

Analysis of VZV and HSV-1 and -2 in different matrices by affigene® kits

Niklas Finnström¹, Karin Bergsten², Helena Ström¹, Sophie Martin¹, Andreas Sauerbrei³, Peter Wutzler³ - 1) Cepheid Europe, Maurens Scopont, France; 2) Cepheid AB, Bromma, Sweden; 3) Institute of Virology and Antiviral Therapy, University of Jena, Germany

Background

Real-time PCR can provide excellent performance for diagnosing varicella zoster virus (VZV) and herpes simplex virus (HSV) -1 and -2 infections. The methodology itself offers high sensitivity and great specificity. However, for good performance the result is also dependent on the efficiency of the sample preparation method used.

Diagnostic methods should be compatible with different sample preparation assays. The preparation method also needs to be flexible in terms of which sample matrix it can handle. The complete procedure, from sample preparation to analysis needs to be validated.

Methods

Retrospective samples from a wide range of different matrices (table 1a and b) were used, 175 samples diagnosed for VZV and 300 samples diagnosed for HSV (291 samples were used for statistical analysis).

DNA from the samples was prepared using Qiagen blood mini kit according to the manufacturer's instruction. Subsequently, the presence of VZV and HSV-1 and -2 was analysed using affigene® VZV tracer and affigene® HSV 1/2 tracer, respectively, on the Mx3000P instrument (Stratagene, La Jolla, USA).

In parallel, 52 samples (51 were used for statistical analysis) were prepared using affigene® DNA extraction according to the manufacturer's instructions and analysed using affigene® HSV 1/2 tracer. All the samples were analysed using Qiagen blood mini kit and a qualitative in-house PCR (1) prior to analysing by affigene® assays.

The agreement between the two different real-time PCR assays and agreement within subsets of matrices were statistically calculated using McNemar Test in the R software (freeware). The agreement between the two different sample preparation methods was calculated using the K_{hi}2-test in the R software.

Table 1 Different matrices analysed for a) VZV and b) HSV-1 or -2.

Table 1a. Matrices for VZV analysis		Table 1b. Matrices for HSV 1/2 analysis	
Matrix	Number	Matrix	Number
CSF	98	vesicle/skin swabs	198
Vesicle/skin swabs	42	Respiratory/eye swabs	36
EDTA blood/plasma	14	CSF	40
Respiratory/eye/ear swabs	11	EDTA blood	4
Tissue/ascites/amniotic fluid	10	Genital swabs	20
		Amniotic fluid	2

Objectives

The aim of this study was to:

- Compare the results from the commercial PCR assays to in-house PCR assays.
- Analyse samples using two different sample preparation methods: the Qiagen blood mini kit (Qiagen, Hilden, Germany), and the affigene® DNA extraction kit (Cepheid AB, Bromma, Sweden) in combination with affigene® tracer assays.
- Analyse a wide range of matrices for detection of VZV, HSV -1 and -2 using the affigene® VZV tracer and the affigene® HSV 1/2 tracer kits (Cepheid AB, Bromma, Sweden), respectively.

Results

Comparing the two PCR assays.

Generally, more positive samples were detected using affigene® assays in combination with the Qiagen blood mini kit preparation system compared to the in-house assays (tables 2a-c). 12.6% of the samples were positive for VZV using affigene® VZV tracer and 7.4% when using the in-house assay (p=0.012). For HSV-1 the corresponding figures were 39.2% for affigene® HSV1/2 tracer and 33.6% for in-house assay (p=0.0018) and for HSV-2 21.6% for affigene® HSV 1/2 tracer and 11.0% for the in-house assay (p=7.7x10e-6).

Comparing different sample preparation methods.

Table 3 shows that there is no difference when using the two complete procedures (Qiagen+in-house vs affigene® extraction + affigene® HSV1/2) with respect to sensitivity.

Comparing different matrices

To be able to compare sensitivity within different matrices they were divided into subsets as shown in Table 1a and b.

As seen in Table 4a and b and Fig 1a and b there were significant differences in sensitivity between affigene® HSV 1/2 tracer and the in-house assay for the following matrices CSF (p=0.0047) and vesicle/skin swabs (p=0.00007). The other matrices and analysis for VZV did not show any significant differences.

The positive agreement, for analysing VZV in all matrices was 85% and the negative agreement was 92%. For HSV-1 the corresponding figures were 97% and 89%, respectively, and for HSV-2 100% and 90%, respectively.

Table 2 Numbers of positives and negatives for a) VZV, b) HSV-1 and c) HSV-2 using Qiagen blood mini kit and in-house PCR assays or affigene VZV tracer/affigene HSV 1/2 tracer assay.

Table 2a		affigene VZV tracer	
		Pos	Neg
Inhouse	Pos	11	2
	Neg	11	151

Table 2b		affigene HSV 1/2 tracer	
		HSV-1 Pos	HSV-1 Neg
Inhouse	HSV-1 Pos	81	3
	HSV-1 Neg	17	149

Table 2c		affigene HSV 1/2 tracer	
		HSV-2 Pos	HSV-2 Neg
Inhouse	HSV-2 Pos	21	0
	HSV-2 Neg	20	149

Table 3 Numbers of positives and negatives for a) HSV-1 or b) HSV-2 using either affigene DNA extraction and affigene HSV1/2 tracer (Cepheid) or Qiagen blood minikit and in-house PCR assay (in-house).

Table 3a		Analysis of HSV-1 using either affigene assay or in-house assay	
		Cepheid	
		Pos	Neg
Inhouse	Pos	1	0
	Neg	6	44

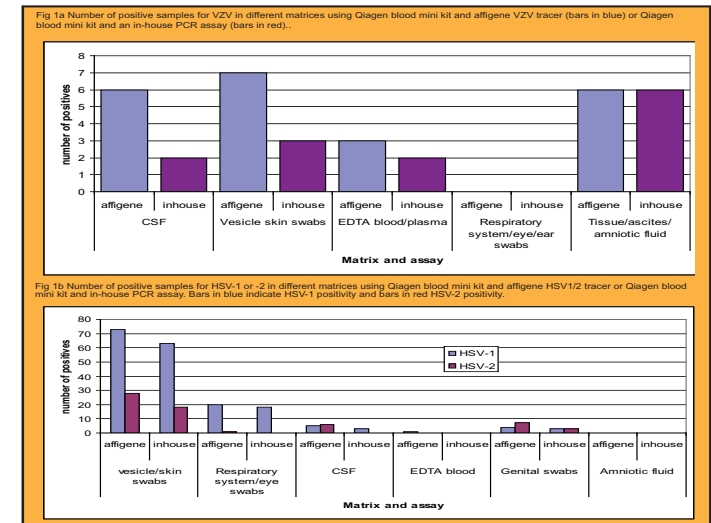
Table 3b		Analysis of HSV-2 using either affigene assay or in-house assay	
		Cepheid	
		Pos	Neg
Inhouse	Pos	0	0
	Neg	2	49

Table 4 Number of positive and negative samples for a) VZV or b) HSV-1 or -2 using Qiagen blood mini kit and affigene VZV tracer/affigene HSV1/2 tracer or Qiagen blood mini kit and an in-house PCR assay.

Table 4a				Table 4b				
Positivity in different matrices for VZV				Positivity in different matrices for HSV-1 and -2				
		Pos	Neg			Pos HSV-1	Pos HSV-2	Neg
CSF	affigene inhouse	6	92	Vesicle/skin swabs	affigene inhouse	73	28	95
		2	96			63	18	115
Vesicle skin swabs	affigene inhouse	7	35	Respiratory/eye swabs	affigene inhouse	20	1	15
		3	39			18	0	38
EDTA blood/plasma	affigene inhouse	3	11	CSF	affigene inhouse	5	6	28
		2	9			3	0	36
Respiratory/eye/ear swabs	affigene inhouse	0	11	EDTA blood	affigene inhouse	1	0	2
		0	11			0	0	3
Tissue/ascites/amniotic fluid	affigene inhouse	6	4	Genital swabs	affigene inhouse	4	7	10
		6	4			3	3	14
				Amniotic fluid	affigene inhouse	0	0	2
						0	0	2

Conclusion

affigene® VZV tracer and affigene® HSV 1/2 tracer are sensitive assays for detection of varicella zoster and herpes simplex viruses. A wide variety of sample matrices can be analysed using either Qiagen blood mini kit or affigene® DNA extraction as sample preparation method.



References

- 1) Sauerbrei et al. J Clin Virol. 2002 Suppl 1:S45-51 i