STATE OF NEW YORK
DEPARTMENT OF HEALTH

Wadsworth Center The Governor Nelson A. Rockefeller Empire State Plaza P.O. Box 509 Albany, New York 12201-0509

Richard F. Daines, M.D.
Commissioner

James W. Clyne, Jr.
Executive Deputy Commissioner

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New York State Human Papilloma Virus (HPV) Proficiency Test 7/2010 Evaluation[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 (HPV031 – HPV035) containing cervical cells in PreservCyt® medium were sent out to every participating laboratory on July 13th, 2010, and the due date for the test results was August 2, 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 two categories, positive (pos), negative (neg), or indeterminate (ind) for high risk HPV screening, and for those laboratories performing genotyping, the genotype(s) present.

Results

In this mailing, 68 test sets were sent out, and valid answers were received from 67 laboratories by the due date. Forty-seven laboratories (70 %) used the Hybrid Capture® method, fifteen (22 %) Cervista® (Invader technology), four (6 %) polymerase chain reaction, and one (2 %) in situ hybridization. Compared with the previous HPV proficiency test event, the proportion of tests performed by the Hybrid Capture® method slightly declined, and that done by the Cervista® (Invader technology) correspondingly increased, while the small numbers of tests performed by polymerase chain reaction and in situ hybridization remained unchanged. The results are broken down by methods in Table 1. High consensus was achieved with the samples HPV031, HPV032, HPV 034 and HPV035 across all methods. The results for sample HPV033 showed much higher variability, ranging from negative through indeterminate to positive. Results for this sample obtained by Cervista® (Invader technology) displayed a higher degree of uniformity (13 negative (87%) vs. 2 positive (13%)) than with the Hybrid Capture® method (26 negative (55%) vs. 18 positive (38%) and 3 indeterminate (6%)). Since the Hybrid Capture® method did not produce a clear consensus (>80 %) for this sample, the results for this sample were not graded, i.e. any answer for them was considered correct. In our own laboratory we obtained a weakly positive (low virus titer) result for this sample with the Hybrid Capture® method, which may explain the inconclusive result. Alternatively, and/or in addition, it is also possible that the apparent positivity is derived from cross reactivity of the Hybrid Capture® method with the low risk HPV genotypes 6 and 11 present in this sample (Table 2). Such cross reactivity of the Hybrid Capture® test has been widely described in the literature. This time we also evaluated our samples by microscopic examination. The two “negative” samples (HPV031 and HPV034) were negative for intraepithelial lesions or malignancy (“NILM”), one “positive” sample

[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.

(HPV032) contained atypical squamous cells of indeterminate significance ("ASCUS"), the other "positive" sample (HPV035) could be diagnosed as low grade squamous intraepithelial lesion ("LG SIL"), all consistent with the HPV consensus results. Finally, no abnormal cells were found in two thin preparations from the indeterminate sample (HPV033), a result that suggests that this sample was a true negative, consistent with the majority of the HPV results.

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

	HPV031	**HPV032**	**HPV033**	**HPV034**	**HPV035**
All methods					
Total	67	67	67	67	67
Negative	64	0	43	67	0
Positive	2	67	21	0	67
Indeterminate	1	0	3	0	0
% Negative	95.5 %	0.0 %	64.2 %	100.0%	0.0 %
% Positive	3.0 %	100.0 %	31.3 %	0.0 %	100.0 %
% Indeterminate	1.5 %	0.0%	4.5 %	0.0 %	0.0 %
Consensus	**NEG**	**POS**	**NO CONS**	**NEG**	**POS**

	HPV031	**HPV032**	**HPV033**	**HPV034**	**HPV035**
Hybrid Capture					
Total	47	47	47	47	47
Negative	46	0	26	47	0
Positive	0	47	18	0	47
Indeterminate	1	0	3	0	0
% Negative	97.9%	0.0 %	55.3 %	100.0 %	0.0 %
% Positive	0.0 %	100.0 %	38.3 %	0.0%	100.0 %
% Indeterminate	2.1 %	0.0 %	6.4 %	0.0 %	0.0 %
Consensus	**NEG**	**POS**	**NO CONS**	**NEG**	**POS**

	HPV031	**HPV032**	**HPV033**	**HPV034**	**HPV035**
Cervista					
Total	15	15	15	15	15
Negative	14	0	13	15	0
Positive	1	15	2	0	15
% Negative	93.3 %	0.0 %	86.7 %	100.0 %	0.0 %
% Positive	6.7 %	100.0 %	13.3 %	0.0 %	100.0 %
Consensus	**NEG**	**POS**	**NEG**	**NEG**	**POS**

	HPV031	HPV032	HPV033	HPV034	HPV035
PCR					
Total	4	4	4	4	4
Negative	3	0	3	4	0
Positive	1	4	1	0	4
% Negative	75.0 %	0.0 %	75.0 %	100.0 %	0.0 %
% Positive	25.0 %	100.0 %	25.0 %	0.0 %	100.0 %
Consensus	**NO CONS**	**POS**	**NO CONS**	**NEG**	**POS.**

	HPV031	HPV032	HPV033	HPV034	HPV035
ISH (N=1)					
Consensus	**NEG**	**POS**	**NEG**	**NEG**	**POS**

Genotyping

Laboratories that do 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 assessed only but not graded. In other words, no penalties were imposed because of potential errors in genotyping. Fifteen laboratories did genotyping using variable methodologies (Table 2). Since the methods for genotyping are not standardized, it is understandable that the results were somewhat divergent. Nevertheless, the high risk types HPV16 and HPV18 were found most frequently and by almost all laboratories in the two clearly positive samples HPV032 and HPV035. In addition, there was fairly good agreement for those two samples in regards to the presence of other high risk genotypes among those labs that employ a more comprehensive panel of detection reagents. Interestingly, some samples of HPV033 and HPV034 contained the low risk types 6, 11, and 53. They clearly screened negative for high risk types.

Table 2. Genotyping results, 15 laboratories:

Method	HPV031	HPV032	HPV033	HPV034	HPV035
HYC		**16**			**16**
INV		**16, 18**			**16, 18**
INV		**16, 18**			**16, 18**
INV		**16, 18**			**16, 18**
INV		**16**			**16, 18**
INV		**16, 18**			**16, 18**
INV		**16, 18**			**16, 18**
INV		**16, 18**			**16, 18**
PCR		**16,31,45, 39/56,51/59,52/58**			**16,18,31,35/68,39/56,45,51/59,52/58**
PCR		**16, 18/45, 31/33/35/39**	**6, 11**	**6, 11**	**6, 11, 16, 18/45, 31/33/35/39**

PCR		**16, 51, 52, 56, 59**			**16, 31, 51, 56, 59**
PCR		**16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68**			**16, 18, 31, 33, 35, 39, 45, 51, 52, 59, 66, 68**
RFL		**16, 31, 53**			**16, 31, 61**
RFL		**16**			**16, 31, 52**
RFL	**53, 83**	**6,11,58,84,CP8304,LVX160**	**6, 53**	**6, 11**	**6,11,18,31,58,66,CP141,CP8304**

INV = Cervista, PCR = polymerase chain reaction, RFL = PCR followed by restriction fragment length polymorphism determination

Conclusions

The results of this HPV DNA proficiency testing event were overall satisfactory. Specimen HPV033 provided variable results with the Hybrid Capture® method since the titer of virus particles was low and/or because of possible cross reactivity with the low risk HPV genotypes 6 and 11 present. In contrast, the more uniform results from the Cervista® method seem to suggest that this method may be less sensitive for low virus titers, or more likely, be better at discriminating high risk from low risk genotypes. The latter possibility is supported by the fact that no high risk genotypes were detected in this sample by genotyping and is consistent with the absence of abnormal cells by microscopic analysis.

The overall good agreement of the genotyping results suggests that there is increasing expertise and proficiency by laboratories in the determination of HPV types.

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 remaining 2010 New York State HPV proficiency test:

Mail-out Date	**Due Date**
October 19, 2010	November 8, 2010

Correspondence:

George K. Nagy, M.D.
Wadsworth Center, Room D206,
Empire State Plaza,
Albany, NY 12201 – 0509.

If you have questions, call or e-mail:

George K. Nagy, M.D., 518-474-3190, gkn02@health.state.ny.us
Halyna Logan, 518-473-8715, hll01@health.state.ny.us
Helen Ling, 518-474-0036, hxl01@health.state.ny.us