



## AIMS

We evaluated the sensitivity and specificity of the *SpeedX PlexPCR™ 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<sup>1</sup>. Furthermore, Varicella-zoster virus (VZV) reactivation (herpes zoster) may occasionally involve genital sites with a similar clinical presentation to HSV infection<sup>2</sup>.

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 PlexPCR™ 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 QIA-symphonySP 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 amplification 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 PlexPCR™ VHS* Assay comprises a pentaplex reaction for HSV1/HSV2/VZV/TP/IC utilising *PlexZyme™*, 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 PlexPCR™ VHS* assay was compared with LDT assays using Panels A-C (n=373), Figures 1 and 2.

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

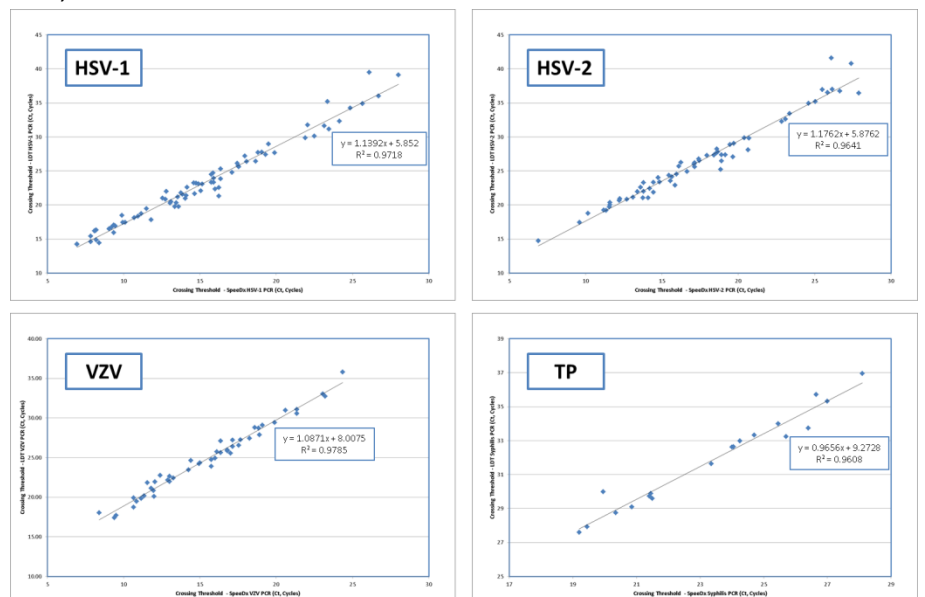
		HSV-1 LDT		HSV-2 LDT	
		POS	NEG	POS	NEG
SpeedX PlexPCR™ VHS	POS	73	3	63	3
	NEG	3	294	2	305

		VZV LDT		TP LDT	
		POS	NEG	POS	NEG
SpeedX PlexPCR™ VHS	POS	47	0	19	5
	NEG	2	324	0	349

SpeedX PlexPCR™ VHS		Diagnostic Sensitivity (95% CI)	Diagnostic Specificity (95% CI)	Cohen's Kappa (95% CI)
	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 PlexPCR™ 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
<b>Herpes Simplex</b>	<b>Total</b>	<b>52</b>	<b>84</b>	<b>46.10%</b>
	HSV-1	28	43	24.07%
	HSV-2	22	41	21.36%
	HSV 1+2	2	0	0.68%

		Male	Female	Prevalence
<b>Syphilis</b>	<b>Total</b>	<b>3</b>	<b>2</b>	<b>1.69%</b>

		Male	Female	Prevalence
<b>VZV</b>	<b>Total</b>	<b>1</b>	<b>0</b>	<b>0.34%</b>

## CONCLUSIONS

The *SpeedX PlexPCR™ 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.