NEW YORK
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HEALTH

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May 16, 2011

Evaluation of the New York State Human Papilloma Virus (HPV) Proficiency Test Event 3-11

March 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 March 2011. A report with your laboratory's score and grade will be sent separately to you by regular mail. Five vials (HPV041 – HPV045) containing cervical cells derived from actual patients in PreservCyt® medium were sent out to every permitted laboratory on March 29th, 2011 and the due date for returning the test results was April 18th, 2011. Each correct answer received 20 points, and an incorrect one 0 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 asked to submit those results too. In addition, we also asked for the raw data, RLU/CO from Hybrid Capture® or FOZ from Cervista®, though these were not used for grading.

A total of 71 test sets were sent out, and valid answers were received from all 71 laboratories. Forty-seven laboratories (66%) used the Hybrid Capture® method, twenty (28%) Cervista® (Invader technology), three (4%) polymerase chain reaction, and one (1%) *in situ* hybridization. Compared with the previous HPV proficiency test event, the proportion of laboratories using Hybrid Capture® slightly decreased, whereas that using Cervista® slightly increased. The small number of laboratories using PCR remained the same and one laboratory continues to use the *in-situ* hybridization method. The results are broken down by methods in Table 1. Furthermore, cytology smears were prepared and evaluated from each sample. Samples HPV041, HPV043 and HPV045 were satisfactory smears "within normal limits"; however, both HPV041 and HPV045 also contained a fungus morphologically consistent with *Candida albicans*. Sample HPV042 contained a few LGSIL cells characteristically consistent with a HPV infection. Sample HPV044 showed an occasional scattering of mildly to moderately dysplastic cells throughout the smears. The cytological diagnosis on all specimens was in agreement with the consensus results of the HPV testing.

Results

Across all methods, samples HPV041 and HPV045 achieved a high consensus result of 93.0% and 91.5% negative, respectively, and a 100% positive consensus result was achieved for both samples HPV042 and HPV044. However, when each method was evaluated separately, samples HPV041 and HPV045 were 100% negative by Cervista®, whereas the small number of positive samples was detected by either Hybrid Capture® or PCR. Both samples were found to be negative for low risk HPV genotypes by Hybrid Capture®, but one lab detected high risk genotypes 35 and 39 (in HPV041) or 35, 58 and 59 (in HPV045) in these samples (Table 2), which could possibly account for the few positive results. Thus, it is possible that there were small numbers of positive cells in these samples; however, it seems questionable whether these

[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.

would be clinically significant. In conclusion, these four samples achieved a clear consensus of HPV negative or HPV positive, respectively, and it is recommended that those laboratories whose results were against the consensus re-examine their results. A limited number of samples are available for this upon request.

One sample, HPV043, did not achieve an overall consensus result, although a plurality of 74.6% of laboratories found this sample to be negative. Again, Cervista® users provided a more consistent response of 85.0% negative with only three laboratories reporting a positive result, and thus reached a consensus that this sample was negative. In contrast, only 70.2% of the Hybrid Capture®-using laboratories found this sample to be negative, with the rest reporting either a positive (17.0%) or low positive (12.8%) result. In addition to the high risk genotypes 52 and 35 detected by genotyping, this sample was also highly positive for low risk HPV by Hybrid Capture®, which could explain the substantial proportion of positive results by this method because of the well known cross-reactivity.

Table 1. Screening Results

	HPV041	**HPV042**	**HPV043**	**HPV044**	**HPV045**
All methods					
Total	71	71	71	71	71
Negative	66	0	53	0	65
Positive	4	71	12	71	2
Low Positive	1	0	6	0	3
Indeterminate	0	0	0	0	1
% Negative	93.0%	0.0%	74.6%	0.0%	91.5%
% Positive	5.6%	100%	16.9%	100%	2.8%
% Low Positive	1.4%	0.0%	8.5%	0.0%	4.2%
% Indeterminate	0.0%	0.0%	0.0%	0.0%	1.4%
Consensus	**NEG**	**POS**	**NO CONS**	**POS**	**NEG**
Hybrid Capture®					
Total	47	47	47	47	47
Negative	44	0	33	0	44
Positive	2	47	8	47	0
Low Positive	1	0	6	0	3
% Negative	93.6%	0.0%	70.2%	0.0%	93.6%
% Positive	4.3%	100.0%	17.0%	100.0%	0.0%
% Low Positive	2.1%	0.0%	12.8%	0.0%	6.4%
Consensus	**NEG**	**POS**	**NO CONS**	**POS**	**NEG**
Cervista®					
Total	20	20	20	20	20
Negative	20	0	17	0	20
Positive	0	20	3	20	0
% Negative	100.0%	0.0%	85.0%	0.0%	100.0%
% Positive	0.0%	100.0%	15.0%	100.0%	0.0 %
Consensus	**NEG**	**POS**	**NEG**	**POS**	**NEG**

	HPV041	HPV042	HPV043	HPV044	HPV045
PCR					
Total	3	3	3	3	3
Negative	1	0	2	0	0
Positive	2	3	1	3	2
Indeterminate	0	0	0	0	1
% Negative	33.3%	0.0%	66.7%	0.0%	0.0%
% Positive	66.7%	100.0%	33.3%	100.0%	66.7%
% Indeterminate	0.0%	0.0%	0.0%	0.0%	33.3%
Consensus	**NO CONS**	**POS**	**NO CONS**	**POS**	**NO CONS**
ISH (N=1)	**NEG**	**POS**	**NEG**	**POS**	**NEG**

Table 2. Genotyping Results from 21 Laboratories

Method	HPV041	HPV042	HPV043	HPV044	HPV045
INV	N/A	16,18	NOTID	16,18	NA
INV	N/A	16,18	N/A	16,18	N/A
INV	N/A	16,18	OTHER	16,18	N/A
INV	N/A	16,18	N/A	16,18	N/A
INV	N/A	16,18	N/A	16,18	N/A
INV	N/A	16	N/A	16	N/A
INV	N/A	16,18	N/A	16,18	N/A
INV	N/A	16,18	N/A	16,18	N/A
INV	N/A	16,18	N/A	16,18	N/A
INV	N/A	16,18	N/A	16,18	N/A
INV	N/A	16,18	N/A	16,18	N/A
INV	N/A	16,18	N/A	16,18	N/A
INV	N/A	16,18	N/A	16,18	N/A
INV	N/A	16,18	N/A	16,18	N/A
PCR	N/A	16,31,35,39,45,51,56,59	52	16,18,31,39,45,51,52,56	N/A
PCR	N/A	16,18,31,33,45,35/68, 39/56,51/59	N/A	16,18,31,45,35/68,39/56, 51/59,52/58	N/A
PCR	N/A	6/11,16,18/45, 31/33/35/39	6/11	16,18/45,31/33/35/39	NOTID
PCR	N/A	16,18,51,52,68	N/A	16,18,51,52,68	N/A
PCR	35,39	16,18,31,33,35,45,51,52, 56,58,66,68	35,52	16,18,31,33,35,39,45,51,5 2,56,58,59,66,68	35,58,59
RFLP	N/A	16,31,72	N/A	16,31,61	N/A
RFLP	84,72, unk	16,11,18,cp141	6	84,18,58,53	53,84,6

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

Genotyping

Laboratories that routinely determine HPV genotypes were also asked to submit those results ("genotyping"). Twenty-one laboratories did genotyping using variable methodologies. Fourteen (66%) used the Cervista® 16/18, and seven used a PCR based method (Table 2). Since not every method detects every genotype and because the samples represent a mixture of patient samples it is understandable that the results were somewhat divergent, and therefore, the genotyping results were not graded. Nevertheless, in sample HPV042 all the laboratories reported the presence of the high-risk type HPV16, and all but three also found HPV18. Of the three laboratories that did not detect the high-risk genotype HPV18 in this sample, two used a PCR based method, whereas one used Cervista®. The data obtained for the other positive sample, HPV044, showed that 1/21 laboratory did not detect the presence of HPV16, and 2/21 other laboratories failed to identify the high-risk HPV18 genotype in this sample. The same laboratories also failed to detect HPV18 in sample HPV042. These laboratories may want to re-examine their results. Overall, there was fairly good agreement for samples HPV042 and HPV044 in regards to the presence of other high-risk genotypes among those laboratories that employ a more comprehensive panel of detection reagents. It is interesting to note that for HPV043 two laboratories reported the presence of low risk HPV11 and/or 6, and two other laboratories detected the high risk genotypes 35 and/or 52, which, as mentioned above, may explain the substantial number of screen positive results for this sample, whether as a result of cross reactivity of the Hybrid Capture® method with the low risk genotypes, and/or the direct detection of the high risk genotypes.

Raw data

Figure 1 shows scatter plots of the raw data from Hybrid Capture® and Cervista®. Though neither of these assays is truly quantitative, the relatively wide scatter of the data is nevertheless somewhat surprising and suggests a fair amount of laboratory-to-laboratory variation in the signal output. Since this is the first time that we collected and analyzed these data we did not attempt to interpret them further. However, we will continue to collect these data.

Conclusions

Overall, there was good agreement among the laboratories for four of the five samples, and the results were consistent with the cytologic features of the samples. For sample HPV043 the results suggest that it is difficult to assess borderline samples unequivocally and that such samples may require special attention and/or frequent follow-up.

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 **failure** and put your lab at risk for an unsuccessful performance.

The schedule for the upcoming 2011 New York State HPV proficiency tests is:

Event	Mail-out Date	Due Date
7-11	July 12	August 1
10-11	October 18	November 7

For questions, comments, or suggestions, call or e-mail:

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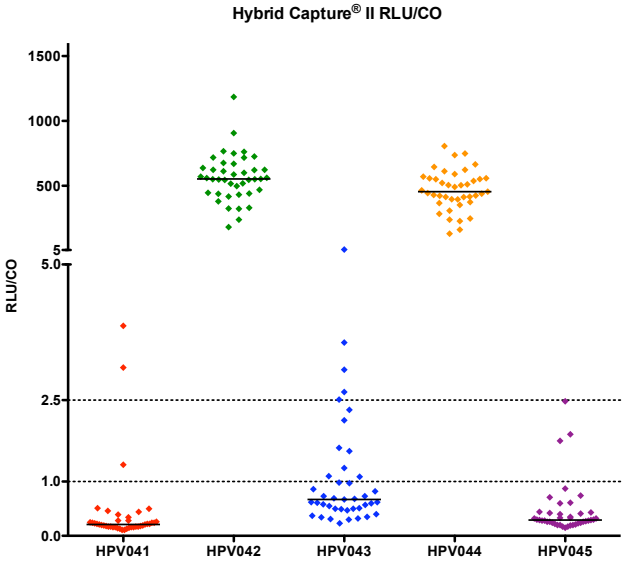
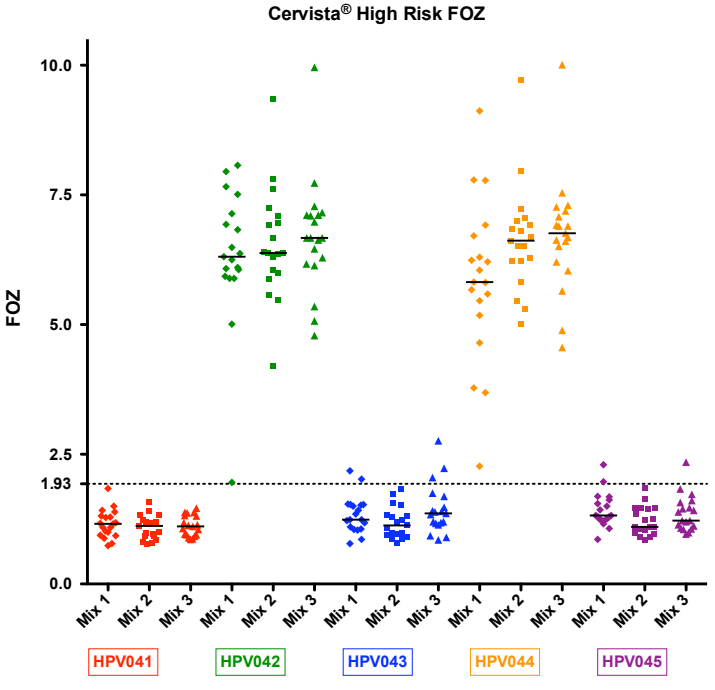
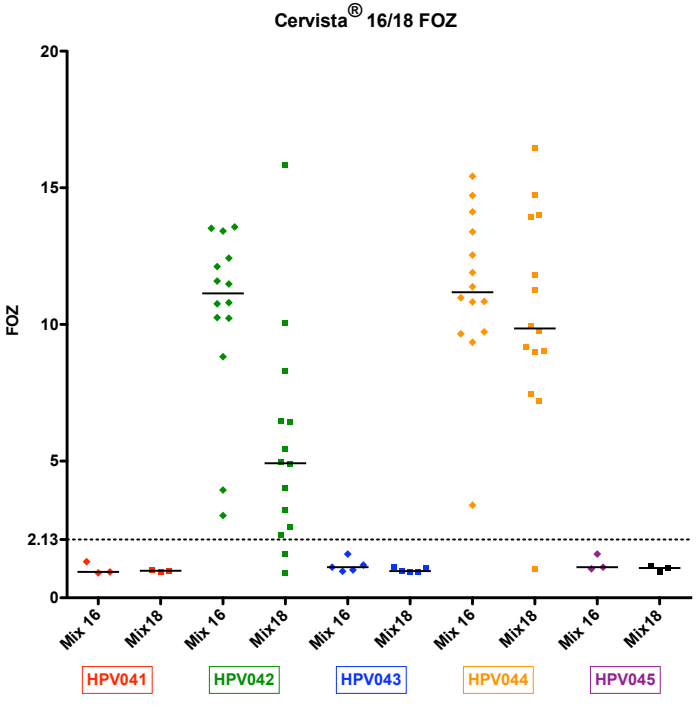
Figure 1


Hybrid Capture® II RLU/CO
RLU/CO
1500
1000
500
5
5.0
2.5
1.0
0.0
HPV041
HPV042
HPV043
HPV044
HPV045


Cervista® High Risk FOZ
FOZ
10.0
7.5
5.0
2.5
1.93
0.0
Mix 1
Mix 2
Mix 3
HPV041
HPV042
HPV043
HPV044
HPV045


Cervista® 16/18 FOZ
FOZ
20
15
10
5
2.13
0
Mix 16
Mix18
HPV041
HPV042
HPV043
HPV044
HPV045