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4.1 GENERAL CYTOLOGY COLLECTION PROCEDURES

The quality of the diagnostic information derived from any cytology test is dependent upon the proper collection and handling of specimens and accompanying forms which are submitted for cytologic evaluation. Not only is correct patient preparation an essential factor in obtaining accurate diagnostic information, but each specimen obtained must also be collected and packaged properly for transport.

4.10 Patient and Specimen Identification

WellSpan has adopted the 2008 National Patient Safety Goal in which at least two patient identifiers (name and preferably date of birth) must be used when providing care, treatment, or services. In some instances a medical record number (MRN) or social security number (SSN) may be used in place of the DOB, but both unique identifiers should be the same on both the specimen and the requisition.

* <u>The Specimen Label Must Include:</u>
    1. Patient's Name (as listed on insurance card)
    2. Patient's Date of Birth
    3. Date and Time of Collection
    4. Specimen Source - must match source listed on test requisition

*** For vials, specimen label should be placed on side of vial, not on lid.**
*** For slides, write above info in pencil on frosted end.**

* <u>The Test Requisition Must Include:</u>
    1. Patient's Name
    2. Patient's Date of Birth
    3. Patient Demographic and Current Insurance Information – may attach separately
    4. Date and Time of Collection
    5. Attending / Ordering Physician's Name and Signature
    6. Office Location (where specimen was collected)
    7. Test Requested
    8. Valid ICD-9 code(s) that support the testing requested
        * Codes must match the test ordered, clinical reason and specimen source
        * For Pap Tests – HPV, GC, or CT testing require supporting ICD codes
        * For NGYN Tests – Submit code and/or narrative history/symptoms/Dx
    9. Specimen Source
    10. Pertinent Clinical Information

*** For Pap tests, clinical info includes: Last Menstrual Period (LMP), Reason for no LMP Pregnancy, Post-Partum, Previous abnormal GYN results, treatments, or surgeries, etc…**

Best Practice Guideline: Before the patient leaves the specimen collection site, show the labeled forms and specimens to them and ask for confirmation of their identifiers. Identification labels or names should be affixed to the vial ***after*** *the specimen has been collected and the accuracy of the label verified.* ***To prevent erroneous patient labeling, do not label vials in advance.***

4.11 Specimen Rejection Criteria

The York Hospital Anatomic Pathology Receiving technician and prep staff are responsible for ensuring that specimens and requisitions submitted to the department contain all required elements before specimen processing begins.

Common reasons why specimens are rejected or unable to be processed at York Hospital:

1. Unlabeled container, vial, slide, or requisition
2. The specimen is not accompanied by a valid order
3. Mismatch between patient identifiers on container or slide and test requisition.
4. The specimen or requisition is missing a second patient identifier
5. The requisition is missing any required information (listed above).
6. Leakage of most or all fluid during transport
7. Slide broken in transit beyond our ability to repair
8. A needle was submitted. The cytology prep lab does not accept needles.

Specimens submitted without positive patient/specimen identification must be resolved, rejected, or discarded and will result in a delay in service. This situation requires either:

1. Corrective action by submitting clinician or other authorized provider OR
2. Specimen re-collection

* Specimens will not be rejected until appropriate measures have been taken to correct the problem. In most instances, a delay in processing is the only direct outcome.

** See the following policies for additional information regarding specimen rejection criteria:*

- ** APC 5.1 – Specimen and Requisition Acceptability Criteria (Cytology)*
- ** A-SP-8 – Specimen Identification Requirements and Rejection Criteria (Lab General)*
- ** AP GENERAL 10 – Specimen, Requisition, and Identification Resolution (AP)*

4.12 Ordering Cytology Tests

GYN (Pap Tests) and Non-GYN tests require a valid attending or ordering physician complete a written or electronic test order prior to specimen processing and case analysis.

* Written Orders and Test Requests:
  - Use York Hospital's Cytopathology Requisition, Form #3007 (gray form)

* Electronic Orders and Test Requests:
  - OUTPATIENT: For those offices using an electronic order system such as MedEnt, Ecare (Allscripts), Emdeon, etc… enter information as required, print the requisition form and submit the form with matching, labeled specimen.

  - INPATIENT: Using PowerChart in Cerner, search for these pneumonics:
    * *CYTO GYN, PATHOLOGY GYN REQUEST*
    * *CYTO NGYN, PATHOLOGY NON-GYN REQUEST*

    Answer all required questions and submit order. An order label will print at the ordering location. Affix label to specimen and send specimen to the clinical lab. The Power order requisition will print at printer AP03 in cytology to alert the staff that a specimen will be coming to cytology.

* Add-On Orders:
  - If additional testing is requested from a specimen that was already submitted to the cytology lab, then a new, updated requisition with physician signature should be faxed to Cytology with all of the required information for the added test.
  - Orders can only be added on if the original specimen is still available and viable.
  - Examples: HPV, GC, CT added to a Pap Test, PCP added to a Lavage, etc…

* *See 4.5 on Page 28 for examples of Form #3007, Power Order requests, and Ecare requests*

4.13 Universal Safety Precautions

Care should be taken with all specimens submitted to the York Hospital Cytology Lab in order to minimize the spread of infection, reduce the risk of chemical exposure, and also to avoid safety-related accidents.

* Biological Hazards:
  - Unfixed body fluid specimens and specimens in vials of Cytolyt (transport medium) may be biohazardous. To promote infection prevention and control, always use personal protective equipment when handling patient specimens.
  - Specimens in Pap vials of PreservCyt (liquid preservative/fixative) are rendered Non-biohazardous after fifteen (15) minutes.
  - Spray fixed or air-dried slides are non-biohazardous after thorough drying. However, caution should be used even with these specimens.

* Sharps Hazards:
  - Needles **must** be removed from collection devices **before** transport to the cytology lab. Submitted needles may be rejected for exposure control.
  - Rinse specimen from needle and syringe into a CytoLyt vial. Seal in bio-bag.
  - You may also safely remove needle from a syringe, cap syringe, Seal in bio-bag.

* Chemical Hazards:
  - Fixatives, preservatives, and other products may contain hazardous chemicals.
  - Take special precautions when handling formalin as this is a known carcinogen.
  - All Product Material Safety Data Sheets (MSDS) can be found on the INET.

4.14 Cytology Fixatives and Supplies

* All supplies furnished by WellSpan must be sent to WellSpan for processing.
* **Off-Site Supply Ordering** – Contact TransLab Mobile Services at 851-1417
* **On-Site Supply Ordering** – Use Lawson system or contact Materials Management
* A limited number of forms, slides, fixative, vials, and collection devices are available in Anatomic Pathology – contact Cytology (851-5010, 851-5004, or 851-5013)

SPECIMEN	FIXATIVE
Cervical/Vaginal Smears (conventional)	Spray fixative
Cervical/Vaginal Smears (ThinPrep Liquid-based)	ThinPrep PreservCyt Solution
Anal-Rectal Pap Smear	CytoLyt Preservative

Body Cavity Fluids -Peritoneal, pleural, pericardial	Fresh, Unfixed, Add Heparin Refrigerate
Cerebral Spinal Fluid (CSF)	Fresh, Unfixed
Fine Needle Aspiration Cytology	Equal # spray-fixed and air-dried slides Cytolyt Preservative
Gastrointestinal Tract Brushings	Spray fixative, Cytolyt Preservative
Nipple Discharge Smears	1 spray-fixed and 1 air-dried slide
Respiratory Cytology	
- Washings	Fresh, Unfixed
- Brushings	Spray fixative (slides), Saline
- Lavage	Fresh, Unfixed
- Sputum	Fresh, Unfixed
Tzanck or Skin Scrapings (for Herpes)	1 spray-fixed and 1 air-dried slide
Urine	Fresh, Unfixed, Refrigerate

4.15 Packaging and Transport of Specimens

PACKAGING

* <u>Fluid Vials and Containers:</u>
  - Tightly close to prevent leakage.
  - Make sure vial or container is properly labeled. Check names / ID
  - Package individually in sealed bio-bags.
  - Place folded requisition in outside pocket of bio-bag.
  - Transport in closed, impervious, leak-proof coolers or bags.
  - Separate Non-GYNs from GYNs and other specimens for delivery

* <u>Slides:</u>
  - Check Names / ID.
  - When dry, place in slide holders and securely close.
  - Wrap requisitions together with matching slide holder(s), seal in Bio-bag

* Special Instructions:
  - **Correlating Surgical Pathology and Cytology** – Package specimens together.
  - **Priority Cases** – Position the priority label or instructions to be readily visible
  - **Complete a Tracking Log** – see 'Delivery' below for specific instructions.
  - **Shared Specimens** – If both cytology and microbiology tests are ordered, send separate sample to each area when possible. If the specimen cannot be split, label as "Shared Specimen," send in a sterile container with all test requests to the microbiology department for triage and distribution.

DELIVERY

* On-Site Specimen Delivery:
  - Non-GYN fluid specimens and SHARED specimens – deliver to first floor Clinical Lab, Central Processing or Hematology
  - Non-GYN specimens without fresh fluid, with cytology orders ONLY, or Pap test vials, deliver to Anatomic Pathology Receiving (APR) drop-off room located in the basement of Century Project.
  - For in-house OR, GI, or after hour courier drops, fill in tracking info on AP in-House Tracking Sheet located on the refrigerator in the APR drop-off room.

* Off-site Specimen Delivery:
  - Contact York Hospital Courier service for delivery of specimens or supplies
  - Call TransLab Mobile Services at (717) 851-1417 for pick up times and hours of operation.
  - Offices and out-patient collection sites should complete a tracking log for each pick up/delivery to Lab.
    * Pap Tests – count the number of total specimens and record on log.
    * Non-GYN Tests – write case-specific information for each specimen delivered

4.16 Result Reporting and Turn-Around Times

* GYN (Pap) Tests:
  - York Hospital Cytology uses a modified Bethesda System with descriptive terminology for Pap interpretation and reporting including Specimen Adequacy, Diagnosis, optional Case Comments and/or recommendations.
  - **TAT** - York Hospital's target turn-around time for GYN reporting is 90% of testing verified within or by four (4) working days from the time of specimen

receipt.
- GYN TATs are monitored and reported to PI committees on a monthly basis.
- Notify Cytology if GYN results must be expedited for any reason.

* HPV Tests:
- When ordered as a co-test, HPV results commonly post before the Pap results.
- When ordered alone, HPV results may post as early as the day after receipt.
- When ordered as a reflex test, HPV results typically take an additional 2 business days to post after the Pap report has been verified.

* Non-GYN Tests:
- Cytology testing includes sample preparation, evaluation, and report.
- Cell blocks, special stains, and/or IHC stains may be ordered at the discretion of the pathologist. These services will be performed at an additional charge.
- **TAT** - York Hospital's expected turn-around time (TAT) for Non-GYN reporting is 90% of testing verified within or by two (2) working days from the time of specimen receipt.
- Non-GYN TATs are monitored and reported to PI committees on a monthly basis.

4.17 Confidentiality of Medical Information

According to the U.S. Department of Health and Human Services,

> *"The HIPAA Privacy Rule provides federal protections for personal health information held by covered entities and gives patients an array of rights with respect to that information. At the same time, the Privacy Rule is balanced so that it permits the disclosure of personal health information needed for patient care and other important purposes.*
>
> *The Security Rule specifies a series of administrative, physical, and technical safeguards for covered entities to use to assure the confidentiality, integrity, and availability of electronic protected health information."*
>
> *(http://www.hhs.gov/ocr/privacy/hipaa/understanding/)*

According to the Wellspan Health Code of Conduct, all staff are responsible for "maintaining the confidentiality of privileged business and personal information, including the protection of information and access to files, records, and electronic systems, and managing the use of keys, passwords, and codes that could enable access to any protected location, system, or information."

Additional administrative privacy policies are available through the INET:

* Policy #106 – Confidentiality

* Policy #113 – WellSpan Health Notice of Information Practices
* Policy #115 – Faxing Confidential Info
* Policy #135 – Privacy Office Protected Health Information Access Audits
* Policy #145 – Release of Patient Information
* Policy #147 – Security of Protected Health Information

4.2 GYNECOLOGIC CYTOLOGY COLLECTION PROCEDURES

4.20 Purpose and Value

The Papanicolaou (Pap) Test is the standard of care for early detection of cervical cancer and its precursors as well as other gynecologic abnormalities. Using liquid-based ThinPrep technology, concurrent sample collections utilizing cytology and aliquot removal for high risk HPV DNA testing as well as certain sexually transmitted diseases (N. gonorrhea and C. trachomatis) may provide added value to the patient and practitioners.

Test value depends critically on the quality of the specimen received. An adequate test sample provides a safe, noninvasive and cost effective screening test procedure. Practitioners should note that with sub-optimal sampling, chance misallocation of diagnostic cells may occur if they are very rare. Aliquot removal from low-cellular specimens may leave insufficient material in the sample vial for a satisfactory Pap test slide or for reflexive HPV testing using the residual specimen. Collection of separate samples for Pap, HPV and/or STD testing may be considered based on patient risk and clinical history as well as specimen suitability (e.g. exudates or bleeding) that can impact diagnostic reliability.

<u>Additional applications of the Pap test which further add value to patient care include:</u>

* Identification of several infectious processes or organisms when abundantly present
  - Examples include fungal forms, trichomonas vaginalis, coccobacilli, Actinomyces, Herpes simplex, etc…
  - ***Refer to Clinical Lab for antibody testing, Refer to Microbiology for cultures.***
* DES Exposure – For patients exposed to DES in utero, monitor for vaginal adenosis or adenocarcinoma. Sample each quadrant of the Vaginal Wall and label accordingly.

Diagnostic accuracy of the Pap test is not absolute. False negative or false positive results are inherent to the procedure and have been known to occur. A negative Pap test does not preclude additional evaluation of abnormal clinical signs and symptoms. If negative results from the specimen do not fit with the clinical impression, a new specimen may be necessary. Regularly

scheduled cervical screening is recognized as the most effective method of reducing the incidence and mortality of cervical cancer.

4.21 Current Pap Test Screening Guidelines for Cervical Cancer

Updated: 2012
Supporting Agencies: American Cancer Society (ACS), American Society of Clinical Pathologists (ASCP), American Society for Colposcopy and Cervical Pathology (ASCCP)

Table 1. Screening Methods for Cervical Cancer: Joint Recommendations of the American Cancer Society, the American Society for Colposcopy and Cervical Pathology, and the American Society for Clinical Pathology ⇦

Population	Recommended Screening Method	Comment
Women younger than 21 years	No screening	
Women aged 21–29 years	Cytology alone every 3 years	
Women aged 30–65 years	Human papillomavirus and cytology co-testing (preferred) every 5 years	Screening by HPV testing alone is not recommended
	Cytology alone (acceptable) every 3 years	
Women older than 65 years	No screening is necessary after adequate negative prior screening results	Women with a history of CIN 2, CIN 3 or adenocarcinoma in situ should continue routine age-based screening for at least 20 years
Women who underwent total hysterectomy	No screening is necessary	Applies to women without a cervix and without a history of CIN 2, CIN 3, adenocarcinoma in situ, or cancer in the past 20 years
Women vaccinated against HPV	Follow age-specific recommendations (same as unvaccinated women)	

Abbreviations: CIN indicates cervical intraepithelial neoplasia; HPV, human papillomavirus.

Modified from Saslow D, Solomon D, Lawson HW, Killackey M, Kulasingam SL, Cain J, et al. American Cancer Society, American Society for Colposcopy and Cervical Pathology, and American Society for Clinical Pathology screening guidelines for the prevention and early detection of cervical cancer. CA Cancer J Clin 2012;62:147–72.

** Taken from the ACOG Practice Bulletin No. 131, November 2012*

The above guidelines are just that, and women who are at high risk for cervical cancer may need to be screened more often.

* Additional Resources:

- U.S. Preventive Services Task Force
  http://www.uspreventiveservicestaskforce.org/
- American College of Obstetricians and Gynecologists (ACOG)
  www.acog.org
- American Society for Clinical Pathology

www.ascp.org
- American Cancer Society
www.cancer.org
- American Society for Colposcopy and Cervical Pathology
www.asccp.org
- WellSpan INET, Clinical Tab, Lab, Laboratory Test Catalog, Testing, Lab Tests Online, search 'Cervical Cancer' or other topics such as 'HPV.'

4.22 Pap Test Methodology

Cells are collected from gynecologic reproductive tract sources for laboratory processing and diagnostic evaluation. Specimens are submitted either as glass slides with cells smeared and affixed (conventional Pap smear) or liquid vials with cells rinsed into preservative solution (ThinPrep Pap) that can also be used for HPV, Gonorrhea, and Chlamydia tests.

* **Conventional Pap Smear**
  - Testing is very limited in scope but significantly more cost effective
  - The collected specimen is directly smeared on a slide and spray-fixed.
  - Ancillary testing cannot be performed with this method.

* **Liquid Based ThinPrep Pap (TPP)**
  - Wellspan Health processes most client Pap tests using TPP technology.
  - Cervical-vaginal cell samples are collected utilizing a plastic spatula and endocervical brush or cervical broom and rinsing the material into the ThinPrep Preservcyt vial.
  - Non-diagnostic material such as white and red blood cells are partially removed during the liquid filtration process, thus improving the sample quality.
  - The cells are homogenized and placed in a thin monolayer on a glass slide and stained for microscopic evaluation.

* **Automated Imaging Technology**
  - All ThinPrep Pap tests at York Hospital are screened with the assistance of the Automated Imaging System, a computer-assisted primary screening system that indicates improved diagnostic capability by using a quantitative stain that measures cellular DNA content and dual slide review.
  - The imaging system prescreens the slides, and then every slide is reviewed and analyzed by a Cytotechnologist.
  - Slides that were analyzed as unsatisfactory, reactive, atypical, or abnormal are then given to a Pathologist for final review.

4.23 Patient Preparation

* Ideally, pre-menopausal patients should schedule their exam appointment for two weeks after the first day of their last menstrual period (LMP), if possible.
* Specimens should not routinely be collected during menstruation since the presence of excessive blood and endometrial cell groups may interfere significantly with the quantity and quality of available diagnostic cells, increasing the likelihood of an unsatisfactory result.
    - ***NOTE: Patients should NOT defer scheduling for symptoms of abnormal bleeding.***
* Patients should refrain from intercourse and not use vaginal medication, tampons, vaginal contraceptives, personal lubricants or douches for 48 hours before the exam.

4.24 GYN Specimen Collection

SUPPLIES

* <u>Conventional Pap Smear:</u>
    - Vaginal speculum, warmed to body temperature
    - Requisition completed with all information
    - Collection devices: spatula and endocervical brush OR cervical broom
    - Frosted end glass slide – include name, date of birth, and source in pencil
    - Spray Fixative (pump or aerosol) – 95% ethyl alcohol or 80% isopropyl alcohol
    - Slide Transport Container

* <u>Liquid ThinPrep Pap:</u>
    - Vaginal speculum, warmed to body temperature
    - Requisition completed with all information
    - Collection devices: plastic spatula and gentle-touch (GT) endocervical brush OR cervical broom
    - 20 mL PreservCyt vial – completely labeled with all required info
        * Alcohol based GYN preservative, transport and antibacterial medium
        * Stable at room temperature for six (6) weeks
        * Check out-dates and rotate supply stock. Do not use expired materials.
    - Small biohazard bag for specimen transport

*NOTE: Off-site providers may obtain Pap supplies and courier services upon request. Contact Laboratory Mobile Services. On-site providers may obtain Pap supplies from the hospital storeroom or from Anatomic Pathology receiving.

GYN SPECIMEN SOURCES

Cervix (C)	epithelial cells from the outer cervix (ectocervix), including the squamocolumnar junction (transformation zone)
Endocervix (E) or (EC)	glandular cells from the endocervical canal
Vaginal Cuff (VC)	sufficient for patients who have had a total hysterectomy (no cervix remains); If patient had a supracervical hyster with cervical stump remaining, the source would be cervix.
Vaginal Pool or Vault (V)	upper vaginal sample; may obtain cells exfoliated from the endometrium, tubes, or ovaries; Recommended for women over age 40, peri- or postmenopausal or at risk for endometrial or ovarian cancer
Vaginal Wall	Sample and label each quadrant to monitor DES-exposed patients
Polyps and Lesions	Obvious visible lesions or abnormal areas should be biopsied for histologic evaluation, although a Pap test from the sampled area may be performed

KEYS TO OPTIMAL SAMPLE COLLECTION

An optimal cervicovaginal specimen includes sampling of the squamous and columnar epithelium which includes the transformation zone where most cervical neoplasia exists. It is important to obtain a specimen that is not obscured by blood, mucus, inflammatory exudates or lubricant in order to avoid an unsatisfactory test result.

* Take the sample prior to the pelvic exam.
* Do **not** clean the cervix by washing with saline.
    - It may result in a low or hypocellular specimen

* Obtain the sample **before** any application of acetic acid.
* Use an appropriate size speculum. Use lukewarm water to warm and lubricate.
* Use of lubricant should wait until after the specimen is collected
    - If an approved water-soluble gel lubricant such as Surgilube, Astroglide, or Crystelle must be used, apply sparingly and only on outer portion of speculum with great care to avoid the tip.
    - Excessive amounts of gel may lead to an unsatisfactory result.
* Fully expose the cervix in order to view the portio and cervical canal, to evaluate and sample the entire squamocolumnar junction, and to optimally tailor the sample collection for each individual patient.
* Gently remove excess cervical mucus or inflammatory exudates with a cotton swab before taking the sample.

PAP COLLECTION PROCEDURE

In order to obtain an adequate sampling of epithelial cells, all equipment and supplies should be readily available prior to beginning the procedure.

* **Liquid ThinPrep Pap Technique *(preferred method)*:**

1. Vaginal Sample – if indicated should be obtained first, prior to introduction of the speculum. Scrape or manipulate spatula in the area of collection to obtain a sampling of cells.
2. Obtain an adequate sampling from the ectocervix using a **plastic** spatula.
    - Insert longer end of the spatula to reach the ectocervix, to fit the external os and edge of endocervical canal, and to cover transformation zone area
    - Rotate the spatula 360 degrees under pressure, pivoting around the entire circumference of the cervical os.
    - Retain the cell sample on the spatula during removal.
3. Immediately rinse the spatula into the PreservCyt Solution vial by swirling the spatula vigorously in the vial 10 times. Discard the spatula.
4. Obtain an adequate sampling from the endocervix using an endocervical brush.
    NOTE: This step may not be warranted for pregnant patients.
    - Insert brush into the cervix until only the bottommost fibers are exposed.
    - Slowly rotate ¼ or ½ turn in one direction. **DO NOT OVER-ROTATE.**
5. Rinse the brush in the PreservCyt Solution by rotating the device in the solution 10 times while pushing against the PreservCyt vial wall to open the bristles and release the cells into the solution.
    - Swirl the brush vigorously again to further release material.
    - Discard the brush.
6. Tighten the cap so that the torque line on cap passes the torque line on the vial.
7. Record patient's name and date of birth on vial and on requisition form.

8. Place the vial and requisition form into a specimen bag for transport to lab.

* **Conventional Pap Smear Slide Preparation Technique:**
  - This technique requires skill and dexterity to avoid drying of cells on slide.
  1. Obtain vaginal sample first, if indicated. Do not smear, but hand the sample Device (spatula) to an assistant.
  2. Obtain cervical sample – Rotate the spatula/ scraper 360 degrees under pressure, pivoting around the entire circumference of the cervical os. Do not smear, but hand the sample device to an assistant.
  3. Obtain endocervical sample, except on pregnant patients. Insert the brush into the endocervical canal. Rotate the brush ¼ to ½ turn. Remove the brush.
  4. Beginning with the endocervical material, smear all three specimens on the slide. Apply samples to same side of slide where patient's name will be. Spread the material in a thin, uniform monolayer over the surface of the slide.
  5. Immediately fix the cells on the slide using an alcohol-based spray fixative.
     - Essential step to prevent air-drying of cellular detail.
     - Shake bottle; hold 10"-12" from the slide; holding thumb over patient name, spray 2-3 times. Dry 10-15 minutes before slide transport.
  6. Write patient name and second identifier on frosted end slide.

4.25 High-Risk Human Papillomavirus (HPV) Testing

The human papillomavirus has been established as the primary cause of cervical cancer. Cervical neoplasia develops in susceptible individuals in response to sexually transmitted HPV infections. Most HPV-infected women will not develop significant cervical abnormalities. Cell changes can take several years to develop. The high risk HPV DNA test can detect high to intermediate-risk HPV types, but it cannot determine the specific HPV type.

CLINICAL INDICATIONS AND METHODOLOGY

* Co-Testing
  - Used adjunctively with cytology Pap test to screen women age 30 and older to assess the presence or absence of high-risk HPV types.
  - *ORDER: HPV with Any PAP Diagnosis*

* Reflex Testing
  - To screen patients with cytology Pap test results of ASC-US to determine the appropriate patient management.
  - *ORDER: HPV Reflex if PAP is ASCUS*

* HPV Testing Only
  - Requested if original HPV test was insufficient or QNS
  - *ORDER: HPV Only*

* No HPV Testing
  - If patient declines the HPV test due to cost or insurance limitations or if the physician does not want the test ordered due to clinical reasons.
  - *ORDER: Pap Only*

CURRENT ASSAYS

* High-Risk HPV Assay

- Current Assay	**Roche Cobas HPV Test** * This FDA-appoved test identifies all 14 hrHPV genotypes. The Cobas test which includes an internal control also simultaneously tests for HPV type 16 and 18
- Testing Facility	**Central Pennsylvania Alliance Lab (CPAL)**
- Requirements	1 mL residual liquid from ThinPrep Pap specimen * Small sample size eliminates QNS results
- Limitations	* WellSpan does not perform low-risk HPV testing as these assays lack clinical utility. * While HPV tests can be performed up to 6 weeks after collection, vials are only stored on-site for approx. 4-5 weeks due to space limitations. **- Call cytology for add-on HPV tests.**

* HPV 16/18 Genotyping

- Testing Facility	**Central Pennsylvania Alliance Lab (CPAL)**
- Requirements	1 mL residual sample from Pap vial
- Requests	HPV 16/18 genotyping is currently performed simultaneously with the regular hrHPV test.

HPV RESULTS AND REPORTING

HPV results can be viewed in PowerChart → Flowsheets → Lab → Infectious Disease

*** Understanding the Results**

RESULT	INTERPRETATION
Detected (Det)	The specimen was positive for hrHPV viral types
Not Detected (Not Det)	The specimen was negative for hrHPV viral types
QNS (Quantity Not Sufficient)	Residual specimen volume too low to perform test
Not Done	Initial HPV test results were inconclusive, but not enough fluid was present for repeat testing

** NOTE: The results for the more specific categories (HPVHR 16 DNA, HPVHR 18 DNA, and Other HPVHR DNA) will post in Powerchart as either "Positive" or "Negative". Please contact Pathology with any questions regarding HPV Genotype reports.*

4.26 Chlamydia and Gonorrhea PCR Testing From ThinPrep

Women of child-bearing age may be simultaneously screened for the presence of (asymptomatic) infection by *Chlamydia trachomatis (CT)* and *Neisseria gonorrhoeae (GC)* using aliquoted specimen from ThinPrep Pap sample collections.

* York Hospital GC and CT Testing Methodology

- PCR Assay	BD Viper ProbeTec using BD Viper XTR ***FDA Approved**
- Collection Device	ThinPrep PreservCyt transport medium solution ***See Microbiology Collection Manual for CT/GC swab collection method**
- Source	Cervix or Endocervix (preferably)
- Volume	Lab aliquots 0.5 mL from vial prior to Pap processing using sterile techniques to avoid contamination.
- Test Order	CHLAM PCR, GC PCR
- Limitations	* Due to the nature of the testing method, NO

Requests for GC/CT testing after ThinPrep slide preparation can be accepted.

* Testing from vial only stable within 8-10 days of collection.

4.27 Information Regarding Medicare Coverage

Medicare currently pays for one Screening Pap test every two (2) years for Low Risk beneficiaries and one screening Pap test every one (1) year for High Risk beneficiaries. (Check Medicare website for updates)

* Advanced Beneficiary Notice (ABN) – Form #8996
  - All screening Pap test patients must read, sign, and date an ABN form at the site of specimen collection.
  - Send one copy with cytology requisition to the lab, give one copy to patient
  - ***See 4.5 on page 28 for an example of Form #8996***

* Screening Pap, Low Risk
  - Patient has no signs, symptoms, or abnormal history, and there is no evidence of high-risk factors.

* Screening Pap, High Risk
  - Patient has no signs, symptoms, or abnormal history, but on the basis of medical history or other findings, is at high risk for developing cervical or vaginal cancer

* Diagnostic Pap
  - Patient has signs, symptoms, or abnormal history
  - Patient does not need ABN authorization as Pap will be covered by Medicare.

Under the most recent Medicare LCD-L32567 issued in November 2012, Medicare currently will not cover high risk HPV, Chlamydia, or Gonorrhea testing when ordered as a screening test. Consult the current LCD code restrictions for a complete listing of Medicare-approved ICD-9 testing codes.

4.3 NON-GYN CYTOLOGY COLLECTION PROCEDURES

4.30 Purpose and Value

Non-gynecologic cytology testing is used primarily for the detection of cancer and pre-cancerous conditions. The nature of possible underlying disease states including primary or metastatic malignancy, benign tumors, and infections or inflammations can often be detected by microscopically visualizing cytologic changes in cells which are exfoliated and captured by fluid extraction from body cavities, brushings, washings, or fine needle aspirations (FNA).

The value of Non-GYN cytology testing depends critically upon the quality of the specimen received. Thorough clinical information is crucial for accurate specimen analysis. Regulations require submittal of relevant clinical information for test procedures needing medical interpretation, including all cytopathology specimens. Providing pertinent clinical notes and patient history such as prior pathology results, a working diagnosis, symptoms, medication, and treatments (past or present) is directly related to the accuracy and timeliness of results. Tests should be submitted with an accurate specimen source being mindful not to abbreviate.

4.31 Anal-Rectal Cytology

* Supplies:
    - Cytobrush or saline-moistened Dacron swab
    - CytoLyt Liquid Transport Medium

* Collection Procedure:

1. Take cell sample prior to use of lubricating gel
2. Insert cytobrush or swab 2 inches into the anal canal to sample both glandular and squamous cells.
3. Use firm pressure and rotate 10-20 revolutions as device is withdrawn.
4. Vigorously shake for 10-15 seconds in Cytolyt vial to dislodge cells.
5. Now, brush or swab over visible perianal lesions, and repeat steps 3 and 4.

* Additional Information:

- High-risk HPV patients include HIV positive individuals, those who engage in anal sexual intercourse, men with anal warts, women with history of anal, vulvar, or cervical warts.
- The Digene HC2 HPV Test is not FDA-approved for sources other than cervico-Vaginal. Contact the Clinical Lab Referrals Desk for additional information.

4.32 Body Cavity Fluids (Serous Effusions)

* Supplies:

- Sterile, Leak-Proof Specimen Container
- Heparin (see ratio below)
- Refrigerator (if specimen will not be delivered to testing site immediately)

* Collection Procedure:

1. To prevent clotting, add heparin to specimen container as follows:
    - Add 3 units of sodium heparin per 1 mL of fluid
    - Add 0.3 mL heparin to every 100 mL fluid collected
2. Fluid aspiration is usually performed by wide-bore needle through the body wall into the cavity where the fluid has accumulated:
    - Pleural fluid (chest cavity thoracentesis)
    - Peritoneal or ascitic fluid (abdominal cavity paracentesis)
    - Pericardial fluid (heart cavity pericardiocentesis)
    - Synovial fluid (joint spaces)
3. As fluid is collected, agitate container gently to mix heparin with fluid.
    - A volume of 50cc to 250cc is optimal for cytology testing
4. Deliver specimen immediately to Clinical Lab, Hematology for specimen processing, triage, or refrigeration; keep specimen cold if transport is delayed.

* Additional Information:

- Washings of the peritoneal cavity with physiologic saline solution at the time of Abdominal surgery may also be collected and submitted for evaluation.
- Submit Cytology and Surgical Pathology specimens together.

4.33 Brushings

(Bronchial, Esophageal, Gastric, Duodenal, Bile Duct, Colon, Urinary Tract)

* Supplies:
  - Endoscopic Brush
  - Cytologic Spray Fixative
  - Frosted End Slides
  -CytoLyt Preservative
  - Non-Sterile Saline (**for bronchial brush specimens**)

* Collection Procedure:
  1. Push brush through the proper instrumentation (scope), visualize the suspect lesion, and brush the area.
  2. Pull brush back into sheath and withdraw both scope and brush.
  3. Spread material from brush thinly and evenly on glass slide
  4. Immediately spray fix the specimen 2-3 times.
  5. If smears are prepared from different areas, label each slide appropriately.
  6. The brush may be cut and submitted in CytoLyt preservative or non-sterile saline (bronchial specimens) for lab processing.

* Additional Information:
  - For triage purposes, bronchial brushings should NOT be submitted in CytoLyt.
  - Cytologic cellular examination is complementary to tissue biopsy.
  - Submit labeled cytopathology and surgical pathology specimens together.

4.34 Cerebral Spinal Fluid (CSF)

* Supplies:
  - Sterile, Leak-Proof Specimen Container

* Collection Procedure:
  1. Obtain CSF fluid via spinal tap procedure.
  2. A volume >5cc is optimal for cytology, however less can be processed

3. Deliver immediately (within 1 hr.) to Clinical Lab and/or refrigerate, but do not freeze.

* Additional Information:
  - Solid brain tissue may be collected by FNA with image-guided techniques
  - At York Hospital, if Flow Cytometry testing is desired, then that can be indicated on the requisition. However, every case is independently evaluated by a Hematopathologist in order to determine if this testing is indicated.

4.35 Nipple Discharge

* Supplies:
  - Cytologic Spray Fixative
  - 2 Frosted End Glass Slides
  - Slide Holder for Transport

* Collection Procedure:
  1. Label slides with patient's name, date of birth, and specimen source.
     - If smears are prepared from both breasts, then label each slide as LEFT or RIGHT, and do not abbreviate.
  2. When secretion occurs, allow a pea-sized drop to accumulate on apex of nipple.
  3. Apply slide directly to the nipple and use a second slide to gently smear the droplet.
  4. Immediately spray fix one slide and label "Fixed."
  5. Allow the second slide to air dry and label "Air."
  6. Repeat steps 1-5 if additional fluid is present. Label as specimen #2.
  7. Allow spray fixative to dry. Package in slide holder for transport.

4.36 Sputum Cytology

* Supplies:
  - Sterile, Leak-Proof Specimen Container

* Collection Procedure:
  - **SPONTANEOUS (Deep Cough)**
    - Patient instruction – before breakfast, rinse mouth several times

- Patient must cough deeply into clean/sterile container
- Refrigerate or deliver specimen promptly to lab.
- Highest diagnostic yield is achieved by collecting and submitting a specimen on each of **three consecutive** mornings.

- INDUCED (Using Heated Aerosol)

- Preferred method for specimen adequacy
- Contact Pulmonary Function Lab for assistance with procedure.

4.37 Tzanck Smear – Skin Scrapings

* Supplies:
  - Swab or collection device
  - 2 Frosted-End Microscope Slides
  - Cytologic Spray Fixative
  - Slide Holder for Transport

* Collection Procedure:
  - Use pencil and label white part of slide with patient's name, date of birth, and specimen source
  - Collect specimen or scrape lesion and spread cellular material evenly on the clear portion of 2 glass slides.
  - Immediately spray fix one slide. Label "Fixed" on white end.
  - Let the specimen on the second slide air-dry. Label "Air" on white end.
  - Once dry, place both slides in slide holder and submit to cytology.

4.38 Urine Cytology

* Supplies:
  - Sterile, Leak-Proof Specimen Container

* Important Instructions:
  - Collection method must be specified (voided, catheterized, cystoscopic) and is essential for medical interpretation
  - Source must be specified (bladder, right or left ureter, renal pelvis, right or left kidney, etc…)
  - A volume of 50cc or more is optimal; however, less can be processed.

* Collection Procedure:

- **VOIDED**
    - First morning specimens, drainage bag samples, and 24-hour urine collections should **not** be submitted.
    - Instruct patient to drink at least two 8 oz. glasses of water one hour before collection in order to remain hydrated.
    - Have patient void urine in sterile container being careful not to contaminate with tissue products.
    - Refrigerate and/or deliver immediately to prevent rapid cellular degeneration, especially if delay over 1 hour is anticipated.
- **CATHETERIZED**
    - When collecting urine, avoid excessive lubricant.
    - Any instrumentation or trauma should be noted

4.39 Washings

(Bronchial Lavage, Bronchial, Pelvic, Peritoneal, Tracheal, Urinary Tract)

*Supplies:

- Sterile, Leak-Proof Specimen Container
- Saline Solution

* Collection Procedure:

- Specimen will be obtained by physician, and collection is obtained using a balanced saline solution.
- After specimen is collected, refrigerate and deliver as soon as possible to lab.

* Additional Information:

- **Pneumocystis carinii pneumonia (PCP) Testing**
    - Acceptable specimens for testing are bronchial washings, bronchoalveolar lavage, and induced sputum (least acceptable)
    - Spontaneous sputum production does not yield a satisfactory specimen for PCP testing.
    - Submit samples to cytology by 2:00p.m M-Th and by 4:00pm F for same day preliminary results.
- Provide all relevant clinical information and patient history.

4.4 FINE NEEDLE ASPIRATION COLLECTION PROCEDURES

4.40 Purpose and Value

Fine Needle Aspiration (FNA) is a cost-effective cell collection method which offers timely diagnosis, and is a less invasive alternative to open surgical biopsy in many cases. Its simplicity and safety facilitate earlier diagnosis. Specimens can be prepared by traditional cytology processes and techniques and ancillary diagnostic testing can be applied. Fine needles can be guided manually or with image-guided fluoroscopy, computed tomography (CT), ultrasound (US), or endoscopic ultrasound (EUS).

4.41 FNA Sources

* Superficial Solid Nodule or Cyst:
  - Breast
  - Lymph Node
  - Neck
  - Salivary Gland
  - Skin Vesicle or Lesion
  - Thyroid

* Deep Seated Mass, Lesion, Nodule, or Cyst:
  - Liver
  - Lung
  - Mediastinum
  - Pancreas
  - Retroperitoneum

4.42 Clinician-Performed FNA Collection Instructions

Have all necessary supplies and equipment readily available when procedure begins. Since a needle is being introduced into the body, FNA procedures should be performed under sterile, aseptic techniques to avoid infection. Having a trained assistant is advantageous. Prior extensive training is needed to perform a fine needle aspiration. This procedure should only be performed by physicians with appropriate training and experience.

NOTE: Contact Pathology at 851-5001 to discuss or request on-site training assistance.

* Supplies:
  - Disposable 22-27 gauge needles
  - 10 mL plastic syringes
  - Syringe holder or pistol device (helpful)
  - Frosted-end Glass Slides
  - Lead Pencil to label slides
  - Cytologic Spray Fixative
  - CytoLyt Preservative
  - Saline Solution – used for bronchial FNAs and any FNA specimen which may require flow cytometry studies.

* Collection Procedure:
  1. After identifying and stabilizing the lesion, using a 23 or 25-gauge needle, insert the needle into the lesion, apply suction to the syringe, and move the needle back and forth.
     - Each thrust motion can be thought of as a “microbiopsy” that drives cellular material into the hub of the needle. Thus, the more time that is invested in moving the needle back and forth within the lesion, the more cellular (and potentially diagnostic) an aspirate sample will be.

2. Release the suction and withdraw the needle.
3. Immediately express the material onto a glass slide.
4. Detach the needle from the syringe, draw some air into the syringe, reattach the needle, and express onto the slide once again. Repeat as necessary.
    - Never draw air into the syringe with the needle still attached.
    - Avoid flooding the slide with blood if the aspirate is particularly bloody (e.g. >0.5 cc of blood collects)
    - If excessive blood is in the syringe, detach the needle from the syringe and empty the bloody contents in to CytoLyt or saline.
5. Place a second slide against the first slide and gently pull apart (smear) once.
6. Immediately spray fix (alcohol fix) the second slide, and leave the first slide to air dry. Write "1" on both slides to denote that this is pass one.
    - It is critical that each pass yield both an air-dried slide (that will be treated with a Diff-Quik stain in the lab) and a fixed slide (that will be treated with a Pap stain in the lab). Label each slide accordingly.
    - Thus, a standard two-pass FNA will generate four direct smear slides (2 air-dried and 2 fixed).
    - It is these direct smear slides that provide the vast majority of diagnostic information for successful cytopathologic interpretation.
7. Rinse the needle vigorously into the CytoLyt (or saline) vial.
8. Move on to the second needle pass (repeat steps 1 through 7). Label these slides with a "2" to denote that this is pass two.

* Additional Information:

- **The submission of FNA specimens entirely into CytoLyt (i.e. without making direct smear slides) is a practice that is diagnostically detrimental and is strongly discouraged.**
    - **One possible exception to this principle may be lesions that appear cystic. If, however, there is any evidence of a solid component to a cystic process, the direct smear technique would be ideal.**
- In order to optimize diagnostic yield, a minimum of two sequential needle Passes is the standard when sampling a lesion by FNA
- A new needle should be used for each pass.
- Because they lessen the effect of hemodilution, 23 and 25-gauge needles are superior to the wider caliber 22-gauge needles
    - **25-gauge needles should be used for aspirates of the thyroid.**

4.43 Image-Guided FNA with Onsite Pathology Assistance

* Specimen collection with Pathology technical assistance and rapid specimen adequacy assessment requires advance scheduling with Imaging Services.

- Availability:	Monday through Friday, 7am-4:30pm
- Contact Number:	717-851-5001

* If onsite pathology assistance is needed after 4:30pm, then call the cytology department in advance or as soon as possible to ensure adequate staffing is available.

* For urgent cases requiring assistance after-hours or on weekends, the on-call pathologist may be contacted by phoning the clerical lab office at 851-2511.

See Chapter 9 of the Cytology Procedure Manual for additional Onsite FNA procedures.

4.44 Flow Cytometry Requests

* When clinically relevant, Flow Cytometry requests will be handled by pathology staff and sent out to a reference lab for testing.

FLOW CYTOMETRY FOR SARCOID

Specimen Type:	Bronchial Wash
Orderable:	Lymphocyte Subset Panel
Background:	Result generates a CD4:CD8 ratio from the T cells in the specimen. A result greater than 2.5 could be consistent with, though not diagnostic of, sarcoid.
Charge:	Approximately $50
Procedure:	Send the specimen with the order to Clinical Lab Referrals for send out.

FLOW CYTOMETRY FOR LYMPHOMA / LEUKEMIA

Specimen Type:	Fine Needle Aspiration, Forceps Biopsy of target lesion

Orderable:	N/A – Consult with Pathology before ordering
Background:	If either lymphoma or leukemia are considered in the differential, quick evaluation of the specimen by a pathologist is important to determine if the process is lymphoid vs. epithelial and if flow cytometry testing is warranted.
Charge:	Approximately $500
Procedure:	Call Pathology (851-5001) to schedule an onsite fine needle aspiration adequacy assessment.

4.5 CURRENT TEST REQUISITIONS

Test requisitions and forms provide a means of communicating patient information necessary for the collection, receipt, preparation, evaluation, billing, coding, and reporting of cytopathology test requests among all persons handling cases.

EXAMPLES AND USAGE

* *Wellspan Cytopathology Requisition – Form #3007*
  Supplied by York Hospital Cytopathology Lab

* *Cerner Millennium PowerChart Orders – Electronic*
  Form generated whenever an order is placed through Cerner for a CYTO GYN or a CYTO NGYN test. Form prints to cytology dept.

* *Ecare (AllScripts) Requisition – Electronic*
  Used by WellSpan Medical Group (WMG) practices and some Non-WMG practices. Form generated when order is submitted.

* *Advanced Beneficiary Notice (ABN) – Form #8996*
  Used at physician office to help Medicare patients make informed choices about lab tests and payments. White copy to Lab; yellow copy to patient