A GUIDE TO

HISTOLOGY, CYTOLOGY and MOLECULAR

SPECIMEN COLLECTION

AND

HANDLING
Histopathology

Tissue Specimens

SPECIMEN COLLECTION

1. Specimen collection is the purview of the clinician, whether biopsy, excision or whole organ removal.
2. Properly label specimen container with patient’s name and source of specimen.
3. Collect tissue immediately and place into 10% buffered formalin. For small biopsy specimens use at least 10 to 20 times the bulk of the tissue. Large tissues and organs are to be placed in large containers with at least an equal amount of 10% buffered formalin. Make sure container does not leak.

ORDERING INFORMATION

Complete requisition with the following information:
1. Patient’s name, age, date of birth, social security number, sex, date of service, and ICD9 code
2. Address
3. Doctors Office and phone number
4. Insurance Information
5. Relevant Clinical History/signs/symptoms (previous surgeries, presence of other masses etc.)
6. Source of Tissue: Including Specimen Type and Location (left, right, quadrant, etc.)
7. Nature of lesion: (solid/cystic, etc.)

SPECIMEN TRANSPORT

1. Place specimen in transport bag and seal.
2. Place the requisition in the pouch outside the sealed bag.
3. Transport specimens to the laboratory by courier.

REJECTION CRITERIA

1. No patient identification on the test requisition
2. No patient identification on slide or specimen container
3. No account/physician name or identification on the test requisition
4. Mismatch between patient name listed on the specimen and the patient name listed on the test requisition
5. No requisition with specimen
Cytopathology

ThinPrep® Pap Test™

SPECIMEN COLLECTION

ThinPrep® Pap Test™

Storage Requirements: Store the vials at room temperature. 15-30 degrees C. (59-86 degrees F.) without cells. Once the vials contain samples, cells are preserved approx. 3 weeks at 4-37 degrees C. (39-98.6 degrees F.) Do not use PreservCyt® solution after the expiration date on the container label. Please do not leave the collection device in the ThinPrep® vial.

Patient Preparation
- Patient abstains from sexual intercourse for 48 hours prior to the examination
- Patient abstains from using vaginal medication, vaginal contraceptives, or douches for 24-48 hours prior to the examination
- The optimal time for a Pap test is mid-cycle. Menses may interfere with Pap test interpretation
- Lubricant jellies should not be used to lubricate the speculum. Excessive amounts may compromise the test

Endocervical Brush/Spatula Protocol
1. Obtain an adequate sampling from the ectocervix using a plastic spatula. On the speculum water, not lubricant and shake off excess. Lubricant jellies often clog the ThinPrep filter making cytologic evaluation difficult or impossible.
2. Rinse the spatula into the PreservCyt® Solution vial by swirling the spatula vigorously in the vial ten times. Discard the spatula.
3. Obtain an adequate sampling from the endocervix using an endocervical brush devise. Insert the brush into the cervix until only the bottom most fibers are exposed. Slowly rotate ¼ or ½ turn in one direction. DO NOT OVER-ROTATE.
4. Rinse the brush into the PreservCyt® Solution by rotating the device in the solution ten times while pushing against the PreservCyt® vial wall. Swirl the brush vigorously to further release material. Discard the brush.
5. Tighten the cap so that the torque line on the cap passes the torque line on the vial.
6. Record the patient’s name on the vial. The name should be placed on the side of the vial, not on the lid.
7. Place the vial and the test requisition in a biohazard specimen bag for transport to the laboratory.

Broom-Like Device Protocol
1. Obtain an adequate sampling from the cervix using a broom-like device. Insert the central bristles of the broom into the endocervical canal deep enough to allow the shorter bristles to fully contact the ectocervix. Push gently and rotate the broom in a clockwise direction five times.
2. Rinse the broom into the PreservCyt® Solution vial by pushing the broom into the bottom of the vial ten times, forcing the bristles apart. As a final step, swirl the broom vigorously to further release material. Discard the collection device.
3. Tighten the cap so that the torque line on the cap passes the torque line on the vial.
4. Record the patient’s name on the vial. The name should be placed on the side of the container, not the lid.

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ORDERING INFORMATION

Complete cytology requisition with the following information:
- Patient’s name, age, date of birth, social security number, sex, date of service, and ICD9 code
- Address
- Doctors Office and phone number
- Insurance Information
- Relevant Clinical History/Signs/Symptoms (LMP, pregnant, postmenopausal, postpartum, hysterectomy, hormonal therapy, pelvic radiation, previous abnormals, etc.)

TRANSPORT SPECIMEN

1. Place specimen in transport bag and seal.
2. Place the requisition in the pouch outside the sealed bag.
3. Transport specimens to the laboratory by courier.

REJECTION CRITERIA

1. No patient identification on the test requisition
2. No patient identification on vial
3. No account/physician name or identification on the test requisition
4. Mismatch between patient name listed on the specimen and the patient name listed on the test requisition
5. No requisition with specimen
6. Expired ThinPrep vials
7. ThinPrep vials received empty or that have leaked in transit
8. ThinPrep vials received more than 3 weeks from the date of collection
**Cytopathology**

**Conventional Pap Test**

**SPECIMEN COLLECTION**

**Patient Preparation**
- Patient abstinences from sexual intercourse for 48 hours prior to the examination
- Patient abstinences from using vaginal medication, vaginal contraceptives, or douches for 24-48 hours prior to the examination
- The optimal time for a Pap test is mid-cycle. Menses may interfere with Pap test interpretation
- Lubricant jellies should not be used to lubricate the speculum. Excessive amounts may compromise the test

**Conventional Pap Test**

1. Using a lead pencil, write the PATIENT’S NAME on the frosted end of the slide (unlabeled slides cannot be accepted.) Labeling on the slide holder is not sufficient.
2. Insert the extended tip of the spatula in the endocervical canal and rotate allowing blunt edge of spatula to scrape the ectocervix. **Do not smear yet.**
3. Insert the cytobrush into the endocervical canal until the bristles are barely visible. Turn 90-180 degrees and remove. **Brush not recommended for use during pregnancy.**
4. Now, in rapid succession:
   - Smear the extended tip spatula (cervical) specimen along the entire length of the slide using only half of the surface
   - Roll the cytobrush (endocervical) specimen along the entire length of the slide using the remaining half of the slide surface. Bending the bristles will help transfer the cells to the slide
5. Immediately apply fixative. Squirt the packet of fixative evenly over the entire slide. If using spray fixative, hold 6-10 inches from the slide. **Do not use commercial hairspray.**
6. Allow slide to dry. Place in pap holder for transport.
7. Record the patient’s name on the slide holder.

**ORDERING INFORMATION**

- See ThinPrep Ordering Information Section

**TRANSPORT SPECIMEN**

1. Place specimen in transport bag and seal.
2. Place the requisition in the pouch outside the sealed bag.
3. Transport specimens to the laboratory by courier.

**REJECTION CRITERIA**

1. No patient identification on the test requisition
2. No patient identification on slide(s)
3. No account/physician name or identification on the test requisition
4. Mismatch between patient name listed on the specimen and the patient name listed on the test requisition
5. No requisition with specimen
6. Slide broken beyond repair upon receipt
Cytopathology

Non-Gynecologic Specimens

Specimen Collection

Appropriate fixatives for Non-gyn Cytology specimens include:
1. CytoLyt®
2. Ethyl alcohol
3. Isopropyl or methyl alcohol may be used as a substitute if none of the above two fixatives are available
4. Cytology spray fixative for smeared slides

Smear
1. Complete the cytology test requisition.
2. Write patient’s name in pencil on frosted end of slide.
3. Submit slide(s) of material from any source that can be evaluated cytologically.
4. Fix slide(s) immediately with cytology spray fixative or immerse in alcohol for 3-5 minutes. Allow fixative to dry thoroughly before packaging slides for transport.
5. Submit in appropriate slide container. Place the container and test requisition in a specimen bag for transport to the laboratory.

Fluid
1. Complete test requisition
2. Submit fluid fixed with a minimum of 10 ml of fixative. Specimens greater than 10 ml should be fixed with a volume of fixative equal to the volume of the specimen.
3. Place fluid/fixative mixture in a tightly capped, leak proof, labeled container (label the container wall, not the lid).
4. Syringes are not acceptable specimen containers.
5. Transportation of needles violates Department of Transportation Federal regulations, 49CFR173.
6. Place the container and test requisition in a bag for transport to the laboratory.

SOURCE - SUBMISSION REQUIREMENTS

Breast Cyst Aspiration - If aspirate is scanty, fluid may be smeared one drop at a time on clean, dry slides and immediately fixed. If aspirate is abundant, mix material with an equal volume of fixative (see specimen collection)

Breast Solid Mass Aspiration – See Fine Needle Aspiration Biopsy below

Breast Secretion (Nipple Discharge) – Drops of fluid from the nipple are smeared directly on clean glass slides and fixed immediately with spray fixative or immersed in alcohol for 3-5 minutes.

Bronchial Brushings – Roll brush(es) over clean, dry slide. Fix immediately with alcohol or spray fixative. The brush(es) used to prepare bronchial brushing slides may be swirled in a container of fixative (see specimen collection) to dislodge additional specimen. Submit slides and liquid specimen together with one test requisition.

Bronchial Washings – Mix with an equal volume of fixative (see above), or put specimen in at least 10 ml.

Effusions – Mix material with an equal volume of fixative (see Fixatives).

Endometrial Washings – Mix material with an equal volume of fixative (see Fixatives).

Esophageal Brushings – Roll brushes over clean, dry slides. Fix immediately with alcohol or spray fixative. The brush(es) used to prepare slides may be swirled in a container of appropriate fixative (see above) to dislodge additional specimen. Submit slides and liquid specimen together with one test requisition.

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**Esophageal Washings** – Mix material with an equal volume of fixative (see fixatives).

**Fine Needle Aspiration Biopsy**
*(Solid Lesion)*

1. Release negative pressure, then remove needle. Specimen should not be drawn up into the barrel of the syringe. Pressure should be released as fluid appears in the needle hub. The cells and tissue fragments obtained from a solid lesion should remain in the barrel of the lesion.
2. Eject specimen directly into container of CytoLyt.
3. The container wall should be labeled with the patient’s name.
4. Flush needle and syringe with fixative.
5. This procedure can be repeated multiple times until the lesion has been thoroughly sampled, adding material obtained with each aspiration to the same container of fixative.

**Slide Technique (alternate method)**

1. Label glass slide(s) with the patient’s name or unique identifier on the frosted end prior to starting the procedure.
2. If local anesthetic is used, insert the anesthetic needle adjacent to but not into the lesion. The anesthetic could dilute or distort the specimen and hinder achieving an accurate diagnosis.
3. Attach a 23, 25, or 27 gauge needle to a syringe.
4. Insert needle into lesion.
5. While applying negative pressure, move needle back and forth traversing the entire lesion.
6. Release negative pressure, and then remove the needle. Specimen should not be drawn up into the barrel of the syringe. Pressure should be released as fluid appears in the needle hub. The cells and tissue fragments obtained from a solid lesion should remain in the barrel of the needle.
7. After withdrawing the needle, eject one drop of specimen onto each of 2-3 slide(s). “Air-dried” slide should be labeled as such.
8. Use another slide to smear the aspirated material.
9. Fix slides immediately (within a few seconds), using cytology spray fixative or immerse in alcohol for 5 minutes.
10. Do not fix slide(s) labeled “air-dried”.
11. This procedure can be repeated multiple times until the lesion has been thoroughly sampled.
12. If blood, fluid or cellular material in excess of three drops is obtained with a needle pass, the excess should be expressed into a container of CytoLyt. The container wall should be labeled with the patient’s name. The needle and syringe should be rinsed with this same fixative. Submit the liquid specimen with the fixed and air-dried slides using one test requisition.

**Gastric Brushings** – Roll brush(es) over clean, dry slide. Fix immediately with alcohol or spray fixative. In order to dislodge additional cells, swirl the brushes used to prepare the slides in a container of fixative (see Fixatives). The container wall should be labeled with the patient’s name. Submit slides and liquid specimen together with one test requisition.

**Gastric Washings** – Mix material with an equal volume of fixative (see Fixatives).

**Lymph Node (Touch Prep)** – Fix immediately in alcohol or use spray fixative. Air-dried slides should be labeled as such.

**Paracentesis (Abdominal Fluid)** – Mix material with an equal volume of fixative (see Fixatives).

**Pericardial Fluid** – Mix material with an equal volume of fixative (see Fixatives).

**Pneumocystis carinii** – Bronchoalveolar lavage or washings are preferred specimens. Bronchial brushings or sputum may be submitted, but diagnostic yield is less. Mix material with an equal volume of fixative (see Fixatives). Use pencil to label frosted end of slide with patient’s name. Fix submitted smears with cytology spray fixative or immerse in alcohol for 3-5 minutes. Submit a minimum of 2 slides. Write “Evaluate for Pneumocystis carinii” on test requisition.

**Skin (Viral) Lesions (Tzanck Smear)** – Remove crust or dome from lesion. Scrape ulceration with a curette. Spread material on alcohol-moistened slide. Spray-fix immediately or fix slides in alcohol.

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Sputum – Submit early morning deep-cough specimen prior to any food ingestion. Have patient rinse mouth with plain water before sputum is collected. Collect separate specimens on 3 consecutive mornings. Do not pool specimens. Mix material with an equal volume of fixative.

Thoracentesis (Pleural or Chest Fluid) – Mix material with an equal volume of fixative (see Fixatives).

Urine (Bladder/Kidney, voided) – Submit all specimens in an equal volume of fixative (see Fixatives). Mark test requisition: “Voided” or “Catheterized” as applicable.

Urine Collected at Home – Instruct the patient to drink three 8-oz glasses of water before bedtime. Provide patient with a premeasured container of CytoLyt®. Have patient collect the second A.M. urine specimen and mix an equal volume with the fixative. Do not submit a 24-hour urine collection for cytologic evaluation.

Urine Collected at Client Office – Have patient void into clean specimen container. A sterile container is not necessary. Voided urine should be added directly to a container of Cytolyt fixative.

ORDERING INFORMATION

Complete requisition with the following information:
♦ Patient’s name, age, date of birth, social security number, sex, date of service, and ICD9 code
♦ Address
♦ Doctors Office and phone number
♦ Insurance Information
♦ Relevant Clinical History/Signs/Symptoms

TRANSPORT SPECIMEN
1. Place specimen in transport bag and seal.
2. Place the requisition in the pouch outside the sealed bag.
3. Transport specimens to the laboratory by courier.

REJECTION CRITERIA
1. No patient identification on the test requisition
2. No patient identification on slide(s) or specimen container
3. No account/physician name or identification on the test requisition
4. Mismatch between patient name listed on the specimen and the patient name listed on the test requisition
5. No requisition with specimen
6. Slide broken beyond repair upon receipt
7. Specimen containers that are empty or have leaked in transit.
Molecular Testing

HPV – Using Female ThinPrep® Pap Test™ vial

HUMAN PAPILLOMA VIRUS, HIGH RISK TYPES, SCREEN
Storage Requirements: Store the vials at room temperature 15-30 degrees C without cells. Once the vials contain samples, cells are preserved several weeks at room temperature, 20-30 degrees C. Do not use PreservCyt® solution after the expiration date on the container label.

PATIENT PREPARATION
- Patient abstains from sexual intercourse for 48 hours prior to the examination
- Patient abstains from using vaginal medication, vaginal contraceptives, or douches for 24-48 hours prior to the examination
- Do not use lubricants or gloves with powder during procedure. These may cause inhibition of test results.
- Obtain the specimen before the application of acetic acid.

SPECIMEN COLLECTION
Refer to Collection of a “ThinPrep® Pap Test™”

ORDERING INFORMATION
Complete requisition with the following information:
- Patient’s name, age, date of birth, social security number, sex, date of collection, and ICD 9 code and clinical information.
- Patient address
- Doctors office and phone number
- Insurance Information
- Test(s) desired

TRANSPORT SPECIMEN
1. Place specimen in transport bag and seal.
2. Place the requisition in the pouch outside the sealed bag.
3. Transport specimens to the laboratory by courier.

REJECTION CRITERIA
1. No patient identification on the test requisition
2. No patient identification on vial
3. No account/physician name or identification on the test requisition
4. Mismatch between patient name listed on the specimen and the patient name listed on the test requisition
5. No requisition with specimen
6. Expired ThinPrep Vials
7. ThinPrep Vials received empty or that have leaked in transit
8. ThinPrep vials received more than 4 weeks from the date of collection
9. Specimen quantity not sufficient for testing
10. Inappropriate specimen for testing (i.e. anal specimen)
Molecular Testing

CT/NG/TRICHOMONAS – Using Female ThinPrep® Pap Test™ vial

CHLAMYDIA TRACHOMATIS, NEISSERIA GONORRHOEAE (CT/NG) AND TRICHOMONAS

Storage Requirements: Store the vials at room temperature, 15-30 degrees C without cells. Do not use PreservCyt® solution after the expiration date on the container label.

Patient Preparation
- Patient abstains from sexual intercourse for 48 hours prior to the examination
- Patient abstains from using vaginal medication, vaginal contraceptives, or douches for 24-48 hours prior to the examination.
- Do not use lubricants or gloves with powder during procedure. These may cause inhibition of test results.
- Obtain the specimen before the application of acetic acid.

SPECIMEN COLLECTION
Refer to Collection of a “ThinPrep® Pap Test™”

ORDERING INFORMATION
Complete requisition with the following information:
- Patient’s name, age, date of birth, social security number, sex, date of collection, and ICD 9 code and clinical information
- Patient address
- Doctors office and phone number
- Insurance Information
- Test desired(s)

TRANSPORT SPECIMEN
1. Place specimen in transport bag and seal.
2. Place the requisition in the pouch outside the sealed bag.
3. Transport specimens to the laboratory by courier.

REJECTION CRITERIA
1. No patient identification on the test requisition
2. No patient identification on vial
3. No account/physician name or identification on the test requisition
4. Mismatch between patient name listed on the specimen and the patient name listed on the test requisition
5. No requisition with specimen
6. Expired ThinPrep Vials
7. ThinPrep Vials received empty or that have leaked in transit
8. ThinPrep vials received more than 30 days from the date of collection
9. Specimen quantity not sufficient for testing
10. Inappropriate specimen for testing (i.e. anal specimen)
Molecular Testing

CT/NG/TRICHOMONAS – Female Endocervical Swab

CHLAMYDIA TRACHOMATIS, NEISSERIA GONORRHOEAE (CT/NG) AND TRICHOMONAS

Endocervical Swab Procedure
This test requires a GenProbe Aptima endocervical swab and transport tube.
Storage Requirements: Once collected, store at 2 to 30 degrees C prior to testing.

PATIENT PREPARATION
- Patient abstains from sexual intercourse for 48 hours prior to the examination
- Patient abstains from using vaginal medication, vaginal contraceptives, or douches for 24-48 hours prior to the examination.
- Do not use lubricants or gloves with powder during procedure. These may cause inhibition of test results.
- Obtain the specimen before the application of acetic acid.

SPECIMEN COLLECTION
1. Remove excess mucus from the cervix with the swab (white shaft swab) provided in the collection kit.
2. Insert the small-tipped specimen swab (blue shaft swab) into the endocervix and rotate the swab for 10 to 30 seconds.
3. Unscrew the cap of the transport tube; insert the swab into the transport tube and break at the score line. Replace the cap securely making sure that the swab fits into the tube.
4. The specimen should remain at room temperature.

ORDERING INFORMATION
Complete requisition with the following information:
- Patient’s name, age, date of birth, social security number, sex, date of collection, and ICD 9 code and clinical information
- Patient address
- Doctors office and phone number
- Insurance Information
- Test desired(s)

TRANSPORT SPECIMEN
1. Place specimen in transport bag and seal.
2. Place the requisition in the pouch outside the sealed bag.
3. Transport specimens to the laboratory by courier.

REJECTION CRITERIA
1. No patient identification on the test requisition
2. No patient identification on specimen
3. No account/physician name or identification on the test requisition
4. Mismatch between patient name listed on the specimen and the patient name listed on the test requisition
5. No requisition with specimen
6. Specimen quantity not sufficient for testing
7. Inappropriate specimen for testing (i.e. anal specimen)
8. Unacceptable storage/transport for test.
Molecular Testing

CT/NG/TRICHOMONAS – Male Urethral Swab

CHLAMYDIA TRACHOMATIS, NEISSERIA GONORRHOEAE (CT/NG) AND TRICHOMONAS
MALE URETHRAL SWAB PROCEDURE
This test requires a GenProbe Aptima Swab Specimen Collection Kit
Storage Requirements: Store at 2-30 degrees C.

PATIENT PREPARATION
- Collect specimen at least 1 hour after patient has urinated.

SPECIMEN COLLECTION
1. Insert the small-tipped specimen swab (blue shaft swab) 2 to 4 cm into the urethra and rotate 2-3
   seconds and withdraw it carefully.
2. Unscrew the cap of the transport tube; insert the swab into the transport tube. Break swab shaft at
3. Store at 2-30 degrees C.

ORDERING INFORMATION
Complete requisition with the following information:
- Patient’s name, age, date of birth, social security number, sex, date of collection, and ICD 9 code
  and clinical information
- Patient address
- Doctors office and phone number
- Insurance Information
- Test desired(s)

TRANSPORT SPECIMEN
1. Place specimen in transport bag and seal.
2. Place the requisition in the pouch outside the sealed bag.
3. Transport specimens to the laboratory by courier.

REJECTION CRITERIA
1. No patient identification on the test requisition
2. No patient identification on specimen
3. No account/physician name or identification on the test requisition
4. Mismatch between patient name listed on the specimen and the patient name listed on the test
   requisition
5. No requisition with specimen
6. Specimen quantity not sufficient for testing
7. Inappropriate specimen for testing (i.e. anal specimen)
8. Unacceptable storage/transport for test
**Molecular Testing**

**CT/NG/TRICHOMONAS – Urine (MALE or FEMALE)**

**CHLAMYDIA TRACHOMATIS, NEISSERIA GONORRHOEAE (CT/NG) AND TRICHOMONAS, URINE**

This test requires a urine collection cup free of any preservatives or contaminates and a GenProbe Aptima Urine Collection and Transport Tube.

Storage Requirements: Store at 2-30 degrees C. until tested.

**PATIENT PREPARATION**

- Collect 20-30 ml of first void urine in proper container without preservatives.

**SPECIMEN COLLECTION**

1. Instruct the patient not to urinate for one (1) hour prior to collection. Females should not cleanse.
2. The patient should collect the first 20 to 30 ml of voided urine (the first part of the stream) in a plastic, preservative-free, urine collection cup.
3. Remove cap from urine specimen transport tube and transfer 2 ml of urine into the tube using disposable pipette provided. The correct volume of urine has been added when fluid is between black fill lines on urine specimen transport tube.
4. Recap specimen tube tightly.
5. Store at 2-30 degrees C. until testing.

**ORDERING INFORMATION**

Complete requisition with the following information:

- Patient’s name, age, date of birth, social security number, sex, date of collection, and ICD 9 code and clinical information
- Patient address
- Doctors office and phone number
- Insurance Information
- Test desired(s)

**TRANSPORT SPECIMEN**

1. Place specimen in transport bag and seal.
2. Place the requisition in the pouch outside the sealed bag.
3. Transport specimens to the laboratory by courier.

**REJECTION CRITERIA**

1. No patient identification on the test requisition
2. No patient identification on specimen
3. No account/physician name or identification on the test requisition
4. Mismatch between patient name listed on the specimen and the patient name listed on the test requisition
5. No requisition with specimen
6. Specimen quantity not sufficient for testing
7. Unacceptable storage/transport for test.