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VZV serology: Evaluation of new anti-VZV IgG and IgM assays

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Introduction

Diagnosis of varicella zoster virus (VZV) previous and acute infection is frequently done by serology. Routine diagnosis mainly includes confirmation of suspected acute infection or confirmation of VZV-specific immunity. We evaluated two new commercial VZV antibody immuno-assays, **VZV-IgG-ELISA PKS medac** and **VZV-IgM-ELA Test PKS medac**. Both assays were evaluated regarding diagnostic and technical performance including suitability for automation.

Material and Methods

- Assays: The indirect, quantitative **VZV-IgG-ELISA PKS medac** and the qualitative **VZV-IgM-ELA Test PKS medac** are both based on purified virus antigen. The IgG assay provides quantitative results without the need for a calibration curve in the test run (single-point quantitation method, SPQ). The assay is calibrated against the WHO 1st International Standard anti-varicella Zoster immunoglobulin. The IgM assay is based on µ-capture format. As reference assays the Enzygnost Anti-VZV/IgG and Enzygnost Anti-VZV/IgM from Dade Behring (Dade Behring Marburg GmbH, Marburg, Germany) were used. All measurements were performed according to the manufacturer´s instructions.
- Sera: 150 sera pre-defined by Prof. G. Enders, Stuttgart with the reference assays and classified as seronegative (n=50), from patients with previous (n=50), and acute infection (n=50) were used for diagnostic evaluation. In addition, 150 sera from blood donors were used to determine VZV-IgG as well as VZV-IgM antibody prevalence. A set of pre-defined sera with different reactivity of medac serum bank was chosen for technical performance evaluation.
- Evaluation experiments: For diagnostic evaluation the 150 pre-defined sera and the blood donor sera were measured in both medac assays. Blood donor sera were also measured in the reference assays.

Interassay and intra-assay variation were investigated both manually and using an automatic device (DSX, Dynex). Moreover, person-to-person variation, lot-to-lot variation, and correlation between quantitative IgG results calculated by SPQ and standard curve were investigated. Dilution linearity of 10 VZV-IgG reactive sera was investigated over the whole measuring range. Suitability for automation was investigated using two different automatic devices (DSX and Behring ELISA Processor III, BEP) parallel to manually performed test runs.

Results

- Diagnostic evaluation: The overall agreement with the interpretation of the pre-defined sera obtained with the VZV-IgG medac assay was 99.3 % and with the VZV-IgM medac assay 96.0 % (see Fig. 1). The *-marked serum (initially considered as negative, turned out to be positive) was from a pregnant woman about 9 weeks after onset of disease. Thus the positive medac result is plausible. The deviant IgM results were obtained with samples from different patients with VZV infection taken 6, 8, 13 or 20 weeks after beginning of disease. Concerning the blood donor sera 0.7 % were positive and 0 % equivocal for VZV-IgM, and for VZV-IgG 98.0 % were positive and 0.7 % equivocal with the medac assays. With the reference assays the respective prevalences were 1.3% (positive) and 6.0 % (equivocal) for IgM, and 98.0 % (positive) and 1.3 % (equivocal) for IgG (see Table 1).
- Precision experiments: All precision experiments revealed good coefficients of variation (CV) with both assays, independent of the way of processing (see Table 2). Dilution linearity of 10 sera showed a correlation between expected and obtained values of R^2=0.84 (see Fig. 2). However, two sera originating from blood donors revealed an underestimation and overestimation of the measured VZV-IgG concentrations, respectively. Correlation of quantitative results obtained using internal standard curve and SPQ was excellent (see Fig. 3). Results of automatically performed test runs were plotted against the corresponding results of parallel, manually performed test runs. For both assays and both devices very good correlations were found, with $R^2 > 0.97$ in each case (see Fig. 4).

Pre-definition

medac / VZV-IgG [mIU/ml]	-	±	+	Σ
-	49	0	0	49
±	0	1	0	1
+	1	0	99	100
Σ	50	1	99	150

Specificity = 98.0 %
Sensitivity = 100.0 %
Concordance: 99.3 %

Pre-definition

medac / VZV-IgM [OD/Cut-off]	-	±	+	Σ
-	99	0	4	103
±	0	0	1	1
+	1*	0	45	46
Σ	100	0	50	150

Specificity = 99.0 %
Sensitivity = 90.0 %
Concordance: 96.0 %
*Sample from patient with confirmed acute VZV infection

Fig. 1: Diagnostic Performance of **VZV-IgG-ELISA PKS medac** and **VZV-IgM-ELA Test PKS medac** determined with 150 pre-defined sera.

Table 1: Blood donor sera. Antibody prevalences and concordance of medac vs. reference assay.

	IgG (n=150)		IgM (n=150)	
	medac	reference	medac	reference
Positive	98.0 %	98.0 %	0.7 %	1.3 %
Equivocal	0.7 %	1.3 %	0.0 %	6.0 %
Concordance (medac vs. reference)	99.3 %		93.3 %	

Table 2: Precision of VZV-IgG-ELISA PKS medac and VZV-IgM-ELA Test PKS medac.

	IgG		IgM	
	CV[a]	N[b]	CV[a]	N[b]
Intra-assay variation (manually) [c](n=22)	3.9 %	8	5.9 %	8
Intra-assay variation (DSX) [c](n=22)	6.1 %	8	9.6 %	8
Interassay variation (manually) [c](n=11)	14.6 %	13	6.2 %	9
Interassay variation (DSX) [c](n=11)	10.0 %	13	7.6 %	9
Person-to-person variation [c](n=3)	9.9 %	13	11.4 %	13
Lot-to-lot variation [c](n=3)	6.7 %	12	16.0 %	12

[a] CV calculation based on mIU/ml (IgG) and OD/cut-off (IgM), respectively. Only the highest CV of reactive sera is shown.
[b] No. of samples.
[c] No. of determinations.

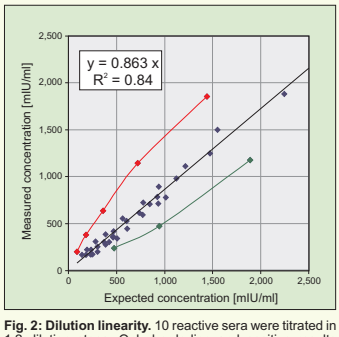

Fig. 2: Dilution linearity. 10 reactive sera were titrated in 1:2 dilution steps. Only borderline and positive results within the measuring range were used for calculation. 2 sera (red and green) behave differently. Linear regression includes all 10 sera (without the 2 nonparallel samples, y=0.856 x, R^2=0.98).

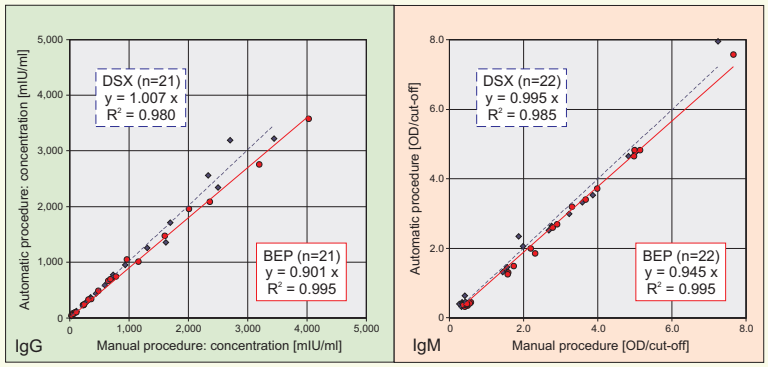

Fig. 4: Automation. Comparison of test results obtained with manually and automatically performed test runs in parallel. IgG concentrations below the measuring range of 40-5,000 mIU/ml were extrapolated.

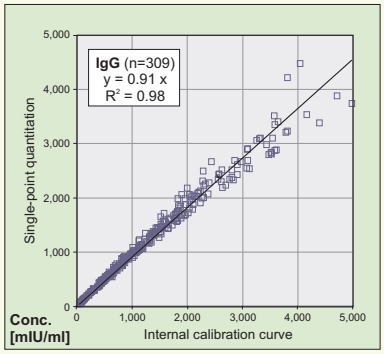

Fig. 3: Single-point quantitation (SPQ). Antibody concentration of 309 sera were calculated by SPQ and using an internal calibration curve (40-5,000 mIU/ml) in one test run.

Conclusions

Our results demonstrate that **VZV-IgG-ELISA PKS medac** and **VZV-IgM-ELA Test PKS medac** fulfill the needs for modern routine diagnosis in laboratories dealing with small and large sample size. The assays are easy to perform, providing precise qualitative (IgM) and quantitative (IgG) results in less than three hours.

The sensitivity of the IgM assay was somewhat lower with samples taken more than six weeks after onset of the disease compared to the reference test. Within the blood donor panel the IgG and IgM antibody prevalences obtained with the medac assays are plausible and demonstrate the excellent specificity of the medac IgM assay. The investigation of the dilution linearity of high titer sera shows that two sera reveal a dilution behaviour which is different from that of the international standard.

The evaluation reveals the suitability of both **VZV-IgG-ELISA PKS medac** and **VZV-IgM-ELA Test PKS medac** for routine diagnosis of VZV immunity status and of acute and previous VZV infection.