



# Guidelines for the Collection and Handling of Cytology, Molecular, and Surgical Specimens

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[www.ThinkOculus.com](http://www.ThinkOculus.com)

601 W. Hwy 6, Suite 111 Waco, Texas 76710 (254) 752-9621

# The Purpose

The manner in which specimens are collected and handled is critical for assurance of valid results. These guidelines are for the purpose of improving the quality of specimen collection and handling which in turn will improve patient monitoring and care.

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# The Purpose Cytopathology

## ROUTINE GYNECOLOGICAL SMEAR (PAP SMEAR)

### CONVENTIONAL SMEAR

#### LABELING SLIDES

1. Use frosted end slides, handle by the edge of the slide only.
2. Write patient's full name and birth date on frosted end.  
Use pencil, ink will "bleed" or wash off.
3. Labeling is a must for proper identification!  
Slides without a name will be returned.

#### REQUEST SLIP

1. All specimens must be submitted with a request form. Please provide all requested information to assure patient's identity and for optimum results.
2. Patient's name, and birth date make retrieval of patient history consistent.
3. The complete address with zip code is needed.
4. Remember the date smeared; this is required for inspection purposes.
5. Please designate doctor; this is a must for returning the report to the proper place.
6. If specimen is from a clinic or hospital, please designate.
7. Billing info:
  - a. Who is to be billed? Patient, clinic, hospital?
  - b. Please provide all insurance information.
  - c. Signed Medicare waiver if applicable.

8. Please give clinical data. FOR ACCURATE DIAGNOSIS, AGE OF PATIENT, LMP AND THE INTAKE OF HORMONES MUST BE STATED.

### COLLECTION PROCEDURE

1. It is recommended that both a cervical scraper and cervical brush be used for sampling.
2. Take the cervical smear by rotating the cervical scraper 360 degrees about the cervical os and ectocervix, while maintaining firm contact with the epithelial surface. Smear the spatula across the slide using one of the listed options for transferring material to slide(s).
3. Insert the cervical brush into the os with gentle pressure and rotate only 90 to 180 degrees to minimize bleeding. Roll the brush across the slide by twirling the handle 360 degrees using one of the listed options for transferring material to slide(s). WARNING, the cervical brush should not be used on pregnant patients or to sample the endometrium.

### TRANSFERRING OF MATERIAL TO SLIDE(S)

1. The object is to spread the cellular material quickly but evenly in a monolayer on the glass slide. If possible, thin-out large clumps but avoid excessive manipulation, which can damage cells. Transfer must take place within a few seconds and a fixative applied to avoid air-drying artifact.
2. One slide option for transferring material to slide:
  - a. Smear the scraper across the upper longitudinal half of the slide; roll the brush across the lower longitudinal half of the slide. Fix immediately.
  - b. Smear the scraper across the slide; roll the brush directly over the previously smeared material. Fix immediately.
  - c. Smear the scraper over the left-hand of the slide; cover the right-hand side with cardboard and fix immediately. Roll the brush material onto the right-hand side of the slide and fix immediately.
3. Two slide option for transferring material to slide:
  - a. Smear the scraper across the slide and immediately fix slide.

- b. Roll the brush material across the slide and immediately fix slide.

#### FIXATION OF SMEARS

Smears are preserved with spray fixative. Hold container 12 inches from the slide to avoid blasting the cells. The slides are immediately ready to be sent to the laboratory and maintain the integrity of the smear for many months.

#### PREPARING FOR LABORATORY PICK-UP

1. After fixation, place slide(s) in a cardboard mailer, plastic slide holder or pap smear kit.
2. Match the appropriate request form for the slide(s) and place in a plastic transport bag.
3. Place in area for pick-up.

NOTE: Also note that the laboratory provides all supplies for the above procedure and requests for these supplies are put in the pick-up area.

#### REJECTION OF SMEARS BY THE LABORATORY

1. Slides will not be processed if there is no identification on the slide or a request slip does not accompany it.
2. If a gross discrepancy exists between the labeled slide(s) and the request slip, the slide(s) will not be processed, and will be returned to the physician's office.
3. Slide(s) cannot be processed if received broken beyond repair. The physician's office will be notified.

#### THINPREP

##### LABELING

1. Write patient's full name on vial
2. Vials without labeling will be returned

## THINPREP COLLECTION TECHNIQUE

1. A plastic spatula, Cytobrush and PreservCyt Solution is needed for collection.
2. Rotate contoured end of spatula 360 degrees around the entire exocervix while maintaining tight contact with the surface
3. Rinse spatula in vial of PreservCyt Solution by swirling vigorously ten times, discard spatula.
4. Insert Cytobrush into the endocervix, slowly rotate one-half turn in one direction, remove device.
5. Rinse brush in the PreservCyt by rotating in the solution ten times while pushing it against the wall of the vial, swirl vigorously to further release material, discard device.

## PREPARING FOR LABORATORY PICKUP

1. Make sure vial lid is tightened and labeled with patient's name and birthdate. (Two identifiers)
2. Complete request form according to instructions above under CONVENTIONAL SMEAR
3. Match the appropriate request form with the vial and place in a plastic transport bag.
4. Place in area for pick-up.

## CYTOLOGY REPORT

1. Oculus Pathology -Waco Division uses The Bethesda System for cervical and vaginal cytology reports. This format is recommended in the CLIA '88 regulations.
2. The format of The Bethesda System addresses each of the following elements.
  - a. A "statement of adequacy" of the specimen for diagnostic evaluation.
    1. Satisfactory indicates the specimen is an adequate specimen that can be evaluated without qualification.

2. Satisfactory but limited by indicates the specimen may provide useful diagnostic information, but is limited by certain conditions, which are explained in the comment area.

3. Unsatisfactory indicates the specimen is not acceptable for diagnostic evaluation.

b. A hormonal evaluation.

c. The presence or absence of endocervical cells.

d. A descriptive diagnosis.

## NON-GYNECOLOGICAL SPECIMENS

### COLLECTION\*\*

#### Sputum

Early morning specimen produced by a deep cough.

#### Bronchial Brush/Wash

A directed brush is obtained during bronchoscopy and immediately smeared on a glass slide and immediately fixed. Washings are obtained by instilling 3 to 5 ml of a balanced salt solution through the bronchoscope and re-aspirating the resulting material.

#### Body Cavity Fluids

Collect into a clear, dry container, which need not be sterile. If all of the fluid is not sent to the lab, at least 50 cc is required.

#### Spinal Fluid

Collector should avoid a bloody tap or aspiration of solid material. Quick delivery to the lab and immediate processing is imperative. Preferred minimum is 3 cc.

#### Gastric Brush Washing

Patient should be fasting overnight but encouraged to drink liberal amounts of water. During the endoscopy, it is recommended to obtain the brush sample before the biopsy due to bleeding. The brush smeared slide should be fixed immediately. Washings may accompany the above.

## Urine

First morning and 24-hour samples not recommended. Mid-stream or catheterized specimen preferred - minimum of 20 cc. Please note collection method - voided vs. catheterization.

## FIXATION

After collection, Saccomanno fluid should be added to the fluid immediately. The ratio being 1-part specimen to 1- part Saccomanno. If Saccomanno is unavailable, 70% alcohol added in the same ratio as above is acceptable. If neither is available, a fresh specimen can be sent to the lab. It must be kept refrigerated and delivered to the lab within 24 hours.

Be sure that the specimen is properly labeled with patient's name, date, physician name and source of specimen. Also, make sure that a cytology request form is completely filled out and sent with specimen. Pertinent clinical information is necessary for diagnosis.

## SMEARS

Smears that are taken from non-gyn sites are treated in the same manner as a Pap smear.

1. Spray fixation must occur immediately.
2. Slide(s) must be labeled with patient's full name and birth date.
3. Completed cytology request form sent with slides.
4. Designate site of specimen.

## FINE NEEDLE ASPIRATES (FNA)

The Fine Needle Aspirate procedure of superficial masses can be performed by a pathologist if desired, Please contact the lab to schedule.

1. Slides are to be laid out and labeled with patient's full name and birth date.
2. After aspiration, a small drop of specimen is expressed on several slides.
3. Remaining slides are used to spread the drops of the aspirate in the following manner
  - a. A second slide is inverted over the drop.
  - b. As the specimen spreads, the slide is either pulled apart or lifted vertically

- c. The idea is to create a monolayer of cells.
- 4. Two of the smears are allowed to air-dry and the remaining slides are immediately fixed in alcohol or sprayed fixed.
- 5. If feasible, drop needle from aspiration syringe into specimen container containing Saccomanno fluid. (The needle will be rinsed and processed at the lab).
- 6. All components are placed in a transport bag.
- 7. Designate site of aspiration and as much history as possible on request slip.

## COLLECTION OF SURGICAL PATHOLOGY

### ROUTINE

1. As soon as the specimen is removed by the physician, immediately place into a container of 10% formalin for fixation.
  - a. This container must be large enough to accommodate the specimen without distortion.
  - b. The container must also be able to contain enough 10% formalin to cover the specimen with a 10:1 ratio (10 parts formalin to 1 specimen).
2. Tighten the lid so that the container does not leak.
3. Make sure the container is labeled.
4. Place specimen container and completed request form into a plastic transport bag.

### FROZEN SECTION

Frozen sections are of great value both to the surgeon and to the patient. The pathologist can often make a diagnosis in a few minutes rather than waiting until the next day for a routine pathology exam.

1. A specimen for frozen section is received in the fresh state; therefore, it must be handled quickly.

2. As soon as the tissue sample is removed from the patient, the sample is:
  - a. Wrapped in moistened gauze or placed in saline.
  - b. DO NOT PLACE IN 10% FORMALIN.
3. Call the lab immediately for pick-up service, stating that the specimen is for frozen section.
4. Place specimen into transport bag with completed request form.

#### MUSCLE AND NERVE BIOPSY

1. Special processing of fresh, unfixed tissue is required.
2. Call laboratory to schedule.

#### BONE MARROW

1. After obtaining core biopsy, immediately place in 10% formalin for fixative. Label as bone marrow biopsy.
2. Carefully place clot in 10% formalin and label as "bone marrow clot".
3. Label bone marrow smears with patient's last name and the letters "BM".
4. Label peripheral smears with patient's last name and the letters "PB". Peripheral smears must be included. Pathologist will not complete case without them.
5. Allow smears to dry before packaging for transport.
6. Attach recent CBC and Histogram to completed request form. Include pertinent clinical data.

#### LYMPH NODE BIOPSY FOR SUSPECTED LYMPHOMA

1. Fresh, unfixed tissue or tissue in RPMI media is required for Flow cytometry studies.
2. For fresh, unfixed tissue wrap in saline moistened gauze. DO NOT ADD FORMALIN.
3. Call the lab immediately for pick-up service, stating that the specimen is a fresh lymph node.

## DIRECT IMMUNOFLUORESCENCE STUDIES

Tissue specimens submitted for Direct Immunofluorescence studies must be submitted in Michel's media, which can be obtained by calling the Laboratory at (254) 752-9621, ext. 417.

## REJECTION OF SPECIMENS BY THE LABORATORY

1. A specimen will not be processed if a request slip does not accompany it or if bottles are not labeled with patient's name.
2. If a gross discrepancy exists between the labeled container and the request slip, the specimen will not be processed.
3. Specimens will be returned to physician's office for corrective action.

## COURIER SERVICES

Depending on the location of the referring Physician, Clinic, Surgery Center, or Hospital, Clinical Pathology Associates will provide courier services utilizing our internal courier staff or a contracted courier service. Locations in Waco may call (254) 752-9621, ext. 417 to request a specimen pick up for special situations.

## REFERENCES

"The Papanicolaou Technique"; NCCLS Document, GP15-T.

Pap-Pak Kit, Medical Packaging Corp.

A Manual of Cytotechnology, 4th Edition, American Society of Clinical Pathologists.

Surgical Pathology/Cytopathology Quality Assurance Manual, American Society of Clinical Pathologists.

"The 1988 Bethesda System for Reporting Cervical/Vaginal Cytological Diagnoses". JAMA, August 18, 1989, pp. 931-934.

Theory and Practice of Histotechnology, 2nd Edition, Sheehan and Hrapchak.