Public Health England

*SpeeDx Plex*PCR™VHS Evaluation

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Aims

We evaluated the sensitivity and specificity of the *SpeeDx Plex*PCR™VHS Assay (HSV-1, HSV-2, VZV and TP) and determined VZV prevalence in genital samples.

Background

Herpes simplex virus (HSV), Syphilis (*Treponema pallidum*, TP) and *Haemophilus ducreyi* (chancroid, HD) are recognised as major aetiological agents of genital ulcers worldwide although in the UK, HD is a relatively rare presentation.

Differential diagnosis typically relies on associated painful lesions in HSV while TP ulcers are regarded as painless. This distinction may be an inaccurate predictor of causative aetiology[1]. Furthermore, Varicella-zoster virus (VZV) reactivation (herpes zoster) may occasionally involve genital sites with a similar clinical presentation to HSV infection[2].

The availability of multiplex PCR tests for HSV, VZV and TP may be cost-effective for simultaneous diagnosis of genital HSV, VZV and TP infections. In this study, we have evaluated the *SpeeDx Plex*PCR™ VHS assay and compared the performance against Laboratory Developed Tests (LDT) for HSV, VZV and Syphilis.

Materials and Methods

Clinical Samples – Three panels of samples were used to undertake the study:-

Panel A: anonymised remnant genital swabs following routine testing for HSV (n= 295).
Panel B: anonymised remnant non-genital skin swabs following routine testing for VZV (n=55).
Panel C: anonymised genital swabs previously tested by PHE Birmingham for Syphilis and Syphilis controls (n=23)

Nucleic Acid Extraction – Swabs samples (200µL) were extracted on the QIAGEN QIAsymphonySP using the DSP Virus Pathogen Mini Kit using the 85µL elution protocol. Samples were spiked with the Internal Controls for both *SpeeDx* test and LDT.

Real Time PCR – Nucleic Acid amplication and detection was performed on the Applied Biosystems® 7500 Fast Real Time PCR System in PCR reactions (20µL) containing 5µL of Nucleic Acid eluate.

The *SpeeDx Plex*PCR™VHS Assay comprises a pentaplex reaction for HSV1/HSV2/VZV/TP/IC utilising *Plex*Zyme™, a novel probe technology.
The LDT assays included a triplex HSV1/HSV2/IC and duplex VZV/IC and TP/IC assays utilising TaqMan™ probes .

Crossing thresholds of reactive samples were determined by manual analysis using Applied Biosystems SDS software (LDT assays) or using an automated software algorithm on exported assay files (*SpeeDx* assay).

Results

The performance of the *SpeeDx Plex*PCR™VHS assay was compared with LDT assays using Panels A-C (n=373), Figures 1 and 2.

Figure 1. Performance of the *SpeeDx Plex*PCR™ Assay compared with LDT assays for HSV-1, HSV-2, VZV and TP.

		HSV-1 LDT	
		POS	NEG
SpeeDX PlexPCR™ VHS	POS	73	3
	NEG	3	294

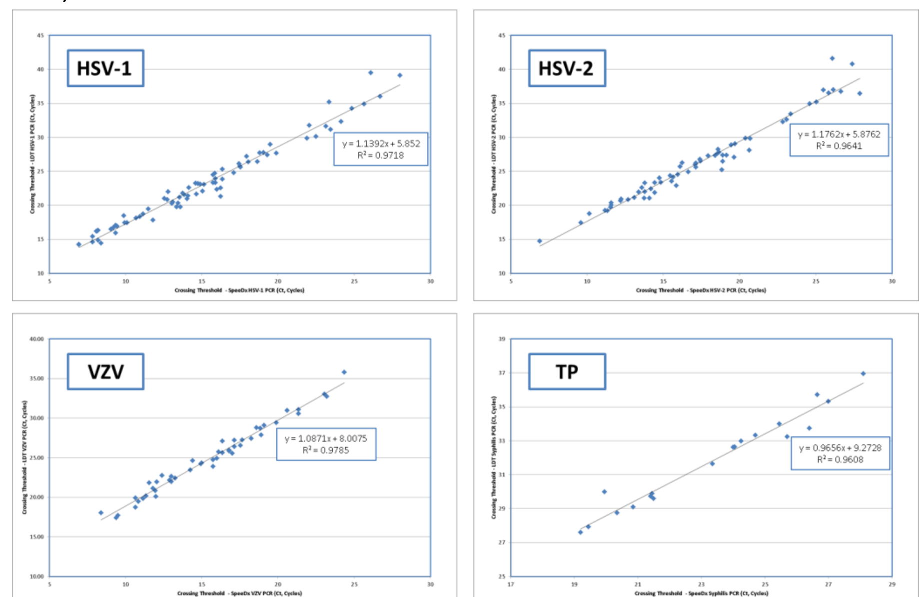
		HSV-2 LDT	
		POS	NEG
SpeeDX PlexPCR™ VHS	POS	63	3
	NEG	2	305

		VZV LDT	
		POS	NEG
SpeeDX PlexPCR™ VHS	POS	47	0
	NEG	2	324

		TP LDT	
		POS	NEG
SpeeDX PlexPCR™ VHS	POS	19	5
	NEG	0	349

		Diagnostic Sensitivity (95% CI)	Diagnostic Specificity (95% CI)	Cohen's Kappa (95% CI)
SpeeDX PlexPCR™ VHS	HSV-1	0.96 (0.92-1.00)	0.99 (0.98-1.00)	0.95 (0.91-0.99)
	HSV-2	0.97 (0.93-1.01)	0.99 (0.98-1.00)	0.95 (0.91-0.99)
	VZV	0.96 (0.90-1.01)	1.00 (1.00-1.00)	0.98 (0.94-1.01)
	TP	1.00 (1.00-1.00)	0.99 (0.97-1.00)	0.88 (0.77-0.98)

Figure 2. Linear correlation of the *SpeeDx* and LDT Assays for HSV1, HSV2, VZV and TP.



The prevalence of each organism as the causative agent of genital ulcers was assessed by the *SpeeDx Plex*PCR™VHS assay from clinical samples in Panel A (n=295), Figure 3.

Figure 3. Prevalence of HSV1, HSV2, VZV and TP in 295 genital swabs from adult patients (>18 years).

		Male	Female	Prevalence
Herpes Simplex	**136**	**52**	**84**	46.10%
HSV-1	71	28	43	24.07%
HSV-2	63	22	41	21.36%
HSV 1+2	2	2	0	0.68%

		Male	Female	Prevalence
Syphilis	**5**	**3**	**2**	1.69%

		Male	Female	Prevalence
VZV	**1**	**1**	**0**	0.34%

Conclusions

The *SpeeDx Plex*PCR™VHS Assay performed well in comparison with LDT assays for individual organisms that are recognised as causative agents of genital ulcers. A multiplex PCR approach is operationally more efficient and may provide a cost effective screen to support clinical management of cases of genital ulcerative infection.

1. Towns et al (2016) Sex Transm Infect. 92 p110-115
2. Birch et al (2003) Sex Transm Infect 79 p298–300