PAP AND HPV TESTING
FREQUENTLY ASKED QUESTIONS

TEST ORDERING

1. **What are the current ordering options for HPV reflex testing?**
   When ordering the Pap Test, select one of the following options for HPV reflex testing:

   1. HPV Co-Testing (recommended in women 30-65yrs.) If Pap is negative reflex to HPV high risk and if positive then HPV Genotyping 16/18.
   2. Reflex HPV High Risk (ASCUS or LSIL Pap results)
   3. Reflex HPV High Risk in younger women (21-29 yrs.) with prior abnormal Pap/biopsy results.
   4. No HPV DNA Reflex

2. **When will the Pap test reflex to HPV testing?**
   HPV reflex testing is completed based on the reflex option selected, the Pap test diagnosis, and the age of the patient. Refer to the tables below for details:

   **#1: HPV Co-Testing (recommended in women 30-65yrs.) If Pap is negative reflex to HPV high risk and if positive then HPV Genotyping 16/18.**

<table>
<thead>
<tr>
<th>Pap Diagnosis</th>
<th>&lt;21</th>
<th>21-29</th>
<th>30-65</th>
<th>&gt;65</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>No HPV</td>
<td>No HPV</td>
<td>Reflex to HPV*</td>
<td>No HPV</td>
</tr>
<tr>
<td>ASCUS or LSIL</td>
<td>No HPV</td>
<td>Reflex to HPV</td>
<td>Reflex to HPV</td>
<td>Reflex to HPV</td>
</tr>
<tr>
<td>HSIL or Above</td>
<td>No HPV</td>
<td>No HPV</td>
<td>Reflex to HPV</td>
<td>Reflex to HPV</td>
</tr>
<tr>
<td>Unsat</td>
<td>No HPV</td>
<td>No HPV</td>
<td>No HPV</td>
<td>No HPV</td>
</tr>
</tbody>
</table>

   *If HPV high risk is positive, will reflex to HPV Genotyping 16/18

   **#2: Reflex HPV High Risk (ASCUS or LSIL Pap results)**

<table>
<thead>
<tr>
<th>Pap Diagnosis</th>
<th>&lt;21</th>
<th>21-29</th>
<th>30-65</th>
<th>&gt;65</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>No HPV</td>
<td>No HPV</td>
<td>No HPV</td>
<td>No HPV</td>
</tr>
<tr>
<td>ASCUS or LSIL</td>
<td>No HPV</td>
<td>Reflex to HPV</td>
<td>Reflex to HPV</td>
<td>Reflex to HPV</td>
</tr>
<tr>
<td>HSIL or Above</td>
<td>No HPV</td>
<td>No HPV</td>
<td>No HPV</td>
<td>No HPV</td>
</tr>
<tr>
<td>Unsat</td>
<td>No HPV</td>
<td>No HPV</td>
<td>No HPV</td>
<td>No HPV</td>
</tr>
</tbody>
</table>

   **#3: Reflex HPV High Risk in younger women (21-29 yrs.) with prior abnormal Pap/biopsy results.**
   Note: Option should only be chosen if patient is 21-29 years old AND has had a prior abnormal Pap or biopsy result.

<table>
<thead>
<tr>
<th>Pap Diagnosis</th>
<th>&lt;21</th>
<th>21-29</th>
<th>30-65</th>
<th>&gt;65</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>No HPV</td>
<td>Reflex to HPV</td>
<td>No HPV</td>
<td>No HPV</td>
</tr>
<tr>
<td>ASCUS or LSIL</td>
<td>No HPV</td>
<td>Reflex to HPV</td>
<td>Reflex to HPV</td>
<td>Reflex to HPV</td>
</tr>
<tr>
<td>HSIL or Above</td>
<td>No HPV</td>
<td>No HPV</td>
<td>No HPV</td>
<td>No HPV</td>
</tr>
<tr>
<td>Unsat</td>
<td>No HPV</td>
<td>No HPV</td>
<td>No HPV</td>
<td>No HPV</td>
</tr>
</tbody>
</table>
3. **Why is the HPV reflex order included on the Pap Order?**

   In accordance with ASCCP guidelines, clinical utility of HPV testing depends on the patient’s age and Pap findings. Ordering a Pap with reflex HPV allows laboratory staff to follow these guidelines and perform HPV testing when necessary.

4. **Can I order an HPV DNA test at the same time that I order the Pap test [HCPATHP]?**

   **NO.** All HPV DNA testing orders should be placed as a reflex within the Pap test order [HCPATHP]. To order HPV testing, answer the HPV Reflex questions in the Pap order. HPV DNA testing should NOT be requested in an order separate from the Pap test.

5. **I have the Pap results on my patient and want to place an add-on order for HPV testing. How long after collecting the specimen can I add-on HPV testing?**

   This is an uncommon situation, as reflex testing is routinely ordered within the Pap. In rare instances when an add-on HPV order is necessary, HPV testing can be performed within 3 weeks of the specimen’s collect date. To order, select the HPV DNA test [HCHPVDNA] with the add-on order class.

6. **When can an HPV DNA test be ordered independent of a Pap test (Not as an add-on order)?**

   1. When HPV testing is requested more than three weeks after a Pap has been collected.  
      **In this situation a new specimen must be collected for HPV testing.**

   2. When there is insufficient specimen remaining to do HPV testing after a Pap result has been issued (HPV results with insufficient genomic DNA).  
      **In this situation a new specimen must be collected for HPV testing.**

   For both instances, use the order class of Normal or Office Collect.

   Send the Thin Prep Vial to the lab with the HPVDNA order. **DO NOT** include another order for a Pap Test.

7. **I want to order HPV testing regardless of the patient’s age or Pap result. Why can’t I do this?**

   1. Testing for HPV on Paps with high grade intraepithelial lesions adds little diagnostic value. The diagnosis of a high grade intraepithelial lesion signifies the presence of HR HPV.

   2. Ordering HPV on negative Paps is useful in patients over age 30 or between the ages of 21 and 29 with a history of an abnormal Pap/Biopsy. HR HPV testing in patients under age 30 without a history of an abnormal Pap/Biopsy has limited clinical significance. Clinical research has confirmed that the HPV viral infection is often transient in patients under age 30 with negative Pap results.

   3. HPV testing **MUST** be ordered within the reflex segment of the Pap order. Submitting a separate HPVDNA order in addition to the Pap order has no added benefit. Laboratory staff will follow the Co-Testing guidelines for reflex HPV testing and the order will be canceled if the request falls outside of those guidelines.
4. Add-on HPV orders (ordered after a Pap has been resulted) can be requested with a HPVDNA order (see # 6). In keeping with the standards of best practice, this request should comply with the ASCCP guidelines summarized in this document.

8. **I want to order HPV testing if the Pap is ASCUS or LSIL, regardless of the patient’s age. Can I do this?**

   NO. HPV status has little added diagnostic value in patients under age 21 with an ASCUS or LSIL Pap result. In keeping with the standards of best practice, the lab will not run the HPV test if the patient does not meet the age criteria.

9. **I want to order HPV testing on a Pap that was signed-out as AGUS. Can I do this?**

   YES. If the atypical glandular cells are endocervical in origin, HPV testing can be done as an add-on order after the Pap result has been finalized. There is a direct association between several types of HR HPV and endocervical glandular lesions.

10. **How do I order HPV co-testing on a patient that is less than 30?**

    In patients age 21-29 HPV Co-testing is only acceptable in patients with a previous abnormal Pap/Biopsy. Select the reflex option Reflex HPV High Risk in younger women (21-29 yrs.) with prior abnormal Pap/biopsy results.

11. **What is the difference between an ECC brushing and a cervical (ectocervical and endocervical) Pap on the Pap order?**

    ECC brushings are used in lieu of endocervical curettage. It is a diagnostic procedure. This should only be selected when a patient is being seen for follow-up of an abnormal Pap.

    Cervical (ectocervical and endocervical) Pap should be selected when sampling the ectocervix and endocervix during a routine Pap collection.

**RESOURCES**

12. **Where can I find instructions on Pap and HPV Test Collection and Reporting?**

    UW Health and U-Connect provide resources within the Laboratory Test Directory. Information can be found by searching “HPV” and “Pap Test”. U-Connect Cytopathology Services and UW Health Cytopathology Services pages include video instructions on Pap collection. Resources on Pap and HPV testing and triage algorithms can be found on the American Society of Colposcopy and Cervical Pathology (ASCCP) website via links on both pages.

**PAP TEST SPECIMEN COLLECTION**

Please Note: Always check the Thin Prep vial’s expiration date before proceeding with Pap collection. Pap specimens collected in expired Thin Prep vials cannot be accepted for testing and will be rejected.
13. **Can I clip-off the cytobrush in the Thin Prep vial and send the specimen to the lab?**

1. **NO.** The instruments in the laboratory cannot process Pap test specimens with the brush in the vial. Leaving the brush in the vial slows down specimen processing and can cause instrument malfunction, impacting turnaround time for multiple Pap test results.

2. Leaving the brush in the Thin Prep vial also reduces the cells available for testing, negatively impacting results.

14. **Can I put the scraper/cytobrush/broom in the Thin Prep vial, complete the exam, and remove the collection devices after the exam?**

1. **NO.** Leaving the collection device in the Thin Prep vial compromises Pap and HPV test results, leading to increased unsatisfactory rates.
   a. Since PreservCyt is methanol based it causes cells to "fix" to whatever substrate is present. Leaving the collection device in the vial will cause cells to bind to the device, making it difficult to dislodge them later. The collection device should be vigorously rinsed in the Thin Prep vial immediately after specimen collection, and then discarded.

   b. For the same reason, brushes should NOT be clipped-off and dropped into the vial before sending the specimen to the lab.

15. **Can I use lubricant during the exam?**

1. The use of lubricant is discouraged.
   a. Numerous studies have shown increased unsatisfactory Pap rates when lubricant is used.
   b. Unsatisfactory Paps cannot be triaged for HPV testing.
   c. Warm water is recommended to insert the speculum.
   d. If lubricant must be used, it should be used sparingly and only applied to the exterior sides of the speculum. SurgiLube is a commonly available lubricant that has been approved for use with the Thin Prep Pap Test. Other lubricants may be used, provided that they do not contain thickening agents such as carbomer or carbopol polymers.

**PAP AND HPV TEST RESULTS**

16. **Why am I getting unsatisfactory Pap results?**

The UWHC Cytopathology Lab follows the Bethesda guidelines for reporting Pap test results. In most patient populations there must be a minimum of 5,000 squamous epithelial cells present to provide a satisfactory Pap test result. Specimens from patients with postmenopausal atrophic changes, radiation/chemotherapy, or that are post-hysterectomy may be deemed satisfactory with as few as 2,000 squamous epithelial cells.

Reasons for unsatisfactory results include:
- Patient physiological conditions
- Collection device clipped into the vial
- Collection device immersed in the Thin Prep vial during the exam
- Excessive blood, inflammation, mucus, lubricant, or other obscuring elements (interferes with specimen processing)
- Cells on the collection device are not vigorously rinsed in the Thin Prep vial
Please Note:
- The UWHC cytopathology laboratory makes every effort to minimize the incidence of unsatisfactory Pap test specimens. Bloody and mucoid specimens are processed using extra steps to eliminate obscuring elements and provide satisfactory results on many compromised specimens.
- All unsatisfactory Pap results are reviewed and resulted by a cytopathologist to provide consistency in reporting.

17. **Why do I get Pap results back without endocervical cells?**
- Postmenopausal, postpartum, and pregnant patients often do not yield an endocervical component (endocervical and squamous metaplastic cells). This is a fairly common and normal finding.
- The UWHC Cytopathology Lab follows the Bethesda guidelines for reporting the presence of an endocervical component on the Pap Test.
- Use of collection device. The cytobrush and spatula combination is the most effective method for sampling the endocervix and ectocervix.

18. **Should I repeat a Pap sooner if endocervical cells are not present?**
- NO - if the patient has had previous negative Paps and a normal clinical history.
- YES - if the patient has an abnormal clinical or Pap history. Repeat within 3-6 months.

19. **Why am I getting QNS and Low DNA content HPV results?**
- The Thin Prep processor requires a set number of cells for Thin Prep processing. This is based on pressure gradients established within the instrument. If there are not enough epithelial cells left in the Thin Prep vial after the Pap is processed, a QNS or low DNA content HPV report is more likely.

20. **Why aren’t HPV results available when the Pap test results are available?**
- HPV testing is reflexed based on the Pap results. Once the Pap is resulted, the remainder of the specimen is sent to the Molecular Lab for HPV testing per ASCCP protocols. HPV results are typically available within 24-48 hours after cytology issues a diagnosis.

21. **Why do I receive amended Pap test reports informing me that the HPV testing comment has been added or removed?**
- Adding the HPV testing comment to Pap test reports is a manual process, with an element of error. The laboratory team makes every effort to reduce errors through the use of multiple checks and monitors. Unfortunately some errors are not immediately detected. We understand that this can cause confusion or concern to the provider and patient. In the patient’s best interest, we are committed to 100% accuracy of reporting.

22. **How will I know that HPV testing is being done on a Pap, when no HPVDNA order is generated in HealthLink?**
- If a Pap test is sent for reflex HPV testing, the HPV testing comment will be included in the report:
  The remainder of the ThinPrep Pap specimen vial was submitted to the Molecular Diagnostics laboratory for high risk HPV DNA analysis. A separate report will be available in the EHR. If appropriate per HPV triage protocols, additional 16/18 genotyping will be performed. A second report will then be available in the EHR.