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December 4, 2015

Evaluation of the New York State Human Papilloma Virus (HPV) Proficiency Test from October 2015

Dear Laboratory Director:

Here is the summary and evaluation of the New York State Proficiency Test for human papilloma virus (HPV) from October 2015. A report with your laboratory's score and grade will be sent separately to you by regular mail. Five vials (HPV091 – HPV095) containing cervical cells derived from actual patients in PreservCyt® medium were sent out to every permitted laboratory on October 13th, 2015, and the due date for submitting the test results was November 2nd, 2015. 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. **Please note:** only samples that tested positive for one or more of the known or suspected high risk genotypes should have been reported as screen positive. Laboratories that perform genotyping were also asked to provide those results. In addition, we asked that you include the raw data with your results, i.e. RLU/CO values from Hybrid Capture®, FOZ values from Cervista®, Ct values from the Roche Cobas®4800 method, or S/CO ratios from the Aptima® methodology, though this information was not used for grading.

Thin prep slides were prepared and evaluated in-house from each of the five test samples. Samples HPV091, HPV092, HPV093 and HPV095 were diagnosed by cytology as "Satisfactory for evaluation", "Negative for intraepithelial lesion or malignancy (NILM)". However, sample HPV091 did contain cells showing reactive cellular changes, and therefore was diagnosed as "NILM with Reactive Cellular Changes". Sample HPV094 was evaluated as "Satisfactory for evaluation" with "Low-grade squamous intra-epithelial lesion (LSIL)" with cells showing evidence of koilocytosis. The cytology evaluations for samples HPV092, HPV094 and HPV 095 were consistent with the consensus or majority HPV screening results. In contrast, samples HPV091 and HPV093, were negative by cytology, but HPV screen positive by majority or consensus, respectively (see below).

Screening Results (Tables 1a,b)

A total of 82 laboratories received samples, and 82 valid answers were submitted by the due date. Eight laboratories (9.8%) used the Hybrid Capture® method, 8 laboratories (9.8%) used the Cervista® method, 28 laboratories (34.1%) used a polymerase chain reaction based method (24 Cobas®4800 and 4 a Laboratory Developed Test) and 38 laboratories (46.9%) used the

Aptima® method (12 laboratories used the Tigris instrument and 26 laboratories used the Panther instrument) .

The results of this last HPV proficiency testing event proved to be interesting in that two of the NILM samples did not produce an all laboratory consensus, one cytology-negative sample was HPV screen positive and there were clear method specific discrepancies across all samples. Sample **HPV094** was the only sample that turned out a unanimous screening result that was 100% positive and matched the corresponding abnormal (LSIL) Pap cytology. For sample **HPV093**, although overall 89% consensus positive, nine discrepant negative answers were submitted (11%), seven of which were from Aptima® assays (7/38), one from a Cervista® assay (1/8) and one from a LDT PCR assay (1/4). Interestingly, this sample was considered NILM by cytology, and thus there is a clear discrepancy between the HPV screening and cytology assessment.

The results for the borderline consensus negative (80.5%) sample **HPV095** showed 16 discrepant positive answers, of which two were from the Aptima® methodology (2/38), one from the Hybrid Capture® methodology (1/8), and 13 from PCR-based methodologies (11/24 from Roche Cobas® and 2/4 from LDT PCR) resulting in a non-consensus outcome for the latter two methods. This sample was classified as NILM by cytology, and the results from the Aptima®, Hybrid Capture® and Cervista® methods all agreed that it was HPV negative. In contrast, the results from the PCR-based methods (Cobas 4800 and LDT) were almost evenly divided between screen positive (13/28) and screen negative (15/28). These results suggest that the PCR-based methods may be calibrated for a somewhat higher sensitivity that may cause borderline samples to be called positive. Indeed, the Ct values derived from Roche Cobas® from those laboratories that called this sample positive ranged from 38.2-40.0 for the pooled channel, and 38.9-40.5 for the HPV16 channel (no laboratory returned a positive result for the HPV18 channel). These values are close to the cut-off of 40.0 and 40.5, respectively, above which a result is considered negative.

Finally, samples **HPV091** and **HPV092** both ended in a non-consensus result across all methods. Sample HPV091 produced a majority (62.2%) positive result, whereas sample HPV092 produced a majority (65.9%) negative result, each with a small number (4.9%) of low positives from Hybrid Capture®. However, in both samples the positive/negative distribution wasn't random, but rather showed some method specificity. Sample HPV091 was classified as consensus positive (94.7%) by Aptima®, whereas results from Cervista® and LDT-PCR were 87.5% and 100% consensus negative, repectively. In contrast, both Roche Cobas® and Hybrid Capture® were almost evenly split between positive and negative, although the positive results from Hybrid Capture® were all in the low positive range that could become negative or positive upon retesting. Sample HPV091 was considered "NILM with Reactive Cellular Changes" by cytology, which raises the question whether the cells exhibiting the reactive cellular changes were low level HPV positive that caused the discrepant positive results, especially from Aptima® that in the other four samples seemed to have a slight preponderance for returning negative results compared to some of the other methods.

"NILM" sample HPV092 also achieved a majority but non-consensus overall outcome across all methods. The Aptima®, Cervista® and LDT PCR methodologies produced a consensus negative response, while the results from Roche Cobas® were consensus positive (83.3%) and those from Hybrid Capture® were 25% positive and 25% negative, with the rest considered low positive that could go either way upon retesting. However, in contrast to sample HPV091, most of the positive results were from Roche Cobas®, whereas all but two of the results from Aptima® were negative.

Overall, it appears that Roche Cobas® tended towards classifying more of the cytologically normal samples as HPV positive, whereas, with the exception of HPV091, Aptima® tended towards a negative classification of those, suggesting a somewhat lower sensitivity, though possibly at a higher specificity. A similar conclusion was also reached by Cuzik et al, BJC 108, 908; 2013, who concluded that "*Positivity rates in cytology-negative specimens were similar for the DNA-based tests, but lower for the APTIMA test suggesting this maintains the high sensitivity of DNA tests, but with better specificity*." The discrepancy between the negative by cytology samples and the high-risk HPV positive results in these may demonstrate a good example of well documented studies that have shown that the sensitivity of the Pap smear is low for the detection of pre-cancerous lesions and lends support to the data from co-testing studies which increases the predictive value of the Pap cytology by detecting women who could possibly be at high risk for developing cervical cancer in the future.

Table 1a: Screening results, all methods combined (82 laboratories)

	HPV091	**HPV092**	**HPV093**	**HPV094**	**HPV095**
All methods					
Total	82	82	82	82	82
Negative	27	54	9	0	66
Positive	51	24	73	82	16
Low Positive	4	4	0	0	0
Indeterminate	0	0	0	0	0
% Negative	32.9%	65.9%	11.0%	0.0%	80.5%
% Positive	62.2%	29.3%	89.0%	100.0%	19.5%
% Low Positive	4.9%	4.9%	0.0%	0.0%	0.0%
% Indeterminate	0.0%	0.0%	0.0%	0.0%	0.0%
Consensus	**No Cons (POS)**	**No Cons (NEG)**	**POS**	**POS**	**NEG**
Cytology	**S/NILM w/ RCC**	**S/NILM***	**S/NILM**	**S/LGSIL***	**S/NILM***

* cytology in agreement with the overall high-risk screen results

Table 1b: Screening results, by method

	HPV091	**HPV092**	**HPV093**	**HPV094**	**HPV095**
Aptima®					
Total	38	38	38	38	38
Negative	2	36	7	0	36
Positive	36	2	31	38	2
% Negative	5.3%	94.7%	18.4%	00.0%	94.7%
% Positive	94.7%	5.3%	81.6%	100.0%	5.3%
Consensus	**POS**	**NEG**	**POS**	**POS**	**NEG**

	HPV091	HPV092	HPV093	HPV094	HPV095
Hybrid Capture®					
Total	8	8	8	8	8
Negative	4	2	0	0	7
Positive	0	2	8	8	1
Low Positive	4	4	0	0	0
Indeterminate	0	0	0	0	0
% Negative	50.0%	25.0%	0.0%	0.0%	87.5%
% Positive	0.0%	25.0%	100.0%	100.0%	12.5%
% Low Positive	50.0%	50.0%	0.0%	0.0%	0.0%
% Indeterminate	0.0%	0.0%	0.0%	0.0%	0.0%
Consensus	**No Cons**	**No Cons**	**POS**	**POS**	**NEG**

	HPV091	HPV092	HPV093	HPV094	HPV095
Cervista®					
Total	8	8	8	8	8
Negative	7	8	1	0	8
Positive	1	0	7	8	0
% Negative	87.5%	100.0%	12.5%	0.0%	100.0%
% Positive	12.5%	0.0%	87.5%	100.0%	0.0%
Consensus	**NEG**	**NEG**	**POS**	**POS**	**NEG**

	HPV091	HPV092	HPV093	HPV094	HPV095
Cobas® 4800					
Total	24	24	24	24	24
Negative	10	4	0	0	13
Positive	14	20	24	24	11
% Negative	41.7%	16.7%	0.0%	0.0%	54.2%
% Positive	58.3%	83.3%	100.0%	100.0%	45.8%
Consensus	**No Cons**	**POS**	**POS**	**POS**	**No Cons**

	HPV091	HPV092	HPV093	HPV094	HPV095
PCR (LDT)					
Total	4	4	4	4	4
Negative	4	4	1	0	2
Positive	0	0	3	4	2
% Negative	100.0%	100.0%	25.0%	0.0	50.0%
% Positive	0.0%	0.0%	75.0%	100.0%	50.0%
Consensus	**NEG**	**NEG**	**No Cons**	**POS**	**No Cons**

Genotyping (Table 2)

Laboratories that routinely determine HPV genotypes were also asked to submit those results. Fifty-nine genotyping results derived with various methods were submitted. Of those, 26 (44.1%) were from the Aptima® method, 24 (40.6%) from the Roche Cobas®4800 method, 4 (6.8%) from the Cervista®16/18 method, and 5 (8.5%) from a laboratory-developed PCR based method followed by DNA sequencing (2), RFLP (1), capillary electrophoresis (1), and a linear array panel (1). However, since not every method equally detects and/or discriminates every genotype and because the samples represent mixtures of patient samples, the genotyping results were not graded. You must, however, compare your results to those of the majority, shown in Table 2, and investigate any discrepancies.

Consensus positive sample **HPV094** showed a consensus (93.2%) for the presence of HPV genotype 16, with Aptima®, Roche Cobas® and Cervista® agreeing. Cervista® (4/4) and Roche Cobas® (23/24) users also agreed that HPV 18 was present, whereas only 4/26 laboratories using Aptima® also detected HPV 18. A majority of Roche Cobas® (19/24) users also included "OTHER" for their result, suggesting that this sample contained a wide variety of HPV genoptypes.

For sample **HPV095**, which was overall consensus screen negative but which 45% (11/24) of Roche Cobas® users called positive, 72% (8/11) of those Cobas® laboratories reported detecting HPV 16 and 82% (9/11) detecting "OTHER" high risk genotypes in their sample.

Sample **HPV093** with a screen positive consensus did not reach a consensus for any of the three major genotyping categories, HPV 16, HPV 18 or 'Other than 16,18', although a majority (69.5%) of laboratories did report the presence of 'Other than 16,18' genotypes. Furthermore, results from Roche Cobas® suggested that there were also HPV 16 and 18 genotypes present, though the results were just shy of the 80% required for a consensus.

Not unexpectedly, there was no clear result from the genotyping of the two samples **HPV091** and **HPV092** that did not reach a screening consensus. Interestingly, although 95% of laboratories using Aptima® classified HPV091 as screen positive, less than 10% of those that also did genotyping were able to detect either HPV 16 or 18. This is consistent with results from Roche Cobas® that indicate that this sample predominantly contained HPV genotypes other than 16 or 18, in agreement with the genotypes reported from the one laboratory that used a linear array but did not find either HPV 16 or 18. Similar results were also obtained for sample **HPV092**, though a quarter of Roche Cobas® users reported finding HPV 18.

In conclusion, in part because the samples comprised a mixture of actual patient samples, there was no clear consensus as to which high risk HPV genotypes were present. However, it seems also clear that there are substantial differences in the detection rate of the two main high risk genotypes HPV 16 and 18 between the different methods.

Table 2. Genotyping results (59 laboratories)

Overall Results	N	HPV091	HPV092	HPV093	HPV094	HPV095
Total 16	59	1	4	25	55	9
Total 18 and/or 45	59	2	7	18	33	0
Total 'Not_16,18 or 'Other'	59	36	20	41	24	11
Results by method						
Total 16 Aptima	26	1	1	2	25	1
Total 16 Roche	24	0	3	19	23	8
Total 16 Cervista	4	0	0	0	4	0
Total 16 Other Methods	5	0	0	4	3	0
Total 18/45 Aptima	26	2	1	0	4	0
Total 18 Roche	24	0	6	17	23	0
Total 18 Cervista	4	0	0	0	4	0
Total 18 Other Methods	5	0	0	1	2	0
Total 'Not_16,18' or 'Other' Aptima	26	15	1	10	1	2
Total 'Not_16,18' or 'Other' Roche	24	20	17	23	19	9
Total 'Not_16,18' or 'Other' Cervista	4	0	0	2	0	0
Total 'Not_16,18' or 'Other' Other Methods	5	1	2	5	3	1

Raw data

Figures 1 and 2 show the distribution of the raw data from the different instruments. While none of the assays is strictly quantitative, these data nevertheless allow a comparison between your results and those of your peers.

Conclusions

Four of the five samples provided variable results presumably because they contained virus titers around the limit of sensitivity for some of the methods, which is affected by the cut-off point settings and/or genotypes present. This can cause discrepancies in the interpretation of the results from different methods.

For laboratories whose results did not match the consensus results for their method and who would like to re-examine their results a limited number of samples are available for retest upon request.

As of January 2016, there will no longer be any New York State HPV proficiency tests offered.

As a result, your laboratory is required to:

1. Enroll in PT offered by a CMS-approved provider that meets the criteria in CFR Title42 Vol5 Part 493 Subpart I for Virology.
2. Authorize the PT provider to release the results to DOH. Failure to do so may result in sanctions for failure to participate in required PT.
3. The following surveys satisfy CLIA PT requirements for HPV testing:
   College of American Pathologists CHPVD, CHPVM, CHPVK, and CHPVJ

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

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Figure 1: Screening data by method

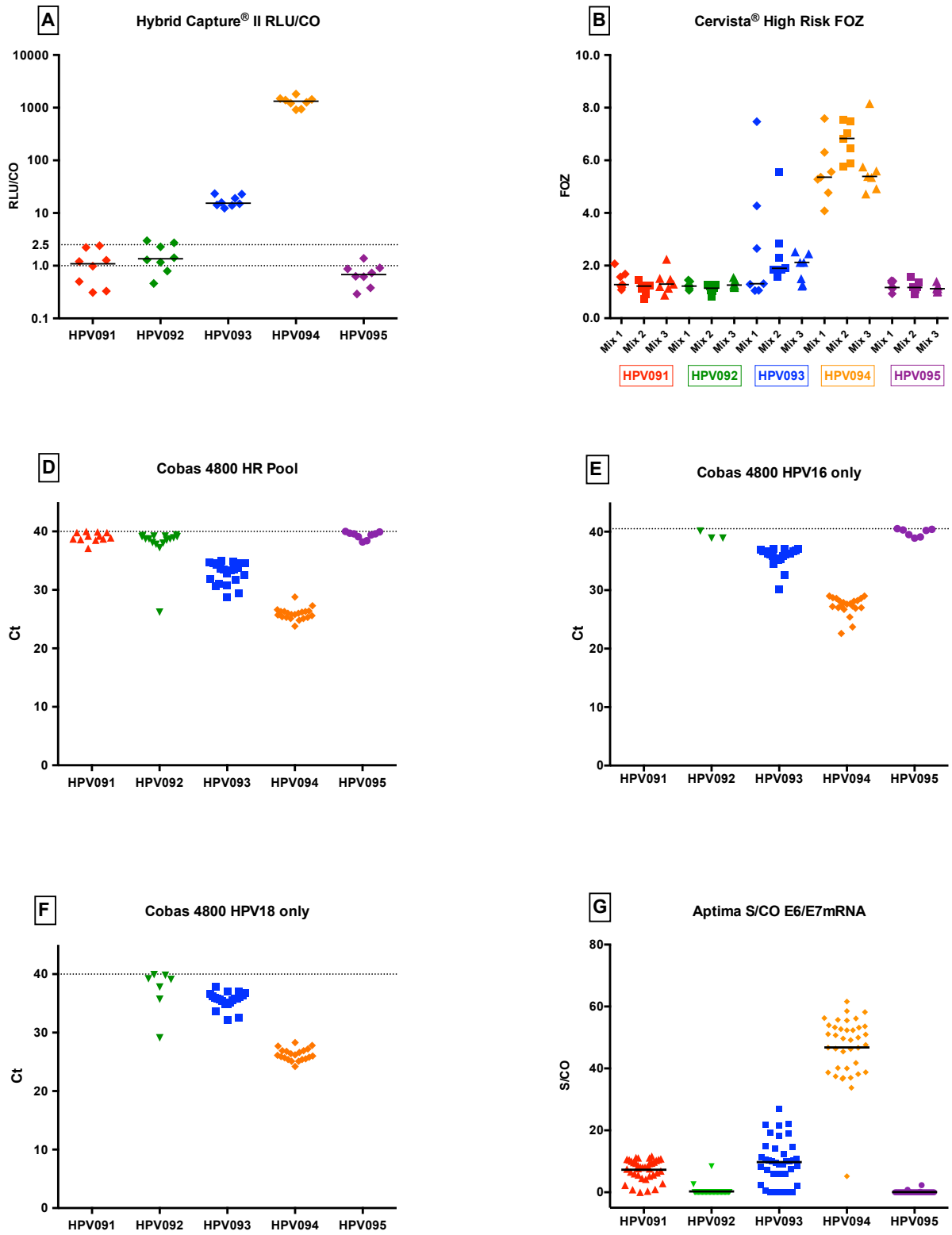

Figure 2: Genotyping data by method