

Yale School of Medicine
Surgical Pathology Outreach Unit



**MANUAL FOR
SPECIMEN COLLECTION
2016-2017**

GENERAL INFORMATION

Laboratory Hours: Monday – Friday
8:30 AM – 5:00 PM

Location: CB5-538

Specimen Receiving Area: Outreach Accessioning Area, CB5-538. After working hours, drop off specimens in Surgical Pathology, East Pavilion 2-616. Rush cases should be directed to the Pathology Resident on call.

Telephone: 785-2636 Cytology Office
737-5492 Cytology Manager

ALL SPECIMENS ARE HANDLED ACCORDING TO UNIVERSAL PRECAUTION GUIDELINES AND STRICT ADHERENCE TO THESE GUIDELINES IS MAINTAINED.

***** REMOVE NEEDLES AND RECAP THE SYRINGE BEFORE SENDING SPECIMEN IN SYRINGES TO LABORATORY *****

INTRODUCTION

This manual is designed to expedite your use of the Outreach Laboratory for the care of your patients. It contains information on how specimens are best collected from various body sites and preserved prior to preparation and examination in Cytology. Please contact the laboratory, Pathology resident, or the laboratory director to discuss tests not included in this manual or other issues.

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GYN SPECIMEN COLLECTION

CONVENTIONAL SMEAR

Materials Needed:

The Cytology Laboratory has several types of materials available for collecting conventional smears, depending on the clinician's preferences.

1. Clear glass slides with single frosted end
2. Spray fixative
3. Cardboard slide holders
4. Clear plastic vials with a colored lid filled with 95% ethanol (ETOH)
5. Cytology Gynecological Requisition (form F-3743) or EPIC Requisition
6. Specimen transport bag

Alternatively, a prepackaged supply kit (PAP PERFECT) is available containing a slide, cytobrush, cervical scraper, fixative, and slide transporter.

All materials are provided by the Cytology Laboratory.

Procedure:

1. Write patient's last name and first name on frosted end of slide with lead pencil only. (Ink and grease pencils will wash off in solutions.)
2. Label slide holder which is being used.
3. Designate specimen source on slide with proper initial. (See below)
 - a. Vaginal smear V
 - b. Cervical smear CX
 - c. Endocervical smear ENDO
 - d. Exocervical smear EXO
 - e. Introital smear I
 - f. Vulvar smear VUL
4. Immediately upon smearing slide, fix specimen using chosen method. Any delay in fixation will cause cellular drying artifact and may render specimen UNSATISFACTORY for cytological evaluation.
5. Place slide in appropriate transport holder.
6. Place in specimen transport bag and close bag.
7. Place completed Cytology Requisition in side pocket of specimen transport bag.
8. Send to Cytology Laboratory.

THINPREP® PAP TEST

Materials Needed:

1. PreservCyt® Solution vial for ThinPrep® Pap Test
2. Medscand Sample Collection Kit (containing spatula and brush)
3. Rovers Cervex-Brush®
4. Cytology Gynecological Requisition (form F-3743)
5. Specimen transport bags

All materials are provided by the Cytology Laboratory.

Procedure:

1. Label PreservCyt® Solution vial with two patient identifiers.
2. Obtain specimen using appropriate sampling device.
3. Rinse as quickly as possible in the PreservCyt® Solution.
 - a. Spatula – swirl vigorously in vial 10 times.
 - b. Endocervical brush – rotate device in solution 10 times while pushing against PreservCyt vial wall. Swirl vigorously to further release material.
 - c. Broom – push broom into bottom of vial 10 times, forcing bristles apart. Swirl vigorously to further release material.
4. Discard device(s).
5. Tighten cap so that the torque line on cap passes the torque line on vial to prevent leakage.
6. Place in specimen transport bag and close bag.
7. Place completed Cytology Requisition in side pocket of specimen transport bag.
8. Send to Cytology Laboratory.

SUREPATH™ PAP TEST

Materials Needed:

1. SurePath™ Preservative Fluid vial
2. Rovers Cervex®-Brush
3. Rovers Endocervical Brush
4. Cytology Gynecological Requisition (form F-3743)
5. Laboratory Transport Bag

All materials are provided by the Cytology Laboratory.

Procedure:

1. Label SurePath™ Preservative Fluid vial with two patient identifiers.
2. Obtain specimen using Rovers Cervex®-Brush.
3. Place brush into Preservative Fluid and remove handle of brush.
4. Cap vial tightly.
5. Place in specimen transport bag and close bag.
6. Place completed Cytology Requisition in side pocket of specimen transport bag.

7. Send to Cytology Laboratory.

MATURATION INDEX – HORMONAL ASSESSMENT

1. Specimen must be prepared from the lateral vaginal wall, or if this is not feasible, from the vaginal fornix. Specimens from the lower third of the vagina, ectocervix, or endocervix are useless for hormonal assessment since metaplasia, cervicitis, ectopia, and other conditions preclude accurate evaluation.
2. Collect specimen following instructions for appropriate type of specimen (Conventional, ThinPrep®, or SurePath™).
3. If more than one slide is submitted, be sure to identify which one is for Maturation Index.
4. Identify on requisition that specimen is for a Maturation Index (MI).

The Maturation Index will be reported in percentages for parabasal, intermediate, and superficial cells present.

CYTOLOGY GYNECOLOGICAL REQUISITION

1. Complete Cytology Requisition with all of the following information:
 - a) Patient's name, medical record number, and visit number
 - b) Patient's address
 - c) Patient's date of birth
 - d) Attending physician
 - e) Service
 - f) Date specimen obtained
 - g) Last menstrual period date to include:
 - (i) Prenatal date
 - (ii) Postpartum delivery date
 - (iii) Postmenopausal – approximate year
 - h) Pertinent clinical history to include:
 - (i) Hormonal therapy, birth control pills, steroids, etc.
 - (ii) IUD in place or recently removed
 - (iii) Prior history of malignancy, surgery, radiation, chemotherapy
 - (iv) Postmenopausal/abnormal bleeding
2. Requisition slip should be placed in the side pocket of specimen transport bag and brought to the Cytology Laboratory, CB5-538, for accessioning.

MOLECULAR TESTING

SPECIMENS FOR HPV TESTING

Yale University uses the Roche Cobas® 4800 HPV DNA Test to determine the presence or absence of the High Risk Human Papilloma Virus (HPV) in cervical specimens. This method is able to detect thirteen high-risk types of HPV DNA, but cannot specify the exact type present.

The possible results of this test are either negative, positive, or indeterminate for any of the 13 HPV types tested.

It must be noted that if clients choose to use the Surepath preservative solution as a form of collection, it is not qualified for use by Roche Cobas® 4800. Yale has developed a protocol for using Surepath specimens with Roche Cobas® 4800 test. Although it has not been cleared or approved by the U.S. Food and Drug Administration, they have determined that such clearance or approval is not necessary.

Materials Needed:

1. 1 of 2 different types of cervical sampler specimens are accepted: ThinPrep PreservCyt solution, or SurePath preservative solution.
2. Cytology Requisition form.

Procedure:

A. HPV Testing with a Pap Smear

1. If a Pap smear is to be collected in one of the liquid based forms (ThinPrep, Surepath), collect the specimen as directed for a routine Pap smear.
2. Make sure two patient identifiers are on the vial.
3. Indicate on the requisition form, on the lower right hand side of the page, that HPV testing is requested by checking the appropriate box. One box must be selected, either “perform HPV test regardless of Pap result” or “if Pap result shows ASCUS, perform HPV DNA test” or this test will not be performed.
4. Send sample to Yale Cytology as you would with a routine Pap smear. The HPV test will be performed on the sample after the Pap smear has been prepared.

NOTE: Cervical specimens should be taken before the use of acetic acid or iodine if using a colposcope during the examination.

B. HPV Testing without a Pap Smear

1. Collect specimen using one of the two indicated collection methods (ThinPrep, SurePath) as you would a routine Pap smear.
2. Fill out a requisition with patient information and label the specimen vial appropriately with two patient identifiers.

3. Indicate on requisition that “HPV testing ONLY” is requested for submitted specimen.
4. Send specimen to Yale Cytology as indicated.

SPECIMENS FOR HPV GENOTYPING

Yale University uses the Roche Cobas® 4800 Test to determine the presence or absence of the High Risk Human Papilloma Virus (HPV) in cervical specimens. We are able to identify types 16,18. The possible results of this test are either negative or positive for either of the 2 HPV types tested.

Materials Needed:

1. 1 of 2 different types of cervical sampler specimens are accepted: ThinPrep PreservCyt solution or SurePath preservative solution.
2. Cytology requisition form.

Procedure:

HPV Genotyping Testing with a Pap Smear

1. If a Pap smear is to be collected in one of the liquid based forms (ThinPrep, Surepath), collect the specimen as directed for a routine Pap smear.
2. Make sure two patient identifiers are on the vial.
3. Indicate on the requisition form, on the lower right hand side of the page, that HPV Genotyping test is requested by checking the appropriate box. One of the HR HPV test boxes must also be selected, either “perform HPV test regardless of Pap result” or “if Pap result shows ASCUS, perform HPV DNA test” or the HPV Genotype test will not be performed.
4. If the Pap smear is Negative and the HPV is positive and a request is made for genotyping we will perform the test.
5. If HPV Genotyping test is to be requested after the pap specimen has already been sent for processing, fax your request on the Yale Additional Testing Request Form to 203-785-3385.

NOTE: Cervical specimens should be taken before the use of acetic acid or iodine if using a colposcope during the examination.

SPECIMENS FOR CHLAMYDIA/GONORRHEA (CTGC/HSV) TESTING

Yale University uses the BD Viper Test to test for the presence or absence of Chlamydia (CT) or Gonorrhea (GC) in cervical specimens and the presence or absence of Herpes Simplex Viruses 1 & 2 in external anovaginal lesions. This test will identify the presence or absence of CHLAMYDIA/GONORRHEA (CTGC), HERPES SIMPLEX VIRUS 1, AND/OR HERPES SIMPLEX VIRUS 2.

*The CTGC test is FDA approved for ThinPrep, SurePath and BD swab.

*The HERPES test is FDA approved for the BD swab only.

Materials Needed:

1. Vial of ThinPrep or SurePath preservative solution or a BD CT/GC or BD HERPES swab.
2. Cytology Requisition form or EPIC Requisition

Procedure:

A. CTGC Testing with a Pap Smear

1. If Pap smear is to be collected in a ThinPrep or SurePath preservative vial, collect specimen as directed for a routine Pap smear.
2. Make sure two patient identifiers are on the vial.
3. Indicate on the requisition form, on the lower right hand side of the page, that Chlamydia/ Gonorrhea testing is requested by checking the appropriate box. If this box is not selected, this test will not be performed.
4. Send the sample to Yale Cytology as you would with a routine Pap smear. The CTGC test will be performed on the sample after the Pap smear has been prepared.
5. If the client is sending a conventional Pap smear but requests CTGC testing, a separate cervical sample must be taken and sent in the BD Swab for CT/GC testing. Indicate on the requisition under “specimens submitted” that a BD swab for CTGC testing is included.

NOTE: Cervical specimens should be taken before the use of acetic acid or iodine if using a colposcope during the examination.

B. CTGC Testing without a Pap Smear

1. Collect specimen using one of the indicated collection methods (ThinPrep, SurePath, or BD Swab).
2. Fill out a requisition with patient information and label the specimen vial with two patient identifiers.
3. Indicate on requisition that “CTGC testing ONLY” is requested for submitted specimen.
4. Send specimen to Yale Cytology, CB5-538, as indicated.

C. HSV Testing

1. Collect specimen using the BD Swab only.
2. Fill out a requisition with patient information and label the specimen vial with two patient identifiers.
3. Indicate on requisition that specimen is for HSV testing.
4. Send specimen to Yale Cytology, CB5-538, as indicated.

BD AFFIRM TESTING

Yale University uses the BD AFFIRM VP III to test for the presence or absence of *Candida* species, *Gardnerella vaginalis*, *Trichomonas vaginalis*. This is a FDA approved test.

Materials Needed:

1. BD Affirm swab
2. Cytology Requisition

Procedure:

1. Collect cervical specimen using the BD Affirm swab only.
2. Fill out the requisition with patient information and label the specimen vial with two patient identifiers.
3. Indicate on requisition that “BD Affirm” is requested for submitted specimen.
4. Send specimen to Yale Cytology, CB5-538, as indicated.

NOTE: BD Affirm specimens are only viable for testing up to 72 hours after collection.

NON-GYNECOLOGICAL SPECIMENS

GENERAL INFORMATION:

1. Complete Cytology Requisition (form F-3741) or use EPIC Requisition with the following:
 - a. Patient's name, medical record number, and visit numbers
 - b. Patient's address
 - c. Patient's birthdate
 - d. **Attending physician – residents are not acceptable due to billing purposes**
 - e. Floor or clinic
 - f. Specimen source
 - g. Date specimen was obtained
 - h. Gender
2. Label specimen container with two patient identifiers and specimen type. All specimens submitted should contain adequate and pertinent clinical history to include the following:
 - a. Reason patient is admitted
 - b. Reason for workup (e.g. abnormal CXR, palpable mass, bleeding, etc.)
 - c. Prior diagnosis of malignancy (if possible give type and primary)
 - d. History of patient being treated with radiation or chemotherapy
 - e. Any pertinent family/social history
3. To expedite the case, relevant beeper numbers and telephone numbers of physicians caring for patient are beneficial.
4. Specimens taken late in the afternoon should be hand-delivered by the house officer, or refrigerated, rather than given to transport. Alternative handling of the specimen may render it inadequate for interpretation. **Do not send cytology specimens through the tube system.**
5. All specimens are handled via Universal Precautions, but this should not preclude an adequate and thorough history of possible infectious cases.

RESPIRATORY SPECIMENS

SPUTUM

Materials Needed:

1. Capped, clean container
2. Label for container
3. Cytology Requisition (form F-3741) or EPIC Requisition
4. Specimen transport bag

Materials are available from the Cytology Laboratory.

Procedure:

1. Patient should expectorate into a clean, empty container. Cap container and label with two patient identifiers.
2. Usually the first early morning deep cough specimen will yield the best cell sample. If it is necessary to take a specimen when the lab is closed, **DO NOT** add any fixative. Refrigerate specimen until it can be brought promptly to the lab for processing.
3. Complete requisition and place in transport bag pocket along with specimen.
4. Send to Cytology via transport, or bring specimen to the Cytology Laboratory, CB5-538.
5. A sputum series will usually consist of 3-5 consecutive early morning deep cough specimens.
6. Histochemical stains for *Pneumocystis carinii* are not performed on standard sputum specimens – only on induced sputa and bronchoalveolar lavage.

Out-Patient Sputa Samples:

Sputa samples collected on an outpatient basis may, if absolutely necessary, be placed in CytoRich® or 70% ethanol as a fixative. Any greater percentage of alcohol added to a fresh specimen is suboptimal, as fixative will often cause difficulty and make specimen preparation impossible. Containers with CytoRich® or alcohol fixative can be obtained from the Cytology Laboratory. If more than one specimen is to be submitted, label containers with date and mark specimen #1, #2, etc.

BRONCHIAL BRUSHINGS

Materials Needed:

1. Glass slides with frosted end (Do not use fully frosted slides)
2. Clear plastic vials with a colored lid filled with 95% ethanol (ETOH)
3. Cytology requisition (form F-3741)
4. Container of CytoRich® Red or 50% ethanol fixative
5. Specimen transport bag

Materials are available from the Cytology Laboratory.

Procedure

1. Label frosted end of slides with two patient identifiers.
2. Specimen must be placed on the same side as the frosted end (top of slide). If specimen is placed on the bottom of slide, it may be lost during processing.
3. **Immediately** upon smearing slide, place in vial containing 95% ethanol fixative.
4. Label vial with patient's name, medical record and visit numbers, and specimen source. It is most important to identify source of brushing on the slide if more than one site is submitted (e.g., RUL, RML, RLL, LUL, LLL).
5. Clip the end of the brush and place in a container of CytoRich® Red or 50% ethanol fixative. Label with two patient identifiers.
6. Complete requisition and place in transport bag pocket along with specimen.
7. Send to Cytology via transport, or bring specimen to the Cytology Laboratory, CB5-538.

NOTE: Any delay in the immersion of slides into the fixative will cause cellular drying artifact which may render the specimen unsatisfactory for cytologic evaluation.

BRONCHIAL WASHINGS/LAVAGE AND TRACHEAL WASHINGS

Materials Needed:

1. Specimen trap tube
2. Cytology Requisition (form F-3741) or EPIC Requisition
3. Specimen transport bag

Requisition and transport bag available in Cytology.

Procedure:

1. Washings should be submitted in a specimen trap with no fixative added. If the specimen cannot be delivered right away, **refrigerate** for cellular preservation.
2. Label container with two patient identifiers, and specimen site (e.g. diffuse wash, right-sided wash, or left-sided wash).
3. Complete requisition and place in transport bag along with specimen.
4. Send to Cytology via transport, or bring specimen to the Cytology Laboratory, CB5-538.
5. **After hours, *Pneumocystis carinii* stain requests should be made by contacting the Chief Resident for instructions at extension 5-2788 or via the page operator for the beeper number of the resident on call.**
6. If clinically suspicious for aspiration pneumonia, clearly request on requisition **Oil Red-O** stain. Do not write lipid (fat) laden macrophages.

***** Please clearly indicate by name which special stains you would like performed, e.g., GMS, Oil Red-O, etc.**

FLUID CYTOLOGY

FLUIDS FROM SEROSAL CAVITIES

1. Pleural fluid
2. Peritoneal fluid
3. Pericardial fluid
4. Synovial fluid
5. Pelvic/Peritoneal Washing

Materials Needed:

1. Capped specimen container (glass containers will not be accepted in accordance with OSHA compliance)
2. Cytology Requisition (form F-3741) or EPIC Requisition
3. Specimen transport bag
4. Optional – 3 units heparin/1ml of fluid to be drawn; must be placed in container prior to specimen itself

Container, requisition, and transport bag available in the Cytology Laboratory.

Procedure:

1. Place specimen in container. **Do not add any fixative.** If there is to be a delay in delivering the specimen, refrigerate for cellular preservation.
2. Label container with two patient identifiers and specimen source.
3. Complete requisition and place in transport bag pocket along with specimen.
4. Send to Cytology via transport, or bring specimen to the Cytology Laboratory, CB5-538.
5. **Cell Blocks** are routinely made if there is adequate material. A cell block requires an additional 24 hours for processing. A preliminary cytologic diagnosis can be made available in most cases prior to the final cell block diagnosis.
6. **Specimens in Pleur-evac containers will not be accepted.**

CEREBROSPINAL FLUID

Materials Needed:

1. Clean, capped container
2. Cytology Requisition (form F-3741)
3. Specimen transport bag

Materials are available in the Cytology Laboratory.

Procedure:

1. Place specimen into container.
2. Label container with two patient identifiers and specimen source.
3. Complete requisition and place in transport bag pocket along with specimen.

4. CSF should be delivered as quickly as possible to the lab for processing as cellular degeneration occurs rapidly in these specimens. If there is an anticipated delay of >30 minutes in delivery, the specimen should be refrigerated, or preferably fixed in CytoRich® Red for preservation.
5. It is most important to identify a CSF specimen as a lumbar tap or as fluid retrieved by a shunt.
6. History is extremely important for the proper evaluation of CSF specimens.

URINE

Materials Needed:

1. Clean, capped container
2. Cytology Requisition (form F-3741) or EPIC Requisition
3. Specimen transport bag

Materials are available in the Cytology Laboratory.

Procedure:

1. Place specimen into container.
2. Label container with two patient identifiers and specimen source. It is important to differentiate between voided and catheterized specimen as this influences the interpretation of the specimen.
3. Complete requisition and place in transport bag pocket along with specimen.
4. It is extremely important to make note on the requisition of recent cytoscopy or intravesical therapy.
5. The first morning voided urine will not yield the best cellular sample as the specimen will be degenerated. Use the second urine specimen.
6. Send to Cytology via transport, or bring specimen to the Cytology Laboratory, CB5-538.

GASTROINTESTINAL TRACT CYTOLOGY

ESOPHAGEAL BRUSHING

Materials Needed:

1. Glass slides with frosted end (Do not use fully frosted slides)
2. Clear plastic vials with colored lid filled with 95% ethanol (ETOH)
3. Cytology Requisition (form F-3741) or EPIC Requisition
4. Container of CytoRich® Red or 50% ethanol fixative
5. Specimen transport bag

Materials are available from the Cytology Laboratory.

Procedure

1. Label frosted end of slides with two patient identifiers.
2. Specimen must be placed on the same side as the frosted end (top of slide). If specimen is placed on the bottom of slide, it may be lost during processing.
3. **Immediately** upon smearing slide, place in vial containing 95% ethanol fixative.
4. Label vial with two patient identifiers and specimen source.
5. Clip the end of the brush and place in a container of CytoRich® Red or 50% ethanol fixative. Label with two patient identifiers.
6. Complete requisition and place in transport bag pocket along with specimen.
7. Send to Cytology via transport, or bring specimen to the Cytology Laboratory, CB5-538.

NOTE: Any delay in the immersion of slides into the fixative will cause cellular drying artifact which may render the specimen unsatisfactory for cytologic evaluation.

GASTRIC BRUSHING

Materials Needed:

1. Glass slides with frosted end (Do not use fully frosted slides)
2. Clear plastic vials with colored lid filled with 95% ethanol (ETOH)
3. Cytology Requisition (form F-3741)
4. Container of CytoRich® Red or 50% ethanol fixative
5. Specimen transport bag

Materials are available from the Cytology Laboratory.

Procedure

1. Label frosted end of slides with two patient identifiers.
2. Specimen must be placed on the same side as the frosted end (top of slide). If specimen is placed on the bottom of slide, it may be lost during processing.
3. **Immediately** upon smearing slide, place in vial containing 95% ethanol fixative.

4. Label vial with two patient identifiers and specimen source.
5. Clip the end of the brush and place in a container of CytoRich® Red or 50% ethanol fixative. Label with two patient identifiers.
6. Complete requisition and place in transport bag pocket along with specimen.
7. Send to Cytology via transport, or bring specimen to the Cytology Laboratory, CB5-538.

NOTE: Any delay in the immersion of slides into the fixative will cause cellular drying artifact which may render the specimen unsatisfactory for cytologic evaluation.

GASTRIC WASHING

Contact the Cytology Laboratory in advance to make an appointment. A technologist will come for the specimen. Specimen container should be placed on ice to retard enzymatic degeneration.

BILE DUCT BRUSHING

Materials Needed:

1. Glass slides with frosted end (Do not use fully frosted slides)
2. Clear plastic vials with colored lid filled with 95% ethanol (ETOH)
3. Cytology Requisition (form F-3741) or EPIC Requisition
4. Container of CytoRich® Red or 50% ethanol fixative
5. Specimen transport bag

Materials are available from the Cytology Laboratory.

Procedure

1. Label frosted end of slides with two patient identifiers.
2. Specimen must be placed on the same side as the frosted end (top of slide). If specimen is placed on the bottom of slide, it may be lost during processing.
3. **Immediately** upon smearing slide, place in vial containing 95% ethanol fixative.
4. Label vial with two patient identifiers and specimen source.
5. Clip the end of the brush and place in a container of CytoRich® Red or 50% ethanol fixative. Label with two patient identifiers.
6. Complete requisition and place in transport bag pocket along with specimen.
7. Send to Cytology via transport, or bring specimen to the Cytology Laboratory, CB5-538.

NOTE: Any delay in the immersion of slides into the fixative will cause cellular drying artifact which may render the specimen unsatisfactory for cytologic evaluation.

REDPATH CASES

At the request of the clinician we will send cases to RedPath for CEA and amylase testing from pancreatic cyst fluid or duct fluid

Materials Needed:

1. RedPath specimen collection kit (provided by Cytology laboratory)

Procedure

Normal Control (Buccal brush)

1. Remove buccal brush from tube. Save the tube.
2. Rub buccal brush firmly on inside of patient cheek 6-8 times to collect cells.
3. Place brush back into tube, cap the tube and label it with two patient identifiers and collection date.

Pancreatic Cyst Fluid (or Duct Fluid)

1. Dispense cyst fluid into a specimen collection vial (maximum of 2ml). Screw cap into vial tightly to avoid leakage. When more than one cyst is present place each into separate vial and label accordingly.
2. Label each vial with collection date, cyst location and two patient identifiers.

NOTE: For the Redpath cases you will need to fill out a separate RedPath requisition which should include the appropriate insurance information to include billing information. Cytology staff will take the specimens to the lab and send out to RedPath for testing.

OTHER SPECIMENS

BREAST/NIPPLE DISCHARGE

Materials Needed:

1. Glass slides with frosted end (do not use fully frosted slides)
2. Clear plastic vials with colored lid filled with 95% ethanol (ETOH)
3. Cytology Requisition (form F-3741) or EPIC Requisition
4. Specimen transport bag

Materials are available from the Cytology Laboratory.

Procedure:

1. Label slide with two patient identifiers and which side specimen is from.
2. Nipple discharge should be smeared on slides.
3. Immediately immerse slide into vial containing 95% ethanol (ETOH) fixative.
4. Label vial with two patient identifiers and specimen source.
5. Complete requisition and place in transport bag pocket along with specimen.
6. Send to Cytology via transport, or bring specimen to the Cytology Laboratory, CB5-538.

CIRCULATING TUMOR CELLS

This test is FDA approved for the identification of circulating tumor cells of breast, prostate and lung in whole blood samples. The Cell Save vial must be used for collection of the sample.

Materials Needed:

1. Cells Save vial
2. Cytology Requisition (form F-3741) or EPIC Requisition
3. Specimen transport bag

Materials are available from the Cytology Laboratory.

Procedure:

1. Label vial with two patient identifiers.
2. Collect blood sample from patient
3. Complete requisition and place in transport bag with specimen
4. Send to Cytology via transport for processing.

NOTE: This sample must not be refrigerated and only has a shelf life of 96 hours after collection. We must receive this sample the same day it was collected if at all possible.

FINE NEEDLE ASPIRATION (FNA) CYTOLOGY

CLINICIAN PERFORMED FNA

Materials Needed:

1. Glass slides with frosted end (do not use fully frosted slides)
2. Clear plastic vials with colored lid filled with 95% ethanol (ETOH)
3. Container of CytoRich® Red or 50% ethanol fixative
4. Cardboard slide holders
5. Cytology Requisition (form F-3742)
6. Syringe
7. Needle (23 or 25 gauge with a length of 1-1/2 inches or longer works well)
8. Specimen transport bag

All materials except syringes and needles are available in the Cytology Laboratory.

Collection of Material:

1. Using a 23 or 25 gauge needle, enter lesion at a slight angle with needle hole down.
2. Slowly move the needle back and forth, being sure to remain in the lesion.
3. Withdraw the needle.
4. Partially fill the syringe with air and attach to needle.

Preparation of Smears:

1. Label slide with two patient identifiers and specimen source.
2. With the needle touching the slide, express a drop of aspirated material.
3. Immediately place another slide over the first and separate in a perpendicular fashion. It is not necessary to apply significant pressure or to rotate the material. Do not spread or smear the material in the manner of a blood smear.
4. Immediately immerse the slides in 95% ethanol fixative. **If specimen source is Thyroid**, place only one slide from the pair into 95% ethanol. Let the matching slide air dry, then place into cardboard slide holder.
5. Rinse the needle in CytoRich® Red fixative. Label with two patient identifiers.
6. Complete requisition and place in transport bag pocket along with specimen.
7. Send to Cytology via transport, or bring specimen to the Cytology Laboratory, CB5-538.

NOTE: If sampling more than one lesion, use a separate container of CytoRich® Red fixative. Make sure that all containers and slides are labeled clearly with proper sources and two patient identifiers.

CYTOTECHNOLOGIST ASSISTED FNA

Assistance by a cytotechnologist is available in order to obtain optimal diagnostic specimens. The cytotechnologist can only determine if adequate cellular material has

been obtained. **A preliminary diagnosis cannot be given.** An appointment should be made at least 24 hours in advance when scheduling a needle aspiration. Call extension 5-2636 to arrange for a cytotechnologist to assist in the procedure. Please request a pathologist if you require a preliminary diagnosis.

PATHOLOGIST PERFORMED FNA

If preferred, a pathologist is available to perform an aspirate and provide a preliminary diagnosis. Please call extension 5-2636 to schedule.

MOLECULAR TESTING FROM FNA SAMPLES

HPV TESTING (HEAD AND NECK CASES)

At the request of the clinician we will perform HPV testing from FNA samples. We will also give the subtypes for 16 and 18.

Materials Needed:

1. Cytology Requisition
2. CytoRich® Red solution (provided by Cytology Laboratory)

Procedure

1. Perform the FNA procedure as you normally would.
2. Wash the syringe as you normally would in the CytoRich® Red solution after each FNA pass and after you make your slides.
3. Take a extra pass from the site and do not make slides and rinse in CytoRich® Red solution.
4. Make the request for HPV testing on the requisition.
5. Send to Cytology for processing.

BRAF TESTING

At the request of the clinician, we will perform BRAF testing from all samples diagnosed as FLUS, atypical, suspicious or positive for malignant cells. This will be reported as addendum to the original case.

Materials needed:

1. Cytology Requisition
2. CytoRich® Red solution (provided by Cytology Laboratory)

Procedure

1. Perform the FNA procedure as you normally would.
2. Wash the syringe as you normally would in the CytoRich® Red solution after each FNA pass and after you make your slides.
3. Take a extra pass from the site and do not make slides and rinse in CytoRich® Red solution. Label with two patient identifiers.
4. Make the request for reflex BRAF testing on the requisition.
5. Send to Cytology for processing.

THYROSEQ Next Generation Sequencing

At the request of the clinician, we will send a provided specimen sample to the University of Pittsburgh Medical Center for testing, on any submitted thyroid FNA. The

test is a panel that will sequence and detect mutations and common chromosomal rearrangements common in key cancer genes.

Materials Needed:

1. Cytology Requisition or EPIC Requisition
2. ThyroSeq collection microtubule (orange cap tube; provided by Cytology)
Special care should be used when handling; preservative solution contains Guanidinium thiocyanate, which can be harmful by inhalation, contact with skin, and if swallowed. Gloves should be worn at all times when handling.
3. CytoRich® Red solution (provided by Cytology Laboratory)
4. Light sensitive storage box (provided by Cytology). Specimens should be stored in this box at all times. *Before Use:* store in light sensitive box at room temperature; *After Addition of Patient FNA Sample:* store in light sensitive box up to 6 hours at room temperature, not longer than 24 hours in the refrigerator, or up to 12 months in the freezer.

Procedure:

1. Perform the FNA procedure as you normally would.
2. Place 1-2 drops of the first pass into the ThyroSeq collection microtubule and use the rest of the pass for Cytology, into CytoRich® Red solution.
3. Wash the needle using the microtubule solution and collect more material if necessary, following the same steps.
4. If the first pass is not cellular (based on cytology) or cellularity cannot be determined immediately and fluid in the microtubule has not changed color, add one-half of the second pass into the SAME tube. Label with two patient identifiers and specimen site, if applicable. **NOTE: if there are multiple sites being requested, they should be submitted in separate ThyroSeq specimen tubes for testing.*
5. Indicate on the patient requisition that ThyroSeq is requested.
6. Send to Cytology for processing. ThyroSeq collection tube preservative solution is light sensitive and should always be stored and transported to Cytology in a light sensitive storage box.

ThyGenX TESTING

At the request of the clinician, we will send a provided specimen sample to Interpace Diagnostics, on any submitted and qualifying diagnostic thyroid FNA (FLUS, atypical, or suspicious for malignant cells) for this gene expression classifying test.

Materials Needed:

1. Cytology Requisition or EPIC Requisition
2. ThyGenX collection microtubule (clear cap; provided by Cytology)
3. CytoRich® Red solution (provided by Cytology)

Procedure:

1. Perform the FNA procedure as you normally would.

2. Immediately add the FNA biopsy to the ThyGenX collection microtubule, washing the needle only, 1-2 times. You can then proceed to the regular FNA collection for cytology testing, collecting the sample in the CytoRich® Red solution.
3. Invert the ThyGenX collection tube 2-3 times after collection, to ensure that all solid material is mixed into the solution. Label with two patient identifiers and specimen site, if applicable. **NOTE: if there are multiple sites being requested, they should be submitted in separate ThyGenX specimen tubes for testing.*
4. Indicate on the patient requisition that ThyGenX testing is requested.
5. Send to Cytology for processing. ThyGenX specimen tubes can be stored and transported at room temperature, both before and after patient specimen collection.

VERACYTE AFIRMA THYROID FNA ANALYSIS

At the request of the clinician, we will send a provided specimen sample to Veracyte Laboratory, on any submitted and qualifying diagnostic thyroid FNA (FLUS, atypical or a Follicular Neoplasm) for this gene expression classifying test.

Materials Needed:

1. Cytology Requisition or EPIC Requisition
2. Veracyte Afirma collection microtubule (blue cap; provided by Cytology)
3. CytoRich® Red solution (provided by Cytology)

Procedure:

1. Perform the FNA as you normally would.
2. Perform 2 passes on the nodule. After completing each pass:
 - 1) disconnect the needle from the syringe
 - 2) aspirate air into the syringe and reconnect the needle,
 - 3) point the needle down and slowly expel all of the specimen into the Veracyte Afirma collection microtubule
 - 4) draw approximately one ml of the solution from the tube back into the syringe, then
 - 5) point the needle down and slowly expel the solution back into the tube.
3. When finished, cap the tube and invert the specimen three times to ensure that all solid material is mixed into the solution. Label the tube with two patient identifiers and specimen site, if applicable. **NOTE: if there are multiple sites being requested, they should be submitted in separate Veracyte Afirma specimen tubes for testing.*
4. Indicate on the patient requisition that Veracyte Afirma testing is requested. The size of the thyroid nodule should also be indicated on the requisition.
5. Send to Cytology for processing. Veracyte Afirma specimen tubes can be stored and transported at room temperature. If not being sent to Cytology the same day, specimen should be stored in the freezer.

OUTREACH HISTOLOGY

OUTREACH SURGICAL BIOPSIES

Materials Needed:

1. 10% neutral buffered formalin
2. Outreach Pathology requisition
3. Specimen transport bag

Materials are available from the Outreach Laboratory.

Procedure:

1. All specimens submitted to the outreach pathology laboratory must be accompanied by a pathology requisition slip that contains the patient's demographics, (including patient's name, medical record number [if applicable], date of birth), clinical history, type of specimen and clinical impression that guide the pathologist toward an issue of clinical significance.
2. All requisitions must be printed clearly and must identify the authorizing requesting MD ordering the request testing.
3. Outreach Surgical Pathology specimens are collected using many different techniques; hence, patient preparation is at the discretion of the clinician or surgeon collecting the tissue sample.
4. Surgical Pathology specimens should be collected in formalin containers (which are provided to all clinicians by the pathology department) if the specimen is intended for routine surgical pathology evaluation. Any specimen that is submitted for specialized studies should direct collect method questions to the specific laboratory for handling conditions (e.g., FISH, FLOW CYTOMETRY, ELECTRON MICROSCOPY OR ANY SPECIALIZED LABORATORY TESTING). All specimen containers must have two patient identifiers and the exact anatomic site, which was sampled. For surgical pathology cases it must include the organ, the laterally of the specimen (right or left), and any additional designation.
5. All specimen requisitions must have two patient identifiers and the exact anatomic site, which was sampled. For surgical pathology cases it must include the organ, the laterally of the specimen (right or left), and any additional designation. All individual specimens from one patient must be listed on the requisition form.
6. The first and last name of the submitting physician must be on the requisition. Any other physicians needing a copy must be clearly listed.
7. The date of collection must be on the requisition form.
8. All specimen containers must be sealed tightly. Urine specimens will completely leak out of containers, and surgical specimens could also be lost if the container is not tight. Containers must be labeled with the patient's demographics and specimen site designation. Specimens are to be placed in sealable biohazard bags for transport with the requisition in the front pocket.

9. All pertinent clinical information must be included on the requisition, in order for the best interpretation to be rendered for the best interpretation to be rendered for the patient.

PROTOCOL ACCEPTANCE GUIDELINES

PROTOCOL FOR SPECIMEN RECEIPT

1. Freshly collected specimens are ideal for cytological processing. Any specimens that can be obtained during the hours the lab is open (8:30-5:00 PM) should be brought by YNHH transportation service or by other personnel directly to Cytology, CB5-538. When a cytotechnologist's assistance is requested, a cytotechnologist may go to the site of collection to assist in obtaining the specimen.
2. If there is to be a delay in sending the sample to the lab, refrigeration, not freezing, of the sample is essential.
3. Body cavity fluids, washes, or aspirations that must be collected at night or on the weekend should be refrigerated (see Gill, p. 2-1).
4. Sputum should consist of an early morning, deeply coughed sample. Do not send a 24 hour combined sample.
5. Since the cells in urine, gastric lavages, and cerebro-spinal fluids deteriorate rapidly it is essential they be sent to the lab immediately after collection (see Gill, p. 6-14); do not send a 24 hour urine specimen.

PROTOCOL FOR SPECIMEN REJECTION

1. Improperly identified or collected specimens [i.e., no fixative, leaking container, improperly labeled/unlabeled slides or containers (e.g., specimen and protocols have different names, specimen with no protocol, specimen with protocol which is inadequately complete, etc.)] may be rejected for processing by the lab. Attempts will be made by the accessioning secretary or cytotechnologist to locate a responsible physician to obtain proper identification and information. If it is deemed possible to properly identify the specimen, the responsible physician may do so after signing the requisition.
2. Slides received broken and beyond repair will be rejected and the referring physician will be notified. Any questions regarding the feasibility of repairing broken slides should be brought to the attention of the supervisor or pathologist in charge.

If proper identification is not possible, the specimen/slides will be discarded and a formal report stating the nature of the problem and the final disposition of the case will be submitted. A record of this case will be maintained per the Quality Assurance protocol (see Appendix A).

REPORTING OF RESULTS

In general, results on cytology specimens are available the day after the specimen is received in the laboratory. Special histochemical stains or immunohistochemistry may add an additional day or two to the sign-out process. Electron Microscopy usually takes 5 working days for tissue processing.

We welcome house staff to come personally to the Outreach Laboratory, CB5-538, and review their patients' specimens. This is best accomplished after 9 a.m. Please call 785-5430 in advance so that the slides will be available for review.

ACCEPTABLE PATIENT IDENTIFIERS

We would like to remind you that **two** acceptable patient identifiers must be present on both the specimen(s) and the test requisition and may include:

- first and last name
- date of birth
- medical record number or patient ID number