A Novel Molecular-Based Diagnostic Screening Test Utilizing the NanoCHIP® Microarray Technology for Simultaneous Detection of Major Sexually Transmitted Infections

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Abstract

Objectives. Sexually transmitted infections (STI) are widely abundant all over the world, presenting constantly a yearly growing trend and consequently increased annual health costs. STIs may cause acute symptoms, chronic infections, infertility, ectopic pregnancy, blindness, cervical cancer, susceptibility to HIV infection and death. Most cases are treatable, however no or late treatment may lead to severe health complications. This is further emphasized in view of the common carriage of STIs in asymptomatic state. Therefore, there is a need for detection of early stages of STIs is in order to control and prevent these diseases. Savyon Diagnostics has recently been engaged with development of a novel molecular-based screening test for simultaneous detection of the most abundant STIs utilizing its proprietary NanoCHIP®XL molecular electronic microarray system. The panel of pathogens is composed of *Chlamydia trachomatis*, *Neisseria gonorrhea*, *Trichomonas vaginalis*, *Ureaplasma urealyticum/parvum, Mycoplasma genitalium* and *Mycoplasma hominis*. The *Ureaplasma urealyticum/parvum* results are reported in a semi-quantitative manner, in order to better differentiate between colonization and acute states, and by that avoiding unnecessary antibiotic treatment. The aim of this work is to demonstrate the utility of the newly developed test for screening purposes of STIs especially in high-risk populations, demonstrating a highly performing, time-saving and cost-effective test.

Methods. DNA was extracted from characterized urethral samples or urine swab specimens using a variety of readily available manual and automatic methods. Specific bacterial and parasitic genes were amplified through multiplex PCR and subjected to the NanoCHIP® detection and analysis system. The generated amplicons were electronically addressed to discrete loci on the NanoCHIP® cartridge, pre-activated with specific capture oligonucleotides. Detection was achieved through specific fluorescent reporter oligonucleotides. The molecular method used in the testing laboratory, and STD6 ACE Detection kit of Seegene were used as reference methods.

Results. The NanoCHIP® results were in accordance with the characterizations of the tested samples in terms of clinical sensitivity and specificity. The Nanochip® multiplex analysis provided clear results about the identity of the pathogen, either bacterium or parasite, within a working day time frame.

Conclusions. The NanoCHIP® based test has proven to be a useful system for medium-high throughput screening of urine and swab samples for reliably detection of STIs. The NanoCHIP® technology presents significant advantages, mainly in terms of minimal hands-on time, improved laboratory workflow and turnaround time, enabling flexibility and saving costs.

Materials & Methods

DNA was extracted from characterized urethral samples or urine swab specimens using a variety of validated available manual and automatic methods. Specific bacterial and parasitic genes were amplified through multiplex PCR and subjected to the NanoCHIP® detection and analysis system. The generated amplicons were electronically addressed to discrete loci on the NanoCHIP® cartridge, pre-activated with specific capture oligonucleotides. The efficiency of the purification was monitored by adding human epithelial cells into each tested sample. Then human β-globin gene was amplified in the same multiplex PCR and analyzed simultaneously in the NanoCHIP® system. The molecular method used in the sample provider laboratory, and STD6 ACE Detection kit of Seegene were used as reference methods

The NanoCHIP® System is an **automated multiplex** platform capable of detecting and analyzing **multiple targets** together with **multiple samples** on the same run utilizing the **electronic micro-array technology**
More information on the system and its function can be found at Booth #81 and at:
www.nanochipxl.com **and** www.savyondx.com

Background

Sexually transmitted infections (STI) are widely abundant all over the world, presenting constantly a yearly growing trend and consequently increased annual health costs. STIs may cause acute symptoms, chronic infections, infertility, ectopic pregnancy, blindness, cervical cancer, susceptibility to HIV infection and death. Most cases are treatable, however no or late treatment may lead to severe health complications. This is further emphasized in view of the common carriage of STIs in asymptomatic state. Therefore, there is a need for detection of early stages of STIs is in order to control and prevent these diseases. Savyon Diagnostics has recently developed a novel molecular-based screening test for simultaneous detection of the most abundant STIs utilizing its proprietary NanoCHIP®XL molecular electronic microarray system. The panel of pathogens is composed of *Chlamydia trachomatis*, ***Neisseria gonorrheae, Trichomonas vaginalis*, *Ureaplasma urealyticum/parvum, Mycoplasma genitalium* and *Mycoplasma hominis*.** The system is able to analyze various specimen types, such as urine, vaginal and urethral swabs, as well as culture isolates. The DNA from these specimens may be extracted by various available automated nucleic acids extraction instruments, but an extraction protocol has been developed in which no instrumentation is needed at the same extraction efficiency. The usage of the NanoCHIP® platform enables high multiplexing capabilities together with testing multiple patient samples in the same run, and thus offers a powerful and unique medium-high throughput screening tool, demonstrating a highly performing, time-saving and cost-effective test.

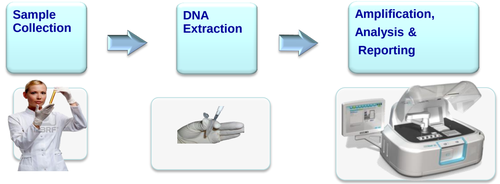
The test provides:

- ❖ Automated testing process from sample to result ; No need for culture enrichment
- ❖ Compatibility with the most abundant automatic nucleic acids extraction instruments, as well as with instrument-free extractions
- ❖ Versatility: the user may choose to test the complete panel, or any combination of organisms composing the panel
- ❖ Detection of bacteria and parasites in the same run
- ❖ Minimal hands-on time, vast reduction in the workload of the lab
- ❖ Same day results
- ❖ Up to 96 samples per run
- ❖ Cost-effectiveness

Objective

The aim of this work is to present the newly developed tests and their utility for screening purposes of Sexual Transmitted Infections especially in high-risk populations

The NanoCHIP® Workflow



Results

Table 1. STI3 and STI6 Panels Composition

Panel/Pathogen	*Chlamidia Trachomatis*	*Trichomonas Vaginalis*	*Neisseria Gonorrhoeae*	*Ureaplasma urealyticum/parvum*	*Mycoplasma hominis*	*Mycoplasma genitalium*
STI3	+	+	+	-	-	-
STI6	+	+	+	+	+	+

Table 2. Typical STI6 Panel Raw Results and Interpretation

Reference Characterization	*Trichomonas vaginalis* Reporter	*Chlamydia trachomatis* Reporter 1	*Chlamydia trachomatis* Reporter 2	*Ureaplasma urealyticum/ parvum* Reporter	*Mycoplasma hominis* Reporter	*Mycoplasma genitalium* Reporter	*Neisseria gonorrhoeae* Reporter 1	*Neisseria gonorrhoeae* Reporter 2	Internal Control	NanoCHIP® Interpretation
Tv,Uu/p	45890	622	141	46052	628	823	1640	388	13289	*Trichomonas vaginalis, Ureaplasma urealyticum/parvum*
Tv	8895	589	88	9075	657	602	776	408	5616	*Trichomonas vaginalis*
Tv,Uu/p	92665	6930	692	37885	237	938	2965	751	13066	*Trichomonas vaginalis, Ureaplasma urealyticum/parvum*
Tv,Uu/p	68253	926	81	24871	397	315	1938	411	23655	*Trichomonas vaginalis, Ureaplasma urealyticum/parvum*
Ct	866	74391	21266	1058	601	826	1497	570	14843	*Chlamydia trachomatis*
Ct	216	64738	14218	2189	427	626	1361	518	14024	*Chlamydia trachomatis*
Ct,Tv,Uu/p	12865	74199	38699	40220	2084	5763	1523	302	15990	*Chlamydia trachomatis, Trichomonas vaginalis, Ureaplasma urealyticum/parvum*
Ct,Uu/p	563	77662	27992	42128	829	817	2242	499	29664	*Chlamydia trachomatis, Ureaplasma urealyticum/parvum*
Ct,Uu/p	160	68072	14465	51116	1068	649	2311	443	19995	*Chlamydia trachomatis, Ureaplasma urealyticum/parvum*
Ct	418	72841	21123	7361	1674	757	1976	275	20377	*Chlamydia trachomatis*
Uu/p,Ct	283	13549	141	35886	264	260	1574	1196	15141	*Ureaplasma urealyticum/parvum, Chlamydia trachomatis*
Uu/p,Ct	2633	19338	6721	40233	455	842	2352	600	20345	*Ureaplasma urealyticum/parvum, Chlamydia trachomatis*
Uu/p	2092	1301	201	17234	2028	584	5803	1657	18954	*Ureaplasma urealyticum/parvum*
Uu/p	742	963	470	50357	149	680	1618	287	17495	*Ureaplasma urealyticum/parvum*
Mh,Ct,Mg	7898	27203	6026	3314	43994	13148	1806	604	9018	*Mycoplasma hominis, Chlamydia trachomatis, Mycoplasma genitalium*
Mh,Tv,Ct	10795	20990	1822	2118	12603	935	1477	439	15258	*Mycoplasma hominis, Trichomonas vaginalis, Chlamydia trachomatis*
Mh,Uu/p,Ct	140	20279	3080	18319	51093	885	1633	485	9450	*Mycoplasma hominis, Ureaplasma urealyticum/parvum, Chlamydia trachomatis*
Mh	2318	673	2492	1364	61863	330	2256	636	13478	*Mycoplasma hominis*
Mg,Uu/p	1087	974	197	43508	436	9902	1778	154	19657	*Mycoplasma genitalium, Ureaplasma urealyticum/parvum*
Ng,Uu/p	252	1031	109	13555	640	707	25414	8229	13750	*Neisseria gonorrhoeae, Ureaplasma urealyticum/parvum*
Ng	248	736	213	536	383	1029	30132	301	6686	*Neisseria gonorrhoeae*
Ng	143	674	182	809	675	1414	68563	16378	13432	*Neisseria gonorrhoeae*
Ng,Uu/p	4573	717	109	11012	154	769	53818	17924	16917	*Neisseria gonorrhoeae, Ureaplasma urealyticum/parvum*
Cutoff	8,000	9,000	9,000	9,000	9,000	7,500	9,000	7,000	5,000	

Samples were characterized according to various RT-PCR based tests that are used in the laboratories that provided the samples

NanoCHIP® results show complete accordance with the reference results

Table 4. STI3 Panel Performance

Pathogen	No. of detected samples / No. of tested samples	% of Detection
Chlamydia trachomatis	50/50	100%
Trichomonas vaginalis	40/40	100%
Neisseria gonorrhoeae	21/21	100%

All the samples were detected in the STI3 system according to their characterization

Table 5. STI6 Panel Performance

Pathogen	No. of detected samples / No. of tested samples	% of Detection
Chlamydia trachomatis	18/18	100%
Trichomonas vaginalis	14/14	100%
Neisseria gonorrhoeae	13/13	100%
Ureaplasma urealyticum/parvum	16/16	100%
Mycoplasma hominis	14/14	100%
Mycoplasma genitalium	12/12	100%

All the samples were detected in the STI6 system according to their characterization

Results (cont.)

Table 3. Typical STI3 Panel Raw Results and Interpretation

Sample #	Reference Characterization	*Chlamydia trachomatis* Reporter 1	*Chlamydia trachomatis* Reporter 2	*Trichomonas vaginalis* Reporter	*Neisseria gonorrhoeae* Reporter 1	*Neisseria gonorrhoeae* Reporter 2	Internal control	NanoCHIP Interpretation
1	N	665	237	311	518	986	10,458	Negative
2	N	498	182	263	218	854	12,831	Negative
3	CT	51,644	16,372	226	141	510	10,078	*Chlamydia trachomatis*
4	CT	67,789	10,967	279	381	563	43,140	*Chlamydia trachomatis*
5	CT	68,170	39,069	162	475	826	93,389	*Chlamydia trachomatis*
6	CT	75,431	18,024	205	4,655	646	21,643	*Chlamydia trachomatis*
7	CT	53,687	41,031	157	390	677	12,517	*Chlamydia trachomatis*
8	TV	1,464	484	37,384	323	512	23,036	*Trichomonas vaginalis*
9	TV	2,491	1,059	20,165	359	565	65,746	*Trichomonas vaginalis*
10	TV	489	2,504	35,627	2,280	504	8,549	*Trichomonas vaginalis*
11	TV	2,362	346	29,054	886	564	12,293	*Trichomonas vaginalis*
12	CT	56,079	53,511	284	461	705	7,849	*Chlamydia trachomatis*
13	CT	65,952	43,989	2,007	244	584	41,340	*Chlamydia trachomatis*
14	CT	44,917	2,662	1,596	257	571	60,874	*Chlamydia trachomatis*
15	CT	68,130	8,241	346	1,287	738	43,490	*Chlamydia trachomatis*
16	TV	1,362	402	49,485	645	762	41,199	*Trichomonas vaginalis*
17	TV	613	289	54,277	407	897	52,161	*Trichomonas vaginalis*
18	TV/CT	31,436	5,519	54,264	299	625	65,639	*Trichomonas vaginalis, Chlamydia trachomatis*
19	TV	822	239	56,515	477	701	76,778	*Trichomonas vaginalis*
20	TV	541	200	49,258	422	456	56,794	*Trichomonas vaginalis*
21	TV	1,202	682	54,339	476	589	65,161	*Trichomonas vaginalis*
22	TV	2,106	476	58,905	490	631	77,459	*Trichomonas vaginalis*
23	TV	1,068	462	55,322	486	691	76,458	*Trichomonas vaginalis*
24	TV	760	217	51,152	376	505	63,464	*Trichomonas vaginalis*
25	TV	943	2,542	55,722	220	580	87,841	*Trichomonas vaginalis*
26	TV	1,538	314	50,005	874	795	45,914	*Trichomonas vaginalis*
27	NG	1,765	560	570	60,269	59,972	49,922	*Neisseria gonorrhoeae*
28	NG	2,236	575	474	59,154	59,768	58,734	*Neisseria gonorrhoeae*
29	NG	1,486	432	423	634	62,501	16,027	*Neisseria gonorrhoeae*
30	NG	1,692	383	595	62,853	60,186	19,656	*Neisseria gonorrhoeae*
31	NG/CT	47,858	12,791	290	20,122	30,975	74,833	*Neisseria gonorrhoeae, Chlamydia trachomatis*
32	NG	2,018	1,239	242	59,061	56,014	73,292	*Neisseria gonorrhoeae*
33	NG	504	48	148	57,568	47,948	56,144	*Neisseria gonorrhoeae*
34	NG	971	190	406	72,105	63,858	43,857	*Neisseria gonorrhoeae*
35	NG	1,381	609	241	489	59,041	54,490	*Neisseria gonorrhoeae*
36	NG	1,774	528	246	65,476	70,824	22,166	*Neisseria gonorrhoeae*
37	NG	606	89	123	50,554	46,786	51,402	*Neisseria gonorrhoeae*
38	NG	1,026	431	1,064	67,049	71,202	46,808	*Neisseria gonorrhoeae*
39	NO DNA	1,037	223	375	678	893	818	NO DNA
40	NO DNA	787	191	420	236	563	591	NO DNA
41	NO DNA	882	216	1,744	362	618	1,758	NO DNA
	cutoff	5,000	5,000	10,000	7,000	7,000	3,000	

Samples were characterized according to various RT-PCR based tests that are used in the laboratories that provided the samples

NanoCHIP® results show complete accordance with the reference results

Discussion & Summary

- ❖ The current NanoCHIP® system provides two versions of diagnostic panels enabling detection of the most abundant STI pathogens
- ❖ The NanoCHIP® results show excellent potency for detection of the target pathogens in both, STI3 as well as STI6 systems, in clinical urethral swabs and in urine specimens, as demonstrated versus various RT-PCR currently used tests
- ❖ Both tests are specific to the detected pathogens and there are no cross reactions between the pathogens in the multiplexed assay
- ❖ The results are clear and enable definite interpretations to be presented conveniently to the end user
- ❖ All in all The NanoCHIP® has proven to be a useful platform for medium-high throughput screening of clinical samples for reliably detection of Sexual Transmitted Infections, and the current tests present significant advantages mainly in terms of minimal hands-on time, improved laboratory workflow and turn around time, enabling flexibility and saving costs