

May 24, 2012

**Evaluation of the New York State Human Papilloma Virus (HPV) Proficiency Test**  
**April 2012<sup>1</sup>**

Dear Laboratory Director:

This is the summary and evaluation of the graded New York State Proficiency Test for human papilloma virus (HPV) determination from April 2012. A report with your laboratory's score and grade will be sent separately to you by regular mail. Five vials (HPV056 – HPV060) containing cervical cells derived from actual patients in PreservCyt<sup>®</sup> medium were sent out to every permitted laboratory on April 17<sup>th</sup>, 2011, and the due date for submitting the test results was May 7<sup>th</sup>, 2012. 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<sup>®</sup>, or FOZ values from Cervista<sup>®</sup>, though this information was not used for grading.

A total of 75 test sets were sent out, and valid answers were received from 74 laboratories by the due date. For screening, 45 laboratories (58%) used the Hybrid Capture<sup>®</sup> method, 25 laboratories (32%) used the Cervista<sup>®</sup> method, of which 4 laboratories (5%) reported results from both of these methods, 5 laboratories (6%) used a polymerase chain reaction based method (2 Cobas<sup>®</sup> 4800, 3 Laboratory Developed Tests), 2 laboratories used the recently approved Aptima<sup>®</sup> method (3%) and 1 laboratory (1%) continued to use the in-situ-hybridization method. The screening results are summarized in Table 1.

Cytology slides were prepared and evaluated in-house from each of the five test samples. Slides from positive samples HPV055, HPV056, and HPV057 all presented with scattered atypical squamous cells and all contained the fungus *Candida albicans*. Smears from the two negative samples, HPV059 and HPV060, were both simply diagnosed as satisfactory, within normal limits. All the cytological diagnoses were in agreement with the HPV consensus results from this proficiency test.

## **Results**

Consensus results from all laboratories and across each method were excellent at 98% (385/390) with only five incorrect sample responses. All laboratories unanimously reported samples HPV057 and HPV058 as positive. Positive sample HPV056 received two discrepant negative answers, one each with Hybrid Capture and Cervista<sup>®</sup>, respectively. The results for sample HPV059 showed one single positive response instead of the consensus negative (1/78) from a Cervista<sup>®</sup> assay. Finally, sample HPV060 received two discrepant answers (2/78) from Hybrid Capture method using laboratories, one positive and one low positive. 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.

<sup>1</sup>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.

**Table 1.** Screening results, 74 laboratories, 78 results submitted:

|                    | HPV056     | HPV057     | HPV058     | HPV059     | HPV060     |
|--------------------|------------|------------|------------|------------|------------|
| <b>All methods</b> |            |            |            |            |            |
| Total              | 78         | 78         | 78         | 78         | 78         |
| Negative           | 2          | 0          | 0          | 77         | 76         |
| Positive           | 76         | 78         | 78         | 1          | 1          |
| Low Positive       | 0          | 0          | 0          | 0          | 1          |
|                    |            |            |            |            |            |
| % Negative         | 2.6%       | 0.0%       | 0.0%       | 98.7%      | 97.4 %     |
| % Positive         | 97.4%      | 100.0%     | 100.0%     | 1.3 %      | 1.3 %      |
| % Low Positive     | 0.0%       | 0.0%       | 0.0 %      | 0.0 %      | 1.3 %      |
| <b>Consensus</b>   | <b>POS</b> | <b>POS</b> | <b>POS</b> | <b>NEG</b> | <b>NEG</b> |

|                       | HPV056     | HPV057     | HPV058     | HPV059     | HPV060     |
|-----------------------|------------|------------|------------|------------|------------|
| <b>Hybrid Capture</b> |            |            |            |            |            |
| Total                 | 45         | 45         | 45         | 45         | 45         |
| Negative              | 1          | 0          | 0          | 45         | 43         |
| Positive              | 44         | 45         | 45         | 0          | 1          |
| Low Positive          | 0          | 0          | 0          | 0          | 1          |
|                       |            |            |            |            |            |
| % Negative            | 2.2%       | 0.0%       | 0.0%       | 100.0%     | 95.6%      |
| % Positive            | 97.8%      | 100.0%     | 100.0%     | 0.0%       | 2.2%       |
| % Low Positive        | 0.0%       | 0.0%       | 0.0%       | 0.0%       | 2.2%       |
| <b>Consensus</b>      | <b>POS</b> | <b>POS</b> | <b>POS</b> | <b>NEG</b> | <b>NEG</b> |

|                  | HPV056     | HPV057     | HPV058     | HPV059     | HPV060     |
|------------------|------------|------------|------------|------------|------------|
| <b>Cervista®</b> |            |            |            |            |            |
| Total            | 25         | 25         | 25         | 25         | 25         |
| Negative         | 1          | 0          | 0          | 24         | 25         |
| Positive         | 24         | 25         | 25         | 1          | 0          |
|                  |            |            |            |            |            |
| % Negative       | 4.0%       | 0.0%       | 0.0%       | 96.0%      | 100.0%     |
| % Positive       | 96.0%      | 100.0%     | 100.0%     | 4.0%       | 0.0%       |
| <b>Consensus</b> | <b>POS</b> | <b>POS</b> | <b>POS</b> | <b>NEG</b> | <b>NEG</b> |

|                  | HPV056     | HPV057     | HPV058     | HPV059     | HPV060     |
|------------------|------------|------------|------------|------------|------------|
| <b>PCR*</b>      |            |            |            |            |            |
| Total            | 5          | 5          | 5          | 5          | 5          |
| Negative         | 0          | 0          | 0          | 5          | 5          |
| Positive         | 5          | 5          | 5          | 0          | 0          |
|                  |            |            |            |            |            |
| % Negative       | 0.0%       | 0.0%       | 0.0%       | 100.0%     | 100.0%     |
| % Positive       | 100.0%     | 100.0%     | 100.0%     | 0.0%       | 0.0%       |
| <b>Consensus</b> | <b>POS</b> | <b>POS</b> | <b>POS</b> | <b>NEG</b> | <b>NEG</b> |

\*includes Roche Cobas® 4800

|                      | HPV056     | HPV057     | HPV058     | HPV059     | HPV060     |
|----------------------|------------|------------|------------|------------|------------|
| <b>APTIMA® (N=2)</b> | <b>POS</b> | <b>POS</b> | <b>POS</b> | <b>NEG</b> | <b>NEG</b> |

|                  | HPV056     | HPV057     | HPV058     | HPV059     | HPV060     |
|------------------|------------|------------|------------|------------|------------|
| <b>ISH (N=1)</b> | <b>POS</b> | <b>POS</b> | <b>POS</b> | <b>NEG</b> | <b>NEG</b> |

## Genotyping

Laboratories that routinely determine HPV genotypes were also asked to submit those results (“genotyping”). Twenty-three laboratories did genotyping using variable methodologies. Seventeen laboratories (74%) used the Cervista®16/18 method, two (9%) used a laboratory developed PCR based methodology, two (9%) used PCR followed by RFLP, one laboratory (4%) used the Hybrid Capture method and one laboratory (4%) used the Cobas® 4800 methodology (Table 2).

As expected, the carcinogenic types 16 and 18 were most frequently observed in the positive samples. Genotyping results for sample HPV057 showed that all the laboratories were in agreement that high-risk HPV genotypes 16 and/or 18 were present in the sample; however, it was interesting to see that among the Cervista®16/18 method users the answers varied, with six laboratories (35%) reporting the high-risk genotype 16 only, ten laboratories (59%) reporting both high-risk types 16 and 18, and one laboratory (6%) being unable to distinguish which of those two genotypes was present in the sample. Responses for sample HPV056 showed an approximate even split between laboratories that found HPV 16 only and laboratories that found both HPV 16 and 18 genotypes, while in sample HPV058 the single carcinogenic genotype 16 was the most prevalent response reported. However, it was surprising that in both samples HPV056 and HPV058 a few laboratories could not identify either of the high-risk genotypes 16 and/or 18 by the Cervista®16/18 method used in their laboratory. These labs should re-examine their procedure. The one laboratory that used the Cobas® 4800 method reported both the high-risk 16 and 18 genotypes and other high-risk positive genotypes present in all the samples including those that it reported as negative by screening. This laboratory should check their results again. Not surprisingly, the PCR methods also identified other high-risk genotypes in each of the positive samples. Table 2 summarizes the genotyping results.

**Table 2.** Genotyping results, 23 laboratories:

| Method     | HPV056                    | HPV057                     | HPV058                    | HPV059                          | HPV060                          |
|------------|---------------------------|----------------------------|---------------------------|---------------------------------|---------------------------------|
| INV        | 16                        | 16                         | 16                        | N/A                             | N/A                             |
| INV        | 16                        | 16                         | 16, 18                    | N/A                             | N/A                             |
| INV        | 16                        | 16, 18                     | NOT ID                    | N/A                             | N/A                             |
| INV        | 16                        | 16, 18                     | NOT ID                    | N/A                             | N/A                             |
| INV        | 16                        | 16, 18                     | 16 or 18                  | N/A                             | N/A                             |
| INV        | 16                        | 16, 18                     | 16, 18                    | N/A                             | N/A                             |
| INV        | 16, 18                    | 16                         | 16                        | N/A                             | N/A                             |
| INV        | 16, 18                    | 16, 18                     | 16                        | N/A                             | N/A                             |
| INV        | 16, 18                    | 16, 18                     | 16                        | N/A                             | N/A                             |
| INV        | 16, 18                    | 16, 18                     | 16                        | N/A                             | N/A                             |
| INV        | 16, 18                    | 16, 18                     | 16                        | N/A                             | N/A                             |
| INV        | 16 or 18                  | 16                         | 16                        | N/A                             | N/A                             |
| INV        | N/A                       | 16 or 18                   | 16 or 18                  | N/A                             | N/A                             |
| INV        | N/A                       | 16                         | 16                        | N/A                             | N/A                             |
| INV        | NOT ID                    | 16                         | NOT ID                    | N/A                             | N/A                             |
| INV        | NOT ID                    | 16, 18                     | NOT ID                    | N/A                             | N/A                             |
| INV        | NOT ID                    | 16, 18                     | 16                        | N/A                             |                                 |
| PCR        | 16, 18                    | 16, 18                     | 16                        | Reactive with HPV generic probe | Reactive with HPV generic probe |
| PCR        | 16, 18, 59, 68            | 16, 18, 59, 68             | 16, 18, 59, 68            | N/A                             | N/A                             |
| RFLP       | 33, 53, 18, 16, 31        | 16, 61, 53, 6, CP141, unk. | LVX160, 16, 59            | 61, 72                          | N/A                             |
| RFLP       | 16, 31, 53, 58            | 16, 58, 61                 | 16, 72, unk.              | N/A                             | N/A                             |
| HYC        | NOT ID                    | 16, 18                     | 16                        | N/A                             | N/A                             |
| Cobas 4800 | 16, 18, other HR positive | 16, 18, other HR positive  | 16, 18, other HR positive | 16, 18, other HR positive       | 16, 18, other HR positive       |

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

### Raw data

Figure 1 shows distribution plots of the raw data, RLU/CO and FOZ, respectively. Though these data are not meant in a strictly quantitative way, Figure 1C clearly shows that a substantial number of results for genotyping Mix 18 for samples HPV056 - 058 are below the threshold for positivity. This is in agreement with the above-discussed genotyping results.

## Conclusions

In general, 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 2012 New York State HPV proficiency tests:

### Mail-out Date

October 16

### Due Date

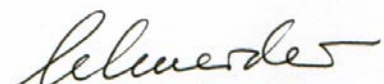
November 5

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

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**Figure 1**

