR
Primer and probes for VZV-DNA-PCR were developed and provided by altona diagnostics, Hamburg, Germany. 12 µl of 2fold concentrated Master-Mix were pipetted into tube 3 of the reagent cartouches for each reaction.
PCR was performed automatically subsequent to DNA-preparation from the BD MAX™ Instrument in Microfluidic cartridges.

Intraassay-Precision



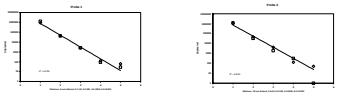
Dilutions from positive clinical samples were prepared to obtain different concentrations of VZV-DNA. Samples 7, 8 and 9 were a pool of negative probes. Sample volume was 700 µl.
9 positive and 3 negative probes were analyzed in triplicate. All negative results remain negative.

Interassay-Precision



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.

Linearity



Two negative sample pool were spiked with a defined concentration of VZV-DNA ($1x10^6$ copies/ml) and diluted to obtain appropriate defined concentrations: $1x10^5$, $1x10^4$, $1x10^3$, $1x10^2$ copies/ml. Each series were tested in duplicate.

Results showed a good recovery and precision as well as a linear decrease of 3 Cts/dilution.

QCMD Proficiency Test

QCMD	QCMD copies/ml	BD Max copies/ml
VZV-DNA10-01	165	211
VZV-DNA10-02	0	0
VZV-DNA10-03	90	97
VZV-DNA10-04	63	0
VZV-DNA10-05	0	0
VZV-DNA10-06	408	69
VZV-DNA10-07	202	190
VZV-DNA10-08	3639	6247
VZV-DNA10-09	21	0
VZV-DNA10-10	1941	344

QCMD proficiency testpanel VZV 2010 was tested with the BD Swab-Kit.

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

Low positive samples (10-04, 10-09) were not detected with the BD MAX™ - Swab-Kit.

Summary & Conclusion

- ✓ The BD MAX™ instrument is a true walk-away system and saves hand on-time in a routine molecular diagnostic laboratory.
- ✓ Clinical specimens (Swabs, plasma, liquor (not shown)) can be processed and analyzed in combination with the altona diagnostics VZV-DNA-Kit.
- ✓ Results showed a good Intra- and Interassay precision as well as a good linear measurement.
- ✓ Proficiency test results from QCMD were confirmed.

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