Color HPV Test for Cervical Cancer Screening

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Executive Summary

HPV (human papillomavirus) is a group of viruses that cause almost all cervical cancers. While not all HPV infections lead to cervical cancer, an active infection with a high-risk HPV (hrHPV) type is considered a substantial risk factor for cervical cancer. When identified in early stages, cervical cancer is largely treatable. Testing for an HPV infection is an effective and recommended first step in screening for cervical cancer. The American Cancer Society (ACS), United States Preventive Services Task Force (USPSTF), and American College of Obstetrics and Gynecology (ACOG) guidelines all recommend HPV testing as a primary screening modality for cervical cancer.[1–3]

Currently, clinician-collected cervical swabs are the primary sample type used for HPV testing in the United States. This screening modality imposes a significant access barrier by requiring an in-person appointment. More than 20% of women are not participating in any in-clinic screening and are at risk of cervical cancer going undiscovered until it is too late.[4] An at-home screening methodology can have a meaningful impact on addressing this persistent health problem.[5]

Studies over the last decade have shown that HPV tests that use self-collected urine have comparable analytic and clinical sensitivity to clinician-collected swabs.[6–8] Self-collection of urine is non-invasive, convenient, and a preferred sample collection method for many patients, compared to an invasive cervical or vaginal swab.

This document describes the development and validation of the Color HPV Test, a laboratory developed test (LDT) that detects hrHPV in first-void urine. The test uses the FDA-approved BD Onclarity™ HPV Assay, extending its use to self-collected first-void urine samples collected in the Novosanis Colli-Pee urine collection device and preserved in UCM DNA stabilization media. We demonstrate through *in vitro* and clinical studies that first-void urine is an acceptable alternative specimen type for the accurate detection of hrHPV.

Introduction

Testing for human papillomavirus (HPV) is a key way to screen for cervical cancer.

Human papillomavirus (HPV) is the cause of almost all cervical cancers, as well as a variety of other cancers. Indeed, over 90% of cervical cancers are caused by HPV.[9] When detected early, cervical cancer is largely treatable, making early detection of HPV crucial for prevention and treatment.[10]

Current guidelines from the the American Cancer Society (ACS), United States Preventive Services Task Force (USPSTF), the American College of Obstetrics & Gynecology (ACOG) recommend testing for hrHPV as the primary screening modality for cervical cancer.[1–3] The American Cancer Society (ACS) recommends hrHPV testing every 3-5 years for people with a cervix beginning at age 25-65. United States Preventive Services Task Force (USPSTF) and American College of Obstetrics and Gynecology (ACOG) guidelines also recommend HPV testing as a primary screening modality for cervical cancer.

Utilization of cervical cancer screening is low.

About 1 in 4 people in the general population are not up-to-date with recommended cervical cancer screening.[4] Uptake of screening is particularly low amongst individuals without a high school degree, falling below the federal poverty level[11], without health insurance, and living in rural communities[4]. For these reasons, cervical cancer has one of the highest degrees of diagnostic and prognostic inequity in medicine.[12] At this point, cervical cancer is primarily suffered by those who cannot access or afford screening.

Alternative sample collection modalities can increase access to screening.

Currently, the gold standard collection modality for HPV testing is a clinician-administered cervical swab. However, sample collection by a clinician can be expensive[13], uncomfortable, inconvenient, and unequally available.[14] These barriers prevent and discourage participation in recommended screening protocols.[4]

The use of self-collected biological specimens for health screening has several advantages:

1. Self-collection can greatly increase access to testing – one of the major bottlenecks to effective screening in the United States.
2. Self-collection can dramatically decrease the amount of time it takes for patients to access testing and results vs in-person screening.
3. Self-collection reduces the workload on healthcare providers to supervise the collection of specimens.
4. Self-collection is less invasive and less uncomfortable, making for a better patient experience.

The National Cancer Institute and the United States Centers for Disease Prevention and Control (CDC) have encouraged exploration of alternative sample collection methods that are cheaper and more widely available. Self-administered vaginal swabs and self-collected urine samples are two options that are increasingly supported by data demonstrating effectiveness.

Urine collection is a screening modality that can further break down access barriers to cervical cancer screening.

The FDA has approved multiple molecular testing platforms that detect HPV and identify hrHPV types. While clinician-collected cervical swabs are the primary sample type used for HPV testing in the United States, studies over the last decade have shown that HPV detection in self-collected urine has comparable analytic and clinical sensitivity. For example, a meta analysis published in 2014 found that HPV testing with urine samples had a sensitivity of 89% and a specificity of 97% compared with cervical swabs.[15] Since this meta analysis, several other studies have demonstrated similar sensitivities and specificities.[16–18]

Test Methodology

Step 1: Self-collection of first-void urine with the Colli-Pee device
Urine samples are self-collected using the Novosanis Colli-Pee device. Importantly, this device facilitates convenient and consistent collection of 'first-void' urine, which is the first portion of the urine stream that contains washed-away mucus and cellular debris from exfoliated cervical cells where HPV can be detected.[8,18] This device collects first-void urine required for testing and then redirects the rest of the urine stream to pass through the device without user intervention. Nucleic acids in the first-void urine are stabilized with a proprietary Urine Conservation Medium (UCM), allowing for ambient temperature storage and shipping of collected samples.

Step 2: Analysis of first-void urine sample with the BD Onclarity™ HPV Assay
First-void urine is analyzed with the BD Onclarity™ HPV Assay. This assay received pre-market approval (PMA) from the FDA on July 22, 2020 and detects 14 hrHPV types in a single analysis.

Step 3: Results of Color HPV Test
The Color HPV Test reports the detection of hrHPV (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68). HPV type information is only reported for HPV 16, 18, and 31. A human DNA control is included to verify specimen integrity (human beta-globin), and samples which fail to yield a positive human beta-globin signal will be reported as 'invalid'. Clinical samples that produce a valid result will be reported as shown in Table 1.

Result	Meaning
Positive for hrHPV (16/18/31)	DNA was detected from one or more of the 3 genotyped hrHPV types: 16, 18, 31
Positive for hrHPV	DNA was detected from one or more of the other 11 hrHPV types: 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68
Negative for hrHPV	DNA was not detected from any hrHPV types
Invalid	Human DNA not detected.

Table 1. Clinical result types of the Color HPV test. Samples positive for HPV 16, 18, or 31 will report genotyping results, and all other listed types will be reported as 'other hrHPV type'.

Assay Validation

The Color HPV Test was evaluated for analytical sensitivity, accuracy, precision and analytical specificity. Here we present the results of the LDT validation study.

Analytical Sensitivity (Limit of Detection)

The Limit of Detection (LoD) is defined as the lowest concentration at which all replicates are positively detected. The LoD of the Color HPV Test was established in a range-finding experiment, using a 2X dilution series of quantitated synthetic HPV DNA spiked into negative first-void urine starting from 10 copies/uL to 0.3125 copies/uL. The initial LoD in this range finding was determined to be 1.25 copies/uL for all accessible types (HPV 16, HPV 18, and HPV 31). This LoD was then verified in 5 replicates (Table 2).

Concentration	HPV 16	HPV 18	HPV 31
1.25 copies/uL	5/5	5/5	5/5

Table 2. LoD confirmation results. 100% of replicates were detected at 1.25 copies/uL in all 3 available types.

Accuracy

A combination of clinical samples, reference samples, and contrived samples were used to evaluate positive and negative percent agreement with the Color HPV Test. The overall approach is summarized in Figure 1. Contrived samples were created by spiking in HPV DNA in first-void urine collected in the Colli-Pee collection device. Reference samples were obtained through two certified proficiency testing agencies.

HPV was detected in 97.5% of all positive samples, and 0.0% of all negative samples (clinical and contrived). Sample details and results are provided in Table 2.

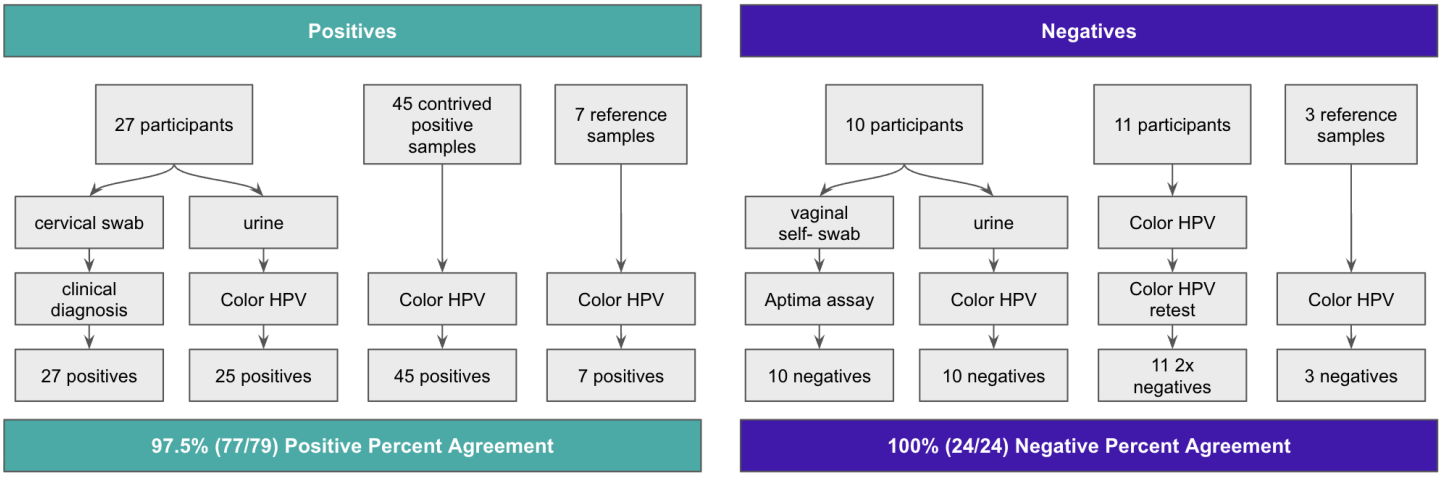

Figure 1. Approach to validating the Color HPV test.

Positive Sample Type		Total Samples	Tested Positive	Positive Percent Agreement (PPA)
Positive Clinical*		27	25	92.6%
Positive Contrived†	HPV 16	15	15	100%
	HPV 18	15	15	100%
	HPV 31	15	15	100%
Reference§		7	7	100%
Total Positive		**79**	**77**	**97.5%**

Table 3. Accuracy results, positive samples. *Positive clinical: Clinical samples consisted of self-collected first-void urine samples from patients diagnosed positive within approximately six months. 'Total Samples' represents cervical samples that tested positive for hrHPV via a clinically approved nucleic acid amplification test; 'Tested Positive' represents hrHPV positive urine samples that tested hrHPV positive on the Color HPV test.
† Positive contrived: Negative donor first-void urine samples spiked with HPV DNA (5 high-titered positives (5X LOD), 5 moderate-titered positives (3X LOD) and 5 low- titered positives (≤2X LOD) for each HPV type).
§ Reference: Proficiency testing specimens obtained through two certified proficiency testing agencies.

Negative Sample Type	Total Samples	Tested Negative	Negative Percent Agreement (NPA)
Negative Clinical*	10	10	100%
Negative Clinical†	11	11	100%
Reference§	3	3	100%
Total Negative	**24**	**24**	**100%**

Table 4. Accuracy results, negative samples. Negative for all hrHPV types tested.
*Negative clinical: Clinical samples were paired self-collected vaginal swab and self-collected first-void urine samples collected within 30 days; 'Total Samples' represents vaginal samples that tested negative for hrHPV via the Hologic Aptima HPV Assay; 'Tested Negative' represents urine samples that tested hrHPV negative on the Color HPV Test.
†Negative clinical: Clinical samples consisted of two self-collected first-void urine samples, collected at least one week apart but within two weeks, from participants with no hrHPV diagnosis or those who had cleared an hrHPV infection for at least six months; 'Total Samples' represents the total number of participants who tested hrHPV negative on the Color HPV Test in their first sample; 'Tested Negative' represents the number of participants who tested hrHPV negative for both urine samples on the Color HPV Test.

Precision

The reproducibility of the Color HPV assay was evaluated using contrived negative and contrived high, moderate and low positive HPV 16 and HPV 18 positive samples, and multiplexed samples that are positive for all individual genotypes and genotype groups. The precision study was conducted across 3 days by different operators.

Sample Type	Within Run	Between Run
Negative	88.9% (8/9)*	88.9% (8/9)*
Low Positives (2X LoD)	100% (18/18)	100% (18/18)
Moderate Positive (3X LoD)	100% (18/18)	100% (18/18)
High Positive (5X LoD)	100% (18/18)	100% (18/18)
Multiplexed Positive	100% (20/20)	95% (19/20)**
Overall Reproducibility	98.8% (82/83)	97.6% (81/83)

Table 4. Precision results. Inter- and intra-run reproducibility is shown. *One sample failed internal control(human beta-globin). **One sample failed to detect HPV 51.

Analytical Specificity

Cross Reactivity

We performed a cross reactivity study by testing a panel of ten organisms that would possibly be present and cause a false positive result in urine specimens. This panel was made up of bacteria, protozoan parasites, yeasts, and viruses. The study was performed by spiking in purified genomic DNA or purified virus into contrived negative first-void urine samples at a concentration of $1x10^6$ copies/mL or ≥ $1.6x10^3$ vp/mL. Each potential cross-reactant was tested independently and 3 times in the study.

Potential Cross-Reactant	Material Tested	HPV Positive Samples
Neisseria gonorrhoeae	Quantified Genomic DNA	0/3
Chlamydia trachomatis	Quantified Synthetic DNA	0/3
Escherichia coli	Quantified Genomic DNA	0/3

Trichomonas vaginalis	Genomic DNA	0/3
Mycoplasma genitalium	Quantified Genomic DNA	0/3
Ureaplasma urealyticum	Quantified Synthetic DNA	0/3
Candida albicans	Quantified Genomic DNA	0/3
HSV1	Quantified Genomic DNA	0/3
HSV2	Quantified Genomic DNA	0/3
EBV-1 (B95-8 Strain)	Virus	0/3

Table 5. Cross-reactivity results. Reactions containing all potential cross-reactants were negative for HPV, demonstrating no cross-reactivity to these organisms.

Interfering Substances

A study was performed to assess 5 interfering substances that can potentially be found in urine specimens. Each interfering substance was added into contrived negative samples with and without spiked-in HPV DNA at 3X LoD. Samples were tested individually, and processed through the workflow 3 times independently.

		HPV type detected			
Potential Interfering Substance	**Concentration**	**HPV 16**	**HPV 18**	**HPV 31**	**Negative**
KY Vaginal Lubricant	6% w/v	3/3	3/3	3/3	0/3
Monistat 3	2% w/v	3/3	3/3	3/3	0/3
Summer's Eve Douche	10% w/v	3/3	3/3	3/3	0/3
Whole Blood	4% v/v	3/3	3/3	3/3	0/3
Clindamycin Vaginal Cream	8% w/v	3/3	3/3	3/3	0/3

Table 6. Interfering substance results. All potential interfering substances did not affect negative or positive results (for all 3 types of HPV tested), demonstrating no interference by these substances.

Stability Studies

The Colli-Pee collection device was previously validated by Novosantis for the preservation of urine samples for 7 days at room temperature, 14 days at 4℃, or significantly longer when stored at -20℃ or -80℃.[19] HPV DNA stability was previously assessed using a validated PCR test with a spike-in at 1000 cp/uL to show that the UCM in Colli-Pee can preserve HPV DNA in urine.[20]

Simulated shipping study

Color performed a sample transport stability study to simulate the condition of sample shipment by mail to Color's laboratory. Contrived positive samples were made by spiking in HPV DNA at 3X LoD into first-void urine samples collected with the Colli-Pee device with UCM. Samples were exposed to 'summer' and 'winter' simulated shipping profiles for up to 7 days – summer temperatures ranged from 22 to 40℃ and winter temperatures ranged from -10 to18℃. The temperatures were chosen based on the FDA COVD-19 home specimen collection shipping profiles (Table 7).[21] Eight HPV 16, eight HPV 18 contrived positive samples, and eight contrived negative samples were tested at each timepoint. One aliquot of each of the three samples were tested on day 0 as the baseline result. The remaining aliquots were subjected to the transportation conditions and then tested at 3 days (72 hours), 4 days (96 hours), and 7 days (168 hours) (Table 8). Samples collected in the Colli-Pee device demonstrated stability up to 7 days (168 hours) under both summer and winter shipping conditions.

Cycle Period	Cycle Period (Hours)	Total Time (Hours)	Summer Temperatures	Winter Temperatures
1	8	8	40℃	-10℃
2	4	12	22℃	18℃
3	2	14	40℃	-10℃
4	44	58	30℃	10℃
5	6	64	40℃	-10℃
6	8	72	30℃	10℃
7	24	96	30℃	10℃
8	6	102	40℃	-10℃
9	18	120	30℃	10℃
10	24	144	30℃	10℃
11	24	168	22℃	18℃

Table 7. Sample transportation conditions. Extreme summer and winter shipping conditions were simulated for up to 7 days (168 hours).

	Summer Conditions			Winter conditions		
Day (Hour)	**HPV 16**	**HPV 18**	**Negative**	**HPV 16**	**HPV 18**	**Negative**
0 (0)	1/1	1/1	1/1	1/1	1/1	1/1
3 (72)	8/8	8/8	8/8	8/8	8/8	8/8
4 (96)	8/8	8/8	8/8	8/8	8/8	8/8
7 (168)	8/8	8/8	8/8	8/8	8/8	8/8

Table 8. Sample transportation study results. Samples collected in the Colli-Pee device demonstrated stability up to 7 days (168 hours) under both summer and winter shipping conditions.

Conclusions

Taken together, we have shown that hrHPV can be detected in first-void, self-collected urine. Validation studies demonstrate 94.4% PPA and 100% NPA, with a stability of 7 days under extreme shipping conditions. The development of this Color HPV Test enables distributed and at-home sample collection, lowering barriers to access and reaching a broader population of individuals who need cervical cancer screening.

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