STATE OF NEW YORK
DEPARTMENT OF HEALTH

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Commissioner

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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 (HPV026 – HPV030) containing cervical cells in PreservCyt® medium were sent out to every participating laboratory on March 30th, 2010, and the due date for the test result was April 19, 2010. 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 64 laboratories by the due date. Forty-eight laboratories (75 %) used the Hybrid Capture® method, eleven (17 %) 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 shown in Table 1. High consensus was achieved with the samples HPV026, HPV028, and HPV030 across all methods. In contrast, samples HPV027 and HPV029, that essentially fell into the "high titer negative" or "indeterminate" range, showed much less uniformity (see Table 1). Only the results obtained by Cervista® (Invader technology) displayed a high degree of uniformity (>90% concordance) and found these samples to be negative (see Table 1). The one lab that reported a positive result for these two samples may want to verify that its results are not due to a contamination.

Since the results for the specimens HPV027 and HPV029, tested either by the Hybrid Capture® or polymerase chain reaction methods did not produce a clear consensus (>80 %), the results for these two samples in these two method groups were not graded, i.e. any answer for them was considered correct.

Table 1. Results obtained using Hybrid Capture®, Cervista®, PCR and ISH methods:

	HPV026	**HPV027**	**HPV028**	**HPV029**	**HPV030**
All methods					
Total	64	64	64	64	64
Negative	0	35	0	47	0
Positive	48	24	48	14	48
Indeterminate	0	5	0	3	0

% Negative	0.0 %	54.7 %	0.0 %	73.4 %	0 %
% Positive	100.0 %	37.5 %	100.0 %	21.9%	100.0 %
% Indeterminate	0.0 %	7.8%	0.0%	4.7 %	0.0 %
Consensus	**POS**	**NO CONS.**	**POS**	**NO CONS.**	**POS**

	HPV026	**HPV027**	**HPV028**	**HPV029**	**HPV030**
Hybrid Capture					
Total	48	48	48	48	48
Negative	0	23	0	33	0
Positive	48	21	48	12	48
Indeterminate	0	4	0	3	0
% Negative	0.0 %	47.9 %	0.0 %	68.8 %	0 %
% Positive	100.0 %	43.8 %	100.0 %	25.0%	100.0 %
% Indeterminate	0.0 %	8.3 %	0.0%	6.3 %	0.0 %
Consensus	**POS**	**NO CONS.**	**POS**	**NO CONS.**	**POS**
	HPV026	**HPV027**	**HPV028**	**HPV029**	**HPV030**
Cervista					
Total	11	11	11	11	11
Negative	0	10	0	10	0
Positive	11	1	11	1	11
Indeterminate	0	0	0	0	0
% Negative	0.0 %	90.9 %	0.0 %	90.9 %	0.0 %
% Positive	100.0 %	9.1 %	100.0 %	9.1 %	100.0 %
Consensus	**POS**	**NEG**	**POS**	**NEG**	**POS**
PCR					
Total	4	4	4	4	4
Negative	0	1	0	3	0
Positive	4	2	4	1	4
Indeterminate	0	1	0	0	0
% Negative	0.0 %	25.0 %	0.0 %	75.0 %	0.0 %
% Positive	100.0 %	50.0 %	100.0 %	25.0 %	100.0 %
% Indeterminate	0.0 %	25.0 %	0.0 %	0.0 %	0.0 %
Consensus	**POS**	**NO CONS.**	**POS**	**NO CONS.**	**POS.**
ISH (N=1)					
Consensus	**POS**	**NEG**	**POS**	**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 the number of laboratories doing it was small, the genotyping results were assessed only but not graded. In other words, no penalties were imposed because of potential errors in genotyping. A few laboratories did genotyping using variable methodologies after the Hybrid Capture method provided positive HPV DNA results.

Since the methods for genotyping are not standardized, it is understandable that the results were widely divergent. However, the high risk types HPV16 and HPV18 were found most frequently and by almost all labs in the three clearly positive samples HPV026, HPV028, and HPV030.

Table 2 summarizes the genotyping results.

Table 2. Genotyping results, 12 laboratories:

Method	HPV026	HPV027	HPV028	HPV029	HPV030
INV	16,18		16,18		16,18
INV	16,18	INDET.	16,18		16,18
INV	16,18		16,18		16,18
INV	16,18	18	16,18		16,18
INV	16,18		16,18		16,18
PCR	16,18/45, 31/33/45(weak)		16,18/45, 31/33/35(weak)		16,18/45
PCR	16,18,45,39/56, 51/59,52/58	16	16,18,31,45,35/68,39/56,51/59, 52/58	16	16,18,39/56,51/59,52/ 58
PCR	16,31,51,52,59		16,31,51,52,56,68		16,18,45,51,52,68
PCR	16,18,31,33,35, 39,45,51,52,56, 58,59,66	16,39	16,18,31,33,35,39,45,51,52,56, 58,59,66	16,39	16,18,31,33,35,39,45, 51,52,56,58,59,66
RFL	18,53,58		18,53,58		18,53,58
RFL	16,61		16,18,61, LVX160		16,52,62, UNK.
RFL	6,16	16	16,61		16,18

INDET.= indeterminate, UNK = unknown, INV = Cervista, PCR = polymerase chain reaction, RFL = PCR followed by restriction fragment length polymorphism determination

Importance of genotyping limited to HPV types 16 and 18

HPV types 16 and 18 were prominently represented in these samples that were derived from mixing variable numbers of HPV-positive patient samples. Genotyping kits are available that are designed to determine only these two HPV types. Published data show that indeed, distinguishing HPV types 16 and 18 identifies those HPV-positive women who are at the greatest risk of developing CIN3 or more serious cervical lesions (Khan MJ et al., 2005). According to this cohort study the 10-year cumulative incidence rate of CIN3 or more serious lesions was 17 % among HPV16+ women, and 14 % among HPV18+ women, but only 3 % among women who were positive for other high risk HPV types. Thus it remains to be seen

whether identifying other than the HPV16 and 18 genotypes is of clinical value, at least in the USA.

Conclusions

In general, the results of this HPV DNA proficiency testing event were satisfactory. Specimens HPV027 and HPV029 provided variable results, presumably because they contained virus titers around the limit of detection for the different methods. Nevertheless, it is interesting that all but one lab using the Cervista method found these samples to be negative, whereas the results from both the HybridCapture and PCR based methods were approximately evenly split between positive and negative. This raises the question whether subtle differences in the cut-point settings exist.

Tentative schedule for the remaining 2010 New York State HPV proficiency tests:

Mail-out Dates	**Due Dates**
July 13, 2010	August 2, 2010
October 19, 2010	November 8, 2010

Reference

Khan MJ et al.: The elevated 10-year risk of cervical precancer and cancer in women with human papillomavirus (HPV) type 16 or 18 and the possible utility of type-specific HPV testing in clinical practice. JNCI 2005;97:1072-1079

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