NEW YORK
state department of
HEALTH

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Commissioner

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Executive Deputy Commissioner

August 29, 2011

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

July 2011 [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 July 2011. A report with your laboratory's score and grade will be sent separately to you by regular mail. Five vials (HPV046 – HPV050) containing cervical cells derived from actual patients in PreservCyt® medium were sent out to every permitted laboratory on July 12th, 2011, and the due date for submitting the test results was August 1st, 2011. 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.

A total of 73 test sets were sent out, and valid answers were received from 71 laboratories by the due date. Forty-seven laboratories (66%) used the Hybrid Capture® method, twenty-one (30%) the Cervista® (Invader technology) method, two (3%) used the polymerase chain reaction, and one (<1%) laboratory used the in-situ-hybridization method. One laboratory submitted results for both the Hybrid Capture® and Cervista® methods. The screening results are summarized in Table 1.

Cytology smears were prepared and evaluated in-house from each of the samples. Slides from samples HPV046, HPV048 and HPV049 were all "within normal limits" (NILM) with *Candida albicans* noted, with sample HPV049 also showing areas of Herpes virus scattered throughout the smears. Slides from sample HPV050 displayed a few "atypical squamous cells of indeterminate significance" (ASCUS) and also present were infections with both *Actinomyces israelii* and *Candida albicans*. Sample HPV047 contained more obvious "low grade squamous intraepithelial lesion" (LGSIL) cells consistent with a HPV infection. Also noted on these smears were infections of Candida and BV (Bacterial vaginosis). All the cytological diagnoses were in agreement with the HPV testing results.

[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.

Results

With the exception of two PCR results and one Hybrid Capture result, all laboratories agreed with the samples' respective consensus, for an overall concordance of 352/355 (99.15%) results across all samples and all methods. Thus, those laboratories that reported results that do not match the consensus, irrespective of the method used, should re-examine their results. A limited number of samples are available for retest upon request. We would like to remind the laboratories that only samples positive for high-risk genotypes should be reported as screen positive in this part of the proficiency test.

Table 1. Screening results, 71 laboratories:

	HPV046	**HPV047**	**HPV048**	**HPV049**	**HPV050**
All methods					
Total	71	71	71	71	71
Negative	70	0	69	71	0
Positive	1	71	2	0	71
Low Positive	0	0	0	0	0
% Negative	98.6%	0.0%	97.2%	100.0%	0.0 %
% Positive	1.4%	100.0%	2.8%	0.0 %	100.0 %
% Low Positive	0.0%	0.0%	0.0 %	0.0 %	0.0 %
Consensus	**NEG**	**POS**	**NEG**	**NEG**	**POS**

	HPV046	**HPV047**	**HPV048**	**HPV049**	**HPV050**
Hybrid Capture					
Total	47	47	47	47	47
Negative	47	0	46	47	0
Positive	0	47	1	0	47
Low Positive	0	0	0	0	0
% Negative	100.0%	0.0 %	97.9 %	100.0 %	0.0%
% Positive	0.0 %	100.0 %	2.1 %	0.0%	100.0 %
% Low Positive	0.0 %	0.0 %	0.0 %	0.0 %	0.0 %
Consensus	**NEG**	**POS**	**NEG**	**NEG**	**POS**

	HPV046	HPV047	HPV048	HPV049	HPV050
Cervista					
Total	21	21	21	21	21
Negative	21	0	21	21	0
Positive	0	21	0	0	21
% Negative	100.0%	0.0 %	100.0%	100.0%	0.0 %
% Positive	0.0 %	100.0%	0.0 %	0.0 %	100.0%
Consensus	**NEG**	**POS**	**NEG**	**NEG**	**POS**

	HPV046	HPV047	HPV048	HPV049	HPV050
PCR					
Total	2	2	2	2	2
Negative	1	0	1	2	0
Positive	1	2	1	0	2
% Negative	50.0 %	0%	50.0%	100.0%	0.0%
% Positive	50.0 %	100.0 %	50.0 %	0.0 %	100.0 %
Consensus	**NO CONS**	**POS**	**NO CONS**	**NEG**	**POS**

	HPV046	HPV047	HPV048	HPV049	HPV050
ISH (N=1)	**NEG**	**POS**	**NEG**	**NEG**	**POS**

Genotyping

Laboratories that routinely determine HPV genotypes were also asked to submit those results ("genotyping"). Twenty laboratories did genotyping using variable methodologies. Fifteen laboratories (75%) used the Cervista® 16/18 method, two (10%) used a PCR based methodology, two (10%) used RFLP based methodology and one laboratory (5%) used a Hybrid Capture® method (Table 2).

As expected, the carcinogenic types 16 and 18 were most frequently observed in the positive samples. However, since not every method detects every genotype and because the samples represent a mixture of patient samples it is understandable that the results may be somewhat divergent. Therefore, the genotyping results were not graded.

Nevertheless, all but one laboratory (95%) agreed that sample HPV050 contained both the high-risk HPV16 and HPV18 genotypes. This one laboratory (5%) using a PCR-RFLP method only detected the high risk genotype 16 in this sample. In contrast, the results for sample HPV047 were slightly more varied. Thirteen laboratories (65%) reported both the high-risk 16 and 18 genotypes, whereas seven laboratories (35%), including the one laboratory that also did not detect HPV18 in sample HPV050, only reported HPV16 genotype as their result for this sample. The laboratory that missed HPV18 in both samples should reexamine its assay's sensitivity for this particular genotype. Laboratories whose method is able to detect a large number of

genotypes included one or more additional subtypes as part their result for these samples. In each of the negative samples, HPV046, HPV048 and HPV049, only one lab detected a few subtypes. Presumably, no other laboratories tested these samples because they were negative by screening. Again, we would like to remind the laboratories that only samples positive for high-risk genotypes should be reported as screen positive. Table 2 summarizes the genotyping results.

Table 2. Genotyping results, 20 laboratories:

Method	HPV046	HPV047	HPV048	HPV049	HPV050
INV	N/A	16	N/A	N/A	16,18
INV	N/A	16,18	N/A	N/A	16,18
INV	N/A	16,18	N/A	N/A	16,18
INV	N/A	16	N/A	N/A	16,18
INV	N/A	16,18	N/A	N/A	16,18
INV	N/A	16,18	N/A	N/A	16,18
INV	N/A	16,18	N/A	N/A	16,18
INV	N/A	16	N/A	N/A	16,18
INV	N/A	16,18	N/A	N/A	16,18
INV	N/A	16	N/A	N/A	16,18
INV	N/A	16,18	N/A	N/A	16,18
INV	N/A	16	N/A	N/A	16,18
INV	N/A	16,18	N/A	N/A	16,18
INV	N/A	16,18	N/A	N/A	16,18
INV	N/A	16,18	N/A	N/A	16,18
PCR	N/A	6,11,16,18,3,1,35,39,45	N/A	N/A	16,18,45
PCR	N/A	16,18,51,52	N/A	N/A	16,18,51,58
RFLP	84,53	16,58,LVX1,60,70,61	84	54,LVX160	16,53,18,5858, LVX160
RFLP	N/A	16	N/A	N/A	16
HYC	N/A	16,18	N/A	N/A	16,18

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

Raw data

The attached Figure 1 shows the raw data from both the Hybrid Capture® as well as the Cervista® assays. Though these assays are not quantitative, the graphs nevertheless show the general distribution of the data in relation to the “cut points” used for a samples’ classification as

positive or negative. In particular, the Cervista® genotyping data for sample HPV047 clearly illustrate why some laboratories did not call this sample positive for the HPV18 genotype. As the distribution of mix 18 shows, the FOZ values are clustered around the "cut-point" of 2.13, with a substantial number of results just below and hence "negative" for the HPV18 genotype for this sample.

Conclusions

Overall, there was high agreement among the laboratories in this proficiency test and the results were consistent with the cytologic features of the 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 last 2011 New York State HPV proficiency test:

Mail-out Date	Due Date
October 18	November 7

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

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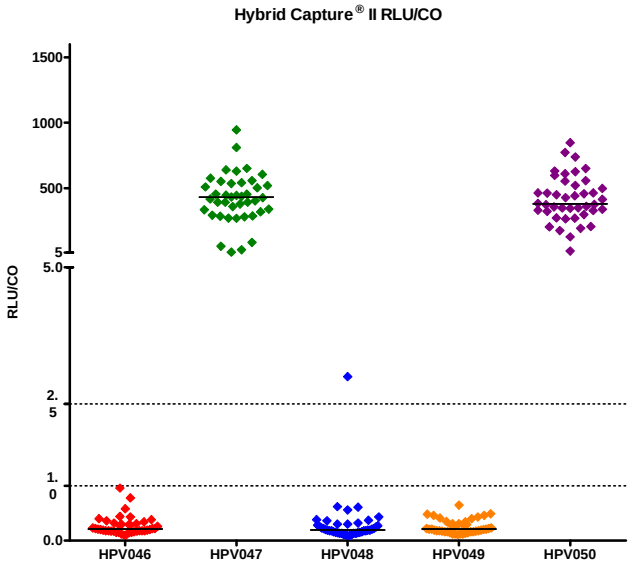
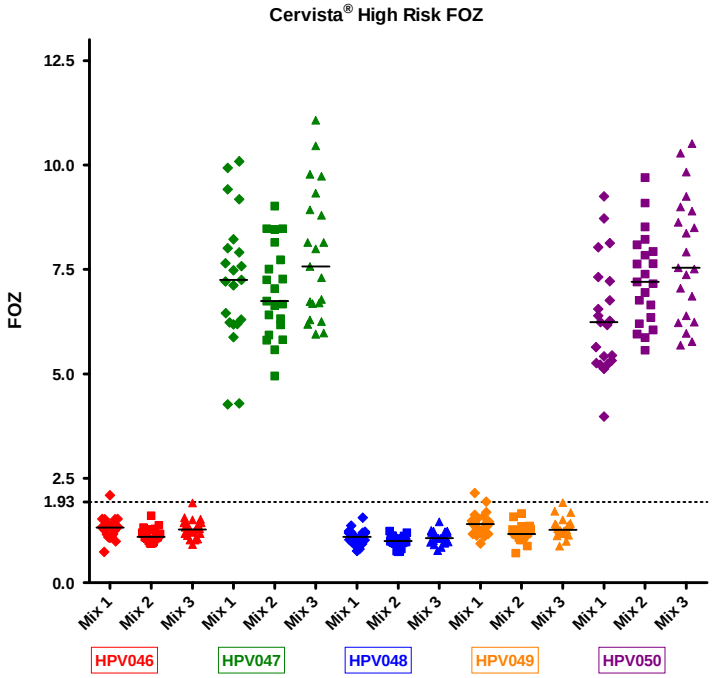
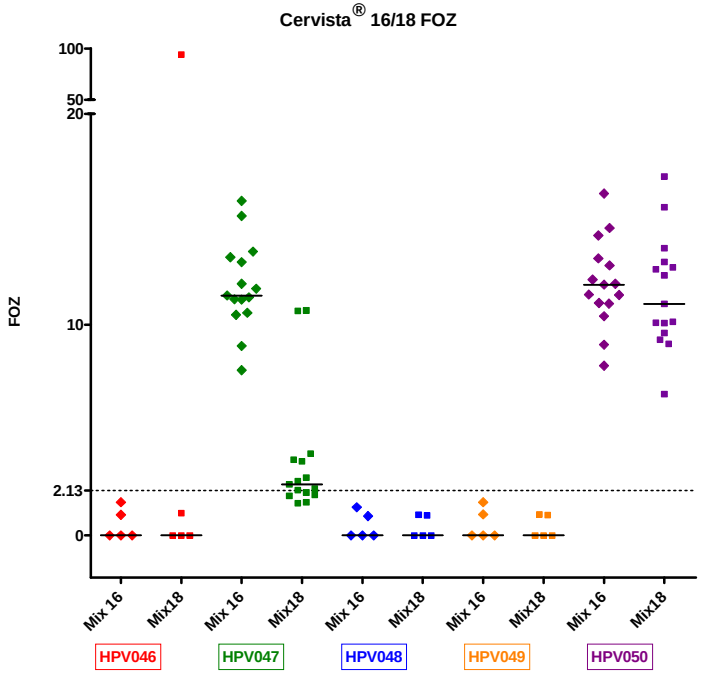
Figure 1


Hybrid Capture® II RLU/CO
RLU/CO
1500
1000
500
5
5.0
2.5
1.0
0.0
HPV046
HPV047
HPV048
HPV049
HPV050


Cervista® High Risk FOZ
FOZ
12.5
10.0
7.5
5.0
2.5
1.93
0.0
Mix 1
Mix 2
Mix 3
HPV046
HPV047
HPV048
HPV049
HPV050


Cervista® 16/18 FOZ
FOZ
100
50
20
10
2.13
0
Mix 16
Mix18
HPV046
HPV047
HPV048
HPV049
HPV050