

Point of Care PCR Testing for Ten Different Sexually Transmitted Diseases

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ABSTRACT

Background: The FilmArray® is an investigational point of care molecular diagnostic device that features minimal sample handling, integrated sample preparation, and a multiplexed PCR output. This fully automated system is capable of detecting many PCR targets from a single specimen in less than one hour. We developed a Sexually Transmitted Disease (STD) Panel for the FilmArray device that detects the following organisms: *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (GC), *Treponema pallidum* (syphilis), *Trichomonas vaginalis*, *Mycoplasma genitalium*, *Ureaplasma urealyticum*, *Ureaplasma parvum*, *Haemophilus ducreyi*, and herpes simplex viruses (HSV-1 and 2).

Methods: The FilmArray STD Panel was used to test 99 clinical specimens collected from patients visiting the Salt Lake County STD Clinic. The STD panel test results were compared to the standard CDC recommended clinical tests on duplicate specimens. Standard testing included gram staining, CT/GC amplification, wet mount examination, viral culture, and (serum) syphilis IgG. Sample types included urine (44), vaginal/cervical swabs (7), urethral swabs (5), ulcer swabs (7), oral swabs (20), and rectal swabs (16).

Results: Concordance between the new STD panel and standard testing was: *C. trachomatis* (79/81, 98%), *N. gonorrhoeae* (81/81, 100%), HSV1 (6/6, 100%), HSV-2 (6/6, 100%), and *T. vaginalis* (5/6, 83%). The STD Panel was able to detect *T. pallidum* in 4 samples from patients who were subsequently diagnosed with syphilis by serology. We detected *Ureaplasma* species in 28 of 99 (28%) and *M. genitalium* in 3 of 99 (3%) specimens. In patients with chronic dysuria where standard clinical testing failed to reveal a cause, we were able to detect infectious agent in 8 of 14 (57%) specimens (2 HSV-1, 5 *Ureaplasma* species, 1 *M. genitalium*). The STD Panel detected single infections in 52% of samples, double infections in 10%, triple infections in 2%.

Conclusion: We conclude that the FilmArray STD Panel is a robust clinical diagnostic tool that has the potential to improve public health by providing highly sensitive and rapid point of care STD diagnostics.

INTRODUCTION

Clinical diagnosis of STDs requires laboratory tests in a majority of patients. Lengthy turnaround time for STD diagnosis is burdensome to patients and clinicians and has led to empirical treatment for several disease presentations. In an effort to expedite the diagnosis and treatment of Sexually Transmitted Diseases, Idaho Technology Inc. has developed a first generation point of care FilmArray STD panel. The FilmArray STD panel was designed to detect and identify the following organisms: *C. trachomatis*, *N. gonorrhoeae*, *T. pallidum*, *T. vaginalis*, *M. genitalium*, *H. ducreyi*, herpes simplex virus 1 & 2, *U. urealyticum*, and *U. parvum*. *U. urealyticum* and *U. parvum* are detected by the same assay but the species are not differentiated. As illustrated in Figure 1, the FilmArray STD panel is a small footprint instrument that uses multiplex nested PCR (Figure 2) and LC Green chemistry. Melt analysis is used for detection which allows increased specificity in organism identification. This panel was used to test 99 clinical specimens collected from patients visiting the Salt Lake County STD Clinic. Results were compared to the standard CDC recommended clinical tests using duplicate specimens.

Figure 1 The FilmArray® Instrument and Pouch

ITI has developed a lab-in-a-pouch system called "FilmArray". It is a medium-scale fluid manipulation system performed in a self-contained, disposable, thin-film plastic pouch. The FilmArray platform processes a single sample, from nucleic acid purification to result, in a fully automated fashion. These system characteristics are ideal for the multiplex testing of pathogens in standard diagnostic sample matrices.



The FilmArray Test System

A FilmArray test is initiated by injecting rehydration solution and a patient blood culture sample into the FilmArray pouch and placing it in the FilmArray instrument. The user enters the sample and pouch type (using a barcode reader) into the software and initiates a run. Results are provided in ~1 hour.

The FilmArray pouch has a filament (see label A) containing all freeze-dried reagents.

The film portion of the pouch has stations for:

1. Cell lysis (Blistar C)
2. Magnetic bead based nucleic acid purification (D & E)
3. First-stage multiplex PCR (F & G)
4. Array of 102, second-stage nested PCR (I)

PCR primers are dried into the wells of the array and each primer set amplifies a unique product of the first-stage multiplex PCR. The second stage PCR product is detected in real-time using a fluorescent-double-stranded DNA binding dye, LCGreen®.

- A. Filament with freeze-dried reagents
- B. Plug-and-deliver reagents to blisters
- C. Sample lysis and bead collection
- D. Wash station
- E. Magnetic bead collection blister
- F. Elution Station
- G. Multiplex Outer PCR blister
- H. Division blister
- I. Inner Nested PCR array



Figure 2 Schematic of Multiplex Nested PCR

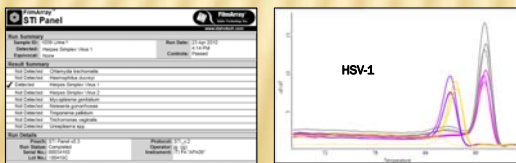
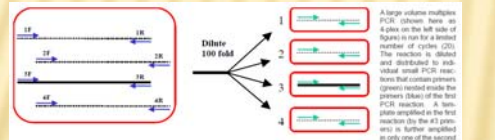


Figure 3 Detection of HSV-1 in a urine sample from a patient with dysuria. The left panel shows run report indicating detection of HSV-1. The right panel shows melt profiles for four assays specifically targeting HSV-1. No other causative agents were detected. Detection of HSV-1 infection at this site (urine) would have been unlikely using standard clinical practices.

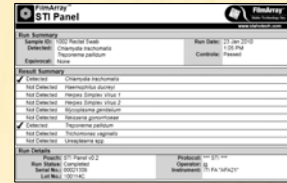


Figure 4. Dual detection of *T. pallidum* and *C. trachomatis* in a rectal swab. The top left panel shows a run summary indicating the detection of *T. pallidum* and *C. trachomatis*. The top right panel shows the melt profiles of two assays targeting *T. pallidum*. The bottom right panel shows the melt profiles of two assays targeting *C. trachomatis*. These results were confirmed by FDA-approved *Chlamydia* amplification and syphilis serologic tests.

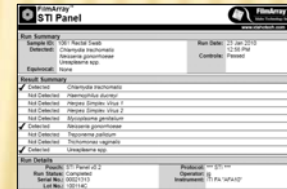


Figure 5. Detection of *C. trachomatis*, *N. gonorrhoeae*, and *Ureaplasma* spp. from a rectal swab. The top left panel shows a run summary indicating the detection of these organisms. Panels on the right show melt profiles for assays targeting the indicated organisms. The results for *C. trachomatis* and *N. gonorrhoeae* were confirmed by an FDA-approved amplification test. Detection of *Ureaplasma* spp. would be unlikely with current clinical practices.

Table 1. STI Detection by Sample Type

Organism	Urine n=44	Urethral Swab n=5	Cervical Swab n=7	Rectal Swab n=16	Oral Swab n=20	Ulcer Swab n=7	Total Positives
<i>N. gonorrhoeae</i>	4	2		2	2		10
<i>C. trachomatis</i>	7	2		3			12
<i>T. pallidum</i>	1			1	1	1	4
<i>T. vaginalis</i>	1		2				3
HSV-1	2						2
HSV-2							0
<i>M. genitalium</i>	3						3
<i>Ureaplasma</i> spp.	17	1	3	4	2	1	28
<i>H. ducreyi</i>							0

Table 2. Concordance of Clinical Samples

Organism	# tested	Standard +/+	FilmArray +/-	Concordance
<i>N. gonorrhoeae</i>	81	10/71	10/71	81/81 (100%)
<i>C. trachomatis</i>	81	11/70	12/69	80/81 (99%)
<i>T. vaginalis</i>	6	2/4	3/3	5/6 (83%)
HSV-1	6	0/6	0/6	6/6 (100%)
HSV-2	6	0/6	0/6	6/6 (100%)

CONCLUSION

The FilmArray STD panel is a robust clinical diagnostic tool that has the potential to improve public health by providing highly sensitive, specific, and rapid point of care STD diagnostics.

ACKNOWLEDGMENT

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