WHITE PAPER

A Novel Approach to Increase HPV Screening Rate by PHASiFY-Next Generation Sequencing Method

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Summary

HPV (Human Papillomavirus) is a prevalent transmitted infection, associated with various health complications, including genital warts and cancers like cervical, anal, and throat cancer. Early and accurate detection plays a pivotal role in reducing cancer incidence and enhancing life expectancy. Different sampling and testing methods exist, such as endocervical Pap smears, vaginal swabs, and urine sampling. Swab-based testing typically offers higher sensitivity but has limited patient compliance due to its invasive nature. In contrast, urine-based tests are non-invasive but have historically been less sensitive. PHASE Scientific developed an HPV laboratory diagnostic that leverages the PHASiFY® Urine extraction process to utilize larger volumes of urine samples for highly sensitive detection of HPV. Furthermore, it was developed on the Oxford Nanopore Technologies sequencing equipment to enable detailed HPV genotyping. Here we present the results of a clinical validation study of 391 patients that evaluated the molecular pathology concordance between self-collected urine samples and professionally collected swabs samples. The results showed a strong overall percent agreement (OPA) between urine and swab sample testing of 98.5% and 97.7% for diagnosis of high-risk HPV (HR-HPV) and general HPV detection, respectively. In addition, we performed a clinical public screening study of 963 female participants where they provided self-collected urine samples for molecular diagnosis of HPV. 351 of the 963 participants returned to have professionally collected endocervical swabs taken for Pap cytology analysis. From the demographic data, we observed a large portion of participants have a poor history of having Pap examinations. This is consistent with data from the Hong Kong Department of Health, which showed that only 22% of participants had received cervical screening within the prior 3 years and more than 50% of participants have never been screened for cervical cancer[1]. Moreover, participants who were informed of having HR-HPV were 55.5% more likely to return for a Pap examination than those who were negative for HPV. Of the HR-HPV positive participants who returned for a Pap exam, 19.5% of the cytology results showed ASCUS +ve, and 9.8% showed +ve for one of the more concerning non-ASCUS abnormalities (AEC-NOS, ASC-H, or LSIL).

Overall, these studies indicate that the improved sensitivity enabled by the PHASiFY® Urine extraction process makes self-collected urine sample testing a reliable and preferred alternative to swab sample based molecular testing, due to the comfort, convenience, and privacy of self-collection. Moreover, being informed of the results motivated participants to follow up with Pap smear testing, which resulted in identification of a significant proportion of concerning cellular abnormalities for high-risk HPV positive participants which would have otherwise gone undetected. Considering the poor adherence to routine health examination, the INDICAID™ HPV Urine Test is a promising approach for increasing early identification of pre-cancerous cellular abnormalities and improving the overall state of reproductive health in Hong Kong.

Methods

Clinical Evaluation of PHASiFY® ONT Urine HPV Assay

- 391 prospective patients from 3 clinics in Hong Kong had samples collected by physician referral.
- The patient cohort included both symptomatic and asymptomatic male and female patients ranging in age from 17 to 70 years old.
- Patients provided a self-collected urine sample and a professional collected endo-cervical swab (female) or proximal urethral swab (male) sample.
- Urine samples were sent to the lab for testing while the swab samples were processed by the collection facilities' normal HPV diagnostic methods.
- The assays used by the three clinical facilities included i) Seegene Anyplex II HPV28 Detection assay, and ii) DiagCor GeneFlow HPV Array Test kit.
- For PHASiFY® Urine extraction, 40 mL of crude urine sample was processed following the manufacturer's instructions to extract DNA.
- The study compared the performance of INDICAID™ HPV Urine Test with the clinical facilities' standard molecular assay for their ability to detect the presence or absence of i) any HPV strain and ii) high risk HPV strains[2].

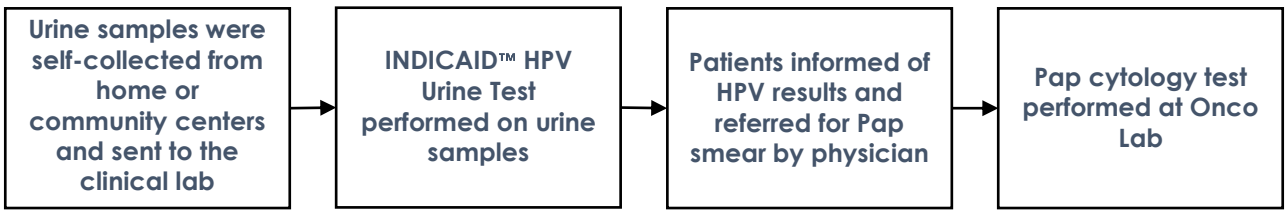

Figure 1. Public screening study design

Clinical Screening Study

- 963 female participants provided self-collected urine samples either at home or at a community center. Patients ages ranged from 18 to 78. They provided background information at the time of collection.
- Urine samples were sent to the lab for HPV testing using the INDICAID™ HPV Urine Test.
- Participants were contacted by a physician and informed of their HPV results. They were referred by a physician for a Pap smear test. 351 participants complied with the request.
- Swab samples were sent to the Onco Medical Laboratory Ltd for Pap cytology analysis (Figure 1).

Results

Comparison of Urine and Swab Sample Testing

When comparing the detection rate for INDICAID™ HPV Urine Test and the reference diagnostic procedure that used swab samples, we see strong concordance for both detection of all HPV and high-risk HPV[2] (Table 1). General HPV was concordantly detected or not detected in urine and swab samples for 382 of the 391 patients, resulting in an overall percent agreement (OPA) of 97.7%. High risk HPV was concordantly detected or not detected in urine and swab samples for 385 of 391 patients, resulting in an overall percent agreement (OPA) of 98.5%.

All HPV Detection		Reference Diagnostic (Swab Sample)		
		Detected	Not Detected	Total
INDICAID™ HPV Urine Test	Detected	97	7	104
	Not Detected	2	285	287
	Total	99	292	391

		95% Confidence Interval	
	Agreement (%)	Lower Limit	Upper Limit
Positive Percent Agreement (PPA)	98.0%	92.9%	99.8%
Negative Percent Agreement (NPA)	97.6%	95.1%	99.0%
Overall Percent Agreement (OPA)	97.7%	95.7%	98.9%

High Risk HPV Detection		Reference Diagnostic (Swab Sample)		
		Detected	Not Detected	Total
INDICAID™ HPV Urine Test	Detected	63	4	67
	Not Detected	2	322	324
	Total	65	326	391

		95% Confidence Interval	
	Agreement (%)	Lower Limit	Upper Limit
Positive Percent Agreement (PPA)	96.9%	89.3%	99.6%
Negative Percent Agreement (NPA)	98.8%	96.9%	99.7%
Overall Percent Agreement (OPA)	98.5%	96.7%	99.4%

Table 1: INDICAID™ HPV Urine Test concordance with Swab DNA Test.

Screening Outcome - Public Health Observations

We see from the participant self-reported data in the clinical screening study that a majority of participants do not adhere to a regular health examination routine. 21.4% of participants had not received a Pap examination within the previous 3 years, while 56.4% had never received a Pap examination (Table 2). These results are in line with previous Hong Kong government findings[1]. The Cancer Expert Working Group (CEWG) recommends that women aged 25-64 who have had sexual experience have cervical a screening every 3 years[3].

Most Recent Pap Test	# of patients Tested	**INDICAID™ HPV Urine Test Results**		
		HR-HPV +ve	LR-HPV +ve	HPV -ve
<3 years	214	28	16	170
>3 years	206	19	15	172
Never	543	49	67	427
Sub-Total	963	96	98	769

Table 2. Correlation of HPV with previous Pap testing history. Participants that were positive for high-risk HPV strains were labeled as 'HR-HPV +ve' regardless of whether they also tested positive for low-risk HPV strains. Participants labeled as 'LR-HPV' only tested positive for low-risk HPV strains and did not test positive for high-risk HPV strains.

Screening Outcomes – HPV and Pap Cytology Results

963 participants were recruited and examined by INDICAID™ HPV Urine test (Table 3); 10.0% were positive for HR-HPV and 10.2% were positive for LR-HPV. All participants were requested to have a Pap smear screening by physician referral. The compliance for the follow up exam was 85.4%, 39.8%, and 29.9% for HR-HPV +ve, LR-HPV +ve, and HPV -ve, respectively. The compliance rate was remarkably higher for participants informed of testing positive for HR-HPV. Of the 82 HR-HPV positive patients who returned for Pap examination, 19.5% of the cytology results showed ASCUS +ve, and 9.8% showed +ve for one of the more concerning non-ASCUS abnormalities (AEC-NOS, ASC-H, or LSIL). As expected, this is notably higher compared to the collective results of LR-HPV +ve and HPV -ve participants, which showed 7.4% and 1.9% positivity for ASCUS and non-ASCUS cytology results, respectively.

Public Screening Results		INDICAID™ Testing			Pap Cytology Results		
		Total Tested for HPV	# Returned for Pap Test	Follow up Compliance	+ve (non-ASCUS)	+ve (ASCUS)	-ve
INDICAID™ HPV Urine Test	HR-HPV +ve	96	82	85.4%	8	16	58
	LR-HPV +ve	98	39	39.8%	3	8	28
	HPV -ve	769	230	29.9%	2	12	216
	Sub-Total	963	351		13	36	302

Table 3. Correlation of the HPV and Pap cytology test results for the public screening study. Participants that were positive for high-risk HPV strains were labeled as 'HR-HPV +ve' regardless of whether they also tested positive for low-risk HPV strains. Participants labeled as 'LR-HPV' only tested positive for low-risk HPV strains and did not test positive for high-risk HPV strains. Participants were labeled as '+ve (Non-ASCUS)' if the Pap cytology report showed AEC-NOS, ASC-H, or LSIL. AEM*

Conclusion and Discussion

The clinical evaluation results of INDICAID™ HPV Urine Test showed very strong performance compared to endo-cervical swab-based diagnostics. The strong performance was enabled by the PHASiFY® Urine extraction process which efficiently processes large volumes of urine samples to extract and concentrate trace amounts of viral DNA. Historically, urine sample based HPV diagnostics have underperformed compared to swab sample diagnostics due to the dilute nature of urine samples and that they are less physiologically relevant with respect to the proximity of urine to the HPV infection location (in female samples). The results of this evaluation demonstrated a strong concordance of 97.7% and 98.5% between the two sample types for general HPV and high-risk HPV detection, respectively (Table 1). This demonstrated that the PHASiFY® technology can provide the same sensitivity and accuracy as professionally collected swab sample-based tests but with the privacy, convenience, and comfort of an at-home urine collection test.

These results have significant implications about the ability of the INDICAID™ HPV Urine Test to improve HPV testing in Hong Kong and globally. In many cultures, there are stigmas related to sexually transmitted infections (STI) and having discussions about sexual health and safe practices is considered taboo. Many women also have concerns about the discomfort and invasiveness of colposcopies and swab-based examinations. These barriers can cause a large portion of the population to not seek proper medical attention[1]. We saw from the clinical screening demographic data that a majority of participants had a poor historical record of getting Pap examinations, with 21.4% not having a Pap exam within the last 3 years and 56.4% never having a Pap exam (Table 2). An at-home urine collection option for HPV detection would provide a more private, convenient, and comfortable alternative approach. This has the potential to not only increase the percentage of people getting proper HPV diagnostic testing, but also motivate them to take the following steps of seeking professional medical advice and examination. We saw from the data that participants who were informed of being positive for high-risk HPV were 55.5% more likely to return for a Pap exam (Table 3). Intuitively this makes sense as these participants were more concerned about their health, but it also highlights the power of HPV result information in motivating a person to seek further medical evaluation. This in turn promoted the discovery of abnormal Pap cytology results that would otherwise have gone undetected. In this study, 19.5% of the HR-HPV participants who returned for examination found ASCUS abnormalities, while another 9.8% found one of the more concerning non-ASCUS abnormalities (AEC-NOS, ASC-H, or LSIL). In conclusion, the convenient sample collection and strong diagnostic performance of the INDICAID™ HPV Urine Test makes it a promising approach to reduce risk of developing cervical cancer for individuals and to improve the state of reproductive health in Hong Kong.

References

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