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HEALTH

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Commissioner

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Executive Deputy Commissioner

December 2, 2011

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

October 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 October 2011. A report with your laboratory's score and grade will be sent separately to you by regular mail. Five vials (HPV051 – HPV055) containing cervical cells derived from actual patients in PreservCyt® medium were sent out to every permitted laboratory on October 18th, 2011, and the due date for submitting the test results was November 7th, 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 72 test sets were sent out, and valid answers were received from 71 laboratories by the due date. For screening, 42 laboratories (59%) used the Hybrid Capture® method, 21 laboratories (30%) used the Cervista® method, 4 laboratories (6%) reported results from both methods, 3 laboratories (4%) used the polymerase chain reaction method, and 1 laboratory (1%) used the in-situ-hybridization method. The screening results are summarized in Table 1.

Thin prep slides were prepared and evaluated in our laboratory from each of the test samples. Slides from samples HPV051, HPV052, HPV054 and HPV055 all presented with Candida albicans on the smears. Slide HPV051 showed cells with "Reactive Cellular Changes" possibly due to the presence of the fungal infection which was noted on the smear. Sample HPV052 was interpreted as "Within Normal Limits" (WNL) with areas of cytologic changes consistent with herpes virus infection. The HPV positive samples HPV053 and HPV055 both contained "atypical squamous cells of undetermined significance" (ASCUS), and in addition, slide HPV053 showed "clue cells" consistent with Bacterial vaginosis. Finally, sample HPV054, also HPV positive, was diagnosed as a low-grade squamous intraepithelial lesion (LGSIL). All the cytological diagnoses were in agreement with the HPV consensus results from this proficiency test.

[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

Regardless of the methods used, the consensus was in general excellent, 369/375 (98.4%) overall, with the exception of two samples, HPV051 and HPV054, for which no consensus was reached by the PCR method of screening (N=3). Among the 230 responses obtained by the Hybrid Capture® method, all but two low positive responses (<1%) for sample HPV052 were in agreement with the consensus results. The results for the Cervista® method were similar with 1/125 responses discrepant, a single negative result instead of the consensus positive for sample HPV055. The 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.

The small number of laboratories using PCR and the lack of standardization among these assays makes it difficult to interpret the overall poor consensus by this method. However, the positive result for HPV051 seems to be based on the more extensive number of HPV genotypes detected that are considered high-risk. In contrast, it is interesting to note that the sole positive PCR-derived result for the positive HPV054 sample came from a laboratory that used the recently FDA cleared Roche Cobas 4800® method.

Table 1. Screening results, 72 laboratories:

	HPV051	**HPV052**	**HPV053**	**HPV054**	**HPV055**
All methods					
Total	75	75	75	75	75
Negative	74	73	0	2	1
Positive	1	0	75	73	74
Low Positive	0	2	0	0	0
% Negative	98.7%	97.3%	0.0%	2.7%	1.3 %
% Positive	1.3%	0.0%	100.0%	97.3 %	98.7 %
% Low Positive	0.0%	2.7%	0.0 %	0.0 %	0.0 %
Consensus	**NEG**	**NEG**	**POS**	**POS**	**POS**

	HPV051	**HPV052**	**HPV053**	**HPV054**	**HPV055**
Hybrid Capture					
Total	46	46	46	46	46
Negative	46	44	0	0	0
Positive	0	0	46	46	46
Low Positive	0	2	0	0	0
% Negative	100.0%	95.7 %	0.0 %	0.0 %	0.0%
% Positive	0.0 %	0.0 %	100.0 %	100.0%	100.0 %
% Low Positive	0.0 %	4.3 %	0.0 %	0.0 %	0.0 %
Consensus	**NEG**	**NEG**	**POS**	**POS**	**POS**

	HPV051	HPV052	HPV053	HPV054	HPV055
Cervista					
Total	25	25	25	25	25
Negative	25	25	0	0	1
Positive	0	0	25	25	24
% Negative	100.0%	100.0%%	0.0%	0.0%	4.0 %
% Positive	0.0 %	0.0%	100.0%	100.0%	96.0%
Consensus	**NEG**	**NEG**	**POS**	**POS**	**POS**

	HPV051	HPV052	HPV053	HPV054	HPV055
PCR					
Total	3	3	3	3	3
Negative	2	3	0	2	0
Positive	1	0	3	1	3
% Negative	66.7 %	100%	0.0%	66.7%	0.0%
% Positive	33.3 %	0.0 %	100.0 %	33.3 %	100.0 %
Consensus	**NO CONS**	**NEG**	**NEG**	**NO CONS**	**POS**

	HPV051	HPV052	HPV053	HPV054	HPV055
ISH (N=1)	**NEG**	**NEG**	**POS**	**POS**	**POS**

Genotyping

Laboratories that routinely determine HPV genotypes were also asked to submit those results ("genotyping"). Twenty-one laboratories did genotyping using variable methodologies. Sixteen laboratories (76%) used the Cervista®16/18 method, two (10%) used a PCR based methodology, two (10%) used PCR followed by RFLP and one laboratory (4%) used a Hybrid Capture® method (Table 2).

As expected, the carcinogenic types 16 and 18 were most frequently observed in the positive samples. However, there appears to be some inconsistency between the reported genotypes and the actual data. For sample HPV054, six laboratories that used the Cervista® genotyping method reported the presence of either HPV16 or HPV18. However, a look at the raw data (Figure 1C) shows that no laboratory found a FOZ ratio above the 2.13 threshold given by the manufacturer as the cut-off point for calling a sample positive. Thus, it is unclear how these laboratories arrived at their genotype call. Indeed, the results from the two PCR laboratories suggest that this sample may have been primarily positive for HPV031, a probe for which is included in the Cervista® screening mix 3 that gave the highest FOZ ratio (Figure 1B). Laboratories are reminded that they must report what they actually find, not what they test for. For samples HPV055 and HPV053 a substantial number of laboratories, 33% and 14% respectively, did not detect HPV18, but otherwise there was good consensus that these two samples contained both of the major high-risk genotypes HPV16 and HPV18. Table 2 summarizes the genotyping results.

Table 2. Genotyping results, 21 laboratories:

Method	HPV51	HPV052	HPV053	HPV054	HPV055
INV	N/A	N/A	16,18	NOT ID	16,18
INV	N/A	N/A	16,18	16 or 18	16,18
INV	N/A	N/A	16,18	16 or 18	16,18
INV	N/A	N/A	16,18	NOT ID	16,18
INV	N/A	N/A	16,18	NOT ID	16,18
INV	N/A	N/A	16,18	16 or 18	16,18
INV	N/A	N/A	16,18	NOT ID	16
INV	N/A	N/A	16,18	NOT ID	16,18
INV	N/A	N/A	16,18	16 or 18	16
INV	N/A	N/A	16,18	N/A	16,18
INV	N/A	N/A	16,18	NOT ID	16
INV	N/A	N/A	16,18	16 or 18	16
INV	N/A	N/A	16,18	NOT ID	16
INV	N/A	N/A	16,18	NOT ID	16,18
INV	N/A	N/A	16,18	16 or 18	16
INV	N/A	N/A	16,18	NOT ID	16,18
PCR	Weak reactive	6/11	6/11,16, 18,31	31	6/11,16,18, 31
PCR	N/A	N/A	16,31, 58,68	31,51	16,18,31, 51,59,68
RFLP	84,53,6	11	18,53,58, 16,6,ukn.	LVX160	18,58,16,61
RFLP	N/A	N/A	6,16, 66,ukn.	LVX160,ukn.	16,31,52
HYC	N/A	N/A	16	NOT ID	16,18

HYC = Hybrid Capture®, INV = Cervista®, N/A = not applicable, PCR = polymerase chain reaction, RFLP = PCR followed by restriction fragment length polymorphism determination, ukn. = unknown, NOT ID = Not identifiable by the method used

Raw data

Figures 1 A-C show a graphical representation of the raw data, some of which have already been discussed above. Despite the fact that the data are not strictly quantitative in nature, it is evident that there is a large spread of the analytical values that have been obtained. Somewhat of a concern are those values that are clearly below the respective cut–off points for a positive call in otherwise clearly positive samples, especially with the Cervista® method. However, because of the mixed nature of these samples this did not result in a false overall call.

Nevertheless, we suggest that the laboratories in question re-examine their analytical procedure.

Conclusions

In general, there was high consensus 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 2012 New York State HPV proficiency tests:

Mail-out Date	Due Date
April 17	May 7
October 16	November 5

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

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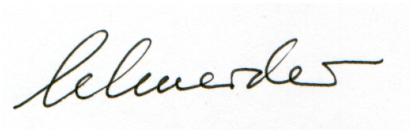

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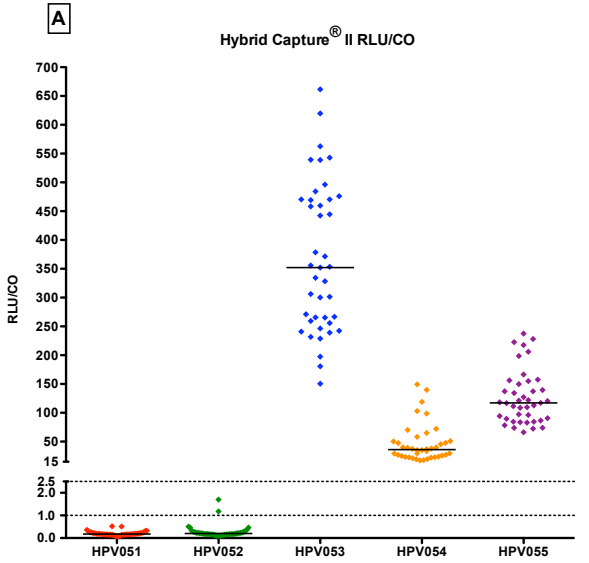
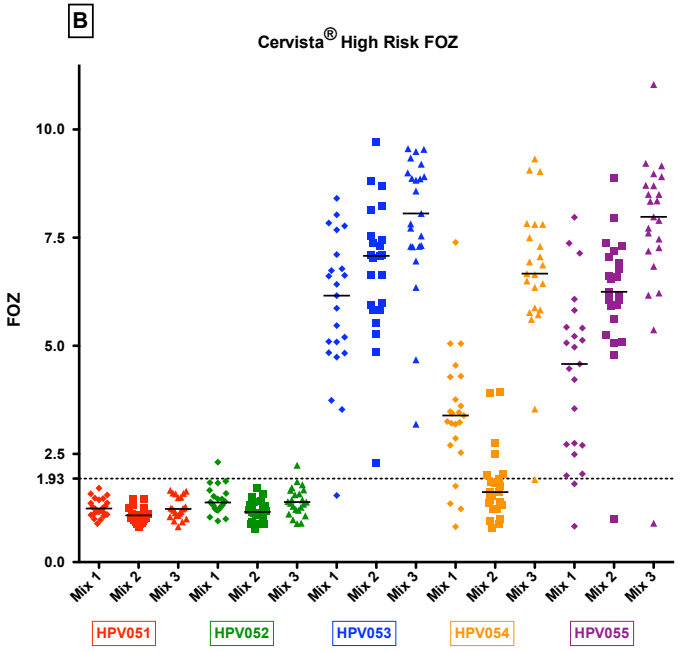
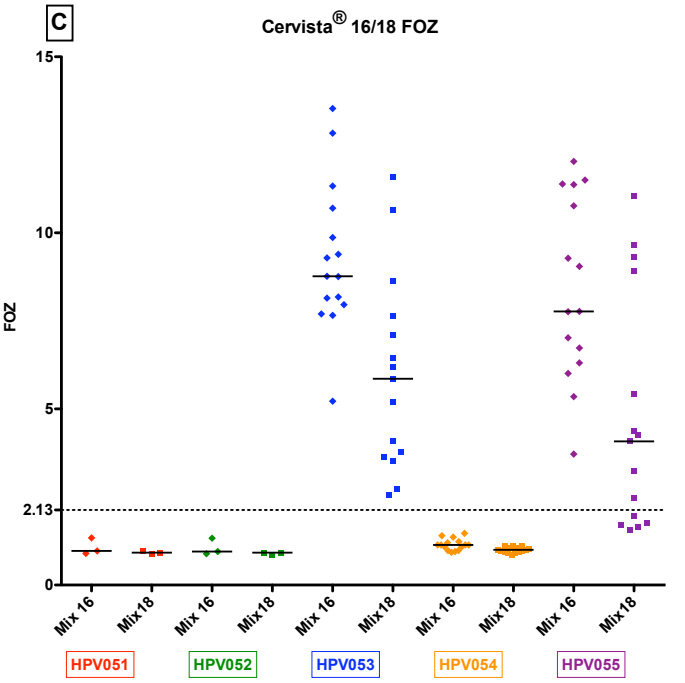
Figure 1


A
Hybrid Capture® II RLU/CO
RLU/CO
700
650
600
550
500
450
400
350
300
250
200
150
100
50
15
2.5
2.0
1.0
0.0
HPV051
HPV052
HPV053
HPV054
HPV055


B
Cervista® High Risk FOZ
FOZ
10.0
7.5
5.0
2.5
1.93
0.0
Mix 1
Mix 2
Mix 3
HPV051
HPV052
HPV053
HPV054
HPV055


C
Cervista® 16/18 FOZ
FOZ
15
10
5
2.13
0
Mix 16
Mix18
HPV051
HPV052
HPV053
HPV054
HPV055