

# NewPath Diagnostics, LLC

## Specimen Collection manual

This manual is written for specimen collections in physician offices of various specialties. While there may be slight differences in each specialty, the overall principle and procedure apply to all submitting clients.

If there is any question regarding specimen collection or other matters, contact us at:

Phone: 718-321-1108

FAX: 718-321-0158

### **Patient Preparation**

There is no unusual/additional requirement for routine office procedure-related specimens other than those adopted by each clinical specialty practices when such procedures are performed.

### **Items Supplied to Referring Physicians**

NewPath Diagnostics, LLC (NewPath), provides specimen collection and preservation supplies to its clients. The following are some of the supplies provided directly to referring physicians. For information on other available supplies, please call 718-321-1108

- Cytology spatulas
- Cytology brushes
- Cytology slides
- Cytology slide cardboard folders
- Cytology spray fixative
- ThinPrep™ Pap Tests™ – collection vials and cytobrush or broom
- Surgical Biopsy Collection Vials – biopsy containers are available in 20mL, 60mL, 120ml and 180 ml sizes with 10% neutral buffered formalin

### **Specimen Labeling and Submission**

Prudent medical-legal practice and our laboratory accrediting agencies have strict guidelines for specimen labeling and submission. They also mandate rejection of improperly completed requisitions or incorrectly identified slides or specimens. For example, some specimens cannot be analyzed because of improper collection, preservation or degradation in transit. Other specimens may have prolonged turn-around-times because of lack of necessary patient information. Still other specimens will, by necessity, be rejected because of inaccurate or absent specimen and/or requisition

labeling. You will be notified of rejected or problem specimens upon receipt. To avoid delayed diagnoses and potential specimen rejection, please observe the following requirements.

## **Surgical Specimens**

### **Requisition:**

1. Patient last, first name
2. Date of birth
3. Collection date
4. Physician and clinic name and address
5. Insurance and/or billing information including name of insured, subscriber number, group number, name and address of insurance company, and IPA group (if applicable) – copy of insurance card preferred
6. Reason for testing/ICD-9 code
7. Test Order
8. Site and type of biopsy
9. Brief clinical history, pre-op diagnosis, etc.
10. Requests for any special stains or studies

### **Specimen container:**

1. Patient last name, first name (last name and first initial are acceptable)
2. Second identifier such as date of birth, chart number, SS# or other unique identifier is required
3. Specimen type and/or location (i.e. skin lesion, left shoulder etc.)

## **Cytology Specimens**

### **Requisition:**

1. Patient last name, first name
2. Date of birth
3. Physician and clinic name and address
4. Insurance and/or billing information
5. Collection Date
6. Reason for testing/ICD-9 code
7. Type of Specimen
8. Brief clinical history including last menstrual period (LMP) or menstrual history
9. Request for any special stains or studies (i.e. HPV, CT/NG, Molecular Tests)

### **Vials/Non-Gyn Slides:**

1. All Pap test and Non-Gyn vials and submitted Non-Gyn cytology smears must be legibly labeled with the patient's name (last name and first initial are acceptable) matching that on the requisition slip. Minimally, the last name must be legible and correctly spelled. Including the patient's first name or first initial on the slide is encouraged. Second identifier such as date of birth, chart number, SS# or other unique identifier is required
2. A #2 lead pencil is recommended for slides. Markers and ballpoint pens are unacceptable for slides because of wash off during processing. Also, a name written

on top of fixative will wash away with the fixative during processing. All smears will be processed as air-dried. If no liquid based fixative is available, a fixed smear is acceptable. Write "Fix" on fixed smears.

## Specimen Preservation

This is a general information section. For detailed and specific information regarding specific preservation guidelines for particular types of biopsies and cytological collections, please see the sections in this handbook relative to the procedure in which you are interested. If you have any questions, please contact NewPath at (718) 321-1108

### Biopsy Specimens

1. Most biopsy specimens should be submitted in 10% neutral buffered formalin with enough fixative to cover the specimen. UniPath will provide containers filled with 10% neutral buffered formalin to our clients.

2. The following specimens should be submitted immediately and may require special processing (Note: We currently do not perform tests for most of the following tests, and this list is for your reference only!!!):

- Frozen sections (submit fresh or in saline)
- Cytogenetics (submit in saline or RPMI)
- Lymph nodes (submit in saline or RPMI)
- Limbs (must be refrigerated)
- Muscle biopsy (submit in saline soaked gauze and refrigerate)
- Kidney biopsy (one biopsy in Glutaraldehyde for electron microscopy and one biopsy in 10% formalin)
- Electron microscopy only (must be submitted in glutaraldehyde)
- Urate crystals (gout) specimens (must be submitted fresh or in 100% alcohol)
- POC (products of conception) for genetic or chromosomes studies, send in a sterile container with no fixative, refrigerate
- Toenails or fingernails for fungus send in formalin and maybe sent with routine plastic bags
- Skin biopsy for immunofluorescence, send one biopsy in Michel's (Zeus) fixative and one biopsy in 10 % formalin. Specimen maybe sent with routine courier.
- Liver biopsy for hemachromatosis (quantitative iron, Fe or Iron) send with no fixative in a metal free container and refrigerate
- Calculi/stones for analysis, send dry
- Nerve biopsy, clinician should speak with a pathologist

3. It is best to confer with a pathologist concerning requests out of the ordinary prior to submission of the specimen

**Note:** Prior to submission of these specimens, The lab should be contacted during business hours to arrange for an unscheduled pick-up (718) 321-1108.

# Surgical Pathology

## **Biopsy – Routine Cervical, Endocervical and Endometrial**

### **Materials Required:**

1. Collection container with 10% formalin
2. Requisition form

### **Procedure:**

1. Label the body of the collection container (not the lid) with the patient's name and tissue identification
2. Complete requisition form including tissue type, patient name, complete address, birth date, date of service, and billing data
3. Provide clinical history, i.e. last menstrual period, prior biopsies or Pap smear information, and any history of hormone use including birth control pills on the requisition form
4. Immediately place the specimen in the fixative container, tightly close container lid and forward to laboratory with requisition

**Note:** Use of gauze pads to hold or place endometrial and endocervical samplings is discouraged because

portions of the specimen are absorbed into the coarse weave and lost. Telfa is acceptable; placing the specimen directly into formalin is preferred.

## **Biopsy – Cone and Leep Conization of Cervix**

### **Materials Required:**

1. Collection container with 10% formalin
2. Requisition form

### **Procedure:**

1. Label the collection container with the patient's name, and tissue identification.
2. Complete requisition form including tissue type, patient name, complete address, birth date, date of service, and billing data.
3. Include on the requisition a history of prior Pap smear or biopsy results.
4. Orient cone specimen with surgical suture material is preferred.
5. Immediately place the specimens in the fixative container, tightly close container lid and forward to laboratory with requisition.

## **Biopsy – Endoscopic (esophagus, gastric, small and large intestine, lung, etc.)**

### **Materials Required:**

1. Collection container with 10% formalin
2. Telfa or sponges
3. Requisition form

### **Procedure:**

1. Label the body of the collection container (not the lid) with patient's name and tissue identification.
2. Complete requisition form including tissue type, patient name, complete address, birth date, date of service, and billing data.
3. Place specimen directly into formalin, close lid tightly and forward to laboratory.

4. If you chose to use Telfa, gently orient and place tissue on Telfa, mucosal surface up with submucosal surface in contact with the Telfa. Slowly enter the Telfa with attached tissue into the formalin, tightly close lid and forward to laboratory with the requisition.

**Note:** Use of gauze pads to hold or place endoscopically obtained specimens is discouraged because portions of the specimen are absorbed into the coarse weave and lost. Telfa is acceptable; placing the specimen directly into formalin is preferred.

## **Biopsy – Skin Excisions**

### **Materials Required:**

1. Collection container with 10% formalin
2. Requisition form

### **Procedure:**

1. Label the collection container (not the lid) with the patient's name and tissue identification
2. Complete requisition form including tissue type, patient name, complete address, birth date, date of service, and billing data
3. Include on the requisition form any clinical history, a gross description of lesion, and history of prior biopsies if available
4. Indicate if specimen is a shave, a punch or an excision
5. Orient specimen as necessary using description or surgical suture material. Immediately place the specimen in the fixative container.
6. Tightly close the container and forward to laboratory with requisition

## **Biopsy – Lymph Node**

### **Materials Required:**

1. Sterile screw top container
2. Sterile isotonic saline
3. Completed requisition form
4. Ice

### **Procedure:**

1. Label the collection container (not the lid) with the patient's name and identification
2. Place the lymph node in the container and moisten with saline. **DO NOT SUBMIT IN FORMALIN.**
3. Fill out requisition and include name of primary care physician or oncologist
4. Place container in biohazard bag filled with ice
5. Call Intergrated Genetics at 1800-447-5816 for STAT pick-up. Specimen should be received within 30 minutes of excision for optimal results.

## **Cytopathology (Non-Gyn)**

### **Cytology – Urine**

#### **Materials Required:**

1. Leak proof collection containers

2. Appropriate fixative (Cabowax, or CytoLyt®)
3. Completed requisition form

**Procedure:**

1. Label collection container (not the lid) with patient's name and second unique identifier such as date of birth or social security number
2. Complete requisition form, including patient name, date of birth, date of service, and billing data. Patient's history of prior kidney or bladder abnormalities should also be noted.
3. Patient Preparation: Instruct the patient to void and discard the first morning specimen. The patient should drink a quart of water an hour or so prior to collecting the urine specimens.
4. Mix an equal amount of fixative with the urine specimen. Forward specimen to laboratory with completed requisition form. Separate specimens should be collected and dated for three consecutive days.

**Comments:**

1. Urine should be identified as to type of sample (i.e. voided, catheterized, right or left ureteral or bladder irrigation fluid).
2. For detection of cancer of ureters or kidneys, a serial collection of specimens spaced over three days has proven to increase diagnostic sensitivity and yield.
3. Sensitivity and yield of urine specimens has also been shown to increase with optimal collection and preservation of specimen.
4. Unpreserved urine results in the rapid degeneration of exfoliated cells and may become useless for diagnosis.

## **Cytology – Nipple Secretions, Smears**

**Materials Required:**

1. Slides with frosted ends
2. Physiologic saline
3. Cotton swab
4. Requisition form

**Procedure:**

1. Label slide with patient's name and site from which sample obtained. Example: R or L Breast, and allow to air-dry.
2. Complete requisition form, including patient name, birth date, date of service, and billing data. Also include any pertinent history on the requisition (especially if pregnant or lactating).
3. If there is no nipple erosion or ulceration, gently "strip" the area of the breast below the nipple and areola with a motion from beneath the areola towards the nipple surface. Do not massage the entire breast. The stripping motion will propel accumulated secretions within the ampulla of the larger excretory ducts.
4. With appearance of fluid on the nipple surface, touch a slide to the drop of fluid and draw the slide quickly across the nipple
5. Using a separate slide, repeat this process for the opposite breast
6. If there is nipple erosion or ulceration, touch a slide to this area three times, with a different part of the slide in contact each time. This is conveniently done, starting with a contact position close to the hand holding the slide and then

moving the application area of the slide further with your hand for the next two samplings.

7. Following touch preparation (#6) of the ulcerated area, try to express fluid (#3) and prepare slides if fluid obtained

8. If no fluid can be expressed, a swab may be dipped in saline and gently rolled and rotated on the ulcerated surface, and applied to a glass slide

9. Place slides in slide container and forward to the laboratory with the completed requisition

**Note:** We recommend completely air-drying smears to avoid incomplete fixation artifact.

## Cytopathology (Fine Needle Aspiration)

### **Principle:**

Fine needle aspiration (FNA) provides a prompt, cost effective, safe, means of evaluating

a mass through cytologic diagnosis. The procedure is generally well tolerated by patients. FNA is often used as an alternative to surgery and may provide a definitive diagnosis

that will determine therapy and/or assist in a planned surgical approach with effective utilization of operating room time. In general, any palpable mass can be evaluated by aspiration techniques. With ultrasound guidance, fluoroscopy and CT, most deep-seated lesions may also be sampled. Lesions that are commonly sampled include thyroid, breast, salivary glands and lymph nodes. Although the technique is relatively

simple, it does require some practice and understanding of principles of aspiration.

### **Materials Required:**

1. Hand grip syringe holder of preference
2. Syringe, screw lock, disposable, 10mL or 20mL plastic with tight fitting barrel
3. Needles of size preference. Most aspirates may be obtained with a needle no larger than 21 gauge. Most highly vascular structures (i.e. thyroid) are best sampled with a 23 or 25 gauge needle.
4. Frosted end labeled slides
5. Specimen container with appropriate fixative as needed for needle washings (CytoLyt®)
6. Alcohol or iodine solutions for sterilization of skin
7. Cotton swabs for sterilization of skin
8. Sterile gauze
9. Adhesive tape
10. Band-Aids
11. Requisition form
12. RPMI for Lymph Node flow

### **Procedure:**

1. Using a pencil (ink or markers wash off during staining) label slides and specimen containers with the patient's name and precise site of aspiration. Add second unique identifier such as date of birth or social security number on the

container.

2. Place labeled slides in rows of four slides, each row for use with a "pass". Open container with fixative for needle washings.
3. Set-up a sterile field including preparation of syringe and syringe holders for initial and repeat passes
4. Select needles and syringes to be used for the procedure
5. Confirm the identity of the patient by asking their name and comparing it with available requisition, insurance documents, etc.
6. Confirm the site and side which the procedure is to be performed on through review of the requisition and patient history
7. Place patient in a comfortable sitting or reclining position that allows easy access and aspiration of the lesion
8. Palpate the mass to identify the depth of the target and its relationship to surrounding structures
9. Assemble the syringe equipment
10. Clean the skin over the aspiration site with an alcohol swab
11. Immobilize the mass with the thumb and index finger of one hand or between two fingers of one hand
12. Take the syringe equipment in the opposite hand and use a one handed withdrawal and release manipulation of the syringe plunger
13. Place the needle against the skin at a determined puncture site and insert it into the mass area with a single quick motion without negative pressure on the syringe
14. Once the needle is in the desired area, retract the plunger of the syringe to create negative pressure in the syringe and needle lumen. Minimal negative pressure is needed for highly vascular organs such as the thyroid while more negative pressure is needed for dense fibrous organs like the breast.
15. Move the needle back and forth several times directing it in the same plane. Gently redirect the direction of the needle to increase the field of sampling but avoid vigorous redirection of the needle, as this tends to produce unnecessary hemorrhage in the lesion.
16. When material appears in the hub of the needle, the aspiration has been completed. Excess blood in the material will dilute the specimen rendering it unsuitable for microscopic diagnosis. One drop of material can usually produce 4 -6 smears.
17. Release the pressure in the syringe by releasing the syringe plunger
18. Gently withdraw the needle from the lesion and apply pressure to the puncture site with sterile gauze

### **Smear Preparation:**

1. After the needle has been removed from the mass, detach the needle from the syringe using a surgical clamp or other appropriate instrument, fill the syringe with air, and reattach the needle to the syringe
2. Place the bevel of the needle against a glass slide



and express a small drop of aspirated material onto the slide

3. If too much material is expressed onto the slide, either re-aspirate a portion of the material by withdrawing the syringe plunger slightly, or spread the material out among additional slides

4. If the cellular material is semi-solid, place a second slide on top of the material and pull the slides gently and quickly apart as the material spreads from the weight of the slide

5. If the aspirated material is diluted by fluid or by blood, use the same smear technique as for blood smears

6. Place a second slide onto the drop of material allowing it to spread. Gently pull the two slides apart.

7. Allow smears to air-dry for Diff Quick staining. Write "air dried" on the end of the smears.

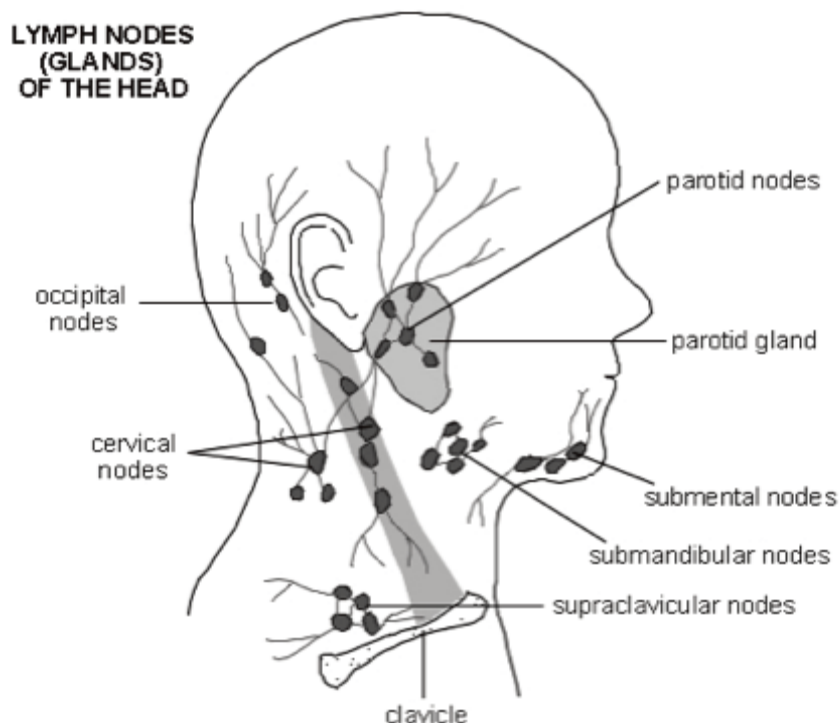
8. Rinse the needle and syringe into the CytoLyt® vial for monolayer preparation

9. If staining and immediate evaluation is available, have the patient remain while adequacy of the aspiration pass is determined and repeat the procedure until the operator is satisfied with the adequacy of the material

10. Complete requisition form. Include patient name, birth date, date of service, and billing data. Provide the exact site of the lesion (i.e. parotid, thyroid, lymph node, etc.).

#### Comments:

1. When the lesion is composed of solid tissue, the needle tip functions as a cutting



instrument. As it is moved back and forth through the tissue, tiny tissue fragments

become dislodged and collect inside the needle. When suction is added to this procedure the previously dislodged fragments are sucked into the needle and the tip of the attached syringe.

2. The needle should never be removed while any negative pressure is in the syringe. Such pressure may force aspirated material from the needle into the syringe. This may make preparation of smears difficult and may start air-drying of the material.
3. It is important not to dilute the cellular material with fluid or blood
4. If a cyst is encountered during the aspiration, evacuate all fluid from the cyst and perform a second aspiration on any residual mass. Express the fluid from the cyst into CytoLyt®, or 50% methyl or ethyl alcohol.
5. If blood is aspirated into the syringe, stop the procedure and prepare slides. Express residual bloody fluid into CytoLyt®, or 50% methyl or ethyl alcohol.
6. If pus is encountered, withdraw as much of the material as possible and perform a repeat aspiration in an adjacent area. (When an infectious process is included in the differential diagnosis, a culture of the aspirated material is often desirable).
7. Necrosis generally occurs in the center of large lesions because of an inadequate blood supply. If the first sample yields only necrotic debris, another sample obtained tangentially to the edge of the mass should be secured.
8. Local anesthesia is rarely required, as the discomfort caused by its application is about the same as the aspiration. Its use is dependent on the lesion location, the discomfort of the patient and the judgment of the operator
9. Passing through layers of muscle while inserting the needle adds significantly to the discomfort of the patient, while making needle placement more difficult and uncertain. Also, small fragments of muscle may plug the needle, jeopardizing subsequent sampling of the target. The muscle and lesion may sometimes be manipulated so that aspiration technique does not involve the muscle.
10. The nipple and the areola of the breast are the areas most sensitive to pain from a needle stick. These areas should be avoided whenever possible. Masses in these areas can sometimes be pushed away from the nipple, immobilized and sampled through adjacent skin.
11. When a mass is located close to the chest wall, there is a possibility that the needle may penetrate and cause a pneumothorax. This can be avoided by moving the mass sideways so it rests on a rib. This not only prevents penetration of the chest wall but also provides good support for immobilization of the target.
12. Complications can vary as to the site of aspiration and cannot all be listed. The aspirator should be aware of these prior to aspiration and communicate them to the patient.
13. When completing the requisition slip it is vital to provide the exact site of the lesion (i.e. Thyroid, lymph node, parotid gland, etc). "Neck Mass" provides insufficient information for the cytopathologists to provide a reasoned diagnosis.
14. For thyroid aspirations, it is important to provide further information. Is there one nodule or several? Is the nodule "cold" or "hot" (if a scan has been performed)?
15. For lymph node aspirations, history of prior malignancy, antibiotic treatment, etc are important. Flow cytometry can be performed on FNA's of lymph nodes if the specimen is properly collected and stored. Please call Intergrated Genetics for specific instructions if a lymphoma is under consideration. Prior planning can often

alleviate the need for a repeat aspiration or biopsy.

## Cytopathology (Gyn)

### **Cytology – ThinPrep® Pap Tests™ by Hologic®, formerly Cytoc**

#### **Principle:**

Automation and new methodologies are dramatically changing gynecological cytology, particularly the Pap test. The FDA has approved the Hologic® ThinPrep® Pap Test™ as

a replacement for the conventional Pap Smear. Studies indicate that it is more accurate than the conventional Pap smear in detecting premalignant and malignant lesions. The advantages of a monolayer slide include:

1. Reduction of obscuring inflammation and blood
2. Elimination of air drying artifact provided the specimen is placed in the fixative immediately after sampling
3. A more uniform and representative sampling of the collected specimen
4. Residual sample available to perform additional testing as needed
5. Lowering of the ASCUS rate since air-drying and obscuring inflammation are not present
6. Increased sensitivity in detecting HIGH GRADE DYSPLASIAS due to the greater ease in recognizing dysplastic cells on the ThinPrep® Pap Test™ monolayer slide

#### **Materials Needed:**

1. The collection kit includes a cervix brush with a spatula or broom, and a collection vial of PreservCyt®
  2. Completed requisition form
- NewPath is equipped to provide this test to you. Contact us to obtain supplies and detailed instructions.*

#### **Procedure:**

1. Patient Preparation
  - a. Schedule patient for sample collection mid cycle (obscuring blood from menstrual smears is a major source of less than optimal smears)
  - b. Instruct patient in advance not to douche for at least 24 hours prior to examination
2. Label PreservCyt® vial with the patient's name and second unique identifier such as date of birth or social security number
3. Complete requisition form including patient name, birth date, date of service, and billing data. Include on the form all clinical information (LMP, hormone use, prior Pap smear and biopsy results).
4. Prior to specimen collection, clean away any visible blood, mucus and/or discharge from the cervix
5. Obtain a vaginal smear from secretions in the posterior fornix. This material, along with the ectocervical scrape should be placed immediately in the PreservCyt® vial and agitated to dislodge the collected cells. Shake vigorously.
6. Sample from the endocervix with a cytobrush or broom. If the brush is used the ectocervix is sampled with the spatula.

7. The brush/spatula or broom is placed into the vial of PreservCyt® and agitated up and down against the bottom of the container to collect as much material as possible. After agitation, discard collection device.

8. Close the vial tightly, and place it with a requisition form into a specimen bag and ship it to the laboratory

**Note:** Although formal maturation indexes (M.I.) cannot be performed on the ThinPrep® Pap Test™ specimen,

estimated estrogen effect can be performed which may provide clinically useful information.

*Due to the significantly higher cost of the ThinPrep® Pap Test™ kit, we are asking you to use the kits we provide only on patients whose specimens will be sent to our lab for processing.*

## **Referral Laboratory Specimen Handling**

In general all specimens are handled similar to described above, and delivered to NewPath. In cases that need to be sent to a reference lab, a requisition is created based on the requirement of that lab, and together with the specimen is pick-up by calling the reference lab courier/client service department. In unusual situation that the specimen may be handled differently, a phone call is made to the reference lab customer support department to ensure proper handling.

Adopted: 8/20/2010

Last updated: 8/28/2012

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