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Evaluation of the New York State Human Papilloma Virus (HPV) Proficiency Test

April 2014[1]

Dear Laboratory Director:

This is the summary and evaluation of the New York State Proficiency Test for human papilloma virus (HPV) from April 2014. A report with your laboratory's score and grade will be sent separately to you by regular mail. Five vials (HPV076 – HPV080) containing cervical cells derived from actual patients in PreservCyt® medium were sent out to every permitted laboratory on April 15th, 2014, and the due date for submitting the test results was May 5th, 2014. Each correct answer received 20 points, and an incorrect one zero points. The passing threshold was set at 80 points (80 percent) for the entire test event. Answers could be provided in three categories, Positive (Pos), Negative (Neg), or Low Positive (LoPos) for high-risk HPV screening. Laboratories that perform genotyping were also asked to provide those results. In addition, we asked that you include the raw data with your submitted results, i.e. RLU/CO values from Hybrid Capture®, FOZ values from Cervista®, Ct values from the Roche Cobas®4800 method, or S/CO ratios from the Aptima® methodology, though this information was not used for grading.

A total of 75 laboratories received samples, and 72 submitted valid answers by the due date. For screening, 23 laboratories (32%) used the Hybrid Capture® method, 16 laboratories (22%) used the Cervista® method, 16 laboratories (22%) used a polymerase chain reaction based method (13 Cobas®4800 and 3 a Laboratory Developed Tests) and 17 laboratories (14%) used the Aptima® method (12 laboratories used the Tigris instrument and 5 laboratories used the Panther System).

Cytology smears were prepared and evaluated in-house from each of the test samples. Samples HPV076, HPV077 and HPV080 were negative and in agreement with the HPV consensus results. Samples HPV077 and HPV080 were diagnosed as "Satisfactory", "Negative" for intraepithelial lesion (NILM); however, Sample HPV076 did contain some cells showing reactive cellular changes, and therefore was signed out as "Satisfactory", "Negative" for intraepithelial lesion (NILM) with Reactive changes". Samples HPV078 and HPV079 presented with abnormal cells with clear evidence of koilocytosis and diagnosed as "Satisfactory for evaluation" with "LGSIL (Low-grade squamous intraepithelial lesion) consistent with HPV infection", which correlated with the positive proficiency test results for those samples. Smears from Samples HPV078 and HPV080 also contained hyphae indicative of the fungus Candida albicans and this finding was included in the report along with the diagnosis.

[1]The use of brand and/or trade names in this report does not constitute an endorsement of the products on the part of the Wadsworth Center or the New York State Department of Health.

Results

Consensus results from all laboratories and across all methods were excellent at 99% (357/360) with only three incorrect sample responses. All laboratories achieved a unanimous result (72/72) for negative samples HPV077and HPV080 and positive sample HPV078. The consensus negative sample HPV076 received two discrepant positive answers (2/72), one each with Cervista® and Roche Cobas®4800, respectively. The results for the consensus positive Sample HPV079 showed one single negative response (1/72) from a PCR assay instead of the consensus positive result. The laboratories that reported results that do not match the consensus, irrespective of the method used, should re-examine their results. A limited number of samples are available for retest upon request.

Table 1. Screening results, 72 laboratories, and 72 results submitted:

	HPV076	HPV077	HPV078	HPV079	HPV080
All methods					
Total	72	72	72	72	72
Negative	70	72	0	1	72
Positive	2	0	72	71	0
Low Positive	0	0	0	0	0
Indeterminate	0	0	0	0	0
% Negative	97.2%	100.0%	0.0%	1.4%	100.0%
% Positive	2.8%	0.0%	100.0%	98.6%	0.0%
% Low Positive	0.0%	0.0%	0.0%	0.0%	0.0%
% Indeterminate	0.0%	0.0%	0.0%	0.0%	0.0%
Consensus	**NEG**	**NEG**	**POS**	**POS**	**NEG**

	HPV076	HPV077	HPV078	HPV079	HPV080
Hybrid Capture®					
Total	23	23	23	23	23
Negative	23	23	0	0	23
Positive	0	0	23	23	0
Low Positive	0	0	0	0	0
Indeterminate	0	0	0	0	0
% Negative	100.0%	100.0%	0.0%	0.0%	100.0%
% Positive	0.0%	0.0%	100.0%	100.0%	0.0%
% Low Positive	0.0%	0.0%	0.0%	0.0%	0.0%
% Indeterminate	0.0%	0.0%	0.0%	0.0%	0.0%
Consensus	**NEG**	**NEG**	**POS**	**POS**	**NEG**

	HPV076	HPV077	HPV078	HPV079	HPV080
Cervista®					
Total	16	16	16	16	16
Negative	15	16	0	0	16
Positive	1	0	16	16	0
% Negative	93.8%	100.0%	0.0%	0.0%	100.0%
% Positive	6.3%	0.0%	100.0%	100.0%	0.0%
Consensus	**NEG**	**NEG**	**POS**	**POS**	**NEG**

Table 1 continued:

	HPV076	HPV077	HPV078	HPV079	HPV080
Cobas® 4800					
Total	13	13	13	13	13
Negative	12	13	0	0	13
Positive	1	0	13	13	0
% Negative	92.3%	100.0%	0.0%	0.0%	100.0%
% Positive	7.7%	0.0%	100.0%	100.0%	0.0%
Consensus	**NEG**	**NEG**	**POS**	**POS**	**NEG**

	HPV076	HPV077	HPV078	HPV079	HPV080
PCR					
Total	3	3	3	3	3
Negative	3	3	0	1	3
Positive	0	0	3	2	0
% Negative	100.0%	100.0%	0.0%	33.3%	100.0%
% Positive	0.0%	0.0%	100.0%	66.7%	0.0%
Consensus	**NEG**	**NEG**	**POS**	**POS***	**NEG**

	HPV076	HPV077	HPV078	HPV079	HPV080
Aptima®					
Total	17	17	17	17	17
Negative	17	17	0	0	17
Positive	0	0	17	17	0
% Negative	100.0%	100.0%	0.0%%	0.0%	100.0%
% Positive	0.0%	0.0%	100.0%	100.0%	0.0%
Consensus	**NEG**	**NEG**	**POS**	**POS**	**NEG**

*Based on all laboratory consensus

Genotyping

Laboratories that routinely determine HPV genotypes were also asked to submit those results. Thirty-eight (53%) laboratories did genotyping using various methodologies. Of those, thirteen (34%) laboratories used the Cobas® 4800 method, eleven laboratories (29%) each used the Aptima® and Cervista®16/18 method, respectively, and three laboratories (8%) used a laboratory developed PCR based method, which one laboratory followed with RFLP and one laboratory followed with Bio-Plex Analysis. Since not every method detects every genotype and because the samples represent a mixture of patient samples, the genotyping results were not graded (Table 2).

Genotyping results for consensus screen positive samples HPV078 and HPV079 showed that most of the laboratories were in agreement that both the high-risk HPV genotypes 16 and 18 along with other high-risk genotypes were present in these samples. For consensus negative sample HPV076, two laboratories submitted a high-risk genotype “NOT 16, 18” response with one laboratory using the Cervista®16/18 method and the other laboratory using the Roche Cobas®4800 method which is consistent with their positive screening result, but clearly against the consensus. One laboratory, however, using the Aptima® method reported a genotype for all three screen negative samples as “Pos, not identified”, even though they did not report these samples as positive by screening. The question arises whether this does not represent a data entry mistake. This laboratory should check their results again. As usual, the laboratories that use a Laboratory Developed Test by PCR were able to identify multiple genotypes. The results are summarized in Table 3.

Table 2. Genotyping results, 38 laboratories:

Method	HPV076	HPV077	HPV078	HPV079	HPV080
Aptima®	N/A	N/A	18/45	16,18/45	N/A
Aptima®	N/A	N/A	16,18	16,18	N/A
Aptima®	N/A	N/A	16,18/45	16,18/45	N/A
Aptima®	N/A	N/A	16,18	16,18	N/A
Aptima®	N/A	N/A	16,18	16,18	N/A
Aptima®	N/A	N/A	16,18PLUSHR	16,18PLUSHR	N/A
Aptima®	N/A	N/A	16,18	16,18	N/A
Aptima®	N/A	N/A	16,18	16,18	N/A
Aptima®	POSNOTID	POSNOTID	16,18	16,18	POSNOTID
Aptima®	N/A	N/A	16,18PLUSHR	16,18PLUSHR	N/A
Aptima®	N/A	N/A	16,18PLUSHR	16,18PLUSHR	N/A
Cobas® 4800	N/A	N/A	16,18	16,18	N/A
Cobas® 4800	N/A	N/A	16,18	16,18	N/A
Cobas® 4800	N/A	N/A	16,18PLUSHR	16,18PLUSHR	N/A
Cobas® 4800	N/A	N/A	16,18PLUSHR	16,18PLUSHR	N/A
Cobas® 4800	N/A	N/A	16,18PLUSHR	16,18PLUSHR	N/A
Cobas® 4800	N/A	N/A	16,18	16,18	N/A
Cobas® 4800	N/A	N/A	16,18PLUSHR	16,18PLUSHR	N/A
Cobas® 4800	N/A	N/A	16,18PLUSHR	16,18PLUSHR	N/A
Cobas® 4800	N/A	N/A	16,18PLUSHR	16,18PLUSHR	N/A
Cobas® 4800	N/A	N/A	16,18PLUSHR	16,18PLUSHR	N/A
Cobas® 4800	N/A	N/A	16,18PLUSHR	16,18PLUSHR	N/A
Cobas® 4800	N/A	N/A	16,18PLUSHR	16,18PLUSHR	N/A
Cobas® 4800	HRNOT16,18	N/A	16,18	16,18	N/A
Cervista®16/18	HRNOT16,18	N/A	16,18	16,18	N/A
Cervista®16/18	N/A	N/A	16,18	16,18	N/A
Cervista®16/18	N/A	N/A	16,18	16,18	N/A
Cervista®16/18	N/A	N/A	16,18	16,18	N/A
Cervista®16/18	N/A	N/A	16,18	16,18	N/A
Cervista®16/18	N/A	N/A	16,18	16,18	N/A
Cervista®16/18	N/A	N/A	16,18	16,18	N/A
Cervista®16/18	N/A	N/A	16,18	16,18	N/A
Cervista®16/18	N/A	N/A	16,18	16,18	N/A
Cervista®16/18	N/A	N/A	16,18	16,18	N/A
Cervista®16/18	N/A	N/A	16,18	16,18	N/A
Bio-Plex Analysis	N/A	N/A	16,18,39 51,56	16,18	N/A
PCR	N/A	N/A	16,18,31,39,51,56	16,18 31 39,51,56,59,68	N/A
RFLP	61	N/A	6,16,61	53,81	N/A

INV = Cervista®, PCR = polymerase chain reaction, polymorphism determination, N/A = not applicable, RFLP = PCR followed by restriction fragment length polymorphism determination

Table 3. Summary of genotyping results:

	HPV076	HPV077	HPV078	HPV079	HPV080
Genotyping results					
POSNOTID	1	1	0	0	1
16, 18	0	0	21	22	0
HRNOT 16, 18	2	0	0	0	0
16, 18/45	0	0	1	2	0
18/45	0	0	1	0	0
16, 18 PLUSHR	0	0	14	13	0
Other	1	0	1	1	0
N/A	34	37	0	0	37
Total	**38**	**38**	**38**	**38**	**38**

HR- High Risk, LR-Low Risk, N/A = not applicable

Raw data

Figure 1 shows the graphical distribution of the raw data from the different instruments.

Conclusions

A high overall consensus of >= 97.2% was achieved among the laboratories in this proficiency test and the results were consistent with the cytologic features of the samples. The results for three of the five samples were in unanimous agreement across all methods. Consensus negative sample HPV076 produced two positive discrepant answers, one by the Cervista® method and the other by the Roche Cobas® 4800 method, while consensus positive sample HPV079 produced one discrepant negative answer by a LDT PCR method. These results indicate that there is good concordance across screening methods when the major high risk genotypes are present in the samples.

Finally an important reminder regarding the data submission process: Be sure your results are submitted. If results are saved but **not submitted**, they will be graded as an administrative **fail** and put your lab at risk for an unsuccessful performance.

Tentative schedule for the next 2014 New York State HPV proficiency test:

Mail-out Date	**Due Date**
October 21	November 10

For questions, comments or suggestions regarding this PT event please call or e-mail:

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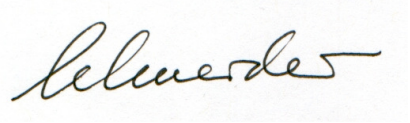

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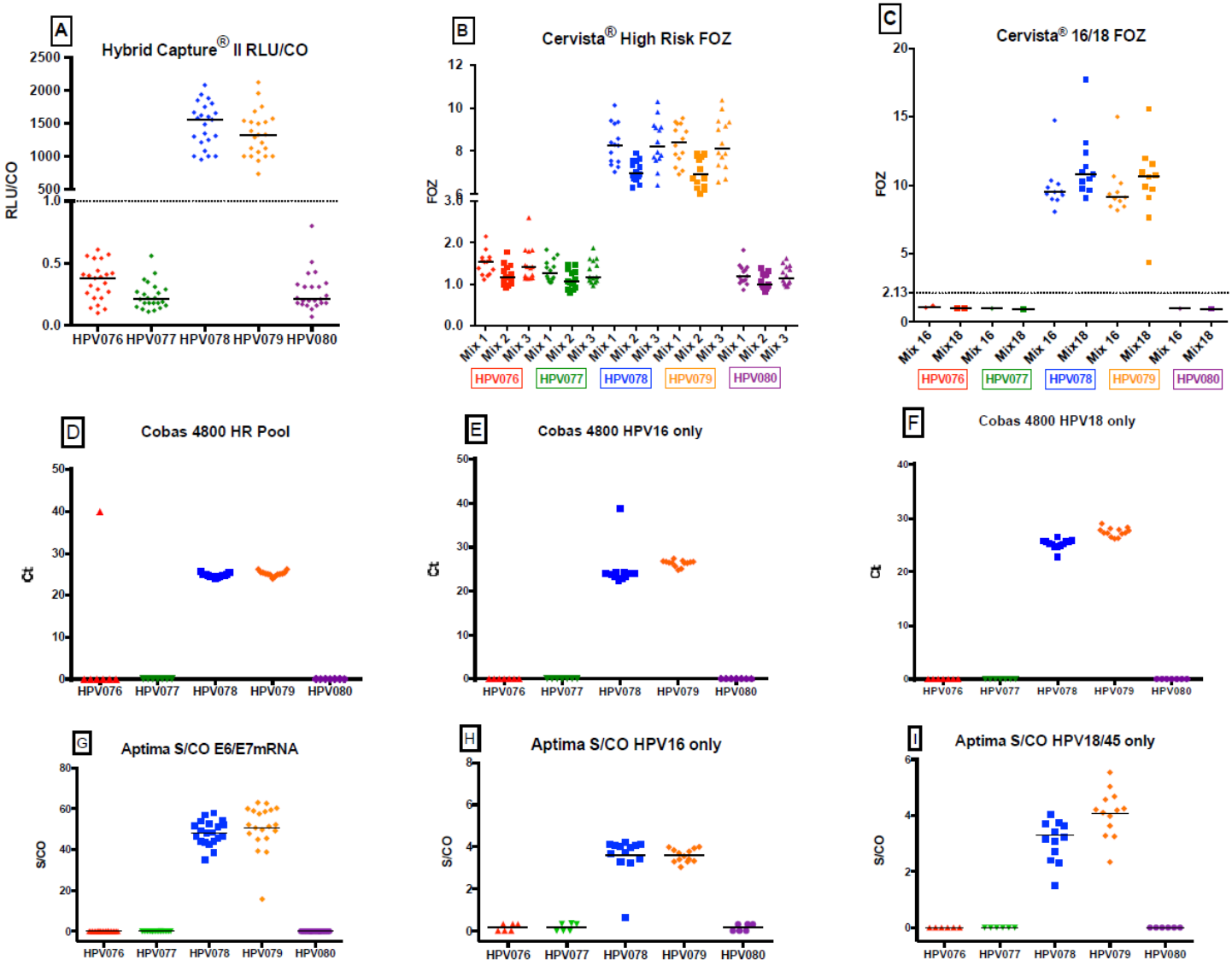
Figure 1


A
Hybrid Capture® II RLU/CO
RLU/CO
HPV076 HPV077 HPV078 HPV079 HPV080
B
Cervista® High Risk FOZ
FOZ
Mix 1 Mix 2 Mix 3
HPV076 HPV077 HPV078 HPV079 HPV080
C
Cervista® 16/18 FOZ
FOZ
Mix 16 Mix18
HPV076 HPV077 HPV078 HPV079 HPV080
D
Cobas 4800 HR Pool
Ct
HPV076 HPV077 HPV078 HPV079 HPV080
E
Cobas 4800 HPV16 only
Ct
HPV076 HPV077 HPV078 HPV079 HPV080
F
Cobas 4800 HPV18 only
Ct
HPV076 HPV077 HPV078 HPV079 HPV080
G
Aptima S/CO E6/E7mRNA
S/CO
HPV076 HPV077 HPV078 HPV079 HPV080
H
Aptima S/CO HPV16 only
S/CO
HPV076 HPV077 HPV078 HPV079 HPV080
I
Aptima S/CO HPV18/45 only
S/CO
HPV076 HPV077 HPV078 HPV079 HPV080