



CATEGORY: Anatomic Pathology, Cytopathology

EFFECTIVE DATE: 03/07/2022

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**SCOPE OF SERVICE / SPECIMEN COLLECTION AND  
SUBMISSION MANUAL**

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VERSION: 1

**SCOPE OF SERVICE**

**Amarillo Pathology Associates** is an anatomic laboratory providing patient diagnostic services to the community and surrounding area. APA produces quality test results in an effective time frame to support treatment. Hours of operation are Monday through Friday 8:00 am to 4:30 pm with 24 hour on-call pathologist coverage for hospital systems. APA maintains accreditation through the College of American Pathologists. Our seven pathologists provide Clinical Laboratory Medical Directorship to multiple hospitals in the Texas panhandle. For any questions concerning the following information, please contact APA at the appropriate phone number:

**Amarillo Pathology BSA office: 806-212-5942**

**Amarillo Pathology Nwth office: 806-354-1754**

**Specific services provided include:**

**Surgical Pathology** – This section provides gross examination of anatomic specimens, documentation of foreign bodies and/or orthopedic hardware, and processing of tissues for light microscopic examination and diagnosis.

**Cytopathology** – This section provides the full range of Cytopathology diagnostic services including a quick examination of a specimen to secure its adequacy at the time of radiological guided aspirates and preparation and interpretation of routine gynecological and non-gynecological materials.

**Autopsy Pathology** – This section establishes, with all possible accuracy, the cause of death and determines the nature and cause of disease processes or other conditions that contribute to death or contributed to other illnesses present at the time of death.

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APPROVED BY: James Hurlly MD

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Page 1 of 14

**Anatomic Pathology**

- Histology processing / staining
- Immunohistochemical staining and professional interpretation
- In situ hybridization staining and professional interpretation
- Frozen section processing, staining and professional interpretation
- Surgical pathology professional interpretation
- Flow Cytometry professional interpretation
- Pathology Consultation
- Autopsy Services
- Appropriate selection of reference laboratory tests
- Provide laboratory statistics to medical staff, administration, and inter-departmental teams as requested

**Cytopathology Services**

- Cytology processing / staining
- Gynecologic interpretation
- HPV, GC/Chlamydia and Trichomonas testing on submitted Thin Prep Pap vial (send-out to Physician’s Preferred Laboratory)
- Non-gynecologic interpretation
- FNA processing / staining
- Rapid on-site evaluation for adequacy of FNA samples (contact laboratory to schedule)

**Turn-Around-Times** – figured using date specimen is received in laboratory to date case is resulted.

<b>Surgical pathology report (routine)</b>	<b>1 Working day</b>
<b>Surgical pathology report requiring peer review</b>	<b>1 Working day (varies)</b>
<b>Surgical pathology report requiring additional stains/studies</b>	<b>2 Working days</b>
<b>Autopsy Preliminary report</b>	<b>2 Working days</b>
<b>Autopsy Final report</b>	<b>60 Working days (or less)</b>
<b>Gyn Cytology (Pap)</b>	<b>1 Working day</b>
<b>Non-Gyn Cytology</b>	<b>2 Working days</b>

## SPECIMEN COLLECTION AND SUBMISSION

### Anatomic / Surgical Pathology Specimen Submission

- **For all specimens submitted to Pathology**
  - All specimens submitted to the Pathology department must have an accompanying pathology requisition including the following information:
    - Patient name, DOB, and sex
    - Name and signature of ordering physician (signature not applicable for interfaced EMR orders)
    - Date and time specimen was collected from the patient
    - Date and time specimen was placed in formalin (if applicable)
    - Specimen/tissue source
    - Clinical information/history
  - All submitted containers must be properly labeled with at least two patient identifiers (EX: Name, DOB, MRN, SS#, etc.).
  - Each surgical tissue is placed in a separately, properly labeled container. Multiple sites and/or organ specimens should not be placed in the same container.
  - Each separate surgical tissue container should be properly labeled with the exact specimen site or organ.
  - Each separate surgical case should be logged into the appropriate Specimen Log with the correct number of containers submitted. Log in information includes specimen type, date, and initials of circulating nurse submitting the specimen(s).
  - Any specimen with incomplete or incorrect identification of the patient will not be accessioned until further identification is established. The pathology department **will not** label/relabel a mislabeled specimen. Only a staff member from the submitting hospital department or physician's office may add/change patient identification.
- **Tissue for routine pathology**
  - Place tissue in specimen container large enough to accommodate specimen and appropriate amount of formalin.
  - The entire specimen should be completely submerged in 10% neutral buffered formalin to approximate specimen volume of 10:1 or higher, or if not feasible (eg, large specimens) at least 4:1.
  - Amputated extremities and specimens too large to place in a formalin container, are to be placed in the appropriate large biohazard bag and properly labeled.
- **Tissue for frozen section diagnosis**

- Do not submerge the tissue in saline, formalin, or any other fluid.
- Keep tissue fresh (not in formalin or any other fixative) and send immediately (STAT) to the Pathology Department.
- Designate on the requisition that the tissue is for “Frozen” and provide a call-back number or OR room number.
- Very small specimens should be placed between two pieces of saline-soaked Telfa pads.
- Prolonged time between collection and receipt in the Pathology department could limit interpretability.
- **Tissue for lymphoma work-up/flow cytometry**
  - Do not submerge the tissue in saline, formalin, or any other fluid.
  - Keep tissue fresh (not in formalin or any other fixative) and send immediately (STAT) to the Pathology Department. Tissue can be placed on saline moistened gauze or saline moistened Telfa pad.
  - Designate on the requisition that the tissue is for lymphoma work-up/flow cytometry.
  - Tissue can be placed directly into RPMI transport media (available through the Pathology Department) by the physician if desired.
  - Peripheral blood and bone marrow aspirate submitted in EDTA or heparinized blood collection vials can also be submitted for flow cytometry.
- **Kidney biopsies for send-out processing**
  - This is a procedure done in CT or Ultrasound.
  - There is a Kidney send-out kit that Pathology can provide to CT or Ultrasound. This kit provides the three containers needed for processing.
  - If three or more tissue cores are obtained, place one core in the 10% neutral buffered formalin container provided, one core in the Michels transport solution container provided, and one core in the Karnovsky’s fixative container provided. Then send the kit including all containers to Pathology.
  - If two tissue cores are obtained, place one core in the 10% neutral buffered formalin container provided, divide the second core and place half in the Michels transport solution container provided and the other half in the Karnovsky’s fixative container provided. Then send the kit including all containers to Pathology.
  - If one core is obtained, submit two 1-2 mm fragments from the ends of the core and place in Karnovsky’s fixative, submit two 1-2 mm fragments from the new ends and place in Michels transport solution, and place the remaining tissue in 10% neutral buffered formalin. Then send the kit including all containers to Pathology.

- **Skin biopsies for immunofluorescence**
  - Obtain two skin biopsies from the same site.
  - Place one skin biopsy in a 10% neutral buffered formalin container (provided by Pathology Department).
  - Place the other skin biopsy in a Michels transport solution container (provided by Pathology Department).
  - Send both containers to the Pathology Department.
  - If formalin and Michels containers are unavailable to the physician, place the skin biopsies on saline moistened gauze or saline moistened Telf pad and send immediately (STAT) to Pathology.
- **Muscle biopsies for send-out processing**
  - This is a surgical procedure and should not be done in CT or Ultrasound.
  - This procedure should be done Monday through Thursday to account for specimen transport. Procedure should not be done on Fridays.
  - The muscle biopsy should be taken from the belly of a moderately affected muscle (avoid EMG and injection sites). The muscle biopsy should be 2.5-3.0 cm in length by 1.0 cm width or wider for an adult or 2.0 cm in length by 0.5 cm width for a child.
  - Do not submerge the tissue in saline, formalin, or any other fluid.
  - Place the muscle tissue on saline moistened gauze or saline moistened Telfa pad and send immediately (STAT) to Pathology.
- **Nerve biopsies for send-out processing**
  - This is a surgical procedure and should not be done in CT or Ultrasound.
  - This procedure should be done Monday through Thursday to account for specimen transport. Procedure should not be done on Fridays.
  - Obtain a segment of nerve approximately 5 cm long.
  - Do not submerge the tissue in saline, formalin, or any other fluid.
  - Place the nerve tissue on saline moistened gauze or saline moistened Telfa pad and send immediately (STAT) to Pathology.
- **Predictive Marker Testing (HER2, ER, PgR) for send-out processing and in-house interpretation**
  - Place tissue in specimen container large enough to accommodate specimen and appropriate amount of formalin.
  - The entire specimen should be completely submerged in 10% neutral buffered formalin to approximate specimen volume of 10:1 or higher, or if not feasible (eg., large specimens) at least 4:1.

- The specimen should be fixed in 10% neutral buffered formalin for at least six hours and up to 72 hours to avoid false test results. This includes the time in formalin during pathology processing.
- Rapid immersion of specimens in fixative is critical and must occur within one hour of the biopsy or resection. (Cold ischemic time)
- If delivery of a resection specimen to the pathology department is delayed (eg., specimens from remote sites), the tumor must be bisected prior to immersion in fixative. In such cases, it is important that the surgeon ensure that the identity of the resection margins is retained in the bisected specimen; alternatively, the margins may be separately submitted.
- **Other biopsies for specialized testing**
  - Contact the Pathology Department prior to collection for submission instructions.

### **Cytopathology Specimen Submission**

#### **Requisition and Identification Requirements:**

All specimens should be clearly labeled with at least two identifiers (patient's name, DOB, soc. security number, etc)., and enclosed in a biohazard bag. A pathology requisition form must accompany the specimen and in addition to name of ordering physician and location of the patient, should contain the following:

1. source of specimen
2. date and time of collection
3. pertinent clinical history

All Pap smears must contain, in addition to the above listed information, the following:

1. Date of last menstrual period.
2. Previous abnormal reports.
3. Treatment and/or biopsy information.

*Specimens will only be accepted when all the above criteria are met and only when ordered by physician or other persons authorized by law.*

#### **Collection and Preservation:**

All specimens should be prepared and submitted to cytology with completed requisitions as soon as possible. If immediate preparation and submission is not possible, it can be preserved in the refrigerator for a period of 24-48 hours.

**Collection of Non-GYN specimens for Cytology:**

**Body Cavity Fluids, Body Washings, Urines and CSF:**

Pleural fluid, Peritoneal fluid, Pericardial fluid:

- specimen is collected by a physician in a clean properly labeled container.
  - specimen container should be properly sealed, not leaking, and not submitted with attached collection tubing/devices.
- send as much fluid as possible, up to 1000 ml. This amount is sufficient for cytological investigation.
  - large volume specimens requiring multiple tests should be sent to different departments (i.e. Microbiology, Hematology, and Chemistry) in separate containers.
  - small volume specimens (preferably between 100-150 ml) that require multiple tests may be sent in a single collection container. The container should be sterile if cultures are ordered.
  - *submitted samples below 100-150ml will yield very little cellular material for review and will affect diagnostic accuracy*
- do not add fixative.

Peritoneal/Gutter Washings, Bronchial Lavages/Washings, Renal Pelvic/Bladder Washings, etc.:

- using normal saline, the specimen is collected according to expected procedural steps by the physician in a clean properly labeled container.
  - if possible, up to 50 cc of fluid should be collected for proper cytologic preparation.
- special orders/studies (such as: PCP, GMS, Silver stain) should be documented on requisition as applicable
  - *preferably, microbiology and cytology should receive separate specimens.*
- information regarding the potential for highly infectious organisms (i.e., TB, COVID-19) must be documented on the requisition as applicable.
- do not add fixative.

Voided Urine:

- specimen is collected by patient. Be sure all specimens are collected “clean-catch” and in properly labeled containers. Make sure to note that specimen is voided urine on completed requisition form.
  - voided specimens should be *first-voided, morning specimens* for optimum cytologic evaluation.

Catheterized Urine:

- specimen is collected by physician or nursing staff in a clean properly labeled container. Make sure to note that specimen is catheterized.

**Cerebrospinal fluid:**

- specimen is collected by a physician in a clean, properly labeled container, with completed requisition indicating site of tap (lumbar, ventricle, omega reservoir) and relevant clinical information.
  - specimens requiring multiple tests should be sent to different departments (i.e. Microbiology, Hematology, and Chemistry) in separate containers.
    - cytology can receive smallest sample collected but preferably no less than 1.0cc of fluid
- do not add fixative.

**Brushings and Sputums:****Brushings (Bronchial and Gastrointestinal Tract):**

- roll (do not smear) the contents of the brush onto a clean, labeled glass slide and spray-fix immediately or place into alcohol.
- cut off brush, leaving approximately one-half inch of wire, and drop brush into labeled PreservCyt® vial.
- send prepared glass slide(s) and PreservCyt® vial to Cytology Laboratory.

**Sputum (Inpatient and Outpatient):**

- specimen must be collected in a clean, sterile, properly labeled container. Specimen should be an *early morning, deep cough* collection, preferably before breakfast, and not saliva.
  - specimens that cannot be delivered to the laboratory within 8 hours should be refrigerated (normally outpatient collections).
- special orders/studies (such as: PCP, GMS, Silver stain) should be documented on requisition as applicable
  - *preferably, microbiology and cytology should receive separate specimens.*
- information regarding the potential for highly infectious organisms (i.e., TB, COVID-19) must be documented on the requisition as applicable.
- do not add fixative.

**Sputum (Induced):**



- heated aerosolized hypertonic saline, or other appropriate solution, is inhaled by the patient for 20 minutes. Have patient cough into a clean, sterile, labeled specimen container, often a “sputum trap”.
- special orders/studies (such as: PCP, GMS, Silver stain) should be documented on requisition as applicable
  - *preferably, microbiology and cytology should receive separate specimens.*
- information regarding the potential for highly infectious organisms (i.e., TB, COVID-19) must be documented on the requisition as applicable.
- Do not add fixative.

**Sputum (24-hour post-bronchial sputum):**

- Collect one good, deep cough specimen at any time during the 24 hours following bronchoscopy. Patient should cough into a clean, sterile, labeled specimen container
- special orders/studies (such as: PCP, GMS, Silver stain) should be documented on requisition as applicable
  - *preferably, microbiology and cytology should receive separate specimens.*
- information regarding the potential for highly infectious organisms (i.e., TB, COVID-19) must be documented on the requisition as applicable.
- Do not add fixative.

**Expressed/Scraped Smears:****Nipple Discharge:**

- Express secretion by gently compressing the full circumference of the areola between thumb and index finger. Smear resulting secretion on a clean, properly labeled glass slide.
  - if secretion is scanty, the slide may be touched to the nipple. If secretion is thick, it may be smeared between two slides.
- *spray-fix slides immediately.*
- place prepared slides in carrier and send to Cytology Laboratory with completed requisition.

**Skin/Tzanck Smear (Herpes Simplex, Varicella-Zoster Virus):**

- physician should identify a fresh, “representative”, vesicle.
- unroof the vesicle and scrape the margins with a scalpel blade. Collected material and debris adherent to the blade onto a clean, properly labeled glass slide.
- *spray-fix immediately.*

- place prepared slides in carrier and send to Cytology Laboratory with completed requisition.

**Fine Needle Aspirate:**

## Aspirates of Superficial Sites:

- prepare aspiration site according to standard protocols. Label at least two (2) clean glass slides, depending on number of aspiration passes to be performed, with the patient's name, specimen source, and part/pass number (i.e., pass 1, 2, 3 or part A, B, C).
- attach a 22-gauge (or 25-gauge in certain sites such as thyroid) needle to a 10-20 cc syringe.
- pass the needle through the skin and into the lesion. After the needle is in the lesions, draw back the plunger of the syringe. Move the needle back and forth in a "Jack Hammer" motion.
  - note: *(solid lesion) material should be aspirated only into the needle and not into the syringe. Once material appears in the hub of the needle, aspiration should be discontinued. Blood is undesirable.*
  - note: *(cystic lesion) the syringe may be filled with fluid. This fluid may be submitted for cytologic examination.*
- once aspiration is completed, release the plunger and allow it to fall back to a neutral position. Remove the needle and syringe from the patient. Remove the needle from the syringe and draw air into the syringe. Replace the needle onto the syringe. Collected material can be submitted in one of two ways:
  1. *with the bevel pointed down, carefully express the material in the needle onto the center of a slide. Immediately place the second slide over the slide with the sample, allowing the sample to spread evenly between the two slides without any smearing/pulling motion.*
  2. *separate the slides using a smooth pulling motion and spray-fix immediately.*
    - a. *if the residual specimen volume allows, needle may be rinsed into a Thin Prep CytoLyt® solution*
  3. *or, express all the material in the needle into a Thin Prep CytoLyt® solution.*
    - a. *needle may be rinsed in the Thin Prep CytoLyt® solution to ensure all material has been transferred into CytoLyt®*
- place the slides/ CytoLyt® in a carrier and send with a completed requisition (clinical information is very useful) to Cytology Lab.
- above procedure may be repeated several times for multiple collection passes.

## Aspirate of Deep Sites:

- deep sites are aspirated under radiologic guidance using a technique similar to that for superficial sites (see above).
- technician spreads the collected sample between at least two (2) labeled slides and *spray-fixes slides immediately* with remaining material being rinsed into the Thin Prep CytoLyt® solution, or all material being expressed into a Thin Prep CytoLyt® solution.
- cytologic preparations and cell blocks are prepared from the CytoLyt® solution. (needle cores can be fixed for histologic sectioning)
- special stains and immunohistochemistry can be performed on cell blocks and tissue cores.
  - tissue to be sent for Flow Cytometry has special transport requirements. *Please contact the Pathology Dept. (BSA 212-5942 or NWTH 354-1754) before starting the procedure for supplies and instructions.*
- Place slides in a carrier and send with any additional specimens along with a completed requisition (to all relevant include clinical information) to Cytology Laboratory.

**Collection of GYN specimens for Cytology:****Collection of Cervical/Vaginal Pap Smear:**

Patient Preparation:

To optimize collection conditions, a patient should (preferably):

1. Schedule an appointment 10-18 days after the first day of her last menstrual period.
2. Not douche 48 hours prior to the test.
3. Not use tampons, birth control foams, jellies or other vaginal creams or vaginal medications for 48 hours prior to the test
4. Refrain from sexual intercourse 24-hours prior to the test.

**Collection Procedure:**

Note: Obtain specimen prior to bimanual examination using a speculum lubricated with saline/water. (approved water-based lubricant may be used *sparingly*).

Preferred Method(s)

**1. Thin Prep® Pap Test™ using broom-like device**

- Record the patient's name and ID number or date of birth on the vial.

- Record the patient information and medical history on the cytology requisition form, indicating on the requisition form which test(s) is to be performed.
- Gently remove mucus exudate or blood from surface of cervix.
- Obtain an adequate sampling from the cervix using the broom-like device by inserting the central bristles of the broom deep enough into the endocervical canal to allow the shorter bristles to fully contact the ectocervix. Push gently and rotate the broom in a clockwise direction five times.
- Rinse the broom into the PreservCyt® solution vial by pushing the broom into the bottom of the vial 10 times, forcing the bristles apart. As a final step, swirl the broom vigorously to further release material. Discard the entire collection device.
  - *do not “snip off” or remove broom device to leave in collection vial; cells will adhere to the broom device and not be adequately collected*
- Tighten the cap so that the torque line on the cap passes the torque line on the vial.
- Place the sample vial and requisition in its own individual specimen bag for transport to the laboratory.

## **2. Thin Prep® Pap Test™ using spatula and endocervical brush**

- Record the patient’s name and ID number or date of birth on the vial.
- Record the patient information and medical history on the cytology requisition form, indicating on the requisition form which test(s) is to be performed.
- Gently remove mucus exudate or blood from surface of cervix.
- Obtain an adequate sampling from the *ectocervix* using a plastic spatula.
- Rinse the spatula into the PreservCyt® solution vial by swirling the spatula vigorously in the vial 10 times. Discard the spatula.
- Obtain an adequate sampling from the *endocervix* using the endocervical brush device. Insert the brush into the cervix until only the bottom-most fibers are exposed. Slowly rotate  $\frac{1}{4}$  or  $\frac{1}{2}$  turn in one direction. **DO NOT OVER-ROTATE.**
- Rinse the brush in the PreservCyt® solution vial by rotating the brush in the solution 10 times while pushing against the vial wall. Swirl the brush vigorously to further release material. Discard the brush.
- Tighten the cap so that the torque line on the cap passes the torque line on the vial.
- Place the vial and requisition in its own individual specimen bag for transport to the laboratory.

### Non-Preferred Method(s)

#### **1. Conventional Pap smear using broom-like device**

- Record the patient's name on the frosted end of the slide using the black pencil (do not use ink).
- Record the patient information and medical history on the cytology requisition form, indicating on the requisition form which test(s) is to be performed.
- Gently remove mucus exudate or blood from surface of cervix.
- Obtain an adequate sampling from the cervix using the broom-like device.
- Insert the central bristles of the broom deep enough into the endocervical canal to allow the shorter bristles to fully contact the ectocervix. Push gently and rotate the broom in a clockwise direction five times.
- "Paint" contents of broom onto the glass slide, ensuring as much homogeneity as possible without aggregating sampled cellular material onto one spot. Discard the collection device.
- *Apply fixative immediately.*
  - *aerosol spray fixatives should be held four to six inches from slide and applied for a few bursts.*
- Place the kit and requisition in its own individual specimen bag for transport to the laboratory.

## **2. Conventional Pap smear using spatula and endocervical brush**

- Record the patient's name on the frosted end of the slide using the black pencil (do not use ink).
- Record the patient information and medical history on the cytology requisition form, indicating on the requisition form which test(s) is to be performed
- Gently remove mucus exudate or blood from surface of cervix.
- Obtain an adequate sampling from the *ectocervix* using the wooden spatula and spread the material quickly and evenly without aggregating sampled cellular material onto uncovered portion of glass slide.
- *Apply fixative immediately.*
  - *aerosol spray fixatives should be held four to six inches from slide and applied for a few bursts.*
- Obtain an adequate sampling from the *endocervix* using the endocervical brush device. Insert the brush into the cervix until only the bottom-most fibers are exposed. Slowly rotate  $\frac{1}{4}$  or  $\frac{1}{2}$  turn in one direction. **DO NOT OVER-ROTATE.**
- Spread the material quickly and evenly by rolling the brush onto the second half of the glass slide.
- *Apply fixative immediately.*
  - *aerosol spray fixatives should be held four to six inches from slide and applied for a few bursts.*
- Place the kit and requisition in its own individual specimen bag for transport to the laboratory.

### **Comments:**

The method of cell collection and concern for detail of collection is crucial. Liquid based collection (Thin Prep®) has been shown to be more effective than the conventional glass smear collection. When using conventional glass slides, two slides have not been shown to be of advantage over one slide.

Some Pap smears are considered atypical (ASCUS), but not clearly neoplastic. This is a very heterogeneous group of smears. HPV testing has shown to be of value in determining the risk factor for developing CIN in women of this diagnostic category. (see below)

We advise a Pap smear for all women starting at the age of first sexual contact and progressing through life as clinically indicated by current published guidelines.

**HPV Testing and GC/Chlamydia (Thin Prep Pap Only):**

Human Papilloma Virus and GC/Chlamydia (and Trichomonas) testing can be ordered at the time of collection by checking the appropriate ancillary testing request box on the requisition:

GC/Chlamydia

High risk HPV testing if ASCUS

High risk HPV testing if ASCUS or Above

High risk HPV testing regardless of pap result

Ancillary Testing: write in "Trichomonas"

*HPV 16,18/45 genotyping can be requested on any positive high risk HPV test by checking the corresponding box after the HPV testing profiles listed above.*

HPV and GC/Chlamydia testing may also be ordered up to 28 days after collection of the Pap smear. HPV and GC/Chlamydia testing are performed utilizing the Hologic Panther® System at Physician's Preferred Laboratory.