Specimen Collection and Preparation

Laboratory test results are dependent on the quality of the specimen submitted. It is important that all specimens and be properly labeled with the name of the patient, collection date, and the origin (source) of the specimen, when applicable.

If there is any doubt or question regarding the type of specimen that should be collected, it is imperative that Barnes-Jewish Hospital Laboratory Customer Service be called to clarify the order and specimen requirements.

Biopsy Specimen Collection

The following is a list of containers available for biopsy specimens. Please see our "Alphabetical Test Listing" for specific requirements.

Formalin Containers: This fixative is available in 90-mL containers and is used for most routine biopsy specimens.

HistoCon (Michels') Fixative: This is the required fixative for all immunofluorescence (IF) specimens. It should be used for renal biopsy specimens and dermatology specimens requiring IF.

Blood Collection

Most laboratory tests are performed on anticoagulated whole blood, plasma, or serum. Please see our "Alphabetical Test Listing" for specific requirements.

Plasma: Draw a sufficient amount of blood with the indicated anticoagulant to yield the necessary plasma volume. Gently mix the blood collection tube by inverting 6 to 10 times immediately after draw.

Plasma for Routine Chemistries (Preferred): The concentrations of most analytes measured in Barnes-Jewish Hospital Clinical Chemistry Laboratory are equal in plasma and serum. However, the concentrations of potassium; protein, total; and phosphorus, inorganic are well documented to be different in plasma and serum. Because plasma represents the more physiologic fluid (ie, coagulation has not occurred) and because these are very commonly ordered tests, plasma is the preferred specimen for most chemistry tests at Barnes-Jewish Hospital. Phosphorus averages 0.3 mg/dL higher in serum than in plasma due to the release of phosphorus during coagulation while protein, total is approximately 0.3 mg/dL lower in serum than in plasma due to fibrinogen consumption during coagulation.[1] For potassium, concentrations average 0.4 mmol/L higher in serum than in plasma due to the release of potassium from platelets during coagulation, but this difference can range from 0.0 mmol/L to 1.4 mmol/L in different patients.[1] The difference between plasma and serum plasma concentrations has been shown to directly correlate with platelet count.[2] Thus, the primary clinical reason for plasma being the preferred specimen for chemistry testing is to assure a physiologic and accurate potassium value. Plasma also provides the advantage of faster turnaround time as specimens can be centrifuged immediately upon receipt rather than waiting for serum to form which can be lengthy in patients receiving anticoagulant therapy.

[1] Ladenson JH: Nonanalytical sources of variation in clinical chemistry results. In Gradwohls Clinical Laboratory Methods and Diagnosis. Edited by A Sonnewirth, L Jarrett. St. Louis, MO, Mosby Publishing, 1980:149-92

[2] Nijsten M, Dofferhoff ASM: Pseudohyperkalemia and platelet counts. NEJM 1991;325:1107

Serum: Draw a sufficient amount of blood to yield the necessary serum volume. Allow blood to clot at ambient temperature.

Order of Draw: When obtaining multiple specimens during a single venipuncture, to avoid possible test result error due to cross contamination, please draw tubes in this order:

1. Blood culture tube
2. Light blue-top tube (coagulation)
3. Red-top or red/grey-top tube
4. Green-top or green/grey-top tube
5. Lavender-top tube
6. Grey-top tube

When only a blue-top tube is needed, a discard tube should be drawn and discarded before drawing the blue-top tube to be submitted for coagulation testing. The discard tube should be collected in a blue-top tube, never a plastic red-top tube. The discard tube does not need to be completely filled

Blood Collection Tubes Available

The following is a list of tubes referred to in "Specimen Required" in the individual test listings.

- *Dark Green-Top (6 mL VACUTAINER® Plus) (Lithium Heparin) Tube*: This tube contains 87 USP units of lithium heparin.
- *Green-Top (10 mL VACUTAINER®) (Sodium Heparin) Tube*: This tube contains 143 UPS units of sodium heparin.
- *Grey-Top (6 mL VACUTAINER® Plus) (Sodium Fluoride/Potassium Oxalate) Tube*: This tube contains 12 mg potassium oxalate and 15 mg sodium fluoride.
- *Lavender-Top (3 mL VACUTAINER® Plus) (EDTA) Tube*: This tube contains spray dried K_2EDTA (5.4 mg).
- *Light Blue-Top (2.7 mL HEMOGARD™) (Sodium Citrate) Tube*: This tube contains 0.109 M/3.2% buffered sodium citrate.
- *Mint Green (PST™)-Top (4.5 mL VACUTAINER® Plus) (Lithium Heparin) Tube*: This tube contains lithium heparin and a gel for separation.
- *Pink-Top (10 mL VACUTAINER®) (EDTA) Tube*: This tube contains 18 mg K_2EDTA.
- *Plain, Red-Top (6 mL VACUTAINER® Plus) Tube*: This tube contains a clot activator.
- *Royal Blue (Purple Label)-Top (7 mL Monoject®) Tube*: This tube contains 10.5 mg of EDTA.
- *Royal Blue (Red Label)-Top (7 mL Monoject®) Tube*: This tube contains no additive.
- *Separator Gel (Red/Grey-Top) (8.5 mL VACUTAINER® SST®) Tube*: This tube contains spray-coated silica and a polymer gel for serum separation.
- *Yellow-Top (8 mL) Tube*: This tube contains ACD Solution A.
- *Yellow-Top (6 mL) Tube*: This tube contains ACD Solution B.

Cerebral Spinal Fluid (CSF) Preferred order of Collection

CSF is typically collected into numbered plastic tubes provided with the sterile lumbar puncture tray. The preferred order of collection for testing is:

1. Tube #1: Chemistry (and Immunology)
2. Tube #2: Hematology
3. Tube #3: Microbiology
4. Tube #4: Hematology

Cytology Specimen Collection

The following is a list of containers available for cytology specimens. Please see our "Alphabetical Test Listing" for specific requirements.

- *Gynecologic - Conventional Pap Smears:* Pap collection kits are provided that include 1 glass slide, endocervical brush, spatula, fixative, and mailer.
- *Gynecologic - SurePath® Pap Smears:* This kit includes collection vial and cervix broom. Endocervical brushes are available on request.
- *Gynecologic - ThinPrep® Pap Smears:* This kit includes the PreservCyt® containers, endocervical brush, and a spatula.
- Non-Gynecologic Specimens: Vials with Cytolyt® fixative solution are available for urine and body fluids. A spray fixative is available for glass slides/smears.

Cervical/Vaginal Smears (Pap Smears) - Conventional Method Collection and Reporting*

A. *Specimen Collection*:

An optimal cervical specimen includes sampling of the squamous and columnar epithelium, encompassing in particular the transformation zone where the majority of cervical neoplasias arise. The specific sampling instrument(s) and sampling technique used should be based on a consideration of individual patient anatomy, particularly the location and configuration of the transformation zone as determined by visual inspection.

1. Collection procedure using wooden spatula and cervical brush.
   a. Label the frosted end of the glass slide with the patient's name before specimen collection.
   b. Choose the contoured end of the spatula that best conforms to the anatomy of the cervix and the location of the transformation zone. Rotate the spatula at least 360° about the circumference of the cervical os and ectocervix while maintaining firm contact with the epithelial surface (Figure 1).

Note: A clockwise rotation beginning and ending at 9 o'clock (or counter-clockwise rotation from 3 o'clock to 3 o'clock) will position the spatula so that the collected material is retained on the upper horizontal surface as the instrument is removed.

Do **not** smear the specimen at this time unless the specimen is to be **immediately** fixed.

c. Insert the cervical brush into the os; some bristles should still be visible. This will minimize inadvertent sampling of the lower uterine segment. With **gentle** pressure, rotate the brush only 90° to 180° to minimize bleeding (Figure 2).
d. To transfer material from the spatula, smear the specimen with a single stroking motion using moderate pressure to thin out clumps of cellular and mucus material. Avoid excessive force or manipulation which will damage cells (Figure 3).
e. To transfer material from the brush, roll the bristles across the slide by twirling the brush handle (Figure 4).
f. **Immediately** fix the specimen by spraying with fixative. Hold the container 12 inches (30.5 cm) from the slide. Spray-fixed or liquid-coated slides must be allowed to dry completely before packaging for transport.

Figure 1.

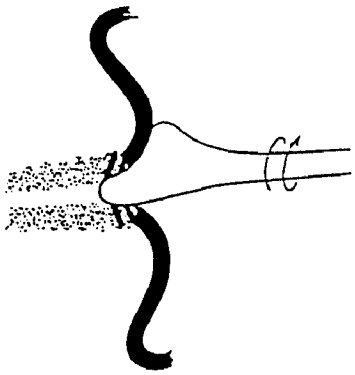


Figure 2.

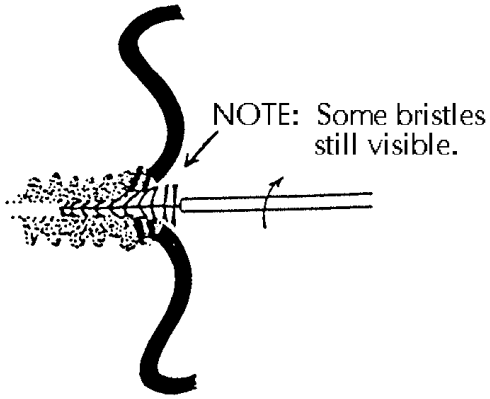


Figure 3.

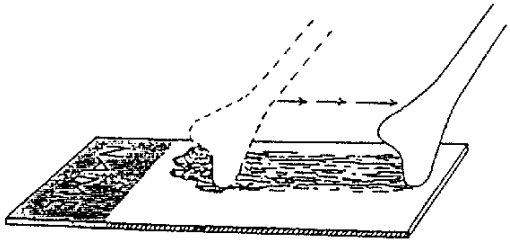
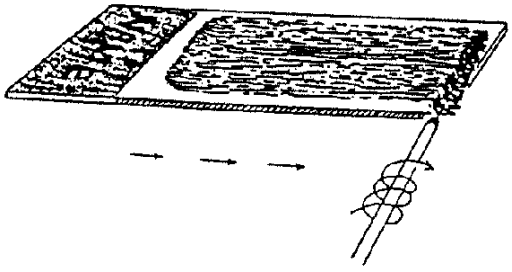


Figure 4.



B. *Specimen Adequacy*:

1.Cervical/vaginal smears will be considered satisfactory for evaluation under the following conditions:
   a. Presence of endocervical cells and/or squamous metaplastic cells
   b. Well preserved specimen, free of artifact or debris that interferes with microscopic evaluation
   c. Presence of squamous and endocervical specimen
   d. Cells present on at least 25% of the smear
   e. Absence of obscuring blood, inflammation, bacteria, or debris that interferes with microscopic evaluation
   f. Enclose the slide in the package provided for specimen transport. Please label the package with the patient's first and last name, date of birth, specimen identification number, or Social Security number.

2. Cervical/Vaginal Smears will be Considered Unsatisfactory for Evaluation Under the Following Conditions:
   a. Obscuring blood
   b. Smears containing partially obscuring blood, inflammation, bacteria, etc.
   c. Too few cells
   d. Excessive air-drying artifact
   e. Absence of squamous epithelial sample, specimen consists entirely of endocervical cells

C. *Rejection Criteria*:

Slides will be considered unacceptable under the following conditions:

1. Any slide or vial not accompanied by a requisition
2. Any slide or vial that is not clearly and unambiguously identified
   Slide or vial must be labeled with the patient's first and last name and date of birth
3. A requisition that is incomplete or contains discrepant information
4. A requisition and slide that do not match
5. Broken slides that cannot be repaired
6. Unfixed slides
7. Leaking vial

D. *Report Terminology*:

All cervical/vaginal smears will be reported using The Bethesda System II (TBSII).

1. General category
   a. Negative for squamous intraepithelial lesion or malignancy
      (1) Normal cytologic findings
      (2) Includes infectious and reactive changes
      (3) Defined by descriptive statement
   b. Epithelial cell abnormality
      (1) Includes all epithelial abnormalities
      (2) Defined by descriptive statement
   c. Other
      (1) Defined by descriptive statement

E. *Requisition:*

1. A Cytology requisition must be accurately completed and accompany each Pap smear. The requisition must include:
   a. Patient name, last and first
   b. Date of birth
   c. Social Security number or
   d. Patient identification number
   e. Date and time of specimen collection
   f. Name of ordering physician
   g. Appropriate insurance information
   h. Source of specimen
   i. Last menstrual period (LMP)
   j. Clinical history
   k. Hormonal status
2. Inaccurately labeled specimens are subject to rejection by the laboratory. Please see "*Rejection Criteria*."

* Portions of this section have been excerpted from NCCLS Publication GP 15-A, Papanicolaou Technique; Approved Guideline. Copies of the current edition may be obtained from NCCLS, 771 East Lancaster Avenue, Villanova, PA 19085.

SurePath® Pap Test Collection and Reporting

The SurePath® Pap Test is an alternative to the conventional Pap smear. The SurePath® Pap Test eliminates many of the limitations of the conventional Pap smear by reducing air-drying artifact, obscuring blood, mucus, and inflammation.

A. *Specimen Collection*:

1. The specimen is collected using a plastic cervical broom and an optional endocervical brush. The cellular material is transported to the laboratory in a liquid transport medium called SurePath® preservative fluid. Once received in the laboratory, the specimen is processed on the SurePath® processor resulting in a 13-mm diameter circle on a glass slide. The slide is microscopically reviewed by a cytotechnologist, and any abnormal findings are identified and referred to a pathologist for diagnosis.
   a. Label a container of SurePath® preservative fluid with the patient's name and date of birth before specimen collection. Other identifying information is also acceptable such as a hospital registration number or Social Security number. **All outpatient specimens must be labeled with patient's first and last name, and date of birth.**
   b. Collect the endocervical and ectocervical specimen using a plastic cervix broom. Insert the bristles of the broom into the endocervical canal until the bristles touch the cervix. Rotate the broom 360° 5 times. Immediately remove the broom head from the handle and drop it into the labeled vial. If using an optional endocervical brush, rotate the brush several times in the endocervical os, remove and break off the head of the brush into the labeled vial.
   c. Close the vial by tightening the cap securely. **This is very important to prevent leakage of the fluid during transport. The laboratory will not process any specimens leaking out of the container.**

B. *Requisition*:

1. A Cytology requisition must be accurately completed and accompany each SurePath® Pap test. The requisition must include:
   a. Patient name, last and first
   b. Date of birth
   c. Social Security number or
   d. Patient identification number
   e. Date and time of specimen collection
   f. Name of ordering physician
   g. Appropriate insurance information
   h. Source of specimen
   i. Last menstrual period (LMP)
   j. Clinical history
   k. Hormonal status
2. Inaccurately labeled specimens are subject to rejection by the laboratory. Please see "*Rejection Criteria*"

C. *Transport*:

Place the SurePath® vial and the requisition in a biohazard bag, only 1 specimen per bag.
Note: Close container completely.

D. *Report Terminology*:

All cervical/vaginal smears will be reported using The Bethesda System II (TBSII).

1. General category
   a. Negative for squamous intraepithelial lesion or malignancy
      (1) Normal cytologic findings
      (2) Includes infectious and reactive changes
      (3) Defined by descriptive statement
   b. Epithelial cell abnormality
      (1) Includes all epithelial abnormalities
      (2) Defined by descriptive statement
   c. Other
      (1) Defined by descriptive statement

ThinPrep® Pap Test Collection and Reporting

The ThinPrep® Pap Test is an alternative to the conventional Pap smear. The ThinPrep® Pap Test eliminates many of the limitations of the conventional Pap smear by reducing air-drying artifact, obscuring blood, mucus, and inflammation.

A. *Specimen Collection*:

1. The specimen is collected using a plastic cervical spatula and an endocervical brush. The cellular material is transported to the laboratory in a liquid transport medium called PreservCyt®. Once received in the laboratory, the specimen is processed resulting in a monolayer of cells placed in a 20-mm diameter circle on a glass slide. The slide is microscopically reviewed by a cytotechnologist, and any abnormal findings are identified and referred to a pathologist for diagnosis.
   a. Label a container of PreservCyt® solution with the patient's name and date of birth before specimen collection. Other identifying information is also acceptable such as a hospital registration number or Social Security number. **All outpatient specimens must be labeled with patient's first and last name, and date of birth.**
   b. Collect the ectocervical specimen **FIRST** using the plastic spatula provided. Rotate the spatula 360° holding the spatula firmly but gently against the cervix. Rinse the spatula in the PreservCyt® vial swirling vigorously 10 times. Discard the spatula.
      Note: A clockwise rotation beginning and ending at 9 o'clock (or counter-clockwise rotation from 3 o'clock to 3 o'clock) will position the spatula so that the collected material is retained on the upper horizontal surface as the instrument is removed.
   c. Collect the endocervical specimen using an endocervical brush. Insert the cervical brush into the endocervical canal and rotate 180°. Rinse the brush in the same PreservCyt® vial as in "step b" above. Swirl the brush vigorously 10 times in the solution, and press against the wall of the vial to remove cellular material. Discard the brush.
   d. Close the PreservCyt® vial by tightening the cap until the torque line on the cap passes the torque line on the vial. **This is very important to prevent leakage of the fluid during transport. The laboratory will not process any specimens leaking out of the container.**

B. *Requisition*:

1. A Cytology requisition must be accurately completed and accompany each ThinPrep® Pap test. The requisition must include:
   a. Patient name, last and first
   b. Date of birth
   c. Social Security number or
   d. Patient identification number
   e. Date and time of specimen collection
   f. Name of ordering physician
   g. Appropriate insurance information
   h. Source of specimen
   i. Last menstrual period (LMP)
   j. Clinical history
   k. Hormonal status
2. Inaccurately labeled specimens are subject to rejection by the laboratory. Please see "*Rejection Criteria*"

C. *Transport*:

Place the PreservCyt® vial and the requisition in a biohazard bag, only 1 specimen per bag.
Note: Close container completely by lining up the black line on the cup and the lid.

D. *Report Terminology*:

All cervical/vaginal smears will be reported using The Bethesda System II (TBSII).
1. General category
   a. Negative for squamous intraepithelial lesion or malignancy
      (1) Normal cytologic findings
      (2) Includes infectious and reactive changes
      (3) Defined by descriptive statement
   b. Epithelial cell abnormality
      (1) Includes all epithelial abnormalities
      (2) Defined by descriptive statement
   c. Other
      (1) Defined by descriptive statement

Non-Gynecologic Cytology Specimens

A. *Specimen Collection*:

For optimal sampling, all specimens should be collected according to the guidelines described in this manual. See individual specimen types. All specimens must be received in a properly labeled container and enclosed in a plastic biohazard bag.

Cytolyt® solution is available for proper fixation of non-gynecologic specimens. Add equal volumes of specimen and fixative. Body fluids may be refrigerated and sent to the laboratory unfixed.

B. *Requisition*:

Each specimen must be accompanied by a Cytology requisition. The requisition must be complete and accurate and include:

1. Patient name, last and first
2. Date of birth
3. Social Security number or
4. Patient identification number
5. Date and time of specimen collection
6. Name of ordering physician
7. Appropriate insurance information
8. Source of specimen
9. Clinical history

C. *Rejection Criteria*:

Specimens will be considered unacceptable under the following conditions:

1. Any specimen not accompanied by a requisition
2. Any specimen which is not properly labeled or for which patient identification is discrepant
3. A requisition that is incomplete or contains discrepant information
4. Specimens grossly leaking into the biohazard bag
5. Broken slides that cannot be repaired