NOVEL HERPES SIMPLEX VIRUS, VARICELLA ZOSTER VIRUS, AND TREPONEMA PALLIDUM QUALITY CONTROL MATERIAL FOR USE WITH GENITAL LESION MOLECULAR DETECTION ASSAYS

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Introduction

The emergence of genital ulcer disease is becoming a global health concern, with over 20 million new cases diagnosed each year[1]. Molecular assays that detect Herpes Simplex Virus (HSV) 1&2, Varicella Zoster Virus (VZV), and *Treponema pallidum* (TP) – the most common etiological agents of genital ulcer disease– are evolving in clinic; however, there is limited quality control material available to support the use of these tests. While native pathogens are available for HSV 1&2, VZV, and TP, the samples are not formulated to mimic patient specimen formats, as genital lesion detection is usually performed with swab-based collection methods. Additionally, the few TP quality control materials on the market are comprised of "naked" synthetic DNA, and thus are not sufficient to control whole workflows, such as sample extraction. To our knowledge, we are the first to develop a multiplex whole-workflow control for genital lesion panels. The sample is formulated on a Copan FLOQSwab®, not only to mimic patient specimen formats, but also to ensure sample compatibility with all assay workflows and elution buffers.

Materials and Methods

Formulate a whole-workflow HSV1&2/VZV/TP sample on a Copan FLOQSwab®

- Native, inactivated HSV1 – *MacIntyre strain*
- Native, inactivated HSV2 – *MS strain*
- Native, inactivated VZV – *VZ-10 strain*
- Synthetic TP, partial genome – target TP47

Evaluate Sample Performance

- Commercial Assays
- Laboratory Developed Tests (LDTs)
- External Quality Assessment (EQA) Pilot Study

Results

1. GUD EQC Performance with Commercial Assays

Table 1: HSV1&2/VZV/TP Sample Performance

Manufacturer	Assay	Target Analytes			
		HSV1	HSV2	VZV	TP
Unable to disclose **Assay is in final stages of development*	HSV1&2/VZV/TP Molecular Assay	+	+	+	+
Seegene	Allplex™ Genital Ulcer Assay	+	+	+	-

Note: *Seegene's Allplex™ Genital Ulcer Assay is not designed to amplify the TP47 gene target for TP detection; therefore, the sample does not test positive for TP.*

2. GUD EQC Performance with LDT

Table 2: HSV1&2/VZV/TP Sample Performance
Cadham Manitoba Provincial Laboratory

Sample	Elution Volume Copan UTM-RT	Target Analytes			
		HSV1	HSV2	VZV	TP
Low Positive N = 9	3 mL	30.13 ±0.19	26.16 ±0.21	28.55 ±0.25	32.28 ±1.94
Medium Positive N = 9	2 mL	30.18 ±0.16	26.23 ±0.09	28.80 ±0.13	30.52 ±1.25
High Positive N = 9	1 mL	29.13 ±0.13	25.10 ±0.07	27.58 ±0.12	29.63 ±0.94

Extraction: *Biomerieux eMAG; 200ul of the sample eluate was used for extraction*
Amplification: *Biorad CFX96 PCR instrument; 5ul of the extracted sample was used*

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3. GUD EQC Performance in an EQA/Proficiency Testing Pilot Study (Table 3-6)

Table 3: HSV1&2/VZV/TP Sample Performance with HSV1&2 Assays

Manufacturer	Assay	No. of Participants	Target Analytes	
			HSV1	HSV2
BIOFIRE BY BIOMÉRIEUX	FilmArray Meningitis/ Encephalitis Panel	1	+	+
DiaSorin	Simplexa HSV1&2 Direct Kit	1	+	+
DNA-TECHNOLOGY	Herpes Simplex Virus 1,2	3	+	+
meridian BIOSCIENCE	Alethia HSV 1&2	1	+	+
QIAGEN	Artus HSV-1/2	3	+	+
		1	-	+
Seegene	Allplex™ Genital Ulcer Assay	3	+	+
VECTOR BEST	RealBest DNA HSV 1,2	1	+	+
Laboratory	In-House PCR	1	+	+

Table 4: HSV1&2/VZV/TP Sample Performance with VZV Assays

Manufacturer	Assay	No. of Participants	VZV
BIOFIRE BY BIOMÉRIEUX	FilmArray Meningitis/ Encephalitis Panel	1	+
DiaSorin	Simplexa VZV Swab Direct Kit	1	+
QIAGEN	Artus VZV	3	+
DNA-TECHNOLOGY	Varicella Zoster Virus	2	+
Seegene	Allplex™ Genital Ulcer Assay	3	+
Laboratory	In-House PCR	1	+

Table 5: HSV1&2/VZV/TP Sample Performance with TP Assays

Manufacturer	Assay	No. of Participants	TP
AmpliSens Biotechnology	AmpliSens Treponema pallidum-FRT PCR Kit	1	+
Seegene	Allplex™ Genital Ulcer Assay	3	- *
Laboratory	In-House PCR	2	- *

**: The assay is not designed to amplify the TP47 gene target for TP detection; therefore, the sample does not test positive for TP.*

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Table 6: Summary of EQA Pilot Study Results

16 Laboratories	13 Assays/platforms	HSV1 Average Success Rate	HSV2 Average Success Rate	VZV Average Success Rate	TP (*TP47 Target) Average Success Rate
		93.3%	100%	100%	100%

Conclusions

- The HSV1&2/VZV/TP swab positive GUD EQC samples successfully monitored laboratory testing performance, procedures and workflows with molecular assays that detect HSV1, HSV2, VZV, and TP (TP47 target). Testing a dilution series with the GUD EQC demonstrated a linear relationship for detection signal for all targets.
- When tested with TP molecular assays that detect the PolA gene target, the samples were determined as "negative", as expected.
- The lack of HSV1 detection with the Qiagen artus HSV-1/2 assay could be attributed to a deviation at one site, since all other participants using the same procedure and test kit successfully detected HSV1 and HSV2. Ultimately, the outcome confirms the intended use of external controls by capturing workflow and performance deviations.
- A future iteration of the GUD EQC formulation will consider incorporating the TP PolA gene target to ensure broader compatibility with TP commercial and laboratory-developed tests on the market.

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