Clinical utility of detecting HPV in urine samples as primary cervical cancer screening including HPV 16/18 genotyping

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Disclosures

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Cervical Cancer Screening – Statistics

Table 71 (page 1 of 5). Use of Pap smears among women aged 18 and over, by selected characteristics: United States, selected years 1987–2015

Excel and PDF versions (with more data years and standard errors when available): http://www.cdc.gov/nchs/hus/contents2016.htm#071.

[Data are based on household interviews of a sample of the civilian noninstitutionalized population]

Characteristic	1987	1993	1999	2000	2005	2008	2010	2013	2015
	Percent of women having a Pap smear within the past 3 years [1]								
18 years and over, age-adjusted [2,3]	74.1	77.7	80.8	81.3	77.9	75.6	73.7	70.4	70.2
18 years and over, crude [2]	74.4	77.7	80.8	81.2	77.7	75.1	73.2	69.4	69.0
Age									
18–44 years	83.3	84.6	86.8	84.9	83.6	81.8	80.4	77.2	76.1
18–20 years	59.4	66.8	65.3	59.8	61.1	57.5	52.0	38.6	34.0
21–44 years	86.1	86.2	89.2	87.8	86.3	84.8	84.0	81.6	81.1
21–24 years	85.3	86.1	85.3	84.1	84.0	80.2	81.1	74.6	69.7
25–44 years	86.3	86.3	89.9	88.5	86.8	85.7	84.6	83.2	83.5
45–64 years	70.5	77.2	81.7	84.6	80.6	78.8	76.9	73.9	75.5
45–54 years	75.7	82.1	83.8	86.3	83.4	81.0	79.9	78.6	79.7
55–64 years	65.2	70.6	78.4	82.0	76.8	76.0	73.2	68.6	71.1
65 years and over	50.8	57.6	61.0	64.5	54.9	50.0	47.1	42.7	42.3
65–74 years	57.9	64.7	70.0	71.6	66.3	61.6	58.0	54.5	52.9
75 years and over	40.4	48.0	50.8	56.7	42.7	37.5	34.6	27.9	28.1

- ***https://www.cdc.gov/nchs/data/hus/2016/071.pdf: Accessed Jan 15, 2018*** [(1)]

- A large proportion of the population is **LOST TO SCREENING PROGRAMS**, either due to poor resources, cultural barriers or avoidance of a pelvic exam.

Current screening guidelines:

Table 1. Screening Methods for Cervical Cancer for the General Population: Joint Recommendations of the American Cancer Society, the American Society for Colposcopy and Cervical Pathology, and the American Society for Clinical Pathology*

Population	Recommended Screening Method	Comment
Women younger than 21 years	No screening	
Women aged 21–29 years	Cytology alone every 3 years	
Women aged 30–65 years	Human papillomavirus and cytology cotesting (preferred) every 5 years Cytology alone (acceptable) every 3 years	Screening by HPV testing alone is not recommended*
Women older than 65 years	No screening is necessary after adequate negative prior screening results	Women with a history of CIN 2, CIN 3, or adenocarcinoma in situ should continue routine age-based screening for a total of 20 years after spontaneous regression or appropriate management of CIN 2, CIN 3, or adenocarcinoma in situ
Women who underwent total hysterectomy	No screening is necessary	Applies to women without a cervix and without a history of CIN 2, CIN 3, adenocarcinoma in situ, or cancer in the past 20 years
Women vaccinated against HPV	Follow age-specific recommendations (same as unvaccinated women)	

- Pap smears every 3 years starting at age 21 or co-testing with pap smears and HPV every 5 years starting at age 30 [(2)].

Primary hrHPV screening

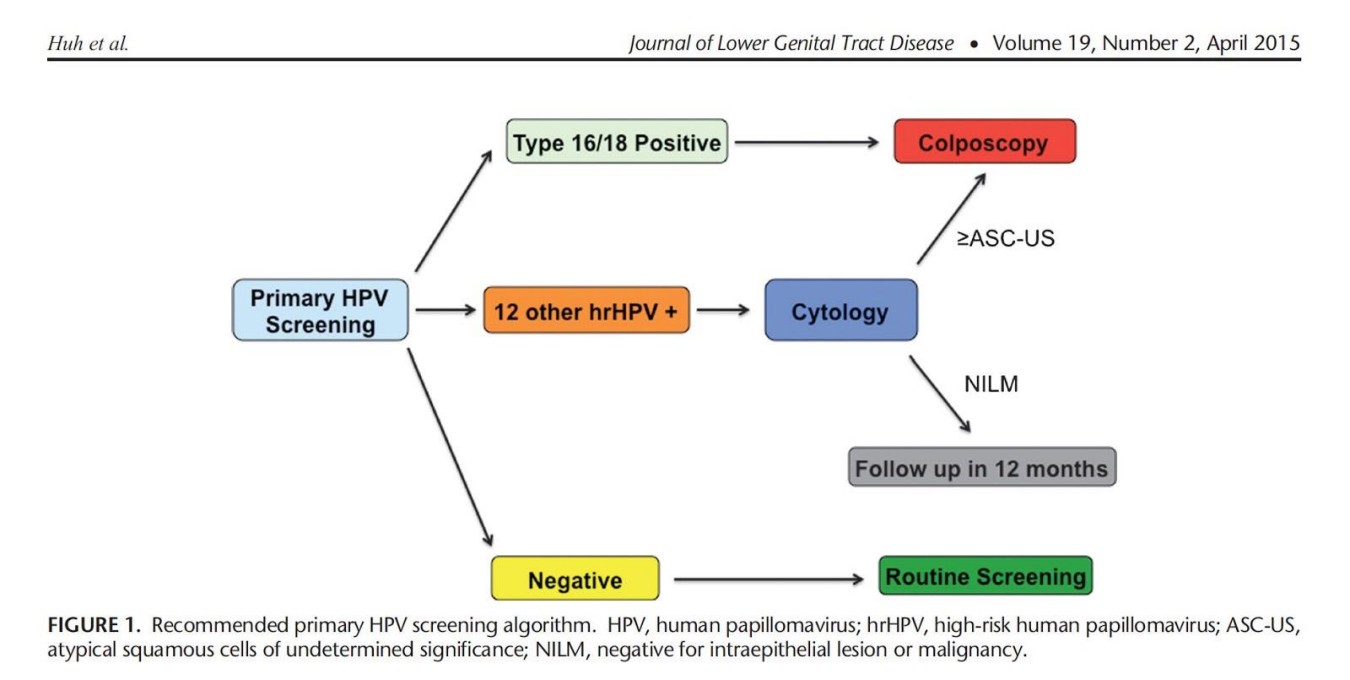


FIGURE 1. Recommended primary HPV screening algorithm. HPV, human papillomavirus; hrHPV, high-risk human papillomavirus; ASC-US, atypical squamous cells of undetermined significance; NILM, negative for intraepithelial lesion or malignancy.

- An interim clinical guideline was published in 2015 on primary HPV screening, which can be considered as **an alternative to cytology alone, co-testing**, and other current US cytology-based cervical cancer screening approaches [(3)].

- For women **> 25 years**: Routine screening: every 3 years [(3)].

Objective

- To detect high-risk human papillomavirus (hrHPV) mRNA **in urine** samples and compare their concordance with hrHPV mRNA **in cervical** samples including HPV 16 and 18/45 genotyping

- **Primary endpoint:**
  - Comparing hrHPV detection in cervical samples to urine samples including HPV 16 and HPV 18/45 genotyping

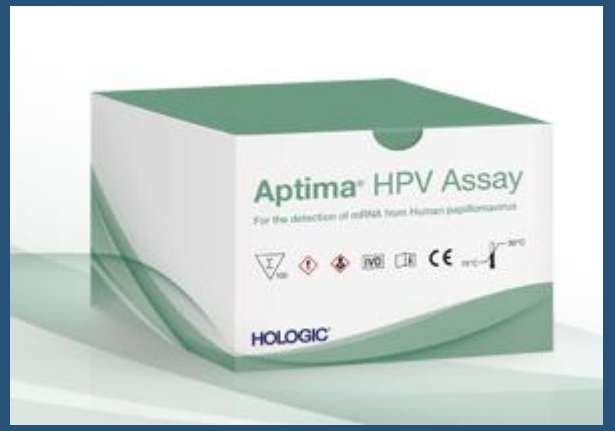
- **Secondary endpoint**
  - Determining the positive predictive value (PPV) for urine hrHPV detection for high grade histologic lesions (≥ CIN 2)

Methods

- **Panther Hologic system**

- **Transcription-mediated** amplification (TMA) system using reverse transcriptase

- **Aptima HPV assay**

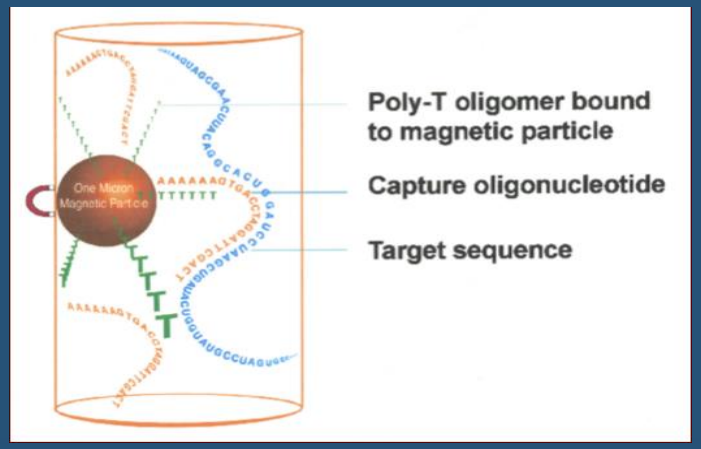


- Detects (**qualitative E6/E7 mRNA**) hrHPV types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68. (Does not discriminate between the 14 high-risk types)

Testing Methodology

1. Target capture

- **Isolates and purifies the target HPV mRNA** by use of capture oligomers that are linked to magnetic micro-particles [4].

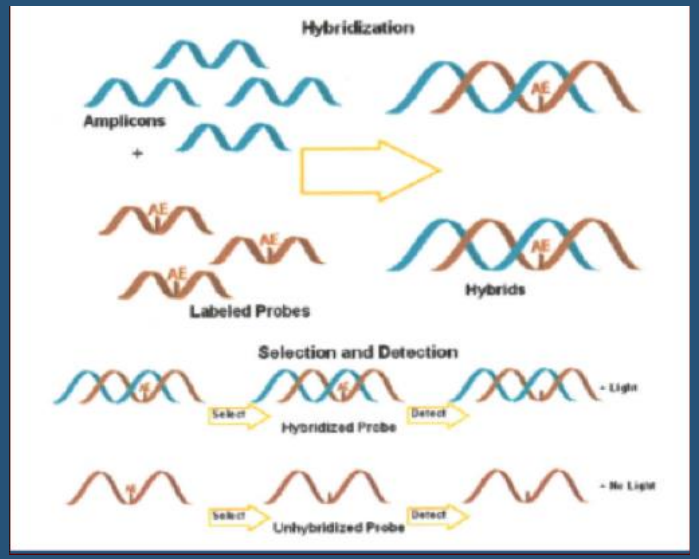


2. Transcription-Mediated Amplification:

- Uses MMLV reverse transcriptase and T7 RNA polymerase to generate multiple copies of **RNA amplicon** from the DNA copy template [4].

3. Hybrid Protection Assay:

- **Hybridizes** the amplicon to single stranded nucleic acids with **chemiluminescent labels** that are complimentary to the amplicon [4].



hrHPV Genotyping

- Urine samples which were positive for hrHPV further underwent hrHPV genotyping using the Aptima HPV 16, 18/45 Genotype Assay (Detects **E6/E7 HPV mRNA**)

- Can differentiate HPV 16 from HPV 18 and/or HPV 45, but **does not** differentiate between HPV 18 and HPV 45.

Power

- A sample size of **186 patients** was calculated to estimate a 95% confidence interval (CI) for the sensitivity of the urinary HPV test for detecting HPV
  - (assuming 80% ± 8% of HPV positive patients will have positive test and the prevalence of HPV in the study population is 50%.)

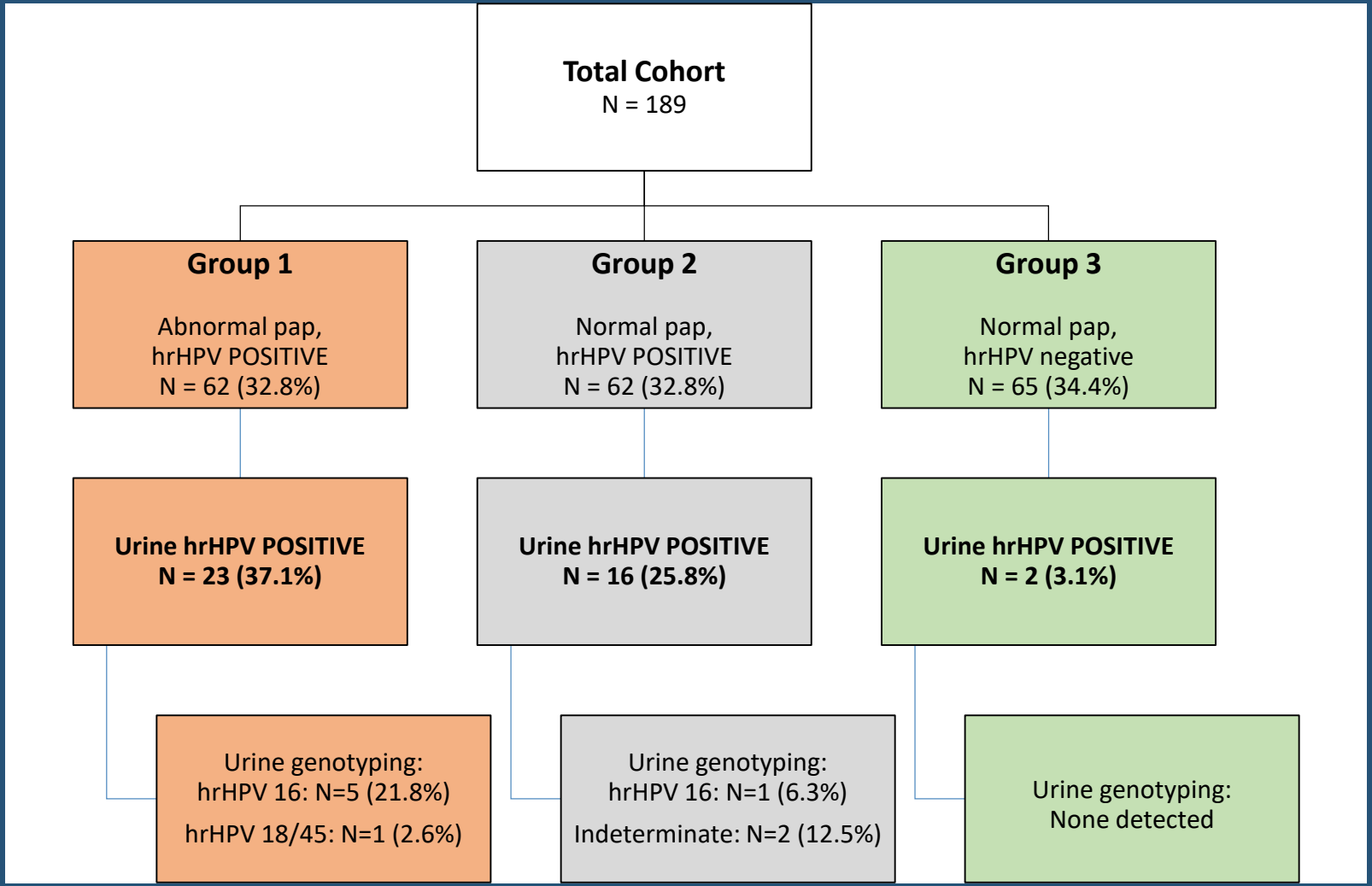
- This equals to **62 patients per group.**
  - With this sample size, the **precision for specificity will be 90%±6%.**

- Results for **189 patients** are presented so far.

Recruitment

- **Location:**
  - Centre for Women's Health at Staten Island University Hospital, NY.
- **Urine collection:**
  - During clinic visits
- **Inclusion criteria:**
  - Age 25 or above
  - Pap smear performed within past 360 days
- **Exclusion criteria**
  - Pregnant at time of urine collection
  - History of HPV vaccine administered

Study Flowsheet



Total Cohort Characteristics

		Total		Group 1 = Abnormal pap, hrHPV POSITIVE		Group 2 = Normal pap, hrHPV POSITIVE		Group 3 = Normal pap, hrHPV negative		p value
		N	%	N	%	N	%	N	%	
N		189		62	32.80%	62	32.80%	65	34.39%	
Age (years)										
	Median	41		36		42		45		
Age Groups (years)										
	<30	24	12.70%	15	24.19%	7	12.28%	2	3.08%	
	≥30-49	108	57.14%	34	62.96%	35	61.40%	39	60.00%	
	≥50	57	30.16%	13	24.07%	20	35.09%	24	36.92%	0.006
Race										
	Hispanic	92	48.68%	29	53.70%	40	70.18%	23	35.38%	
	Non-hispanic	97	51.32%	33	61.11%	22	38.60%	42	64.62%	0.004
Parity										
	0-1	47	24.87%	22	40.74%	8	14.04%	17	26.15%	
	2-4	117	61.90%	34	62.96%	42	73.68%	41	63.08%	
	≥5	15	7.94%	3	5.56%	6	10.53%	6	9.23%	0.080
Smoking										
	Yes	30	15.87%	12	22.22%	10	17.54%	8	12.31%	
	No	159	84.13%	50	92.59%	52	91.23%	57	87.69%	0.553
Hx of past or present sexually transmitted disease (STD)										
	Yes	28	14.81%	14	25.93%	9	15.79%	5	7.69%	
	No	161	85.19%	48	88.89%	53	92.98%	60	92.31%	0.061

Urine hrHPV detection (compared to cervical hrHPV detection)

	Cervical samples		
	hrHPV negative	hrHPV positive	Total
Urine samples			
hrHPV positive	2	39	41
hrHPV negative	63	85	148
Total	65	124	189

Cohen's kappa	95% CI	p value	Overall percent agreement
0.2176	(0.14-0.30)	0.04	54.0%

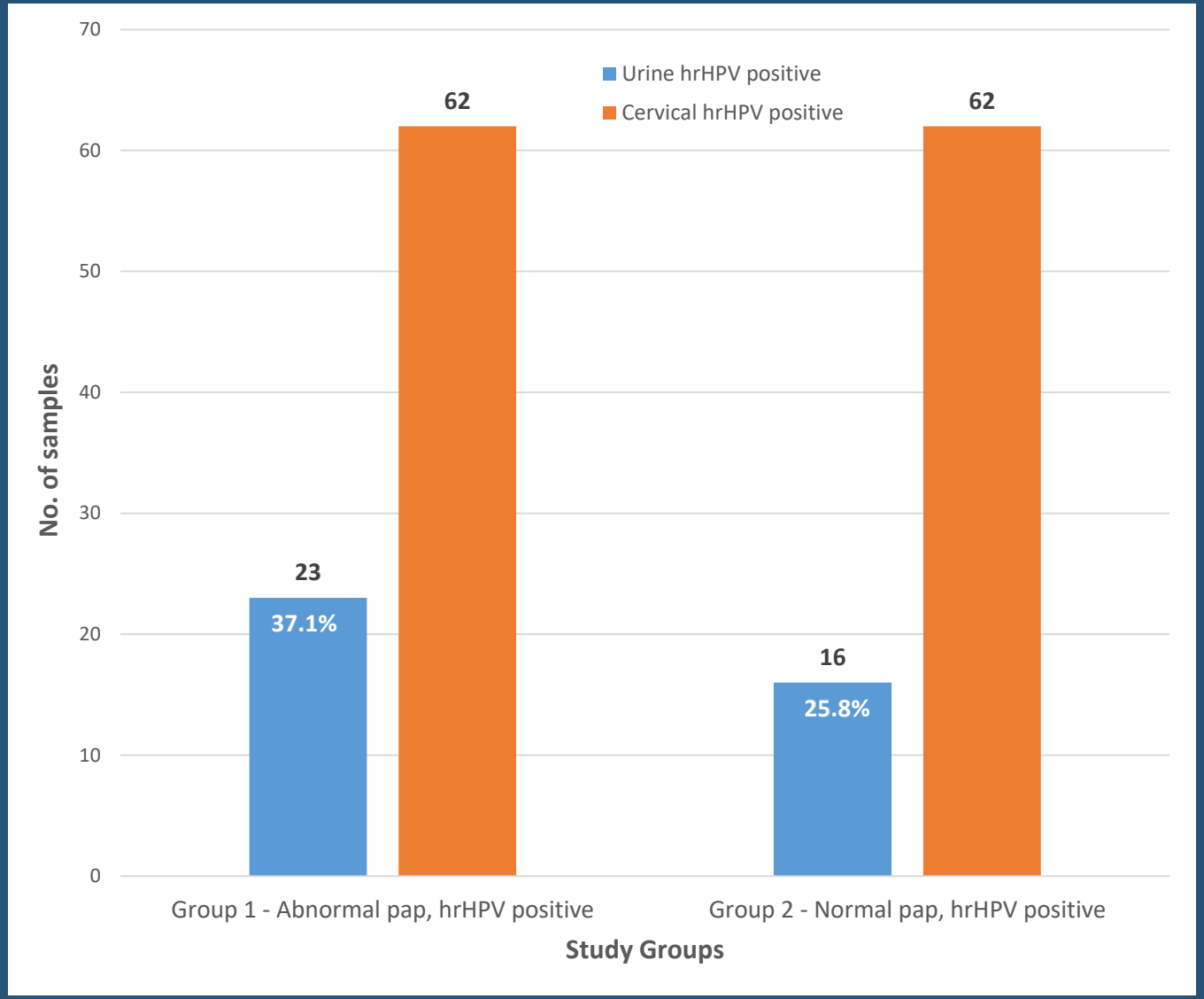
Prevalence (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
65.6% (58.3-72.3%)	31.5% (23.6-40.5)	96.9% (88.4 – 99.5%)	95.1% (82.2-99.1%)	42.6% (34.6-51.0%)

Urine hrHPV detection
(normal versus abnormal pap smear with cervical hrHPV positivity)

	Group 1 – Abnormal pap, hrHPV positive	Group 2 – Normal pap, hrHPV positive	Total
Urine hrHPV positive	23	16	49
Urine hrHPV negative	39	46	85
Total	62	62	124

Prevalence (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
50.0% (38.7-57.8%)	37.1% (25.4-50.3%)	74.2% (61.3 – 84.1%)	60.0% (42.2-74.0%)	54.1% (43.0-64.9%)

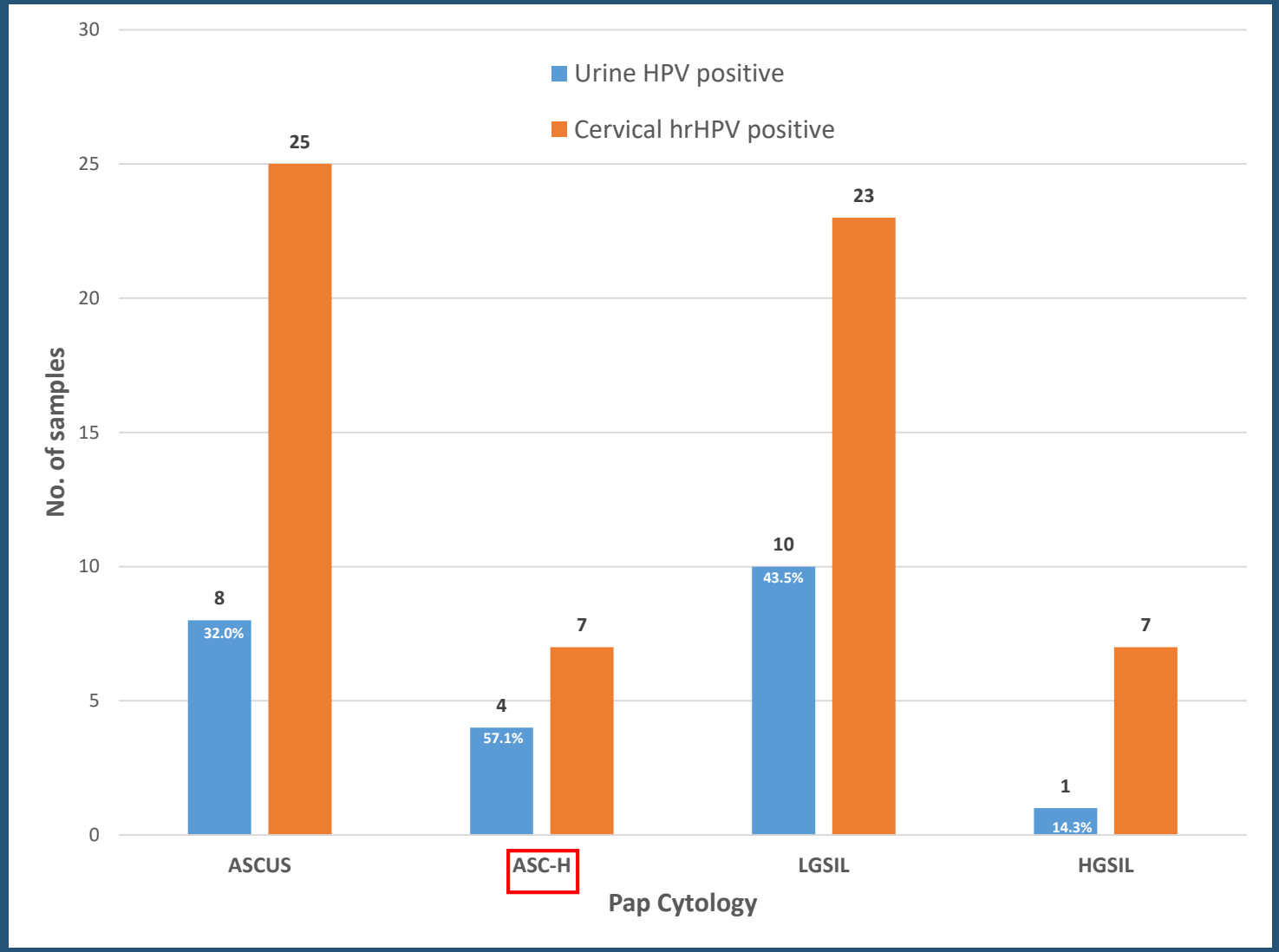
- A **higher proportion** of patients with abnormal cytology and hrHPV positivity had positive urine samples (**37.1**%; 23/62) versus patients with normal cytology and hrHPV positivity (**25.8%;** 16/62).



Urine hrHPV prevalence in Group 1 according to cervical cytology

	Urine hrHPV positive	Cervical hrHPV positive
ASCUS	8	25
ASC-H	4	7
LGSIL	10	23
HGSIL	1	7
Total	23	62

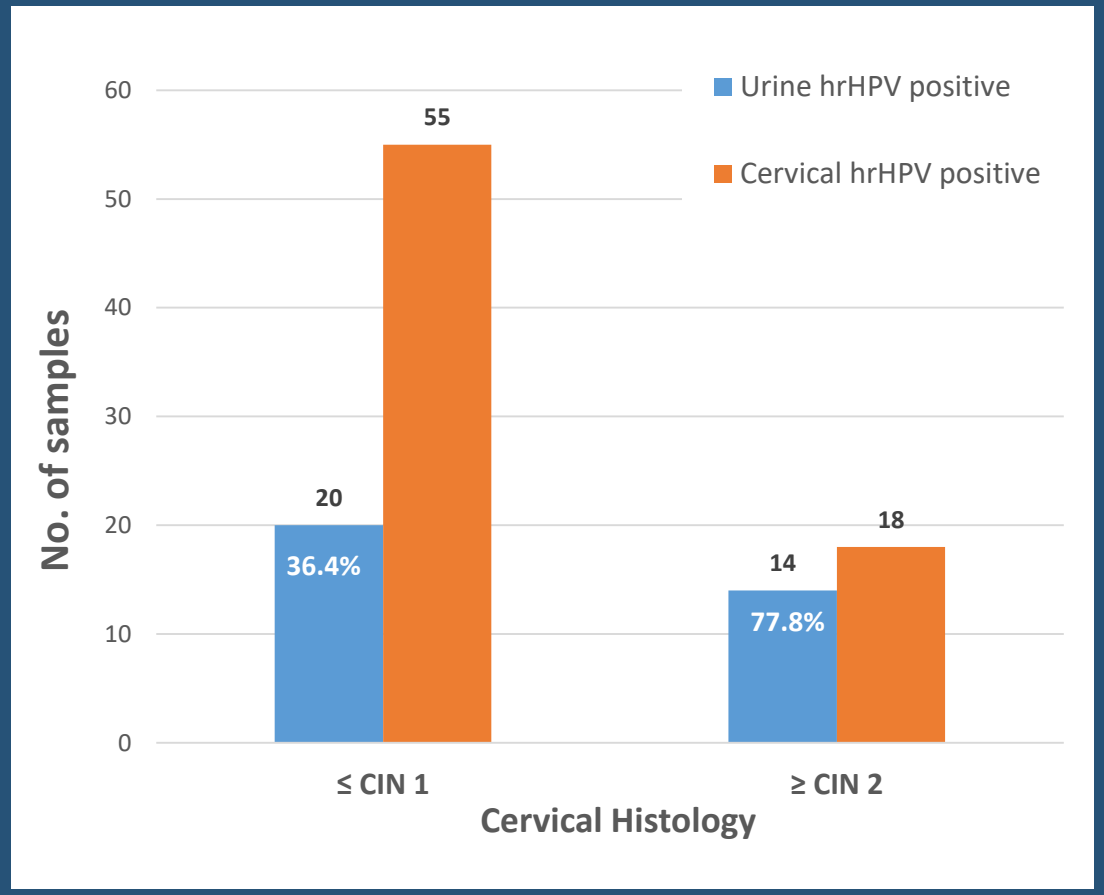
p = 0.64



Urine hrHPV detection for high grade cervical histology (≥ CIN2)

Urine samples	Cervical colposcopy/LEEP biopsies		
	≤ CIN 1	≥ CIN 2	Total
hrHPV positive	20	14	34
hrHPV negative	55	18	73
Total	75	32	107

p = 0.64

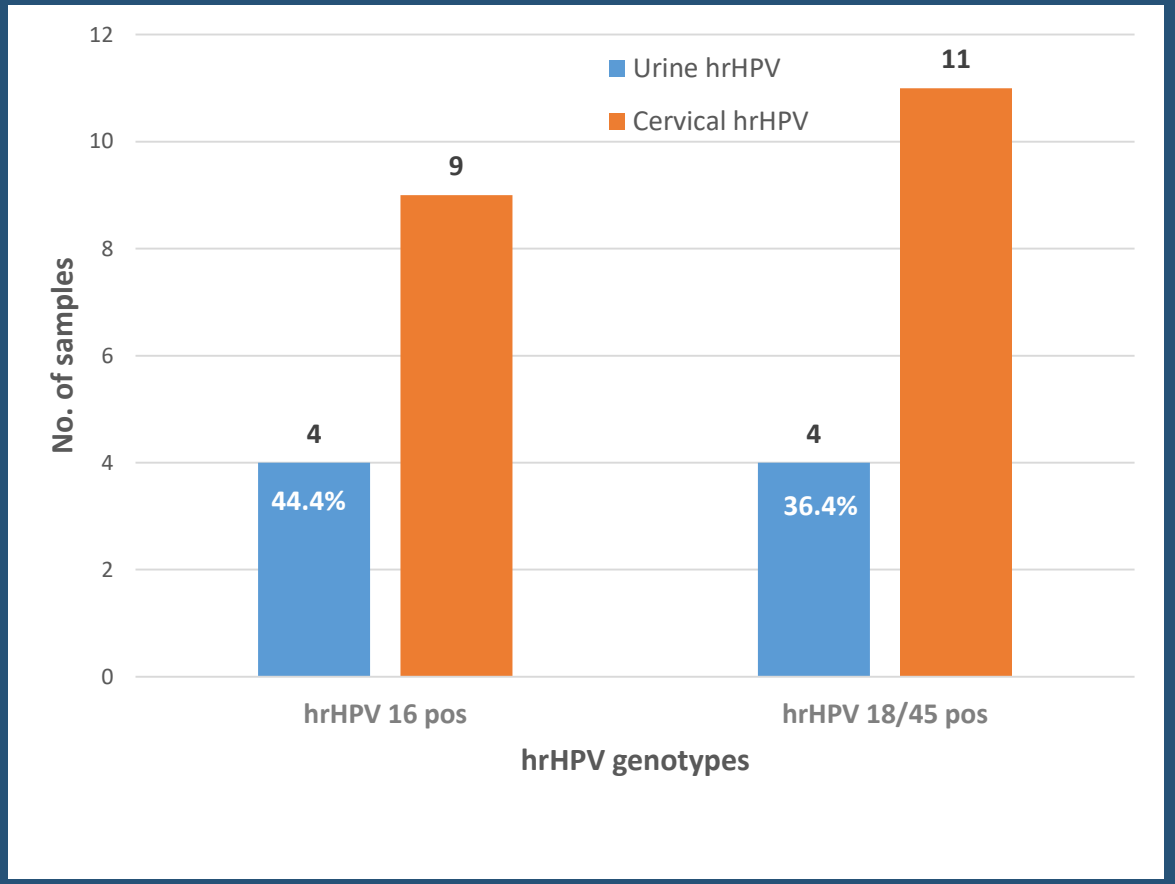


Prevalence (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
29.9% (21.6-39.6%)	43.8% (26.8-62.1%)	73.3% (61.7 – 82.6%)	41.2% (25.1-59.2%)	75.3% (63.7-84.4%)

hrHPV genotyping in urine and cervical samples

- Genotyping was performed on 57 cervical samples, of which **20 (35.1%)** were either hrHPV 16 positive or hrHPV 18/45 positive

	Pap smear hrHPV		Urine hrHPV positive	
hrHPV 16 pos	9	15.79%	4	44.44%
hrHPV 18/45 pos	11	19.30%	4	36.36%



Urine hrHPV Genotype results

Cervical samples			Urine samples			Cytology	Colposcopy / LEEP	Median time (days) from pap collection to urine collection
hrHPV	**HPV 16**	**HPV 18/45**	**hrHPV**	**HPV 16**	**HPV 18/45**			
pos	pos	neg	pos	Detected	Not detected	ASC-H	CIN 3	16
pos	pos	neg	pos	Detected	Not detected	HGSIL	CIN 3	48
pos	pos	neg	pos	Detected	Not detected	NILM	CIN 3	146
pos	neg	pos	pos	Not detected	Detected	LGSIL	CIN 2	0
pos	Not done	Not done	pos	Detected	Not detected	LGSIL	CIN 3	354
pos	Not done	Not done	pos	Detected	Not detected	ASCUS	CIN 2	187
pos	Not done	Not done	pos	Detected	Not detected	LGSIL	CIN 2	116

- The **genotype-specific concordance** for HPV 16 and 18/45 was **100%** for all the urine samples when compared to the cervical samples.

Percent agreement of hrHPV genotyping between urine and cervical samples

	Cervical samples		
	HPV 16 or 18/45 positive	HPV 16 or 18/45 negative	Total
Urine samples			
hrHPV 16 or 18/45 positive	8	0	8
hrHPV 16 or 18/45 negative	12	37	49
Total	20	37	57

Overall agreement	78.9%
Positive percent agreement	40.0%

Paired cervical and urine samples

	Group 1 = Abnormal pap, hrHPV POSITIVE		Group 2 = Normal pap, hrHPV POSITIVE	
N	10		6	
Urine hrHPV positive	4	40.0%	2	33.3%
Genotyping – URINE samples	2	20.0% hrHPV 18/45 pos x 1, hrHPV 16 pos x 1	1	16.7% hrHPV 16 pos
Cytology and HPV status of cervical samples corresponding to urine hrHPV positive samples	1= ASCUS, hrHPV pos (16/18 neg) 2= LGSIL, hrHPV pos (16 neg, 18 pos) 3= LGSIL, hrHPV pos (16 neg, 18 pos) 4= ASC-H, hrHPV pos (16 pos, 18 neg)		1 = NILM, HPV pos (16 pos, 18 neg) 2 = NILM, HPV pos (16 pos, 18 neg)	

- The positive percent agreement for both groups is **37.5%**

Discussion

- In this study, the overall sensitivity of urine hrHPV detection is low at **31.5%,** however the specificity and PPV are above 90% **(96.9% and 95.1%)** respectively.

- There is a **FAIR agreement** between cervical and urine samples (k=0.22, CI 0.14-0.30, p=0.04) with an overall percent agreement of 54%.

- Median time of pap collection to urine collection was **similar** in both Groups 1 and 2 who had hrHPV positivity in cervical samples (122 and 124 days respectively).

Discussion

- A higher percentage of patients with ≥ CIN 2 histology had positive urine HPV results (**77.8%;** 14/18) versus patients with < CIN 2 histology (**36.4%;** 20/55).

- The specificity was **73.3%** with a sensitivity of 43.8%

- The PPV was **41.2%**

Discussion

- For HPV 16 or HPV 18/45 genotyping, **the overall agreement was 78.9%** with a **positive percent agreement of 40.0%.**
  - However, of all the cervical hrHPV positive samples, **less than half** were HPV 16 or HPV 18/45 positive.
  - The genotype-specific concordance was **100%**

- In the group of paired cervical and urine samples, the **positive percent agreement was 37.5%.**
  - This is slightly higher than the positive percent agreement for Groups 1 and 2 (positive cervical hrHPV) at **31.5%**

Current Challenges with urine HPV detection

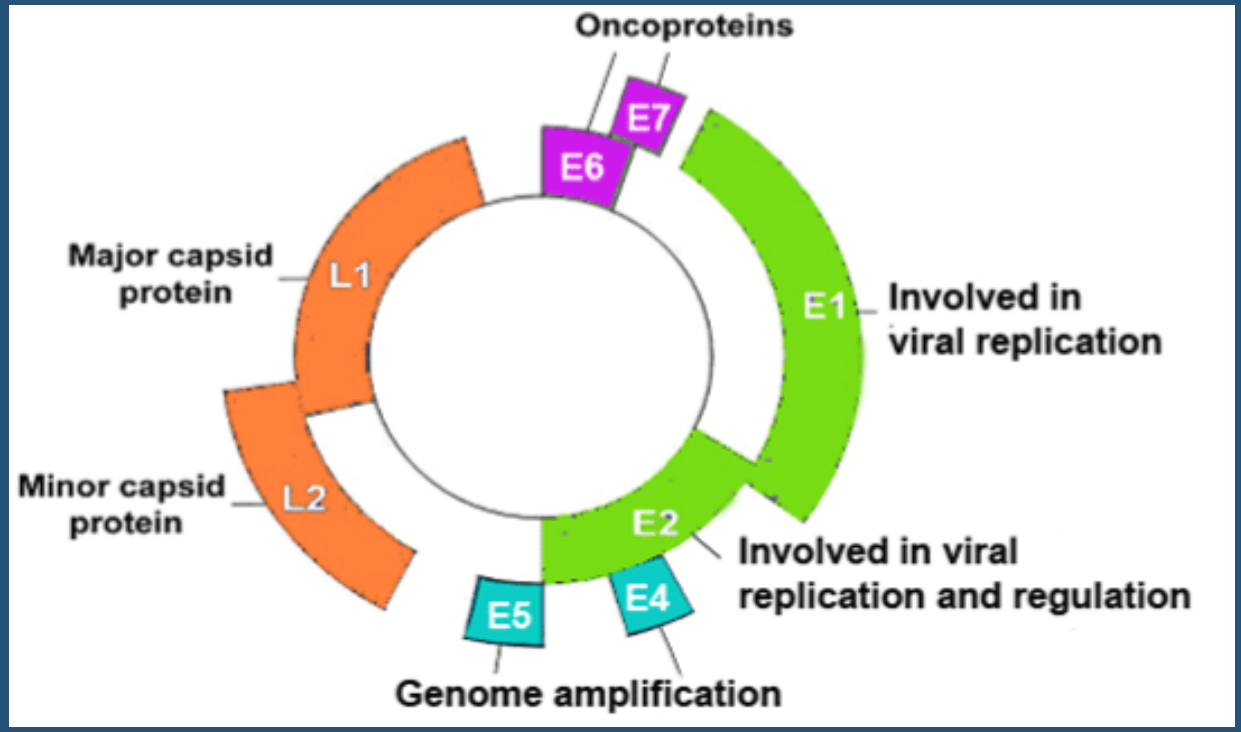
- There is **no consensus** of mode of urine collection (i.e. storage, amount, type of void) and HPV DNA extraction from these samples.

- Some studies have found higher sensitivity and specificity for DNA hrHPV detection in first void samples [5].

- *Senkomago et al (2016):*
  - **"… no difference on HR-HPV detection for first void, initial stream and mid-stream urine for unfractionated and pellet fractions"[6]**

HPV DNA Detection in Urine Samples

- Most HPV DNA tests are based on the **PCR detection of the L1 gene** [7-8].
  - **High sensitivity, BUT low specificity** [7]



Summary of Studies (Worldwide)

Year	Author	Country	Sample size	HPV Detection Test	Sample collection	Urine HPV positive specimens	k agreement (Overall agreement %)	For	Sensitivity (%)	Specificity (%)
2014	Ducancelle et al (9)	France	230	INNO-LiPA HPV genotyping assay	paired urine / cervical samples	98	0.80 (90%)	n/a	n/a	n/a
2014	Combita et al (10)	Columbia	535	E7-MPG, IARC multiplex genotyping assay	paired urine / cervical samples	343	0.81 (79%)	19 HPV genotypes	91	74
2014	Bernal et al (11)	Seville	125	Cobas 4800 HPV Test	paired urine / cervical samples	66	0.76 (88%)	14 HPV genotypes	91	90
2015	Stanczuk et al (12)	Scotland	5318	Cobas 4800 test	paired urine / cervical samples	578	0.9 (n/a)	CIN 2+	63	90
2015	Stanczuk et al (13)	UK	100	Cobas 4800 test	paired urine / cervical samples	78	n/a (84%)	CIN 2+ CIN 3+	80 91	23 27
2015	Lim et al (5)	Korea	100	Roche HPV assay, Abbott HPV assay	paired urine / cervical samples	Roche - 56 Abbot - 69	n/a	HPV 16/18	Roche - 79 Abbott - 82	Roche - 100 Abbott - 100
2015	Burroni et al (14)	Italy	216	INNO-LiPA HPV genotyping Extra	paired urine / cervical samples	51	n/a (80%)	n/a	n/a	n/a
2015	Hagihara et al (15)	Japan	240	Anyplex TM II HPV 28 detection kit	paired urine / cervical samples	98	0.79 (98%)	19 HPV genotypes	68	100
2016	Khunamornpong et al (16)	Thailand	123	Cobas 4800 test	paired urine / cervical samples	30	0.65 (86%)	14 hrHPV genotypes	69	93

Summary of studies (Worldwide)

Year	Author	Country	Sample size	HPV Detection Test	Sample collection	Urine HPV positive specimens	k agreement (Overall agreement %)	For	Sensitivity (%)	Specificity (%)
2017	Cuzick et al (17)	UK	501	Trovagene HPV test	paired urine / cervical samples	396	82.6	CIN 3+ CIN 2+	91 81	(For <CIN 2) - 25
2017	Nilyanimit et al (18)	Thailand	164	HPV GenoArray Assay	paired urine / cervical samples	53	n/a	21 HPV genotypes	57	71
2017	Lee et al (8)	Korea	25	Direct PCR-free colorimetric detection	paired urine / cervical samples	12	n/a	n/a	n/a	n/a
2017	Tshomo et al (19)	Bhutan	89	PCR E7-MPG, G5+/6+	paired urine / cervical samples	PCR E7-MPG - 56 G5+/6+ - 27	n/a	21 HPV genotypes	PCR E7-MPG - 80 G5+/6+ - 58	PCR E7-MPG - 61 G5+/6+ - 89
2017	Vergara et al (20)	Chile	543	PCR-RLB	paired urine / cervical samples	301	0.72 (86%)	30 HPV genotypes	82	94
2017	Leeman et al (21)	Netherlands	91	SPF10-DEIA-LiPA25 assay, GP5+/6+ EIA-LMNX	paired urine / cervical samples	SPF10 - 67,70 GP5+/6+ - 60, 60	SPF10 - 0.83,0.86 (n/a) GP5+/6+ - 0.75,0.78 (n/a)	CIN2+	SPF10 - 95-100 GP5+/6+ - 95	SPF10 - 29-32 GP5+/6+ - 42
2018	Lorenz et al (22)	Brazil	336	HPV-HR (PCR based assay)	paired urine / cervical samples	259	0.363 (80%)	CIN 2+ CIN 3+	83 86	51 36

Summary of Studies: USA

Year	Author	Country	Sample Size	HPV detection Kit	Sample Collection	Urine HPV positive specimens (N)	k agreement (Overall agreement %)	For	Sensitivity (%)	Specificity (%)
2013	Sahasrabuddhe et al (23)	USA	72	Linear array HPV genotyping test assay	paired urine/ cervical samples	54	0.55 (79%)	CIN 2/3	81	53
2014	Sahasrabuddhe et al (24)	USA	72	Trovagene HPV test, linear array HPV genotyping assay	paired urine/ cervical samples	Trovagene - 56 LA-HPV - 47	0.65 (92%)	CIN 2/3 CIN 3 CIN 2/3 CIN 3	Trovagene - 92 Trovagene -100 LA-HPV - 81 LA-HPV - 90	Trovagene - 29 Trovagene - 25 LA-HPV - 42 LA-HPV - 38
2014	Mendez et al (25)	USA	52	Linear Array HPV genotyping assay	paired urine/ cervical samples	22	n/a (76%)	n/a	n/a	n/a
2015	Senkomago et al (6)	USA	37	Trovagene HPV test	paired urine/ cervical samples	21-27	n/a	CIN 2	90	35
2016	Piyathilake et al (26)	USA	502	Reverse line blot method	paired urine/ cervical samples	269	0.66-0.83 (67-91%)	37 HPV genotypes	n/a	n/a

HPV mRNA Detection in Cervical Samples

- E6/E7 mRNA has been studied in cervical samples with overall conclusions of **increased specificity** compared to HPV DNA assays [7, 27,29, 30].

- Theory:
  - Increased expression of **HPV E6/E7 oncoproteins** supports cervical tumorigenesis [7].
  - In active infections, the **hallmark is production of mRNA and proteins** [7].
  - mRNA can be detected by **reverse transcriptase** [7].

APTIMA HPV mRNA detection

- *Asciutto et al* (Sweden, 2018): APTIMA mRNA based hrHPV testing on self collected urine and vaginal samples compared to clinician-taken cervical samples [28]

- For Urine Samples:

• Overall:	Sensitivity: 48.1%, Specificity: 82.8% [28]
• **Compared to this study:**	**Sensitivity: 31.5%, Specificity: 96.9%**
• For detecting HSIL/AIS/cancer:	Sensitivity: 44.8%, Specificity: 61.9% [28]
• **For detecting ≥ CIN2 pathology:**	**Sensitivity: 43.8%, Specificity: 73.3%**

Conclusion

- The utility of mRNA reverse-transcriptase mediated amplification methodology using the APTIMA HPV assay is **suboptimal** in detection of hrHPV mRNA in urine samples and therefore cannot be considered for primary cervical cancer screening.

- However, due to its **high specificity,** mRNA urine HPV detection can be utilized in disease surveillance for patients with ≥ CIN 2 histology.

Strengths

- Power of study
- Comparison of urine HPV detection in distinct groups **with and without cytologic abnormality**

Limitations

- **Small sample si**ze of paired cervical and urine samples
- All hrHPV positive genotypes were **not tested** for on cervical and urine samples

Future Direction

- Standardizing mode of urine collection and storage
- Obtaining larger sample size of paired urine and cervical samples
- HPV mRNA detection in patients with ≥ CIN 2 histology
- Using broad HPV genotyping assays with current methodology.

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Thank you

References

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