Performance Characteristics of the Alinity m HR HPV Investigational Use Only (IUO) Assay and Comparison to the Cobas HPV 6800 Assay

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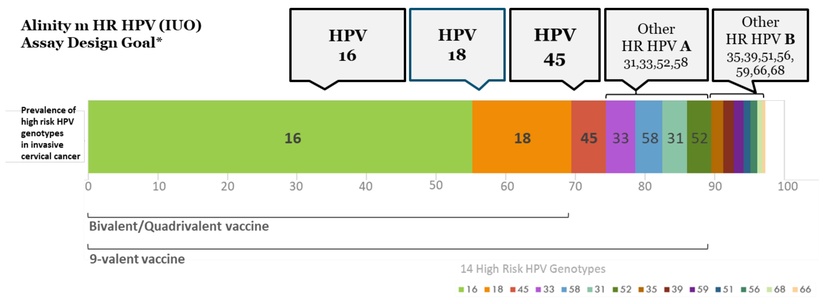
Background

Human papillomavirus (HPV) is a small, non-enveloped, double stranded DNA virus (approximately 8,000 base pairs) that replicates in the nucleus of squamous epithelial cells and induces hyperproliferative lesions. Persistent HPV infection may result in progression to cervical cancer. Genital HPV genotypes are generally classified into high risk (HR) and low risk (LR) groups based on their carcinogenic potential. HR HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) are associated with invasive cervical cancer. Three of the 14 HR HPV genotypes, 16, 18 and 45, are associated with approximately 70-80% of invasive cervical cancer cases worldwide. For adenocarcinoma, which is more difficult to detect, HPV 16, 18, and 45 have been observed in 78-94% of cases. A primary objective of this study was to evaluate the analytical and clinical performance of the Alinity m HR HPV (IUO) assay.

Methods

The Alinity m HR HPV (IUO) test is designed to be a qualitative in vitro assay that targets the conserved sequence within the L1 region of the HPV genome. The Alinity m HR HPV (IUO) assay was developed with a design goal for partial genotyping for clinically relevant risk stratification where 16, 18, and 45 are individually reported and the remaining 11 targeted genotypes are aggregated into two groups A (31/33/52/58) and B (35/39/51/56/59/66/68). Human beta-globin is targeted as a cellular control (CC) for confidence in negative results.



Analytical performance was assessed with external genotype (GT) control material from Microbix consisting of GTs 16/18/45, 31/33/66 and 39/51/52. The control material was prepared in neat ThinPrep PreservCyt Solution and tested in triplicate over 3 days with Alinity m HR HPV (IUO) assay. Cytology results were available for all clinical specimens included in the study. 274 de-identified residual cervical clinical specimens were included in this study. For each specimen, an aliquot was split for Alinity m HR HPV (IUO) assay, Cobas 6800 HPV assay and Hologic Aptima HPV assay. Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) analysis was performed between Alinity m HR HPV (IUO) assay and cobas 6800 HPV assay. Positive Predictive Value (PPV) and Negative Predictive Value (NPV) analysis was performed using the patient infection status (PIS) agreement between the 3 HPV methods in the study.

Results

Table 1 Alinity m HR HPV IUO Precision

Microbix Panel		VP-62-M1 (16/18/45)				VP-62-M2 (31/33/66)			VP-62-M3 (39/51/52)		
HPV GT		16	18	45	CC	Group A 31 / 33	Group B 66	CC	Group A 52	Group B 39 / 51	CC
Day 1	Average CN	24.77	21.75	26.71	25.64	23.95	23.64	26.73	25.07	24.32	24.14
	SD	0.42	0.67	0.37	0.55	0.57	0.42	0.25	0.47	0.35	0.41
	%CV	1.70	3.08	1.40	2.14	2.37	1.79	0.92	1.89	1.43	1.72
Day 2	Average CN	24.18	21.40	26.09	24.99	24.05	23.46	26.66	24.43	23.56	22.97
	SD	0.19	0.26	0.17	0.15	0.23	0.28	0.17	0.41	0.35	1.14
	%CV	0.78	1.20	0.63	0.60	0.97	1.18	0.62	1.66	1.51	4.95
Day 3	Average CN	24.18	20.98	26.67	25.02	23.69	23.31	26.46	24.34	23.43	23.28
	SD	0.13	0.19	0.22	0.11	0.28	0.45	0.12	0.35	0.51	0.49
	%CV	0.53	0.91	0.83	0.44	1.19	1.95	0.46	1.42	2.17	2.11
Totals	Average CN	24.38	21.38	26.49	25.22	23.90	23.47	26.62	24.61	23.77	23.46
	SD	0.38	0.50	0.38	0.43	0.38	0.37	0.20	0.50	0.55	0.84
	%CV	1.56	2.34	1.43	1.71	1.57	1.56	0.76	2.02	2.30	3.56

Within laboratory precision contains the average CN, SD, and %CV for each day, along with the three-day statistics (Table 1).

Table 2

Cytology Result	HPV16	HPV18	HPV45	Other HR HPV A	Other HR HPV B	HR HPV Detected	Not Detected	Total
ASC-US	7	5	4	18	35	69	51	120
ASC-H	0	0	1	0	0	1	1	2
AGC	0	0	0	0	0	0	1	1
LSIL	3	2	1	7	18	31	12	43
HSIL	3	0	0	2	1	6	0	6
>=ASC-US	13	7	6	27	54	107	65	172
NILM	0	0	0	1	6	7	95	102
Total	13	7	6	28	60	114	160	274

> 93% of samples with a positive HPV result by either Alinity m or Cobas had a cytology result ≥ ASC-US. Table 2 outlines Alinity m HR HPV (IUO) results associated with cytology categories.

Table 3a

		Cobas Result HR HPV Detected	Cobas Result Not Detected	Total		
Alinity Result	HR HPV Detected	106	1	107	Cytology	PPA
	Not Detected	10	55	65	≥ ASC-US	91.4%
	Total	116	56	172		

		Cobas Result HR HPV Detected	Cobas Result Not Detected	Total		
Alinity Result	HR HPV Detected	2	5	7	Cytology	NPA
	Not Detected	1	94	95	NILM	94.9%
	Total	3	99	102		

Table 3b

		Cobas Result 16/18 Positive	Cobas Result 16/18 Negative	Total		
Alinity Result 16/18	Positive	20	0	20	Cytology	PPA
	Negative	5*	147	152	≥ ASC-US	80.0%
	Total	25	147	172		

* 3 ASC-US, 1 ASC-H, 1 LSIL

		Cobas Result 16/18 Positive	Cobas Result 16/18 Negative	Total		
Alinity Result 16/18	Positive	0	0	0	Cytology	NPA
	Negative	1	101	102	NILM	100.0%
	Total	1	101	102		

Overall PPA in ≥ ASC-US was 91.4% and NPA in NILM was 94.9% (Table 3a). GT 16/18 PPA in ≥ ASC-US was 80% and NPA in NILM was 100% (Table 3b). Discordant specimens between the two methods were near the clinical cutoff for the two assays.

Table 4

Cytology	Category	PPV (%) (95% CI)	PPV (%) n/N	NPV (%) (95% CI)	NPV (%) n/N
≥ ASC-US	Alinity vs PIS	100 (96.5,100)	107/107		
	Cobas vs PIS	95.7 (91.1,98.1)	111/116		
NILM	Alinity vs PIS			100.0 (96.1,100)	95/95
	Cobas vs PIS			99.0 (97.6,99.8)	98/99

Alinity m HR HPV (IUO) PPV in ≥ ASC-US was 100% and NPV in NILM was 100% and Cobas 6800 HPV PPV in ≥ ASC-US was 95.7% and NPV in NILM was 99.0% (Table 4).

Conclusions

Overall PPA between the two HPV methods in ≥ ASC-US cytology was 91.4% and NPA in NILM cytology was 94.9%. Discordant specimens between the two methods were near the clinical cutoff for the two assays. Alinity m HR HPV (IUO) PPV in ≥ ASC-US cytology was 100% and NPV in NILM cytology was 100%. Cobas 6800 HPV PPV in ≥ ASC-US cytology was 95.7% and NPV in NILM cytology was 99.0%.

Acknowledgments & Disclosures

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- Alinity m HR HPV (IUO) reagents were provided by Molecular Diagnostics of Abbott (Des Plaines, IL, USA) for the completion of this study.
- In the US, Alinity m HR HPV Assay is pending FDA review and not commercially available; the performance characteristics of this product have not been established as it is IUO.