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HEALTH

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Evaluation of the New York State Human Papilloma Virus (HPV) Proficiency Test October 2013[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 2013. A report with your laboratory's score and grade will be sent separately to you by regular mail. Five vials (HPV071 – HPV075) containing cervical cells derived from actual patients in PreservCyt® medium were sent out to every permitted laboratory on October 15th, 2013, and the due date for submitting the test results was November 4th, 2013. 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®, FOZ values from Cervista®, or CT values from the Roche Cobas®4800 method, though this information was not used for grading. In the future, we will also ask for the raw data to be provided from the laboratories that use other instruments.

A total of 75 laboratories received samples, and 79 valid answers were submitted from 72 laboratories by the due date. For screening, 30 laboratories (38%) used the Hybrid Capture® method, 21 laboratories (26.5%) used the Cervista® method, 17 laboratories (21.5%) used a polymerase chain reaction based method (12 Cobas®4800 and 5 Laboratory Developed Tests) and 11 laboratories (14%) used the Aptima® method (9 laboratories used the Tigris instrument and 2 laboratories used the Panther System).

Thin prep slides were prepared and evaluated in our laboratory from each of the test samples. Positive test samples HPV071, HPV072 and HPV074 all contained mildly dysplastic cells and were diagnosed as "Satisfactory for evaluation" with "Epithelial cell abnormalities consistent with LSIL" (Low-grade squamous intraepithelial lesion). The consensus negative sample HPV075 was diagnosed "Satisfactory", "Negative for intraepithelial lesion" (NILM). Slides from test sample HPV073 presented with cells consistent with reactive changes. Reactive cellular changes are benign in nature and can be associated with inflammation or other nonspecific causes. This case was diagnosed as "Satisfactory", "Negative for intraepithelial lesion (NILM) with Reactive changes". The cytological diagnoses were in agreement with the HPV consensus results with the exception of Sample HPV073 for which a no consensus result for HPV was obtained.

[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 Sample HPV073, a high overall consensus of >= 97.5% was achieved for all samples across all methods (Table 1). In contrast, the results for sample HPV073 were somewhat inconsistent and only achieved an overall majority of 64.6% negative. Laboratories using the Hybrid Capture® (10/30) and the Cobas®4800 (12/12) methods reported a high proportion of positive results for this sample, whereas a larger majority of results obtained by the Cervista® (17/21) and Aptima methods (10/11), respectively, was negative. While the exact reasons for this discrepancy are difficult to establish, it suggests that the various methods have different analytical sensitivities, especially against some of the rarer genotypes that may have been present in this sample (see below). Note: because of the inconsistent results, this sample was not graded and each laboratory received automatic credit. Sample HPV071 achieved an overall consensus of 97.5% (77/79) positive; however, both the Cervista® (1/21) and Aptima (1/11) methods produced a single discrepant negative response for this sample and those laboratories should re-examine their results. For sample HPV075, 100% consensus negative results were achieved by all the methodologies with the exception of the Hybrid Capture® method (1/30), which produced a single discrepant positive response. Unanimous (100%) positive results for samples HPV072 and HPV074 were achieved across all methods. For laboratories whose results did not match the consensus results and who would like to re-examine their results a limited number of samples are available for retest upon request

Table 1. Screening results, 72 laboratories, 79 results submitted:

	HPV071	HPV072	HPV073	HPV074	HPV075
All methods					
Total	79	79	79	79	79
Negative	2	0	51	0	78
Positive	77	79	25	79	1
Low Positive	0	0	2	0	0
Indeterminate	0	0	1	0	0
% Negative	2.5%	0.0%	64.6%	0.0%	98.7%
% Positive	97.5%	100.0%	31.6%	100 %	1.3%
% Low Positive	0.0%	0.0%	2.5 %	0.0 %	0.0 %
% Indeterminate	0.0%	0.0%	1.3%	0.0%	0.0%
Consensus	**POS**	**POS**	**NO CONS**	**POS**	**NEG**

	HPV071	HPV072	HPV073	HPV074	HPV075
Hybrid Capture®					
Total	30	30	30	30	30
Negative	0	0	19	0	29
Positive	30	30	8	30	1
Low Positive	0	0	2	0	0
Indeterminate	0	0	1	0	0
% Negative	0.0%	0.0%	63.3%	0.0%	96.7%
% Positive	100.0%	100.0%	26.7%	100.0%	3.3%
% Low Positive	0.0%	0.0%	6.7%	0.0%	0.0%
% Indeterminate	0.0%	0.0%	3.3%	0.0%	0.0%
Consensus	**POS**	**POS**	**NO CONS**	**POS**	**NEG**

Table 1 continued:

	HPV071	HPV072	HPV073	HPV074	HPV075
Cervista®					
Total	21	21	21	21	21
Negative	1	0	17	0	21
Positive	20	21	4	21	0
% Negative	4.8%	0.0%	81.0%	0.0%	100.0%
% Positive	95.2%	100.0%	19.0%	100.0%	0.0%
Consensus	**POS**	**POS**	**NEG**	**POS**	**NEG**

	HPV071	HPV072	HPV073	HPV074	HPV075
Cobas® 4800					
Total	12	12	12	12	12
Negative	0	0	0	0	12
Positive	12	12	12	12	0
% Negative	0.0%	0.0%	0.0%	0.0%	100.0%
% Positive	100.0%	100.0%	100.0%	100.0%	0.0%
Consensus	**POS**	**POS**	**POS**	**POS**	**NEG**

	HPV071	HPV072	HPV073	HPV074	HPV075
PCR					
Total	5	5	5	5	5
Negative	0	0	5	0	5
Positive	5	5	0	5	0
% Negative	0.0%	0.0%	100.0%	0.0%	100.0%
% Positive	100.0%	100.0%	0.0%	100.0%	0.0%
Consensus	**POS**	**POS**	**NEG**	**POS**	**NEG**

	HPV071	HPV072	HPV073	HPV074	HPV075
APTIMA					
Total	11	11	11	11	11
Negative	1	0	10	0	11
Positive	10	11	1	11	0
% Negative	9.1%	0.0%	90.9%	0.0%	100.0%
% Positive	90.9%	100.0%	9.1%	100.0%	0.0%
Consensus	**POS**	**POS**	**NEG**	**POS**	**NEG**

Genotyping

Laboratories that routinely determine HPV genotypes were also asked to submit those results. Thirty-three (46%) laboratories did genotyping using various methodologies. Of those, thirteen (39.4%) laboratories each used the Cervista®16/18 or the Cobas® 4800 methods, respectively, four laboratories (12.1%) used the Aptima method and three laboratories (9.1%) used a laboratory developed PCR based method, which one laboratory followed with RFLP and one laboratory followed with Bio-Plex Analysis. Since not every method detects every genotype and because the samples represent a mixture of patient samples, the genotyping results were not graded (Table 2).

Genotyping results for consensus screen positive samples HPV072 and HPV074 showed that most of the laboratories were in agreement that both the high-risk HPV genotypes 16 and 18 along with other high-risk genotypes were present in these samples. In contrast, for sample HPV071 the results were mixed. Whereas all but two laboratories (94%) agreed that this sample contained HPV16, 11/13 laboratories using the Cervista® method and 2/4 laboratories using the Aptima® method did not also detect HPV 18. Overall, 42% detected only HPV 16, 6% did not detect HPV 16, and 52% detected a mixture of HPV16, 18 plus other high-risk genotypes. Interestingly, sample HPV073 did not appear to contain any of the high-risk genotypes 16, 18 and/or 45, yet was found screen positive by 35% of the laboratories, with substantial discrepancies between the methods, as discussed above. For consensus negative sample HPV075, one laboratory submitted a high-risk genotype "NOT 16, 18" response and one PCR laboratory submitted the low-risk genotype 11 as their response. As usual, the laboratories that use a Laboratory Developed Test by PCR were able to detect multiple genotypes including the intermediate-risk genotypes. The results are summarized in Table 3.

Note for Cobas®4800 users: if a sample is positive in all three channels you must use "16, 18 PLUS OTHER HR" from the drop down menu.

Table 2. Genotyping results, 33 laboratories:

Method	HPV071	HPV072	HPV073	HPV074	HPV075
INV	16	16,18	HR NOT 16,18	16,18	HR NOT 16,18
INV	16 OR 18	16,18	N/A	16,18	N/A
INV	16	16,18	N/A	16,18	N/A
INV	16	16,18	N/A	16,18	N/A
INV	16	16,18	HR NOT 16,18	16	N/A
INV	16	16,18	N/A	16,18	N/A
INV	16	16,18	N/A	16,18	N/A
INV	16,18	16,18	N/A	16,18	N/A
INV	16	16,18	N/A	16,18	N/A
INV	16	16,18	N/A	16,18	N/A
INV	16	16,18	N/A	16,18	N/A
INV	16	16,18	N/A	16	N/A
INV	16	16,18	N/A	16,18	N/A
Cobas 4800	16,18	16,18	HR NOT 16,18	16,18	N/A
Cobas 4800	16,18	16,18	HR NOT 16,18	16,18	N/A
Cobas 4800	16,18 PLUS OTHER HR	16,18 PLUS OTHER HR	HR NOT 16,18	16,18 PLUS OTHER HR	N/A
Cobas 4800	16,18	16,18	HR NOT 16,18	16,18	N/A
Cobas 4800	16,18 PLUS OTHER HR	16,18 PLUS OTHER HR	HR NOT 16,18	16,18 PLUS OTHER HR	N/A
Cobas 4800	16,18 PLUS OTHER HR	16,18 PLUS OTHER HR	HR NOT 16,18	16,18 PLUS OTHER HR	N/A
Cobas 4800	16,18 PLUS OTHER HR	16,18 PLUS OTHER HR	HR NOT 16,18	16,18 PLUS OTHER HR	N/A
Cobas 4800	16,18 PLUS OTHER HR	16,18 PLUS OTHER HR	HR NOT 16,18	16,18 PLUS OTHER HR	N/A
Cobas 4800	16,18	16,18	HR NOT 16,18	16,18	N/A
Cobas 4800	16,18	16,18	HR NOT 16,18	16,18	NA
Cobas 4800	16,18 PLUS OTHER HR	16,18 PLUS OTHER HR	NA	16,18 PLUS OTHER HR	NA
Cobas 4800	16,18	16,18	HR NOT 16,18	16,18	NA
Cobas 4800	16,18	16,18	HR NOT 16,18	16,18	NA

APTIMA	16	16	NA	16	NA
APTIMA	16, 18/45	16, 18/45	POS NOT ID	16	NA
APTIMA	16	16, 18	N/A	16	NA
APTIMA	18/45	16	NA	16	NA
PCR	31,51,56, 68	16,18,31,51,56,58, 59	N/A	16,18, 31, 51,56	NA
RFLP	16, 31,61	16,82	N/A	16, 31, 61	11
Bio-Plex Analysis	16	16,18	NA	16	NA

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

Table 3. Summary of genotyping results:

	HPV071	**HPV072**	**HPV073**	**HPV074**	**HPV075**
Genotyping results					
HPV 16	14	2	0	7	0
HPV 16 ,18	8	22	0	18	0
HPV 16 or 18	1	0	0	0	0
HPV16 ,18/45	1	1	0	0	0
HPV 18/45	1	0	0	0	0
HPV16,18 PLUS OTHER HR	6	6	0	6	0
HR NOT 16,18	0	0	14	0	1
HPV 11 (LR)	0	0	0	0	1
N/A	0	0	18	0	31
Other	2	2	1	2	0
Total	**33**	**33**	**33**	**33**	**33**

HR- High Risk, LR-Low Risk, N/A = not applicable

Raw data

Figure 1 shows the graphical distribution of the raw data from the different instruments.

Conclusions

The overall results of this HPV proficiency test were satisfactory. Two of the five samples, HPV072 and HPV074, were unanimously positive across all methods. Sample HPV073 produced variable responses depending on the method used, which resulted in a non-consensus result, probably due to the presence of a high-risk genotype(s) other than 16, 18 and/or 45 in the sample. The consensus positive sample HPV071 produced two discrepant answers, one by the Cervista® method and the other by the Aptima® method, but no consensus as to the genotypes present, and sample HPV075 produced one discrepant positive answer by the Hybrid Capture® method. Together, these results indicate that there is good concordance across screening methods when the major high risk genotypes are present, but raise the question of how well some methods detect the rarer high-risk genotypes as well as how well the individual genotypes are identified.

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 next 2014 New York State HPV proficiency tests:

Mail-out Date	**Due Date**
April 14	May 5
October 21	November 10

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

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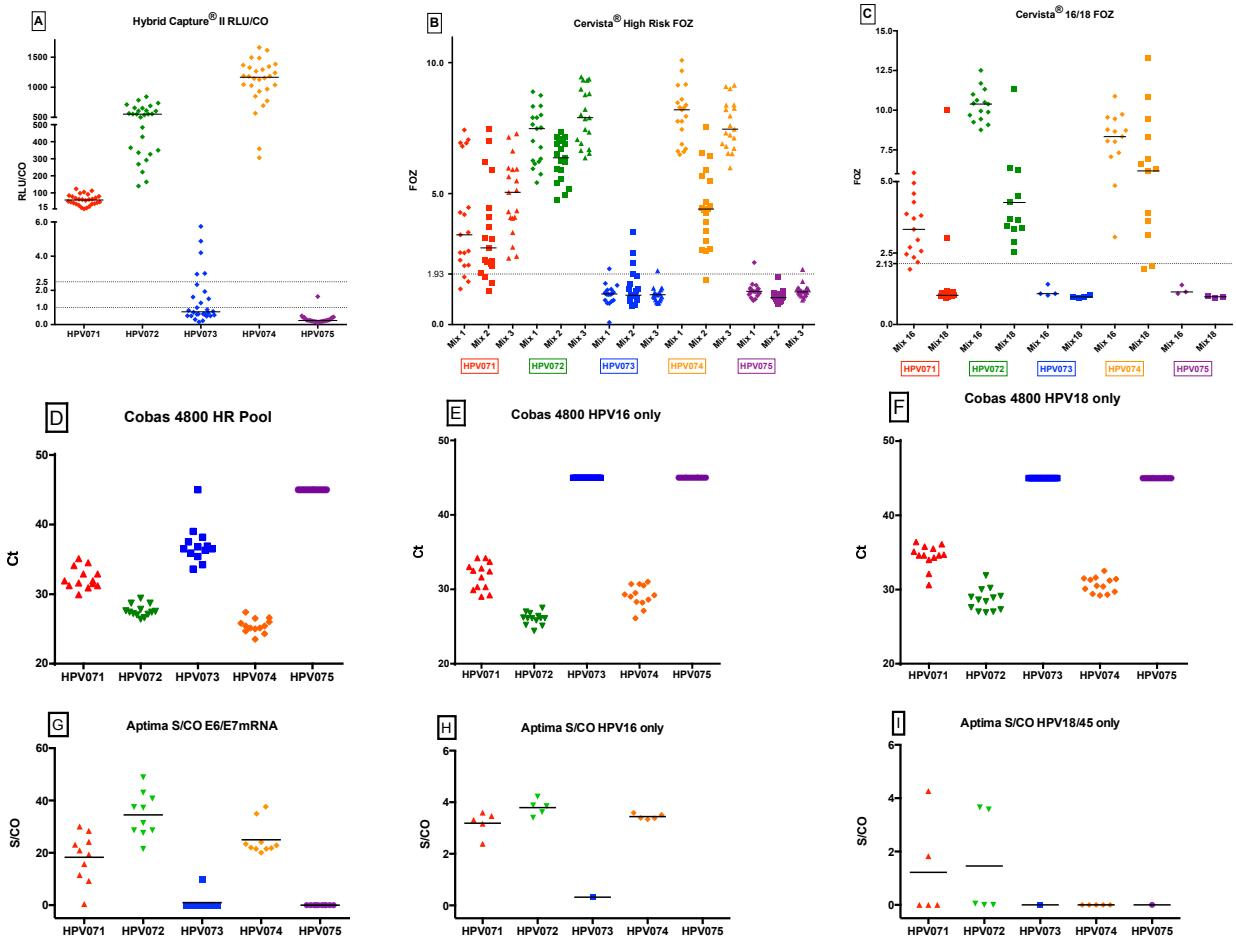
Figure 1


A
Hybrid Capture® II RLU/CO
RLU/CO
HPV071
HPV072
HPV073
HPV074
HPV075
B
Cervista® High Risk FOZ
FOZ
1.93
Mix 1
Mix 2
Mix 3
C
Cervista® 16/18 FOZ
FOZ
2.13
Mix 16
Mix 18
D
Cobas 4800 HR Pool
Ct
E
Cobas 4800 HPV16 only
Ct
F
Cobas 4800 HPV18 only
Ct
G
Aptima S/CO E6/E7mRNA
S/CO
H
Aptima S/CO HPV16 only
S/CO
I
Aptima S/CO HPV18/45 only
S/CO