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HEALTH

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December 14, 2012

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

October 2012 [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 October 2012. A report with your laboratory's score and grade will be sent separately to you by regular mail. Five vials (HPV061 – HPV065) containing cervical cells derived from actual patients in PreservCyt® medium were sent out to every permitted laboratory on October 16th, 2012, and the extended due date for submitting the test results was November 12th, 2012. A few laboratories affected by hurricane Sandy notified us that they were having problems submitting their results due to electrical problems caused by the storm; therefore, under this circumstance these laboratories were granted an extension for submission until November 16th. A few other laboratories were completely unable to test because of the super-storm and were excused from participating in this event. 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®, or FOZ values from Cervista®, 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 77 laboratories received samples, and 78 valid answers were submitted from 73 laboratories by the due date. For screening, 40 laboratories (51%) used the Hybrid Capture® method, 25 laboratories (32%) used the Cervista® method, of which 4 laboratories reported results from both of these methods, 9 laboratories (12%) used a polymerase chain reaction based method (6 Cobas®4800, 3 Laboratory Developed Tests), 3 laboratories used the recently approved Aptima® method (4%) and 1 laboratory (1%) continued to use the in-situ-hybridization method. The screening results are summarized in Table 1.

Thin prep slides were prepared and evaluated in our laboratory from each of the test samples. All the cytological diagnoses were in agreement with the HPV consensus results from this proficiency test. Negative samples HPV061 and HPV064 were negative/satisfactory smears both containing the fungus *Candida albicans*. All three positive test samples contained endocervical cells and were therefore considered satisfactory. Smears from sample HPV062 were diagnosed as ASCUS, with areas of atypical squamous cells present (ASCUS) on the slides. Samples HPV063 and HPV065 both contained low grade dysplastic squamous cells (LGSIL) on the pap smears; however, koilocytosis was evident in the cells from sample HPV065.

Results

Consensus results from all laboratories for four of the five samples were good, with a high consensus of ≥97.4%, with the exception of sample HPV064 (see below). Sample HPV063 was unanimously reported (100%) positive across all methods. Consensus positive sample HPV062 was given a single discrepant negative answer by the laboratory using in-situ-hybridization (1/78). For sample HPV065, two negative responses instead of the consensus positive were submitted, again a negative response by the laboratory using in-situ-hybridization and the other negative response given by a laboratory using the Cervista®

[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.

method (2/78). Sample HPV061 also reported two discrepant answers; however, in this case, two positive answers were given instead of the consensus negative, one provided by the laboratory using in-situ-hybridization and the other by a laboratory using Hybrid Capture® (2/78). Thus, results by the laboratory using in-situ-hybridization were discrepant in 3/4 samples with otherwise high consensus.

Results for sample HPV064 proved to be very interesting with an overall majority of 79.5% negative (62/78) just below the 80% required for consensus; eleven responses were positive (14.1%) and five were low positive (6.4%). Interestingly, all six laboratories using the Roche Cobas®4800 method reported this sample as positive, and five of those laboratories identified the genotype as high-risk HPV16 only. In contrast, the three laboratories that used a laboratory developed PCR method reported this sample as negative. In addition, five low positive responses (12.5% of HCII) and two positive responses (5.0% of HCII) were given by the Hybrid Capture® method and the remaining three positive responses (12.0% of Cervista) for this sample were given by laboratories using the Cervista® method. The exact reasons for these discrepancies are difficult to establish; however, the possible low levels of high-risk genotypes present in sample HPV064 could explain why five low positive (6.4%) and five positive (6.4%) results were submitted for this sample using methodologies other than Roche Cobas®.

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

Table 1. Screening results, 73 laboratories, 78 results submitted:

	HPV061	**HPV062**	**HPV063**	**HPV064**	**HPV065**
All methods					
Total	78	78	78	78	78
Negative	76	1	0	62	2
Positive	2	77	78	11	76
Low Positive	0	0	0	5	0
Indeterminate	0	0	0	0	0
% Negative	97.4%	1.3%	0.0%	79.5%	2.6 %
% Positive	2.6%	98.7%	100.0%	14.1 %	97.4 %
% Low Positive	0.0%	0.0%	0.0 %	6.4 %	0.0 %
% Indeterminate	0.0%	0.0%	0.0%	0.0%	0.0%
Consensus	**NEG**	**POS**	**POS**	**NEG***	**POS**

*79.5% majority

	HPV061	**HPV062**	**HPV063**	**HPV064**	**HPV065**
Hybrid Capture®					
Total	40	40	40	40	40
Negative	39	0	0	33	0
Positive	1	40	40	2	40
Low Positive	0	0	0	5	0
Indeterminate	0	0	0	0	0
% Negative	97.5%	0.0%	0.0%	82.5%	0.0%
% Positive	2.5%	100.0%	100.0%	5.0%	100.0%
% Low Positive	0.0%	0.0%	0.0%	12.5%	0.0%
% Indeterminate	0.0%	0.0%	0.0%	0.0%	0.0%
Consensus	**NEG**	**POS**	**POS**	**NEG**	**POS**

Table 1 continued:

	HPV061	HPV062	HPV063	HPV064	HPV065
Cervista®					
Total	25	25	25	25	25
Negative	25	0	0	22	1
Positive	0	25	25	3	24
% Negative	100.0%	0.0%	0.0%	88.0%	4.0%
% Positive	0.0%	100.0%	100.0%	12.0%	96.0%
Consensus	**NEG**	**POS**	**POS**	**NEG**	**POS**

	HPV061	HPV062	HPV063	HPV064	HPV065
Cobas® 4800					
Total	6	6	6	6	6
Negative	6	0	0	0	0
Positive	0	6	6	6	6
% Negative	100.0%	0.0%	0.0%	0.0%	0.0%
% Positive	0.0%	100.0%	100.0%	100.0%	100.0%
Consensus	**NEG**	**POS**	**POS**	**POS**	**POS**

	HPV061	HPV062	HPV063	HPV064	HPV065
PCR					
Total	3	3	3	3	3
Negative	3	0	0	3	0
Positive	0	3	3	0	3
% Negative	100.0%	0.0%	0.0%	100.0%	0.0%
% Positive	0.0%	100.0%	100.0%	0.0%	100.0%
Consensus	**NEG**	**POS**	**POS**	**NEG**	**POS**

	HPV061	HPV062	HPV063	HPV064	HPV065
APTIMA					
Total	3	3	3	3	3
Negative	3	0	0	3	0
Positive	0	3	3	0	3
% Negative	100.0%	0.0%	0.0%	100.0%	0.0%
% Positive	0.0%	100.0%	100.0%	0.0%	100.0%
Consensus	**NEG**	**POS**	**POS**	**NEG**	**POS**

	HPV061	HPV062	HPV063	HPV064	HPV065
ISH (N=1)	**POS**	**NEG**	**POS**	**NEG**	**NEG**

Genotyping

Laboratories that routinely determine HPV genotypes were also asked to submit those results ("genotyping"). Twenty-nine laboratories did genotyping using variable methodologies. Of those, twenty laboratories (69%) used the Cervista®16/18 method, six laboratories (21%) used the Cobas® 4800 methodology and three laboratories (10%) used a laboratory developed PCR based methodology (Table 2).

As expected, the carcinogenic types 16 and 18 were most frequently observed in the positive samples. Genotyping results for samples HPV062 and HPV063 showed that all the laboratories were in agreement that the high-risk HPV genotypes 16 and/or 18 were present in these two samples. However, whereas all but two laboratories agreed that HPV 16 was present in each of these two samples, only 17/29 (55%) and 19/29 (65.5%) of laboratories, respectively, also detected HPV 18. Other genotypes detected by the two laboratories whose methods are designed to do so are HPV 31, 59 and 68 for sample HPV062, and HPV 31, 51, 68 and possibly 59 for sample HPV063.

Upon review of these data, it is remarkable to see that laboratories that use the same Cervista®16/18 method were not able to obtain consistent genotyping results for the same samples. For example, for HPV062, 50% of the laboratories using Cervista® reported a combination of HPV16 and 18 genotypes present, while 40% of the laboratories reported only the HPV16 genotype present, one laboratory only reported HPV18 and one laboratory could not identify either genotype present. Likewise, for sample HPV063, 65% of the laboratories reported the presence of both HPV16 and 18 genotypes, while 30% reported only the single HPV genotype 16 and again, the same laboratory that was not able to identify either of those two genotypes in the previous sample had the same problem in this sample. In contrast, all laboratories using the Roche Cobas® 4800 were in agreement that both high-risk HPV 16 and 18 were present in both of these samples. For the positive sample HPV065 all laboratories that identified the genotype were in agreement that only the single strain high-risk HPV genotype 16 was present, although two laboratories were not able to identify the high-risk genotype(s) present. For sample HPV064, which all six Roche Cobas® 4800-using laboratories reported positive for high-risk HPV, five of the six reported the presence of HPV16 whereas one laboratory was not able to specifically identify which high risk genotype(s) was/were present. In addition, none of the three Cervista® laboratories who also reported this specimen as positive could identify the genotype(s) present by the Cervista®16/18 technique; finally, one PCR laboratory reported the presence of a low-risk HPV6 genotype in this sample, though one may question whether this does not represent a data entry mistake. Table 2 shows the genotyping results and Table 3 summarizes these results.

Table 2. Genotyping results, 29 laboratories:

Method	HPV061	HPV062	HPV063	HPV064	HPV065
INV	N/A	16	16,18	N/A	16
INV	N/A	16	16,18	N/A	16
INV	N/A	16	16	N/A	16
INV	N/A	16,18	16,18	N/A	16
INV	N/A	16,18	16,18	N/A	16
INV	N/A	16,18	16,18	NOT ID	16
INV	N/A	16,18	16	N/A	16
INV	N/A	16,18	16,18	N/A	16
INV	N/A	16	16	N/A	NOT ID
INV	N/A	16	16,18	NOT ID	16
INV	N/A	18	16	N/A	16
INV	N/A	16,18	16,18	NOT ID	16
INV	N/A	16	16,18	N/A	16
INV	N/A	16	16	N/A	NOT ID
INV	N/A	16,18	16,18	N/A	N/A
INV	N/A	NOT ID	NOT ID	N/A	16

INV	N/A	16,18	16,18	N/A	16
INV	N/A	16,18	16	N/A	16
INV	N/A	16,18	16,18	N/A	16
INV	N/A	16	16,18	N/A	16
Cobas 4800	NOT ID	16,18	16,18	16	16
Cobas 4800	N/A	16,18	16,18	NOT ID	16
Cobas 4800	N/A	16,18	16,18	16	16
Cobas 4800	N/A	16,18	16,18	16	16
Cobas 4800	16	16,18	16,18	16	16
Cobas 4800	N/A	16,18	16,18	16	16
PCR	**Weak reactivity to probe**	16,18	16	6	16,31
PCR	N/A	16,31,59,68	16,31,59,68	N/A	16,31,59
PCR	NOT ID	16, 31,51,59,68	16, 31,51,68	NOT ID	16

INV = Cervista®, PCR = polymerase chain reaction, polymorphism determination, N/A = not applicable, NOT ID = Not identifiable by the method used

Table 3. Summary of genotyping results:

	HPV061	**HPV062**	**HPV063**	**HPV064**	**HPV065**
Genotyping results					
HPV 16	1	8	7	5	24
HPV16 + High-Risk other than 18	0	2	2	0	2
HPV16 and 18	0	17	19	0	0
HPV 18	0	1	0	0	0
N/A	25	0	0	18	1
NOT ID	2	1	1	5	2
Other	1	0	0	1	0
Total	**29**	**29**	**29**	**29**	**29**

N/A = not applicable, NOT ID = Not identifiable by the method used

Raw data

Figure 1 shows the raw data from both the Hybrid Capture® and Cervista® methods. Sample HPV062 and HPV063 clearly represented mixed infections, as evidenced by the fact that the FOZ values for all three Cervista® mixes are above the triple positive cut-off of 1.93. In contrast, for sample HPV065 only Cervista® mix 3 that contains probes for HPV 16, 31, 33, 35, 52 and 58 was positive, which is consistent with the genotyping results that identified HPV 16, but not HPV 18 in this sample.

Conclusions

With the exception of sample HPV064, there was high agreement among the laboratories in this proficiency test and the results were consistent with the cytologic features of the samples. In contrast, sample HPV064, which appeared to possibly have a low level of infection, posed a greater challenge, suggesting that there are some differences in the cut-offs used by the various methods.

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 2013 New York State HPV proficiency tests:

Mail-out Date	Due Date
April 16	May 6
October 15	November 4

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

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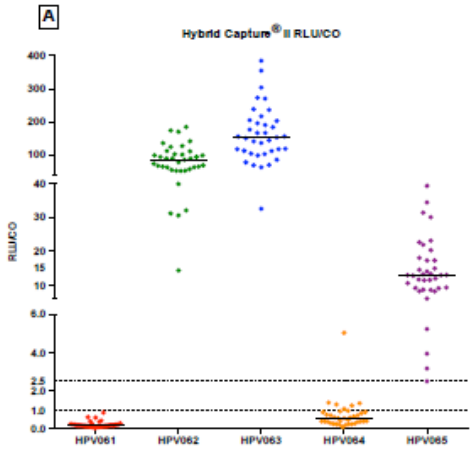
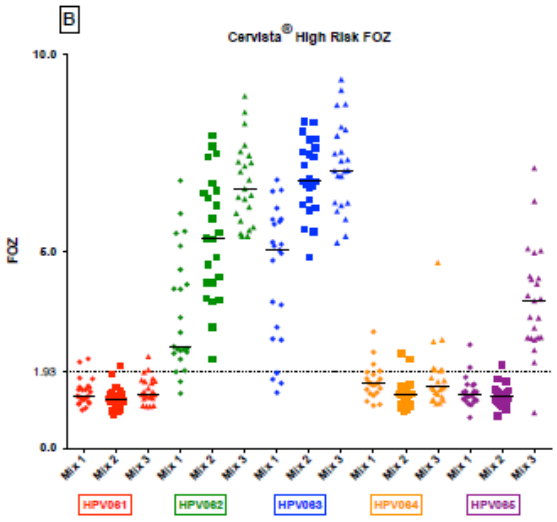
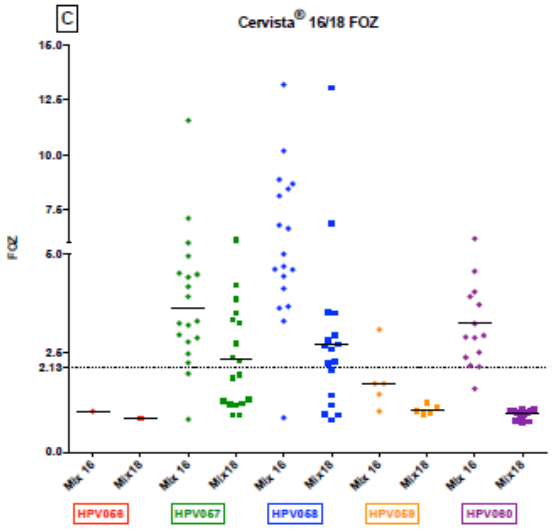
Figure 1


A
Hybrid Capture® II RLU/CO
RLU/CO
400
300
200
100
40
30
20
15
10
6.0
4.0
2.5
2.0
1.0
0.0
HPV061
HPV062
HPV063
HPV064
HPV065


B
Cervista® High Risk FOZ
FOZ
10.0
6.0
1.93
0.0
Mix 1
Mix 2
Mix 3
HPV061
HPV062
HPV063
HPV064
HPV065


C
Cervista® 16/18 FOZ
FOZ
16.0
12.5
10.0
7.5
6.0
2.5
2.13
0.0
Mix 16
Mix18
HPV068
HPV067
HPV066
HPV069
HPV060