STATE OF NEW YORK
DEPARTMENT OF HEALTH

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December 3, 2010

Evaluation of the New York State Human Papilloma Virus (HPV) Proficiency Test October 2010 [1]

Dear Laboratory Director:

This is the summary and evaluation of the graded New York State Proficiency Test for human papilloma virus (HPV) determination. Five vials (HPV036 – HPV040) containing cervical cells in PreservCyt® medium were sent out to every permitted laboratory on October 19th, 2010, and the due date for the test results was November 8th, 2010. The samples contained a mixture of actual patient samples. 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, and for those laboratories performing genotyping, the genotype(s) present.
A total of 68 test sets were sent out, and valid answers were received from 67 laboratories by the due date. Forty-seven laboratories (69 %) used the Hybrid Capture™ method, seventeen (25%) Cervista™ (Invader technology), three (4%) polymerase chain reaction, and one (2%) in situ hybridization. Compared with the previous HPV proficiency test event, the proportion of laboratories using the Hybrid Capture™ method remained the same, whereas those using Cervista™ increased slightly. Only a small number of laboratories used either PCR (slight decrease) or in situ hybridization (unchanged). The results are broken down by methods in Table 1. Cytology smears were prepared and evaluated from each of the samples. Sample HPV036 showed characteristic ASCUS cells, and possibly a few LGSIL cells scattered throughout the smears. Sample HPV039 contained more obvious LGSIL cells. Slides from sample HPV038 displayed definite ASCUS cells, but cytologic changes for herpes simplex infection were also observed. Sample HPV037 and HPV040 were satisfactory smears “within normal limits”.

Results

With the exception of Sample HPV036, a high consensus of > 95% was achieved for all samples across all methods (Table 1). Thus, those laboratories that reported results that do not match the consensus, irrespective of the method used, may want to re-examine their results. A limited number of samples are available for retesting upon request. In contrast, the results for specimen HPV036 were somewhat inconsistent and only achieved an overall consensus of 75% positive, with a clear difference between methods. Similar to what was observed in previous PT events, laboratories using

[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.

Hybrid Capture™ reported a substantially higher proportion of positive results than those using Cervista™ (83.0% vs. 58.8%). While the exact reasons for this discrepancy are difficult to establish, it is conceivable that the reported cross-reactivity of the Hybrid Capture™ method with the low-risk genotypes 6, 11 and 53 present in this sample (Table 2) contributed to this result.

. **Table 1. Results with Hybrid Capture™, Cervista™, PCR and ISH methods**

	HPV036	HPV037	HPV038	HPV039	HPV040
All methods					
Total	68	68	68	68	68
Negative	12	65	0	2	67
Positive	51	2	68	66	1
Low Positive	5	1	0	0	0
% Negative	17.6%	95.6%	0.0%	2.9%	98.5 %
% Positive	75.0%	2.9%	100.0%	97.1 %	1.5 %
% Low Positive	7.4%	1.5%	0.0 %	0.0 %	0.0 %
Consensus	**NO CONS**	**NEG**	**POS**	**POS**	**NEG**
Hybrid Capture					
Total	47	47	47	47	47
Negative	3	46	0	0	46
Positive	39	0	47	47	1
Low Positive	5	1	0	0	0
% Negative	6.4%	97.9%	0.0 %	0.0 %	97.9 %
% Positive	83.0 %	0.0 %	100.0 %	100.0%	2.1 %
% Low Positive	10.6 %	2.1 %	0.0 %	0.0 %	0.0 %
Consensus	**POS**	**NEG**	**POS**	**POS**	**NEG**
Cervista					
Total	17	17	17	17	17
Negative	7	16	0	1	17
Positive	10	1	17	16	0
% Negative	41.2%	94.1%	0.0 %	5.9 %	100.0 %
% Positive	58.8 %	5.9%	100.0 %	94.1%	0.0 %
Consensus	**NO CONS**	**NEG**	**POS**	**POS**	**NEG**
PCR					
Total	3	3	3	3	3
Negative	1	2	0	0	3
Positive	2	1	3	3	0
% Negative	33.3 %	66.7%	0.0%	0.0%	100.0%
% Positive	66.7 %	33.3 %	100.0 %	100.0 %	0.0 %
Consensus	**NO CONS**	**NO CONS**	**POS**	**POS**	**NEG**
ISH (N=1)	**NEG**	**NEG**	**POS**	**NEG**	**NEG**

Genotyping

Laboratories that routinely determine HPV genotypes were also asked to submit those results ("genotyping"). The methods used for genotyping were diverse, and since not every method detects the same panel of genotypes, the genotyping results were not graded. Eighteen laboratories reported results from various methodologies (Table 2). The most prevalent high-risk types HPV16 and/or HPV18 were found by all laboratories in the two clearly positive samples HPV038 and HPV039. Interestingly, a majority of laboratories detected only HPV16 but not HPV18 in sample HPV039. In addition, there was fairly good agreement for samples HPV038 and HPV039 in regards to the presence of other high-risk genotypes among those laboratories that employ a more comprehensive panel of detection reagents.

As mentioned above, in sample HPV036 several laboratories detected the low-risk types HPV 6,11, and 53, whereas most did not find any high-risk genotypes. However, despite the potential cross-reactivity of the Hybrid Capture™ method with the low-risk genotypes, a majority of laboratories still found this sample to be positive by screening. It is possible that high-risk genotypes other than HPV16 or 18 contributed to this result, which would not be identified by the Cervista™ HPV 16/18 assay used by 11/18 laboratories. Interestingly, one lab detected HPV16 or 18 in all five samples, but did not report sample HPV040 as positive by screening. This laboratory should go back and re-evaluate its results.

Table 2. Genotyping results, 18 laboratories:

Method	HPV036	HPV037	HPV038	HPV039	HPV040
INV	NOT ID	N/A	16,18	16	NA
INV	16 or 18	N/A	16,18	16	N/A
INV	16 or 18	16 or 18	16,18	16,18	16 or 18
INV	N/A	N/A	16,18	16	N/A
INV	NOT ID	N/A	16,18	16	N/A
INV	NOT ID	N/A	16,18	16	N/A
INV	N/A	N/A	16,18	16,18	N/A
INV	N/A	N/A	16,18	16	N/A
INV	N/A	N/A	16,18	16	N/A
INV	N/A	N/A	16,18	16	N/A
INV	NEG	N/A	16,18	16	N/A
PCR	6	6	6,16,18	16	6
PCR	51/59,6/11	N/A	16,18,39/56,51/59,52/58	16,18,45,39/56,51/59	N/A
PCR	51	N/A	16,18,51,52,59	16,18,59	N/A
PCR	45,51,68	N/A	16,18,31,33,35,39,45, 51,52,56,58,59,66,68	16,18,31,33,35,39,45, 51,52,56,59,66,68	N/A
RFLP	53,6,11	83,6	16,18,53,6	16,84,58,CP141	11
RFLP	11	N/A	16,18,52,53	16, LVX160	N/A
HYC	NOT ID	N/A	16,18	16	N/A

HYC = Hybrid Capture™, INV = Cervista™, N/A = not applicable, NOT ID = not identified, PCR = polymerase chain reaction, RFLP = PCR followed by restriction fragment length polymorphism determination

Conclusions

Overall, there was good agreement among the laboratories and the results were consistent with the cytologic features of the samples. However, the somewhat inconsistent results for sample HPV036 suggest that samples containing relatively low titers of the human papilloma virus and/or genotypes other than the most prevalent HPV16 or 18 can cause discrepancies in the interpretation between the different 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.

If you have questions or wish to discuss some of the issues alluded to you may contact us at the address below.

Tentative schedule for the upcoming 2011 New York State HPV proficiency test:

Mail-out Date	Due Date
March 29	April 18
July 12	August 1
October 18	November 7

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