NEW YORK
state department of
HEALTH

Nirav R. Shah, M.D., M.P.H.
Commissioner

Sue Kelly
Executive Deputy Commissioner

May 31, 2013

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

April 2013[1]

Dear Laboratory Director:

This is the summary and evaluation of the graded New York State Proficiency Test for human papilloma virus (HPV) determination from April 2013. A report with your laboratory's score and grade will be sent separately to you by regular mail. Five vials (HPV066 – HPV070) containing cervical cells derived from actual patients in PreservCyt® medium were sent out to every permitted laboratory on April 16th, 2013, and the due date for submitting the test results was May 6th, 2013. 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®, or CT values from the Roche Cobas®4800 method, though this information was not used for grading. In the future, we will also ask for the raw data to be provided from the laboratories that use other instruments.

A total of 76 laboratories received samples, and 80 valid answers were submitted from 74 laboratories by the due date. For screening, 34 laboratories (43%) used the Hybrid Capture® method, 25 laboratories (31%) used the Cervista® method, 14 laboratories (19%) used a polymerase chain reaction based method (11 Cobas®4800, 4 Laboratory Developed Tests with 1 laboratory using 2 different in-house PCR based methods), 5 laboratories used the Aptima® method (6%) and 1 laboratory (1%) used the in-situ-hybridization method. The screening results are summarized in Table 1.

Cytology smears were prepared and evaluated in house from each of the test samples. Consensus negative sample HPV067 was diagnosed satisfactory, within normal limits. Samples HPV068 and HPV069 were also satisfactory, negative smears but both contained the fungus *Candida albicans*. Consensus positive test sample HPV066 showed cells consistent with Low-grade squamous intraepithelial lesion (LGSIL) and cytology smears from the consensus positive sample HPV070 were diagnosed as ASCUS-atypical squamous cells present (ASCUS). Both of these positive test sample cases also contained the fungus *Candida albicans* on their slides. All the cytological diagnoses were in agreement with the HPV consensus results from this proficiency test.

Results

In general, consensus results from all laboratories for all samples were very good, with an overall consensus across all samples of 98.25% and ≥93.8% per individual sample. Although consensus negative sample HPV068 was overall ≥93.8% negative, it resulted in a non

[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.

consensus result for the laboratories using the Roche Cobas®4800 method with 3/11 (27.3%) positive results. However, the Ct values for all three positive results were close to the cut-off (Fig 1D) and thus these results most likely represent false positives. Furthermore, one positive result was reported with the Hybrid Capture® and ISH methods, respectively, for this sample. The consensus negative sample HPV069 had two discrepant answers among the Hybrid Capture® users (2/34) with one laboratory submitting its result as a low positive, while the other laboratory submitting its result as positive. In contrast, all laboratories reported samples HPV066 and HPV070 as positive across all methods (100%) and results for sample HPV067 were unanimously reported (100%) as negative by all methodologies.

For laboratories whose results did not match the consensus results for their method and who would like to re-examine their results a limited number of samples are available for retest upon request.

Table 1. Screening results, 74 laboratories, 80 results submitted:

	HPV066	HPV067	HPV068	HPV069	HPV070
All methods					
Total	80	80	80	80	80
Negative	0	80	75	78	0
Positive	80	0	5	1	80
Low Positive	0	0	0	1	0
Indeterminate	0	0	0	0	0
% Negative	0.0%	100.0%	93.8%	97.5%	0.0%
% Positive	100.0%	0.0%	6.3%	1.3 %	100.0%
% Low Positive	0.0%	0.0%	0.0 %	1.3 %	0.0 %
% Indeterminate	0.0%	0.0%	0.0%	0.0%	0.0%
Consensus	**POS**	**NEG**	**NEG**	**NEG**	**POS**

	HPV066	HPV067	HPV068	HPV069	HPV070
Hybrid Capture®					
Total	34	34	34	34	34
Negative	0	34	33	32	0
Positive	34	0	1	1	34
Low Positive	0	0	0	1	0
Indeterminate	0	0	0	0	0
% Negative	0.0%	100.0%	97.1%	94.1%	0.0%
% Positive	100.0%	0.0%	2.9%	2.9%	100.0%
% Low Positive	0.0%	0.0%	0.0%	2.9%	0.0%
% Indeterminate	0.0%	0.0%	0.0%	0.0%	0.0%
Consensus	**POS**	**NEG**	**NEG**	**NEG**	**POS**

	HPV066	HPV067	HPV068	HPV069	HPV070
Cervista®					
Total	25	25	25	25	25
Negative	0	25	25	25	0
Positive	25	0	0	0	25
% Negative	0.0%	100.0%	100.0%	100.0%	0.0%
% Positive	100.0%	0.0%	0.0%	0.0%	100.0%
Consensus	**POS**	**NEG**	**NEG**	**NEG**	**POS**

Table 1 continued:

	HPV066	HPV067	HPV068	HPV069	HPV070
Cobas® 4800					
Total	11	11	11	11	11
Negative	0	11	8	11	0
Positive	11	0	3	0	11
% Negative	0.0%	100.0%	72.7%	100.0%	0.0%
% Positive	100.0%	0.0%	27.3%	0.0%	100.0%
Consensus	**POS**	**NEG**	**NO CONS**	**NEG**	**POS**

	HPV066	HPV067	HPV068	HPV069	HPV070
PCR					
Total	4	4	4	4	4
Negative	0	4	4	4	0
Positive	4	0	0	0	4
% Negative	0.0%	100.0%	100.0%	100.0%	0.0%
% Positive	100.0%	0.0%	0.0%	0.0%	100.0%
Consensus	**POS**	**NEG**	**NEG**	**NEG**	**POS**

	HPV066	HPV067	HPV068	HPV069	HPV070
APTIMA					
Total	5	5	5	5	5
Negative	0	5	5	5	0
Positive	5	0	0	0	5
% Negative	0.0%	100.0%	100.0%	100.0%	0.0%
% Positive	100.0%	0.0%	0.0%	0.0%	100.0%
Consensus	**POS**	**NEG**	**NEG**	**NEG**	**POS**

	HPV066	HPV067	HPV068	HPV069	HPV070
ISH (N=1)	**POS**	**NEG**	**POS**	**NEG**	**POS**

Genotyping

Laboratories that routinely determine HPV genotypes were also asked to submit those results ("genotyping"). Thirty-two laboratories did genotyping using variable methodologies. Of those, eighteen laboratories (56.2%) used the Cervista®16/18 method, eleven laboratories (34.4%) used the Cobas® 4800 methodology and three laboratories (9.4%) used a laboratory developed PCR based methodology (Table 2). The results are summarized in Table 3.

Genotyping results for consensus positive samples HPV066 and HPV070 showed that most of the laboratories were in agreement that both the high-risk HPV genotypes 16 and 18 were present in these two samples. However, for HPV066 one laboratory reported the presence of HPV 16 and 51 with the Cervista ®16/18 method, which is analytically impossible and thus likely is a reporting error. Another laboratory only reported HPV 51, which suggests a problem with their assay's detection of HPV 16 and 18. Similarly, for HPV070, a majority of the laboratories reported both high-risk types 16 and 18 present with the exception of one laboratory that reported HPV 16 as the only high-risk genotype detected in that sample; another laboratory reported HPV 16 together with several other high-risk genotypes, but did not report HPV 18. These laboratories may want to re-evaluate the performance of their HPV 18 assay. Again, the laboratories that use a Laboratory Developed Test by PCR were able to detect multiple genotypes. For sample HPV068 the three positive answers from the Roche Cobas®4800 method were in the pooled probe channel with a Ct value just below the cut-off. Finally, for

consensus negative sample HPV069 one laboratory did report the presence of the low-risk genotype HPV 6 by PCR.

Note for Cobas®4800 users: if a sample is positive in all three channels you must use "16,18 PLUS OTHER HR" from the drop down menu.

Table 2. Genotyping results, 32 laboratories:

Method	HPV066	HPV067	HPV068	HPV069	HPV070
INV	16,18	N/A	N/A	N/A	16,18
INV	16,18	N/A	N/A	N/A	16,18
INV	16,18	N/A	N/A	N/A	16,18
INV	16,18	N/A	N/A	N/A	16,18
INV	16,18	N/A	N/A	N/A	16,18
INV	16,18	N/A	N/A	N/A	16,18
INV	16,18	N/A	N/A	N/A	16,18
INV	16,51	N/A	N/A	N/A	16,18
INV	16,18	N/A	N/A	N/A	16,18
INV	16,18	N/A	N/A	N/A	16
INV	16,18	N/A	N/A	N/A	16,18
INV	16,18	N/A	N/A	N/A	16,18
INV	16,18	N/A	N/A	N/A	16,18
INV	16,18	N/A	N/A	N/A	16,18
INV	16,18	N/A	N/A	N/A	16,18
INV	16,18	N/A	N/A	N/A	16,18
INV	16,18	N/A	N/A	N/A	16,18
Cobas 4800	16,18	N/A	HR NOT 16,18	N/A	16,18
Cobas 4800	16,18	N/A	HR NOT 16,18	N/A	16,18
Cobas 4800	16,18	N/A	N/A	N/A	16,18
Cobas 4800	16,18	N/A	N/A	N/A	16,18
Cobas 4800	16,18 PLUS OTHER HR	N/A	N/A	N/A	16,18 PLUS OTHER HR
Cobas 4800	16,18 PLUS OTHER HR	N/A	N/A	N/A	16,18 PLUS OTHER HR
Cobas 4800	16,18	N/A	N/A	N/A	16,18
Cobas 4800	16,18 PLUS OTHER HR	N/A	N/A	N/A	16,18 PLUS OTHER HR
Cobas 4800	16,18 PLUS OTHER HR	N/A	HR NOT16,18	N/A	16,18 PLUS OTHER HR
Cobas 4800	16,18	N/A	N/A	N/A	16,18
Cobas 4800	16,18	N/A	N/A	N/A	16,18
PCR	16,18	REACTIVITY TO GENERIC PROBE	REACTIVITY TO GENERIC PROBE	6	16,18
PCR	51	N/A	N/A	N/A	16,39,52,58,59
PCR	16,18,31,51,56,58,68	N/A	N/A	N/A	16,18,31,39,51,56,58,59,68

INV = Cervista®, PCR = polymerase chain reaction, polymorphism determination, N/A = not applicable,

Table 3. Summary of genotyping results:

	HPV066	HPV067	HPV068	HPV069	HPV070
Genotyping results					
HPV 16	0	0	0	0	1
HPV 16 + other HR	1	0	0	0	1
HPV16,18+other HR	5	0	0	0	5
HPV 16 and 18	25	0	0	0	25
HR NOT 16,18	1	0	3	0	0
HPV 6(LR)	0	0	0	1	0
N/A	0	31	28	31	0
Other	0	1	1	0	0
Total	**32**	**32**	**32**	**32**	**32**

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

Raw data

Figure 1 shows the raw data from the three methods Hybrid Capture®, Cervista®16/18, and Roche Cobas®4800. Overall there was clear separation between positive and negative results. However, there are a few values close to the cut-off of their retrospective method and these laboratories may wish to review their results and analytical performance of their method.

Conclusions

The overall results of this HPV DNA proficiency test were satisfactory. Three of the five samples were unanimously in agreement across all methods. Consensus negative sample HPV069 produced two discrepant answers by the Hybrid Capture® method, while sample HPV068 produced five discrepant positive answers which resulted in a non consensus outcome for the Roche Cobas®4800 methodology. All cytology diagnoses were also consistent with the consensus results of these ONCO/HPVT 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 2013 New York State HPV proficiency test:

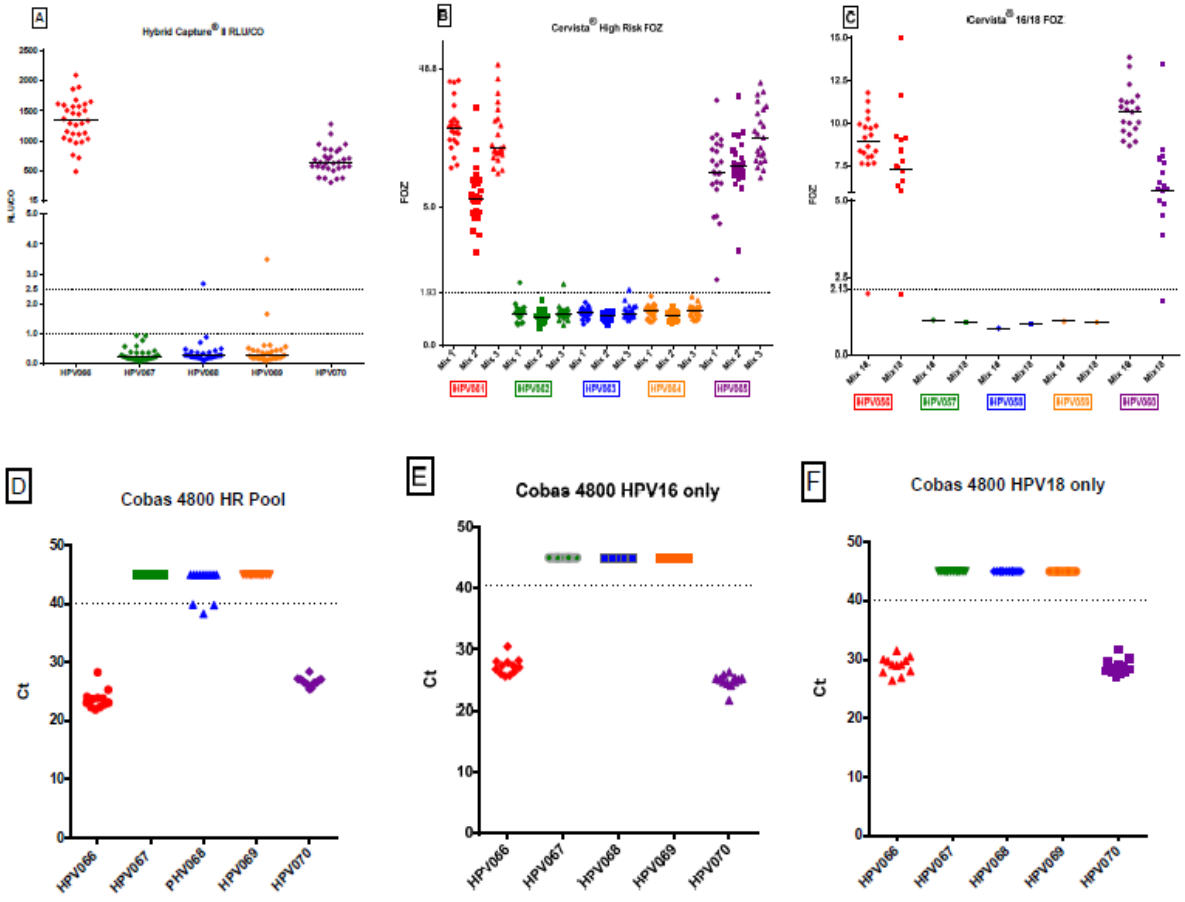
Mail-out Date	Due Date
October 15	November 4

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

Erasmus Schneider, 518-474-2088, schneid@wadsworth.org
Halyna Logan, 518-473-8715, hll01@health.state.ny.us
Helen Ling, 518-474-0036, hxl01@health.state.ny.us

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

Figure 1


A
Hybrid Capture® II RLU/CO
RLU/CO
HPV066
HPV067
HPV068
HPV069
HPV070
B
Cervista® High Risk FOZ
FOZ
1.00
HPV061
HPV062
HPV063
HPV064
HPV065
C
iCervista® 16/18 FOZ
FOZ
2.15
HPV066
HPV067
HPV068
HPV069
HPV070
D
Cobas 4800 HR Pool
Ct
HPV066
HPV067
PHV068
HPV069
HPV070
E
Cobas 4800 HPV16 only
Ct
HPV066
HPV067
HPV068
HPV069
HPV070
F
Cobas 4800 HPV18 only
Ct
HPV066
HPV067
HPV068
HPV069
HPV070