

# COMPREHENSIVE MOLECULAR TESTING for ORAL SEXUALLY TRANSMITTED INFECTIONS (STIs)

## Verification of a Multiplexed STI Pathogen Microarray Test From Saliva Collected With BioMAX SDNA-1000 Kit

The rise in oral sex as a common sexual practice has led to an increased risk of transmitting sexually transmitted infections (STIs) through this route. However, the existing methods for diagnosing STIs often overlook the potential infections resulting from oral sexual activities.<sup>1,2</sup> The consequences of undiagnosed and untreated oral STIs as well as missed coinfections, extend beyond individual health, impacting public health as a whole. Undetected infections including can facilitate the silent spread of STIs within communities, leading to a higher burden of disease and associated complications. Furthermore, certain oral STIs, such as HPV, are strongly linked to the development of various cancers, emphasizing the importance of early detection and prevention.

This knowledge gap in testing strategies poses a significant challenge in effectively identifying and treating oral STIs. As a result, there is a pressing need to develop innovative and reliable diagnostic approaches specifically designed for the detection of oral STIs. Identification of infected persons, both symptomatic and asymptomatic, is one of the five major STI prevention and control strategies goaled by the CDC.<sup>3</sup>

By focusing on the unique challenges associated with oral transmission and leveraging advancements in diagnostic microarray technologies, we can improve our ability to detect and diagnose oral STIs accurately.<sup>4</sup> This will enable timely intervention, appropriate treatment, and effective prevention strategies, ultimately contributing to better sexual health outcomes for individuals and the overall population. For example, saliva-based testing has been clinically shown to be highly effective in identifying individuals infected with Mpox (formerly known as Monkeypox).<sup>5,6</sup>

Efforts should be made to raise awareness, promote regular testing, and integrate oral STI screening into routine healthcare practices to effectively address emerging public health challenges.



SPECTRUM  
SIMPLYTEST™

BACKGROUND



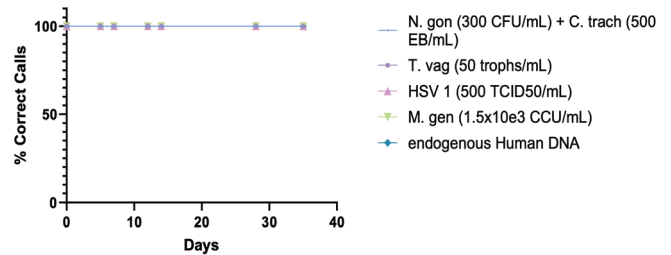
# RESULTS



**FIGURE 1**

## Plot of sample stability of Saliva and Urine in sample in SDNA-1000 preservation buffer.

Nine samples were used per time point for each sample. 3 samples went through low temperature excursions (3x freeze thaw cycles), 3 remained at room temperature, and 3 went through a high temperature excursions to 40° C before being aged at room temperature over 35 days. Samples in preservation buffer were all stable out to 35 days.



**TABLE 1**

## Analytical Sensitivity Competition of Targets

The effect of multiple detectable analytes (co-infection states) within a given sample were evaluated by testing combinations of analytes at varying concentrations at 3 times the LoD of each analyte. Further Testing demonstrated up to 5 organisms can be detected simultaneously and that agents of co-infection should be readily detected.

#	Analyte 1 at 1X LoD	Analyte 1 Detected (Y/N)	Analyte 2 at 1X LoD	Analyte 2 Detected (Y/N)	Competition of Targets (in Saliva)		Analyte 4 at 1X LoD	Analyte 4 Detected (Y/N)	Analyte 5 at 1X LoD	Analyte 5 Detected (Y/N)
					Analyte 3 at 1X LoD	Analyte 3 Detected (Y/N)				
31	M. genitalium	Y	T. vaginalis	Y	N. gonorrhoeae	Y				
32	T. vaginalis	Y	N. gonorrhoeae	Y	T. pallidum	Y				
33	N. gonorrhoeae	Y	C. trachomatis	Y	HSV1	Y				
34	C. trachomatis	Y	HSV1	Y	HSV 2	Y				
35	HSV1	Y	HSV 2	Y	Human (DNA)	Y				
36	HSV 2	Y	Human (DNA)	Y	U. urealyticum	Y				
37	Human (DNA)	Y	U. urealyticum	Y	M. genitalium	Y				
38	U. urealyticum	Y	M. genitalium	Y	T. vaginalis	Y				
39	M. genitalium	Y	T. vaginalis	Y	HSV1	Y				
40	T. vaginalis	Y	N. gonorrhoeae	Y	C. trachomatis	Y				
41	Mpox	Y	HSV 2	Y	U. urealyticum	Y				
42	T. pallidum	Y	Human (DNA)	Y	C. trachomatis	Y				
43	N. gonorrhoeae	Y	U. urealyticum	Y	HSV 2	Y				
44	C. trachomatis	Y	Mpox	Y	Human (DNA)	Y				
45	HSV1	Y	T. pallidum	Y	Mpox	Y				
46	M. genitalium	Y	T. vaginalis	Y	N. gonorrhoeae	Y	C. trachomatis	Y		
47	T. vaginalis	Y	N. gonorrhoeae	Y	C. trachomatis	Y	HSV1	Y		
48	N. gonorrhoeae	Y	C. trachomatis	Y	HSV 2	Y	HSV 2	Y		
49	C. trachomatis	Y	HSV1	Y	HSV 2	Y	Human (DNA)	Y		
50	HSV1	Y	HSV 2	Y	T. pallidum	Y	U. urealyticum	Y		
51	Mpox	Y	U. urealyticum	Y	Human (DNA)	Y	N. gonorrhoeae	Y		
52	T. vaginalis	Y	M. genitalium	Y	U. urealyticum	Y	Human (DNA)	Y		
53	Mpox	Y	N. gonorrhoeae	Y	M. genitalium	Y	HSV 2	Y		
54	M. genitalium	Y	T. vaginalis	Y	N. gonorrhoeae	Y	C. trachomatis	Y	HSV1	Y
55	T. vaginalis	Y	N. gonorrhoeae	Y	C. trachomatis	Y	T. pallidum	Y	HSV 2	Y
56	N. gonorrhoeae	Y	C. trachomatis	Y	HSV1	Y	HSV 2	Y	Human (DNA)	Y
57	C. trachomatis	Y	HSV1	Y	HSV 2	Y	Human (DNA)	Y	U. urealyticum	Y
58	HSV1	Y	HSV 2	Y	Human (DNA)	Y	U. urealyticum	Y	M. genitalium	Y
59	HSV 2	Y	T. pallidum	Y	U. urealyticum	Y	M. genitalium	Y	Mpox	Y
60	Mpox	Y	T. vaginalis	Y	N. gonorrhoeae	Y	HSV1	Y	Human (DNA)	Y

**TABLE 2**

## Limit of Detection in Clinical Matrices

The data for verification of extractability of all analytes from pooled male and female urine, throat swabs, and saliva demonstrate comparative extractability and LoDs at or one log dilution above the LoDs obtained in Amies.

Sexually Transmitted Infection (Strain)	Abbreviation	Analytical LoD (Amies buffer)	Urine	Saliva	Throat Swab
Neisseria gonorrhoeae (2017)	N. gon	30 CFU/mL	30 CFU/mL	250 CFU/mL	30 CFU/mL
Chlamydia trachomatis (Z054; D-UW3)	C. trach	50 EB/mL	50 EB/mL	500 EB/mL	50 EB/mL
Trichomonas vaginalis (Z070)	T. vag	5 Trophs/mL	5 Trophs/mL	5 Trophs/mL	N/A
Herpes Simplex Virus 1 (MacIntyre)	HSV1	50 TCID <sub>50</sub> /mL	50 TCID <sub>50</sub> /mL	50 TCID <sub>50</sub> /mL	N/A
Herpes Simplex Virus 2 (MS)	HSV2	50 TCID <sub>50</sub> /mL	50 TCID <sub>50</sub> /mL	50 TCID <sub>50</sub> /mL	50 TCID <sub>50</sub> /mL
Mycoplasma genitalium (SEA-1)	G. gen	150 CCU/mL	150 CCU/mL	1500 CCU/mL	N/A
Ureaplasma urealyticum (ATCC N8K9Q2CL)	U. urea	5000 CCU/mL	5000 CCU/mL	5000 CCU/mL	N/A
Treponema pallidum (CDC N8K9Q2CL)	T. pal	500 Treponemes/mL	500 Treponemes/mL	5000 Treponemes/mL	500 Treponemes/mL
Mpox_B5prb (USA/MA001/2022)	Mpox	150 copies/mL	150 copies/mL	150 copies/mL	150 copies/mL
Mpox_N3Rprb (USA/MA001/2022)	Mpox	1500 copies/mL	1500 copies/mL	1500 copies/mL	1500 copies/mL

**TABLE 3**

## Exclusivity on Analyte Detection

When challenged with organisms known to be found in the vagina, urethra, or oral cavity the SimplyTest Oral STI assay demonstrates 97% specificity when testing the listed organisms. In addition, when challenged with both on-panel organisms and off-panel organisms, only the on-panel organisms were detected. Vaccinia virus was detected as Mpox, but this is expectable due to the high genetic similarity of orthopoxviruses.

Organism Tested	Strain	Concentration	Detected/Not Detected
Lactococcus lactis	ATCC 11454	1x10e5/mL	Not Detected
Lactobacillus acidophilus	ATCC 4356	1x10e5/mL	Not Detected
Gardnerella vaginalis	ATCC 49145	1x10e5/mL	Not Detected
Veillonella parvula	ATCC 10790	1x10e5/mL	Not Detected
Fusobacterium nucleatum	ATCC 25586	1x10e5/mL	Not Detected
Haemophilus influenzae	ATCC 49247	1x10e5/mL	Not Detected
Streptococcus pyogenes	ATCC 19615	1x10e5/mL	Not Detected
Clostridium perfringens	ATCC 13124	1x10e5/mL	Not Detected
Moraxella catarrhalis	ATCC 25240	1x10e5/mL	Not Detected
Fusobacterium necrophorum	ATCC 25286	1x10e5/mL	Not Detected
Peptostreptococcus anaerobius	ATCC 27337	1x10e5/mL	Not Detected
Candida glabrata	ATCC 2001	1x10e5/mL	Not Detected
Candida albicans	ATCC 18804	1x10e5/mL	Not Detected
Bacteroides fragilis	ATCC 25285	1x10e5/mL	Not Detected
Streptococcus pneumoniae	ATCC 49619	1x10e5/mL	Not Detected
Cytomegalovirus	AD-169	2.3x10e4	Not Detected
Atopobium vaginae	Z242	1x10e5/mL	Not Detected
Mobiluncus curtisii	Z245	1x10e5/mL	Not Detected
Mobiluncus mulieris	Z366	1x10e5/mL	Not Detected
Vaccinia virus	CF Vero	1x10e5/mL	Detected
Streptococcus agalactiae	ATCC 13813	1x10e5/mL	Not Detected
Escherichia coli	ATCC 10536	1x10e5/mL	Not Detected
Enterococcus faecalis	ATCC 19433	1x10e5/mL	Not Detected
Enterococcus faecium	ATCC 700221	1x10e5/mL	Not Detected
Staphylococcus aureus	ATCC 25923	1x10e5/mL	Not Detected
Neisseria cinerea	Z104	1x10e5/mL	Not Detected
Neisseria sicca	Z043	1x10e5/mL	Not Detected
Neisseria subflava	Z119	1x10e5/mL	Not Detected
Neisseria elongata	Z071	1x10e5/mL	Not Detected
Neisseria perflava	ATCC 14799	1x10e5/mL	Not Detected
Neisseria mucosa	ATCC 49233	1x10e5/mL	Not Detected
Neisseria meningitidis	ATCC 13090	1x10e5/mL	Not Detected
Neisseria lactamica	ATCC 23970	1x10e5/mL	Not Detected
Neisseria flavescens	Z061 - High	1/2 High	Not Detected

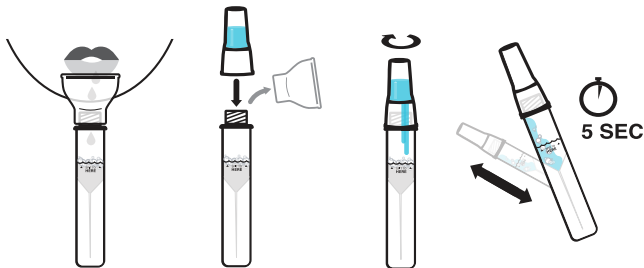
# METHODS

SimplyTest™ Oral STI is a multiplex laboratory developed test for the identification of STI pathogens present in clinical specimens. Saliva specimens collected in SDNA-1000 devices, extracted, purified, amplified (multiplex PCR), labeled, and hybridized onto a microarray with target-specific 50mer capture probes. Bound amplicons are fluorescently tagged and detected on a microarray scanner. Validation included analytical sensitivity (Limit of Detection (LOD), competition of organisms, and inclusivity), sample stability, and analytical specificity (exclusivity, precision, and accuracy are not part of analytical specificity – they are standalone studies). Pathogens evaluated were gonorrhea, chlamydia, syphilis, herpes, Genitalium, Urealyticum, syphilis, trichomonas, and Mpox.

**FIGURE 2**

### User Directed Collection of a Saliva Sample.

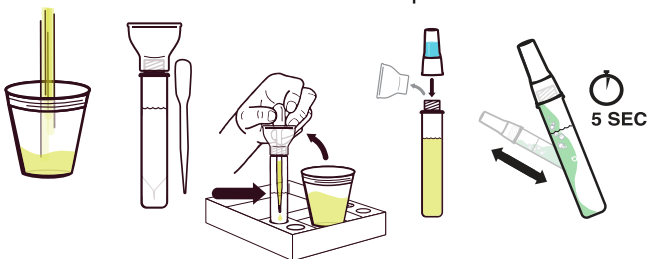
1 mL of saliva is expressed into the collection funnel and mixed with preservation buffer.



**FIGURE 3**

### User Directed Collection of a First Catch Urine Sample.

1 mL of urine is transferred from a urine cup to the collection tube using a pipet and mixed with preservation buffer.

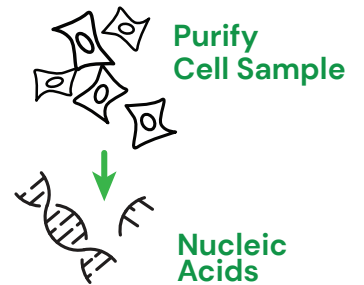


**FIGURE 4**

### Graphical Depiction of Microarray Assay Workflow.

#### Samples:

- Saliva in BioMax SDNA-1000 Device
- First Catch Urine
- Throat swab



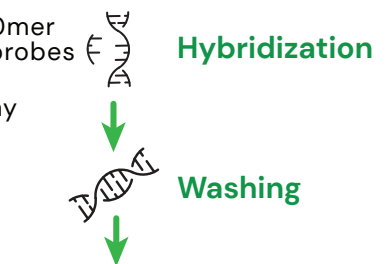
#### Sample Preparation:

- DNA/RNA extraction and purification
- Multiplexed PCR amplification with pooled primers

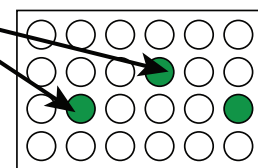


#### Microarray

- Hybridization with 50mer microarray capture probes
- Fluorescent tagging
- Reading on microarray scanner



#### Detected targets/pathogens





# CONCLUSION

Clinical diagnosis alone is unreliable in STI coinfection due to the overlap in symptoms related to STI pathogens. Control of STI associated with oral sex is dependent upon the demonstration of all pathogens present.

Saliva collected in the SDNA-1000 device provides a high degree of sensitivity, precision, and accuracy, as well as the ability to detect multiple analytes (co-infection) within a given sample.

The failure to demonstrate all organisms infecting a given patient may result in incomplete therapy which may result in poor outcomes including sustained communicability, extension of infection beyond

the genital tract, and transmission to an unborn or newborn child. The self-collected saliva specimen is comparable to the provider collected specimen and can improve frequency of testing when a patient is unable or unwilling to visit a clinical office.

Saliva is a clinically informative specimen source useful for the diagnosis, monitoring, and management of patients with oral and systemic disease.

## Summary of Validation Results

Analytical Sensitivity	Result
<b>Competition of Organisms:</b> Ability to co-detect multiple analytes in given sample	Up to five analytes within a given sample
Analytical Specificity	Results
<b>Inclusivity:</b> Ability to accurately detect subspecies and serotypes of given analyte	Related strains detectable at 1XLOD
<b>Exclusivity:</b> Ability to detect analyte when present in samples with high concentrations of closely related analytes predictive of normal flora	97% specificity
<b>Precision</b>	100%
<b>Accuracy</b>	100%



# REFERENCES

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