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HEALTH

Howard A. Zucker, M.D., J.D.
Acting Commissioner of Health

Sally Dreslin, M.S., R.N.
Executive Deputy Commissioner

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

October 2014[1]

Dear Laboratory Director:

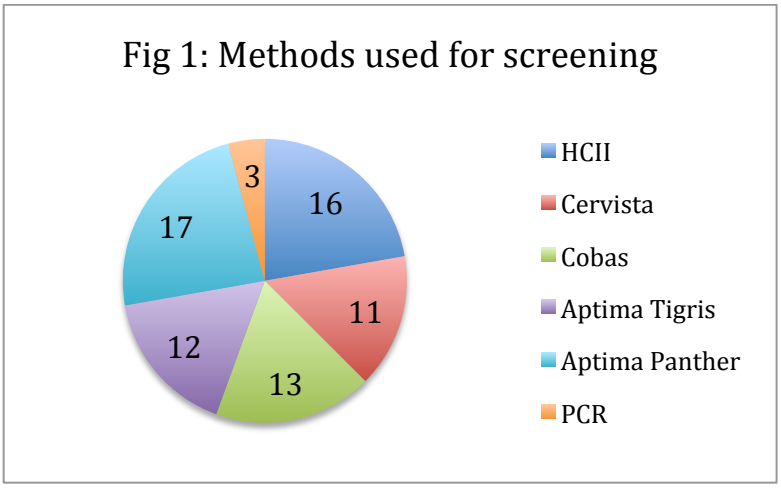
Here is the summary and evaluation of the New York State Proficiency Test for human papilloma virus (HPV) from October 2014. A report with your laboratory's score and grade will be sent separately to you by regular mail. Five vials (HPV081 – HPV085) containing cervical cells derived from actual patients in PreservCyt® medium were sent out to every permitted laboratory on October 21st, 2014, and the due date for submitting the test results was November 10th, 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.

Thin prep slides were prepared and evaluated in our laboratory from each of the test samples. Samples HPV082 and HPV085 were diagnosed as "Satisfactory", "Negative" for intraepithelial lesion (NILM); however, sample HPV085 did contain some cells showing reactive cellular changes and therefore was signed out as "Satisfactory", "Negative for intraepithelial lesion (NILM) with reactive changes". Sample HPV081 was evaluated as "Satisfactory" with "Atypical cells of undetermined significance" (ASCUS), and finally, samples HPV083 and HPV084 presented with abnormal cells with clear evidence of koilocytosis and were both diagnosed as "Satisfactory for evaluation" with "LGSIL (Low-grade squamous intraepithelial lesion) consistent with HPV infection". These diagnoses were consistent with the HPV proficiency test results for those samples (see below).

Results (Tables 1a, b)

A total of 77 laboratories received samples, and 72 submitted valid answers by the due date. For screening, 16 laboratories (22.2%) used the Hybrid Capture® method, 11 laboratories (15.3%) used the Cervista® method, 16 laboratories (22.2%) used a polymerase chain reaction based method (13 Cobas®4800 and 3 a Laboratory Developed Test) and 29 laboratories (40.3%) used the Aptima® method (17 laboratories used the Tigris instrument and 12 laboratories used the Panther instrument) (Fig 1).

[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.



The results from this HPV proficiency testing event produced an overall consensus from all laboratories across all methods of 97.5% (351/360). All laboratories unanimously reported samples HPV083 and HPV084 as positive. The results for consensus positive HPV081 showed one single discrepant negative response (1/72) from a laboratory-developed PCR assay. For consensus negative sample HPV082 six discrepant positive answers were submitted (6/72), two from Roche Cobas®4800 assays (2/13), and four from Cervista® assays (4/11), which resulted in a non-consensus result for this method for this sample. No genotyping results that could have corroborated the screening results were submitted from three of these laboratories, but remarkably, one laboratory did submit a N/A response as their genotyping answer for this sample, indicating that it considered this sample negative and thus not amenable to genotyping, in contradiction to the laboratory's reported screening result. This laboratory should verify its data entry. Finally, consensus negative sample HPV085 had two discrepant positive answers (2/72), one from a Hybrid Capture® assay and the other from an Aptima® Panther assay. In this case, however, both labs also detected the high-risk genotypes HPV16, 18 and/or 45 in this sample, thus internally corroborating their screening result. The exact reasons for these discrepancies are difficult to establish, however, the possibility of low levels of cells with high-risk genotypes present in these samples could explain the random positive results.

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 1a: Screening results, all methods combined (72 laboratories)

	HPV081	HPV082	HPV083	HPV084	HPV085
All methods					
Total	72	72	72	72	72
Negative	1	66	0	0	70
Positive	71	6	72	72	2
Low Positive	0	0	0	0	0
Indeterminate	0	0	0	0	0
% Negative	1.4%	91.7%	0.0%	0.0%	97.2%
% Positive	98.6%	8.3%	100.0%	100.0%	2.8%
% Low Positive	0.0%	0.0%	0.0%	0.0%	0.0%
% Indeterminate	0.0%	0.0%	0.0%	0.0%	0.0%
Consensus	**POS**	**NEG**	**POS**	**POS**	**NEG**

Table 1b: Screening results, by method

	HPV081	HPV082	HPV083	HPV084	HPV085
Hybrid Capture®					
Total	16	16	16	16	16
Negative	0	16	0	0	15
Positive	16	0	16	16	1
Low Positive	0	0	0	0	0
Indeterminate	0	0	0	0	0
% Negative	0.0%	100.0%	0.0%	0.0%	93.8%
% Positive	100.0%	0.0%	100.0%	100.0%	6.3%
% Low Positive	0.0%	0.0%	0.0%	0.0%	0.0%
% Indeterminate	0.0%	0.0%	0.0%	0.0%	0.0%
Consensus	**POS**	**NEG**	**POS**	**POS**	**NEG**

	HPV081	HPV082	HPV083	HPV084	HPV085
Cervista®					
Total	11	11	11	11	11
Negative	0	7	0	0	11
Positive	11	4	11	11	0
% Negative	0.0%	63.6%	0.0%	0.0%	100.0%
% Positive	100.0%	36.4%	100.0%	100.0%	0.0%
Consensus	**POS**	**NoCons**	**POS**	**POS**	**NEG**

	HPV081	HPV082	HPV083	HPV084	HPV085
Cobas® 4800					
Total	13	13	13	13	13
Negative	0	11	0	0	13
Positive	13	2	13	13	0
% Negative	0.0%	84.6%	0.0%	0.0%	100.0%
% Positive	100.0%	15.4%	100.0%	100.0%	0.0%
Consensus	**POS**	**NEG**	**POS**	**POS**	**NEG**

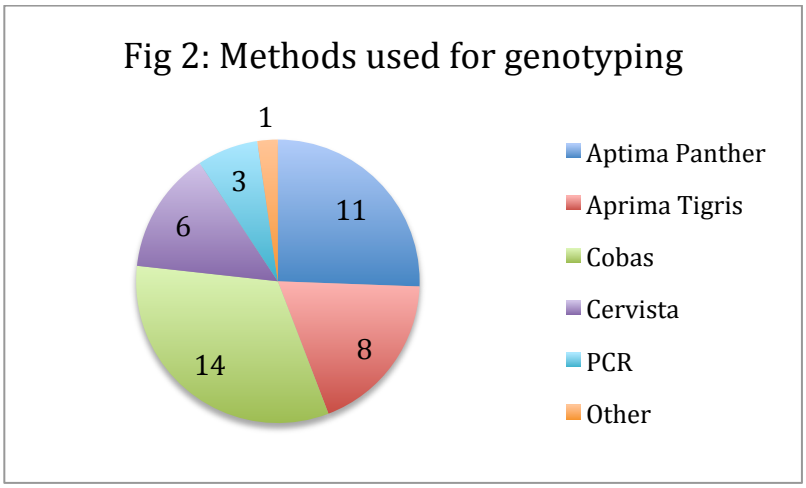
	HPV081	HPV082	HPV083	HPV084	HPV085
PCR (LDT)					
Total	3	3	3	3	3
Negative	1	3	0	0	3
Positive	2	0	3	3	0
% Negative	33.3%	100.0%	0.0%	0.0%	100.0%
% Positive	66.7%	0.0%	100.0%	100.0%	0.0%
Consensus	**POS***	**NEG**	**POS**	**POS**	**NEG**

	HPV081	HPV082	HPV083	HPV084	HPV085
Aptima®					
Total	29	29	29	29	29
Negative	0	29	0	0	28
Positive	29	0	29	29	1
% Negative	0.0%	100.0%	0.0%%	0.0%	96.6%
% Positive	100.0%	0.0%	100.0%	100.0%	3.4%
Consensus	**POS**	**NEG**	**POS**	**POS**	**NEG**

*Based on all laboratory consensus

Genotyping (Tables 2a, b)

Laboratories that routinely determine HPV genotypes were also asked to submit those results. Forty-three (60%) laboratories did genotyping using various methodologies. Of those, nineteen (44%) laboratories used the Aptima® method, fourteen laboratories (33%) used the Roche Cobas®4800 method, six laboratories (4%) used the Cervista®16/18 method, three laboratories (7%) used a laboratory-developed PCR based method, which one laboratory followed with RFLP and another with Bio-Plex analysis, and one laboratory (2%) performed a not further defined laboratory-developed test (Fig 2). However, since not every method equally detects and/or discriminates every genotype and because the samples represent mixtures of patient samples, the genotyping results were not graded.



For samples HPV081 and HPV083, respectively, the results from the laboratories using the Aptima® method were consistent with 18/19 reporting only HPV high-risk genotype 16 for sample HPV081, and 18/19 reporting a combination of HPV high-risk genotypes 16 and 18/45 for sample HPV083. In contrast, the results for sample HPV084 were somewhat variable, with 7/19 reporting a mixture of genotypes 16 and 18/45, whereas 12/19 only detected HPV 16. Results from the Roche Cobas®4800 method indicated that all three screen positive samples HPV081, HPV083 and HPV084 contained a mixture of HPV 16 and 18 and possibly other high risk genotypes. While these findings are consistent with those from Aptima for samples HPV083 and HPV084, there appears to be a difference between the two methods for HPV081. Whereas all labs using the Cobas 4800 instrument detected at least a mixture of HPV 16 and 18, all but one lab using the Aptima method only detected HPV 16 in this sample. While the number of laboratories using Cervista for genotyping is small, a majority of those only found HPV 16 in sample HPV081 and HPV084, and a mixture of HPV 16 and 18 in sample HPV083. In conclusion, there was near unanimous consensus that sample HPV083 contained a mixture of at least genotypes HPV 16 and 18. In contrast, for samples HPV081 and HPV084 there was a nearly even split between laboratories detecting only genotype HPV 16 and those detecting a mixture of genotypes. The exact reason for these discrepancies are unclear, but may possibly be caused by different sensitivities for the various genotypes between different manufacturers' methods. Furthermore, there appears to have been some confusion on how to enter the genotyping results, which may have contributed to some of the variation in the reported genotypes. While the cover letter contained the appropriate instructions we will try to make the data entry more self-explanatory for the next event and would welcome any suggestions you may have on this.

Table 2a. Genotyping results, all methods combined (43 laboratories)

	HPV081	HPV082	HPV083	HPV084	HPV085
Screening results	Pos	Neg	Pos	Pos	Neg
Genotypes					
16	22	1	2	16	
16, 18	6		11	8	
16, 18/45	1		18	7	
18/45					
16,18,45	1		2	1	1
OTHER	1			1	
16,OTHER	1		1	3	
16,18,OTHER	9		9	6	
16,18,45,OTHER	0			1	
N/A	2	42			42
Total	**43**	**43**	**43**	**43**	**43**

OTHER= other high-risk genotypes, not identified, N/A = not applicable because screening result was negative

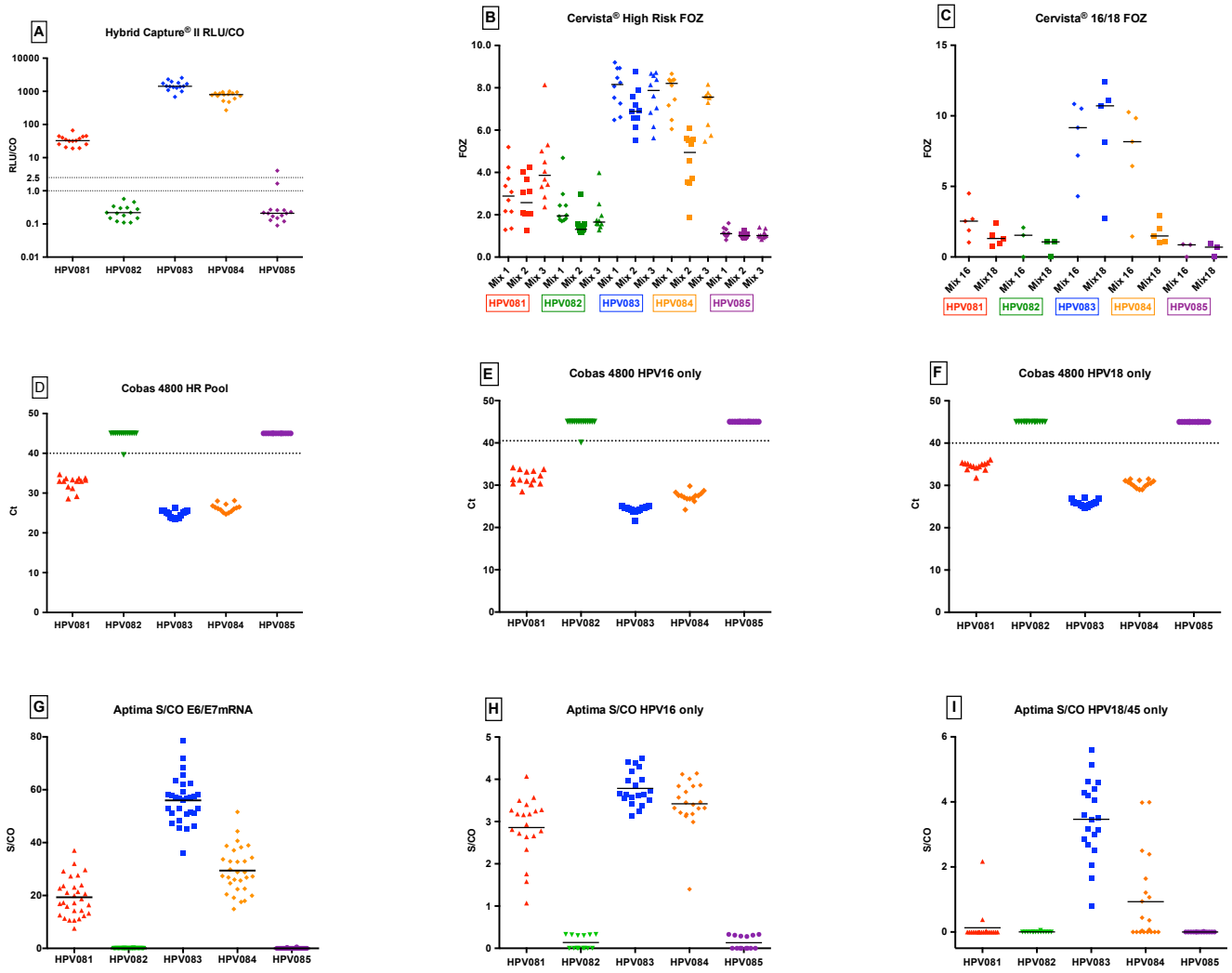
Table 2b. Genotyping results, by method

Method	Genotypes	HPV081	HPV082	HPV083	HPV084	HPV085
Aptima®	16	18		1	12	
Aptima®	16, 18/45	1		18	7	1
Cobas® 4800	16, 18	5		6	6	
Cobas® 4800	16, 18, other	9		8	7	
Cobas® 4800	16		1			
Cobas® 4800	16, other				1	
Cervista®	16	3		1	4	
Cervista®	16, 18	1		4	1	
Cervista®	Other	1			1	
Cervista®	16, 18, 45			1		
PCR		16,31,51,39		16,18,31,39,51,56,59,68	16,31,51,56,58	
PCR/Bio-Plex		16		16,18	16,18	
PCR/RFLP				16,53,61	16,70	
LABDEVT		16,18,45		16,18,45	16,18,45	16,18 45

OTHER= other high-risk genotypes, not identified, N/A = not applicable because screening result was negative
PCR/Bio-Plex = PCR followed by Bio-Plex analysis
PCR/RFLP = PCR followed by restriction fragment length polymorphism determination

Raw data

Figure 3 shows the graphical distribution of the raw data from the different instruments. For Roche Cobas4800 “negative” values were arbitrarily set at a Ct of 45.



Conclusions

In general, the results of this HPV DNA proficiency testing event were satisfactory and consistent with the cytologic features of the samples. Consensus positive samples HPV083 and HPV084 were unanimously reported as positive and sample HPV081 reported only one discrepant negative response. Consensus negative samples HPV082 and HPV085 had a total of eight discrepant positive answers, which were corroborated in three laboratories by their genotyping results that showed high-risk genotypes present in these samples, possibly because they contained virus titers around the limit of detection. This raises the question whether subtle differences in the cut-point settings exist.

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 2015 New York State HPV proficiency tests:

Mail-out Date	**Due Date**
April 7	April 27
October 13	November 2

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

Erasmus Schneider, 518-473-4856, schneid@wadsworth.org
Halyna Logan, 518-473-0203, halyna.logan@health.ny.gov
Helen Ling, 518-474-0036, helen.ling@health.ny.gov

Erasmus Schneider, Ph.D.
Director, Oncology Section
Clinical Laboratory Evaluation Program
Wadsworth Center
Empire State Plaza
Albany, NY 12201-0509