Novel NanoCHIP® XL-Based Molecular Tests For Screening and Genotyping The Most Abundant Human Papillomavirus (HPV) High- and Low-Risk Strains

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Abstract

Objectives. Persistent human papillomavirus (HPV), a highly prevalent sexually transmitted infection, is necessary for the development of cervical intraepithelial lesions and invasive carcinoma. Cervical cancer is the third most common cancer among women and the second female cancer-related cause of death worldwide. HPV types are classified into low- and high-risk groups according to their potential for oncogenesis, when the High-risk types 16 and 18 together account for about 70% of all cervical cancers. Therefore, current immunization against these carcinogenetic HPV strains does not exempt women from further regular cervical screening. The present gold standard the Pap staining has limited sensitivity for detecting abnormal cervical epithelial cells in the early course of their development. Savyon Diagnostics is engaged with development of the concept of two separate and consecutive molecular-based tests, i.e. screening and genotyping of HPV high- and low-risk strains on our proprietary NanoCHIP®XL molecular electronic microarray system. The reagents used in the tests were developed by Master Diagnóstica (Spain) as part of collaborative efforts in the project. The aim of this work is to demonstrate the usefulness of the newly developed tests in providing a comprehensive solution to the need for efficient screening, early detection and HPV genotyping.

Methods. The first HPV kit allows full genotyping of the most prevalent HPV types 16 and 18 and screening of 16 other high risk HPV types together with the most abundant low risk types 6 and 11. The second HPV kit allows specific detection of 18 high-risk types of HPV and 18 low-risk HPV types. Both tests were compared to the microarray test Papillocheck (Greiner, Germany) and to HPV Direct Flow CHIP (Master Diagnóstica, Spain), utilizing a cohort of characterized liquid-based cytology (LBC) specimens and DNA purified from clinical specimens.

Results. The results of the screening as well as the genotyping tests were in accordance with both molecular-based tests that were used for the evaluation. The accordance is demonstrated in the purified DNA as well as in LBC specimens. For screening purpose the system has proven to efficiently process 96 samples within 8 hours with minimal hands-on time and eliminating the need to extract the DNA from the specimens.

Conclusions. The newly developed NanoCHIP®XL based tests constitute a concept in which efficient screening is followed by identifying the etiological strains on the same platform. The sensitivity of the system is expected to enable early detection of pre-cancerous cervical lesions, which is currently an apparent limitation of the Pap smear-based diagnosis. The effective screening is predicted to provide a better negative predictive value, thus allowing for longer screening intervals.

Background

Persistent human papillomavirus (HPV), a highly prevalent sexually transmitted infection, is necessary for the development of cervical intraepithelial lesions and invasive carcinoma. Cervical cancer is the third most common cancer among women and the second female cancer-related cause of death worldwide. HPV types are classified into low- and high-risk groups according to their potential for oncogenesis, when the High-risk types 16 and 18 together account for about 70% of all cervical cancers. Therefore, current immunization against these carcinogenetic HPV strains does not exempt women from performing regular cervical screening. Early detection of HPV is crucial for disease prevention and patient management, and the first step to achieve these purposes is an efficient screening system. The present gold standard for detecting abnormal cervical epithelial cells is the Pap staining, using microscopic analysis of conventional cervical smears or cell suspensions from liquid cytology medium. This method has limited sensitivity for detecting abnormal cervical epithelial cells in the early course of their development. Molecular diagnostic tests for HPV offer greater sensitivity for the early detection of pre-cancerous cervical lesions in comparison to Pap smears and therefore has been recommended as a valuable tool as a screening method. Savyon Diagnostics is engaged with applying a comprehensive concept in the diagnosis of HPV, composed of two consecutive stages, first screening, followed by discrete genotyping of HPV high- and low-risk strains. According to this approach, Savyon has recently developed two separate molecular-based tests, for screening and genotyping, utilizing its proprietary NanoCHIP®XL molecular electronic microarray system. The reagents used in both tests were developed by Master Diagnóstica (Spain) as part of collaborative efforts in the project. Both tests are based on a highly multiplexed PCR that can simultaneously detect multiple HPV types with high throughput, accuracy and short turnaround time from specimen preparation to results. The system is able to analyze a wide range of clinical specimens (i.e. cytological dry swabs, liquid-based cytology specimens, and paraffin embedded-tissue sections) without a DNA purification step. All-in-all 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 tests provide:

- Automated testing process from sample to result
- No need for DNA purification step
- Minimal hands-on time, vast reduction in the workload of the lab
- Short turnaround time from sample to result
- Cost-effectiveness

Objective

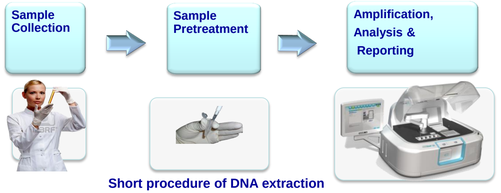
The aim of this work is to present the newly developed HPV screening and genotyping tests and their usefulness in providing a comprehensive solution to the need for efficient screening for HPV, early detection and genotyping

Materials & Methods

DNA was extracted from characterized Liquid Based cytology (LBC) samples using both, validated available manual methods and by newly in-house developed crude-cell-extract protocol which does not require DNA purification. The Purified DNA and crude cell extracts were used as template to amplify HPV L1 viral region through multiplex PCR and were subjected to the NanoCHIP® system. An additional fragment of the beta-globin gene was co-amplified during the multiplex PCR to assure the quality of the input starting material. The generated amplicons were electronically addressed to discrete loci on the NanoCHIP® cartridge, pre-activated with specific capture oligonocleotides. Detection was achieved through HPV-type specific fluorescent reporter oligonucleotides. HPV Direct Flow CHIP (Master Diagnostica) and PapilloCheck® HPV-Screening (Greiner Bio-One) served as reference methods.

Work Flow

Figure 1. The crude cell extract protocol for direct analysis by the NanoCHIP®XL from clinical samples



Short procedure of DNA extraction

Results

Table 1. HPV Screening and Genotyping Panels

HPV Screening kit		
	Full detection	Pool detection
High Risk types	16,18	,26,31,33,35,39,45,51,52 ,53,56,58,59,66,68,73,82
Low risk types	-	6,11
Internal Control		Beta-globin

HPV genotyping kit	
	Full detection
High Risk types	16,18,26,31,33,35,39,45,51, 52,53,56,58,59,66,68,73,82
Low risk types	6,11,*(40,42,43,44,54,55,61, 62, 67,69,70,71,72,81,84,89)
Internal Control	Beta-globin

Table 2. Typical HPV screening test raw results (A) and Interpretation (B)

A

*Samples	HPV-18	HPV-16	HPV 6/11	HPV High-Risk types	Beta-Globin
HPV 18,33,51,53, 26	33837	844	666	74296	38773
HPV16	50	50984	390	2960	23097
HPV16	62	76524	750	1584	41246
HPV 11	73	228	85594	2039	1924
HPV 6, 82	38	177	87326	22436	50118
HPV 33	89	1269	497	74398	39670
HPV 35	38	334	301	9204	19044
HPV 39	154	458	206	46187	47587
HPV 45	50	195	183	67876	6357
HPV 51	143	311	206	22842	50496
HPV 51	64	451	202	65587	41355
HPV 53	63	283	182	41556	50052
HPV 56	121	285	192	20635	45303
HPV 56	62	383	157	71777	44887
HPV 58,33	42	935	121	78348	43034
HPV 59	164	375	249	19335	47520
HPV 66, 26	219	362	187	81088	44025
HPV 73, 58	52	383	194	81673	42821
HPV 82, 58	46	765	140	20885	35751
HPV-NEG	422	274	201	4203	46641
no dna	34	174	188	1069	161
no dna	77	180	185	1881	437

B

Sample #	Sample ID	Result
1	HPV 18,33,51,53,26	HPV 18 , HPV High-Risk types
2	HPV 16	HPV 16
3	HPV 16	HPV 16
4	HPV 11	HPV low risk type: 6 or 11
5	HPV 6,82	HPV High-Risk types , HPV low risk type: 6 or 11
6	HPV 33	HPV High-Risk types
7	HPV 35	HPV High-Risk types
8	HPV 39	HPV High-Risk types
9	HPV45	HPV High-Risk types
10	HPV 51	HPV High-Risk types
11	HPV 51	HPV High-Risk types
12	HPV 53	HPV High-Risk types
13	HPV 56	HPV High-Risk types
14	HPV 56	HPV High-Risk types
15	HPV 58,33	HPV High-Risk types
16	HPV 59	HPV High-Risk types
17	HPV 66,26	HPV High-Risk types
18	HPV 73,58	HPV High-Risk types
19	HPV 82,58	HPV High-Risk types
20	HPV-NEG	Negative
21	no dna	no dna
22	no dna	no dna

Cut off for HPV16 HPV18, HPV 6/11 and for HPV high risk mix - 9000

Raw results of the HPV screening panel demonstrating specific detection of the high risk types 16 and 18 with pool detection of 16 HPV high risk types and pool detection of low risk types: 6 and 11

***Samples were characterized according to either HPV Direct Flow CHIP - (Master Diagnostica) and PapilloCheck® HPV-Screening (Greiner Bio-One)**

Table 4. Detection of HPV characterized samples by the NanoCHIP® system

HPV-type detected	No. of detected samples/ total No. of samples	Percentage of Detection (%)
HPV type 16	16/16	100
HPV type 18	8/8	100
16 X HPV High risk types (pool detection)	*51/53	96
HPV low risk types (6 and 11 – pool detection)	5/5	100
HPV negative	22/22	100

The results show full detection in all the detailed configurations

Samples were characterized according to the reference methods as detailed above

Results (cont.)

Table 3. The efficiency of avoiding DNA purification

*Samples	HPV16	HPV18	HPV 6/11	HPV High risk types	HPV 56	HPV 53	HPV 51	HPV 58	HPV 82	Beta Globin	Nanochip Genotyping	Nanochip Screening
HPV 16 - conventionally purified	248	30760	254	3525	162	235	233	494	56	45383	16	16
HPV 16 - without purification	102	17995	222	2308	66	159	398	265	-5	38941	16	16
HPV 16 - conventionally purified	98	36488	160	2625	162	235	233	494	56	46404	16	16
HPV 16 - without purification	166	46779	191	2660	68	205	271	179	92	44285	16	16
HPV 16 - conventionally purified	243	53829	267	3578	147	454	178	114	163	40131	16	16
HPV 16 - without purification	169	49918	208	3201	134	279	515	18	177	38950	16	16
HPV 16,56,53 weak -conventionally pur	280	32156	280	37312	12257	2974	273	369	370	43651	16,56,53weak	16+HR pos
HPV 16,56,53 weak - without purificatio	192	30823	269	28653	9005	3465	190	174	350	40438	16,56,53weak	16+HR pos
HPV 56 - conventionally purified	123	270	221	76079	38624	260	507	133	20	35415	56	HR pos
HPV 56 - without purification	59	267	99	63376	45801	185	220	-102	-458	29900	56	HR pos
HPV 53 - conventionally purified	140	367	216	54922	144	26654	252	288	564	47620	53	HR pos
HPV 53 - without purification	50	558	554	41825	198	17220	215	-159	235	31462	53	HR pos
HPV 51 - conventionally purified	123	501	245	81278	532	148	55024	57	47	39592	51	HR pos
HPV 51 - without purification	222	454	305	89816	448	266	52565	142	99	42053	51	HR pos
HPV 58 - conventionally purified	289	356	297	73634	581	265	150	49772	206	36770	58	HR pos
HPV 58 - without purification	143	350	277	67650	87	175	206	50523	126	34147	58	HR pos
HPV 82 - conventionally purified	138	825	128	41798	198	165	1225	-28	18994	44851	82	HR pos
HPV 82 - without purification	218	1158	313	19684	65	207	184	109	7796	41491	82	HR pos
HPV NEG - conventionally purified	74	737	1249	3007	560	211	272	-6	-444	42325	NEG	NEG
HPV NEG - without purification	145	807	365	3702	575	273	394	63	-331	39252	NEG	NEG
HPV NEG - conventionally purified	71	316	155	3297	340	178	246	356	-300	45444	NEG	NEG
HPV NEG - without purification	128	462	201	3749	180	244	326	503	-125	45741	NEG	NEG
No DNA	181	709	464	3222	124	219	211	86	37	809		No DNA

Cut off values : HPV16, HPV18, HPV6/11 and HPV high risk mix – 9000 ; other types - 2000

Test results are not compromised due to avoiding a separate DNA purification step by applying a newly developed protocol for extracting DNA from the clinical sample

***Samples were characterized according to HPV Direct Flow CHIP - (Master Diagnostica) and PapilloCheck® HPV-Screening (Greiner Bio-One)**

Discussion & Summary

- In the current presentation we offer two NanoCHIP® based complementary HPV detection systems, screening and genotyping, both cover the most abundant high and low risk types
- The NanoCHIP® results show excellent potency for screening and genotyping of HPV in clinical samples as compared to commonly used reference methods
- Test results are not compromised by applying the newly in-house developed DNA extraction protocol without the need of separate purification step. This procedure significantly simplifies the process and saves time and costs
- 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 and efficient platform for medium-high throughput screening and genotyping of HPV in clinical samples. 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

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