APTIMA® HPV Laboratory Evaluation Study
Protocol Number: Tachezy/hpv2010

General comments

In the manual the order of procedures is not clearly explained. However, upon the instruction from the company person we were able to easily perform test. The Czech version of the manual needs a language revision.

For the test it is absolutely necessary to have physically separated pre- and post-amplification rooms since the tubes are uncovered (or covered with aluminum foil which gets into contact with the tips) through the whole procedure.

The test is quite robust. The equipment needs quite a lot of space which might be of a disadvantage for laboratories limited in space. Quite unusual is the transfer of samples to the reaction tubes. Punching of the aluminum foil need some skill and strength. As a negative we also see the manipulation with thermoblocks. It is easy to push the bottom acidentally twice and in this way skip one step of the procedure and it is impossible to return.

Materials and methods

This study was performed according to the protocol from Exhibit A.

Population studied

Women referred to Centre of Gynecological Oncological Prevention for abnormal cytological findings were examined by expert colposcopy. Cytological smear for classical cytology analyses and a smear for HPV detection (PreservCyt) were collected. When atypical colposcopical findings were observed small punch biopsy has been taken for histological analyses.

Altogether we have collected samples of 72 women.

Cytology, colposcopy, histology

The cytological results were classified as normal, atypical (atypical squamous cells of unknown significance (ASC-US), atypical glandular cells not otherwise specified (AGC-NOS), LSIL, HSIL or atypical glandular cells favor neoplasia (AGC-NEO) or atypical squamous cells cannot exclude HSIL (ASC-H)).

Similarly, on colposcopy, the findings were categorized as an atypical transformation zone (ATZ), LGL, and HGL.
The histological findings were classified as mild dysplasia (DI) equal CIN I, moderate dysplasia (DI-II, DII) equal CIN II, severe dysplasia (DII-III, DIII) equal CIN III and invasive carcinoma.

HPV detection

Samples of 72 patients were analyzed for the presence of 13 HR HPV types by Hybrid capture 2 tests (Qiagen). Based on the RLU/CO score we have than selected out of 65 positive samples 10, 12, 12 and 11 samples with RLU/CO <12; 12-100; 101-300; >300, respectively. Out of the 7 HC2 HR HPV negative samples we have selected 5 for additional testing.

Fifty selected samples were consequently tested by Aptima HPV test three times in two days by two operators. Two different sets but with the same Lot number were used.

The specification of HPV types present in 40 samples positive on HC2 test has been done by Linear array test (Roche).

Results

Out of 72 samples collected, 90.2% (65/72) were positive for HR HPV by HC2 test specific for 13 HR HPV types.
Medium RLU/CO values of HC2 test in correlation with histology results are summarized in Table 1.

Table 1. Medium RLU/CO values of HC2 test in correlation with histology results.

Histology	Median RLU/CO
≤DI (CIN I)	2.885
DII (CIN II)	90.485
>DII (CIN III)	166.76*

*One patient with invasive carcinoma RLU/CO 4.68

In repeated examination done three times with Aptima testing we had 5 samples (10%) with discrepant results between the runs (Table 2). All of these samples had very low viral load (median RLU/CO of these samples on HC2 was 2.83). For additional analyses these samples were scored as negative by Aptima HPV testing.

Table 2. Results of samples tested by Aptima HPV with discrepant outcomes from different runs and RLU/CO values of HC2 testing.

ID	AHPV S/CO	AHPV HPV positivity	AHPV S/CO	AHPV HPV positivity	AHPV S/CO	AHPV HPV positivity	FINAL AHPV HPVpositivity	HC2 RLU/CO
468/10L	4.12	1	0	0	0.33	0	0	0
575/10L	0	0	11.04	0	0	0	0	6.89
586/10L	10.2	1	0	0	0	0	0	2.94
601/10L	10.2	1	0	0	0	0	0	1.63
609/10L	0	0	0.5	0	11.2	1	0	2.83

Overall agreement of HC2 and Aptima HPV test was 88%, good to excellent (Kappa= 0.5652, 95%CI=0.27-0.86). Positive agreement was 86.7% and negative agreement 100% (Table 3).

Table 3. Agreement between Aptima HPV and HC2 Assay.

Test		HC2		
		Positive	Negative	Total
Aptima HPV	Positive	39	0	39
	Negative	6	5	11
	Total	45	5	50

There were only 6 discrepant samples. All of them were positive by HC2 but Aptima HPV negative and all of them contained HPV types which are in the range of Aptima HPV detection. Except for one, all of these samples contained multiple types. Three of these 6 discrepant samples were those which came out with discrepant results on repeated runs of Aptima HPV test. In four of them normal findings or mild dysplasia on histology was detected, two patients had mild to moderate dysplasia finding.

Table 4a. Analyses of samples positive on HC2 and negative on Aptima HPV.

ID	HC2 (RLU/CO)	Aptima HPV	LA HPV type	Histology
475/10L	19.8	0	31, 52	DI-II (CIN2)
549/10L	3.31	0	16, 66	normal
575/10L	6.89	0 **	16, 31, 51, 54, 68	DI (CIN1)
586/10L	2.94	0	6, 51	DI (CIN1)
601/10L	1.63	0 **	16, 68	DI-II (CIN2)
609/10L	2.83	0 **	16	DI (CIN1)

** see Table 2

Table 4b. Analyses of samples positive on HC2 and negative on Aptima HPV.

			HPV by LA test		
	Histology	**N**	**negative**	**LR HPV**	**HR HPV**
HC2+/AptimaHPV-	Negative	1			1
	CIN1	3			3
	CIN2	2			2
	CIN3	0			
	CA	0			

The comparison of the prevalence of HR HPV as assessed by HC2 and Aptima HPV in patients according to their cytological, colposcopical and histological findings is summarized in Tables 5, 6 and 7 and Figures 1, 2 and 3.

Table 5. The comparison of the prevalence of HR HPV as assessed by HC2 and Aptima HPV in patients according to their cytological findings.

Cytology	**Number of patients**	**Aptima HPV HPV positive**	**HC2 HPV positive**
		N (%)	**N (%)**
Normal	**5**	**1 (20.0)**	**2 (40.0)**
ASC-US/AGC-NOS	**7**	**5 (71.4)**	**7 (100.0)**
LSIL	**25**	**21 (84.0)**	**23 (92.0)**
HSIL/AGC-NEO/ASC-H	**13**	**12 (92.3)**	**13 (100.0)**
Total	**50**	**39 (78.0)**	**45 (90.0)**

Figure 1. The comparison of the prevalence of HR HPV as assessed by HC2 and Aptima HPV in patients according to their cytological findings.

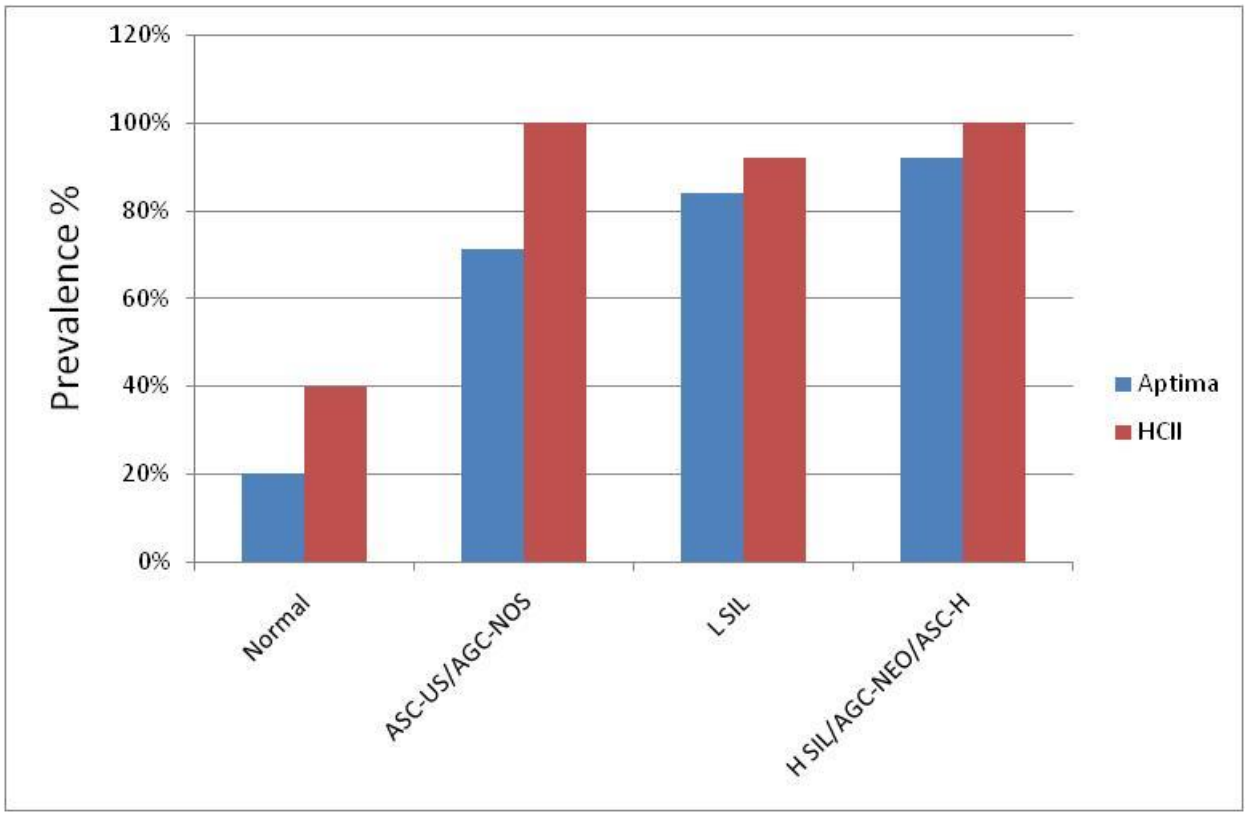
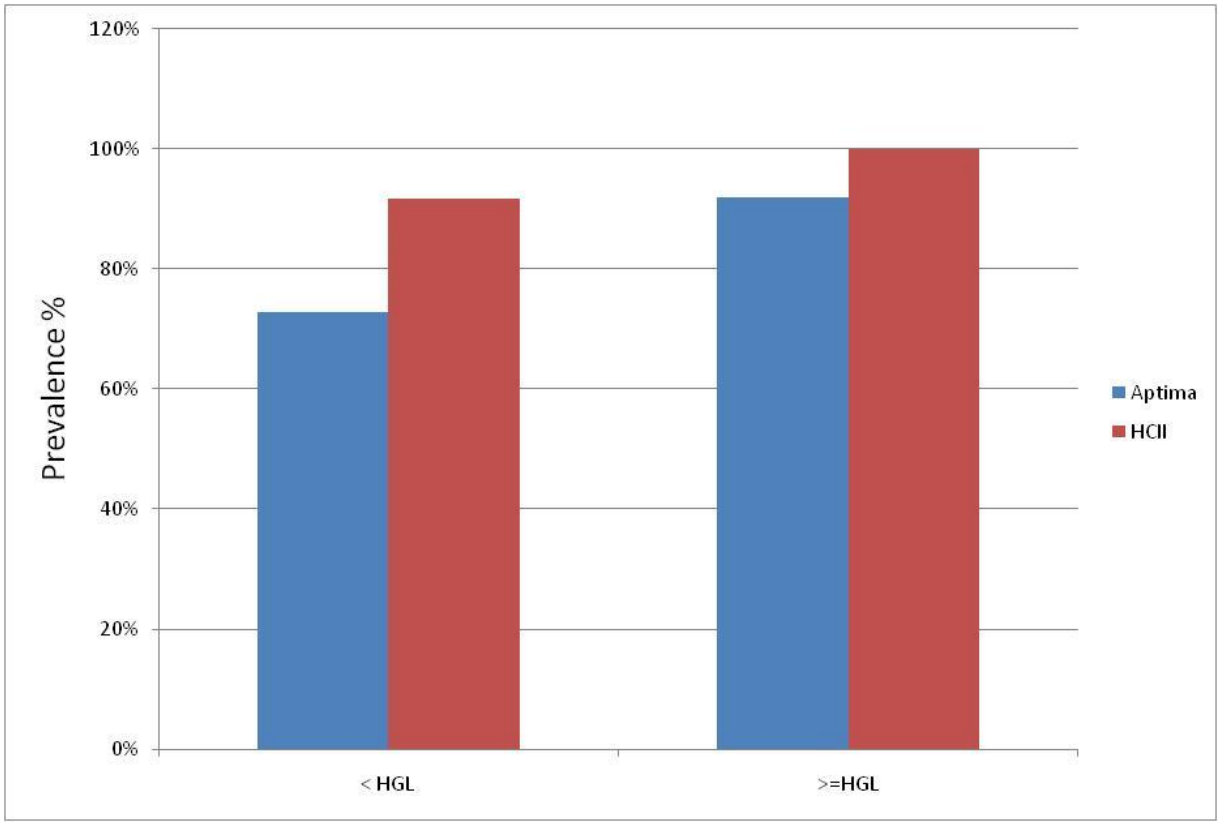


Table 6. The comparison of the prevalence of HR HPV as assessed by HC2 and Aptima HPV in patients according to their colposcopical findings.

Colposcopy	Number of patients	Aptima HPV HPV positive	HC2 HPV positive
		N (%)	N (%)
ATZ	**1**	**1 (100.0)**	**1 (100.0)**
LGL	**37**	**27 (73.0)**	**32 (86.5)**
HGL	**12**	**11 (91.7)**	**12 (100.0)**
Total	**50**	**39 (78.0)**	**45 (90.0)**

Figure 2. The comparison of the prevalence of HR HPV as assessed by HC2 and Aptima HPV in patients according to their colposcopical findings.



<HGL includes ATZ and LGL findings

Table 7. The comparison of the prevalence of HR HPV as assessed by HC2 and Aptima HPV in patients according to their histological findings.

Histology	Number of patients	Aptima HPV HPV positive	HC2 HPV positive
		N (%)	N (%)
≤DI	11	3 (27.3)	7 (63.6)
DII	20	17 (85.0)	19 (95.0)
>DII	19	19 (100.0)	19 (100.0)
Total	50	39 (78.0)	45 (90.0)

Figure 3. The comparison of the prevalence of HR HPV as assessed by HC2 and Aptima HPV in patients according to their histological findings.

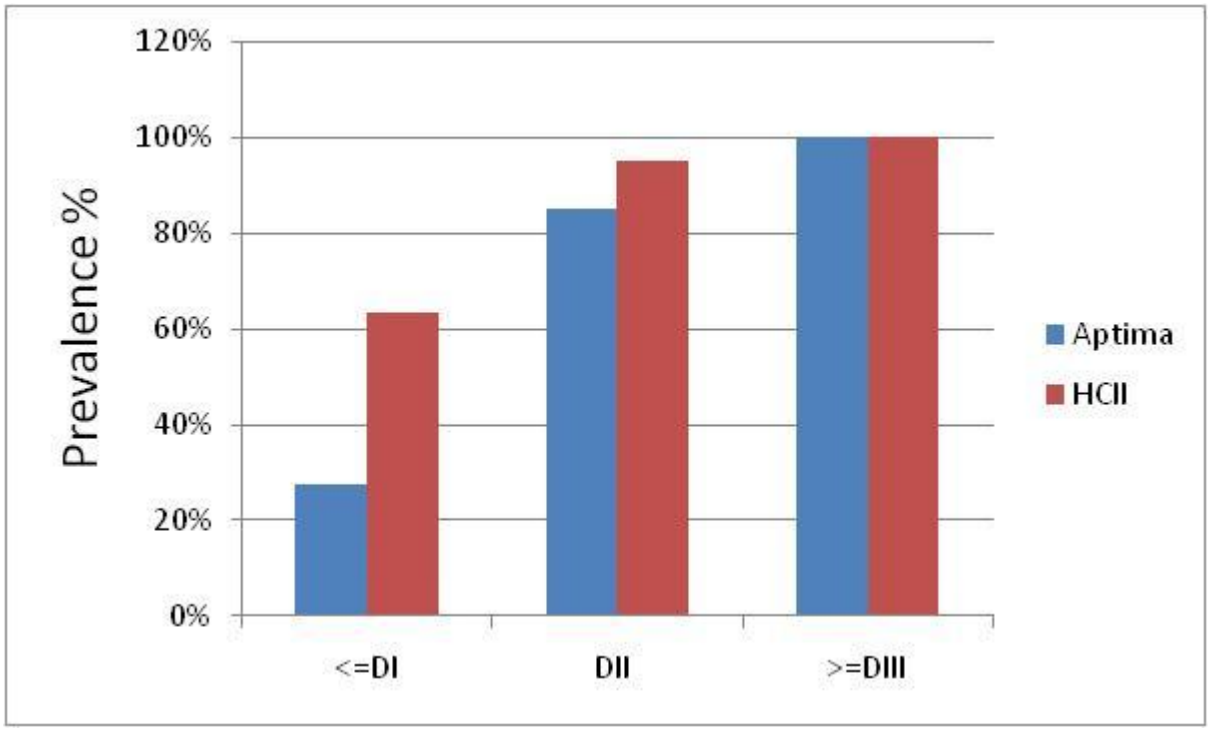


Table 8. Sensitivity and specificity, PPV and NPV of cytology, HC2 test and Aptima HPV test for detection of patients with CIN2+ and CIN3+*.

Clinical end point	Test	Sensitivity (95%CI)	Specificity (95%CI)	PPV (95%CI)	NPV (95%CI)
CIN2+	Cytology (ASC-US+)	97% (86.5-100.0)	36% (11.0-69.2)	84% (71.0-93.5)	80% (28.4-99.5)
	HC2	97% (87.0-100.0)	36% (11.0-69.0)	84% (71.0-93.5)	80% (28.4-100.0)
	Aptima HPV	92% (79.0-98.0)	73% (39.0-94.0)	92% (79.0-98.4)	73% (39.1-94.0)
CIN3+	Cytology (ASC-US+)	95% (74.0-99.9)	13% (3.4-30.0)	40% (25.7-55.7)	80% (28.4-99.5)
	HC2	100% (82.4-100.0)	16% (5.5-33.7)	42% (28.0-57.9)	100% (48.0-100.0)
	Aptima HPV	100% (82.4-100.0)	36% (32.4-65.3)	49% (32.4-65.3)	100% (71.5-100.0)

*CIN2+ includes patients with normal findings, mild to moderate dysplasia (DI, DI-DII, DII)

CIN3+ includes women with severe dysplasia (DII-III, DIII) and invasive cervical carcinoma

Most of the patients from this study underwent treatment with loop excision and the histology of the resected tissue will be available for final evaluation of data.

Conclusions

In conclusion Aptima HPV test is a robust test which is quite demanding in terms of laboratory space. It is necessary that the laboratory has an experience with molecular biological procedures. Even though the volume of samples tested in this study was limited, histological analysis was available for all subjects and therefore the evaluation of the clinical sensitivity and specificity for the detection of CIN2+ and CIN3+ was possible, as well as correlation with the cytological and colposcopical findings. Furthermore, the data were compared to results of HC2 and the type specificity has been confirmed by the Linear array test. The performance characteristics of the test in this study were very good, comparable or better than characteristics of the test currently used in the recommendation as a "gold standard" for the use in primary screening for cervical cancer prevention (Meijer et al., 2009) and cytology widely used as a test of choice for primary screening for cervical cancer in the Czech Republic. The sensitivity of Aptima HPV for CIN3+ was the same as for HC2 and higher than for cytology, specificity of Aptima HPV was higher than for HC2 and cytology as well. For CIN2+ the sensitivity of Aptima HPV was much lower but specificity much higher than for HC2 and cytology as well. Relatively high number of discrepant samples between runs points to the necessity of specification of a "grey zone" for this assay.