

# PSIP

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PUGET SOUND  
INSTITUTE OF  
PATHOLOGY

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## SERVICE MANUAL

**PSIP**  
**PUGET SOUND INSTITUTE OF PATHOLOGY**  
**SERVICE MANUAL**  
**PATHOLOGY, CYTOLOGY, AND**  
**FLOURESCENCE IN SITU HYBRIDIZATION (FISH)**

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## **INTRODUCTION**

The purpose of this manual is to acquaint PSIP clients with the range of our services and to aid in proper collection of specimens and interpretation of reports. However, it is impossible to address all problems and situations. Our staff pathologists are always available to discuss techniques and procedures. We are always willing to provide interpretational assistance if needed.

Puget Sound Institute of Pathology (PSIP) is a multidimensional anatomic pathology laboratory offering general surgical pathology, dermatopathology, hematopathology, gyn cytology and non-gyn cytology. We have a full service laboratory and provide a wide range of special stains including immunohistochemistry. All pathologists are board certified by the American Board of Pathology in anatomic pathology or in combined anatomic and clinical pathology. In addition, subspecialty boards in cytopathology, hematopathology, and dermatopathology are represented. Our cytotechnologists are all experienced professionals certified by the ASCP. PSIP is inspected and accredited by the Collage of American Pathologists and by the State of Washington. We meet or exceed all standards outlined in the 1988 Federal Clinical Laboratory Improvement Act (CLIA '88). In addition to our Harbor Island facility, PSIP is responsible for several hospitals located around Puget Sound and Southeastern Alaska including St. Joseph Medical Center, St. Francis Hospital, St. Clare Hospital, St. Anthony Hospital, Auburn Regional Medical Center, Valley General Hospital and Forks Hospital. Pathologists are on-site Monday through Friday during business hours. A pathologist is on call 24 hours a day.

**PSIP - GENERAL INFORMATION**

**Main Laboratory Location**

1001 Klickitat Way SW, Suite 205  
Seattle, WA 98134

**CAP#**            **2462401**  
**CLIA #**           **50D0633091**

Phone:            206-622-7747  
Toll Free:        1-800-234-7224  
Fax:               206-467-1470  
Website:          www.psip.com

**Please Send Invoices and Billing Inquiries to:**

PSIP  
P.O. Box 34245  
Seattle, WA 98124-1245

**After Hours - Pathologist On-call: (206)622-7747 Answering Service will page**

**To Request Reports or Materials:**

Pathology/Transcription  
- Histology Reports  
- Non-GYN Cytology Reports  
- Histology Slides and/or blocks

Cytology/Data Entry  
- GYN Cytology Reports  
- GYN Cytology Slides

General Supplies – Stephanie George

**Key Personnel:**

Laboratory Director	Douglas Hansen, M.D.
PSIP Operations Manager	Russell Kuehn
Supplies	Stephanie George
Cytology Director	Michael Kalnoski M.D.
Cytology Supervisor	Annie Campbell
Histology Supervisor	Lucinda Royce
Billing Supervisor	Betty Keefe-Shawgo
Transcription Supervisor	Janell Walters
Gross Room (FHS)	Brent Stoner

**Franciscan Health System - Pathology    (253)426-6719**

## PSIP BILLING REQUIREMENTS

For proper billing and reimbursement we **MUST** have the requisitions filled out completely with the following information and submitted at the time of the specimen:

- Patient's full name, clearly written or typed
- Patient's **complete** address with apt #'s and/or PO Box #
- Patient's date-of-birth and Social Security number
- Any pertinent billing instructions, i.e. bill doctor, patient, or insurance
- Complete insurance information:
  1. Claims address.
  2. ID/subscriber number with alpha prefix, if applicable
  3. Group number, if applicable
  4. If possible, copy of front and back of card
  5. If Medicaid, a copy of patient's Provider One card
  6. If Medicare, have patient sign the Medicare waiver on the back of the requisition or a separate Advanced Beneficiary Notice (ABN) provided by PSIP (you may obtain a copy of this form from our website [www.psip.com](http://www.psip.com)).
- ICD9 code
- Clinical history
- Name of the referring physician (be prepared to give doctor's NPI if we call)
- Date specimen collected

## **PSIP CERVICAL/VAGINAL (PAP SMEAR) CYTOLOGY SERVICES**

PSIP provides clinicians with all necessary Pap smear supplies. When a smear is received it is processed, interpreted and reported as timely as possible. Pap smears are picked up by courier in all locations where this service is available. Some remote locations require use of U.S. Mail. Reports are delivered by courier, mailed, faxed or electronically transmitted to the client to optimize turnaround time.

At the end of each month, a patient summary is sent to each client. This includes patient names and diagnoses. In addition, a patient recall report is sent out monthly. Patients with significant epithelial abnormalities or unsatisfactory smears are sent a recall report one month after the smear was interpreted. Recall reports for patients with mild abnormalities, such as squamous atypias of undetermined significance, are sent three months after the initial diagnosis and patients with normal smears receive a recall report twelve months after the initial interpretation.

Recall cards are also available on request. These are self-contained mailers which include the patient's name and address and a notice to the patient that they are due for their Pap smear. They also contain a separate attachment to be placed in the patient's record.

The PSIP information system is quite versatile and does allow access to selective information if needed for studies or other client-specific information.

The Pap smear report will include the Bethesda System Classification 2000, descriptive diagnoses (including presence or infectious/inflammatory agents), and specimen adequacy (including presence of endocervical cells). Maturation index is provided on request if vaginal material is supplied. The PSIP report will always include the most up-to-date classification systems including the most current versions of the Bethesda System.

Recommendations for follow-up may occasionally be given on a report if the diagnosis is unusual or uncertain. However, this is not standard. A table of recommended follow-up is provided in this manual (see PSIP Table of Comparable Diagnoses and Recommended Follow-up); and is available on request as a separate sheet.

It should be noted, the Pap smear is a screening test, not a diagnostic test. Negative or normal results do not necessarily exclude disease, while positive or abnormal findings require confirmation by repeat smear, colposcopy and/or biopsy. Pap smear results must always be correlated with the clinical history and physical examination. Every effort will be made at PSIP to correlate tissue findings with those of previous or concurrent Pap smears.

## **PAP SMEAR COLLECTION PROCEDURE**

### **Conventional Pap Smear Collection Procedure**

Specific instructions for preparation of the Pap smear are given in Pap-Paks supplied by PSIP. In summary, they are as follows: The cervical smear is obtained by rotating the cervical scraper (modified Ayer spatula) around the ectocervix with the emphasis on the squamo-columnar junction. Material is spread evenly on one portion of the glass slide.

The endocervical smear is obtained by gently inserting the cytology brush into the distal endocervical canal at, or just beyond the squamo-columnar junction. The brush is rotated one full turn and gently removed. This material is spread evenly by rotating the cytology brush back and forth over a different area of the glass slide.

Finally, the vaginal smear is taken from the posterior fornix with the spatula end of the cervical scraper smeared on the last remaining portion of the glass slide.

Material on the slide should be fixed immediately, either with the cytology fixative supplied in the Pap-Paks, spray fixative, or immersion in alcohol. Immediate fixation is essential to prevent air-drying artifact which may render the smear uninterpretable. All slides should be labeled with the patient's name. Under most circumstances, a single slide is adequate for a complete Pap smear.

The Pap smear requisition should be filled out as completely as possible. Essential information includes the patient name, birth date, last menstrual period and specimen source. Any special studies or special information desired should be indicated on the requisition such as maturation index. Social security number should be indicated as well; it is the most reliable way of retrieving previous patient data.

### **Liquid Based Pap Collection Procedures**

#### **THINPREP PAP TEST: (Also see Hologic's ThinPrep Quick Reference Guide)**

##### **A. Endocervical Brush and Spatula Protocol:**

1. Obtain an adequate sampling from the ectocervix using a plastic spatula.
2. Rinse the spatula as quickly as possible in the PreservCyt solution vial by swirling the spatula vigorously in the vial 10 times. Discard the spatula.
3. Obtain an adequate sampling from the endocervix using an endocervical brush device. Insert the brush into the cervix until only the bottom most fibers are exposed. Slowly rotate  $\frac{1}{4}$  or  $\frac{1}{2}$  turn in one direction. **DO NOT OVER ROTATE.**

4. Rinse the brush as quickly as possible in the PreservCyt solution by rotating the device in the solution 10 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 and ID number on the vial. Also, record the patient information and medical history on the cytology requisition form.
7. Place the vial and requisition in a specimen bag for transport to the laboratory.

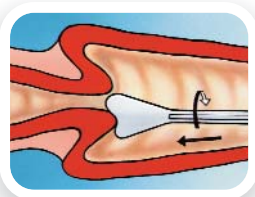
#### **BROOM-LIKE DEVICE PROTOCOL:**

1. Obtain an adequate sampling from the cervix by inserting 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 broom in a clockwise direction 5 times.
2. Rinse the broom as quickly as possible into the PreservCyt solution vial by pushing the broom into the bottom of the vial 10 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 and ID number on the vial. Also, record the patient information and medical history on the cytology requisition form.
5. Place the vial and requisition in a specimen bag for transport to the laboratory.

**All ThinPrep Pap slides are screened using the Hologic Corporation ThinPrep Imaging System.** This system is a device that uses computer-imaging technology to assist in the detection of abnormal cells during primary cervical cancer screening of ThinPrep slides. This technology greatly improves the detection of high-grade cervical intraepithelial lesions.

# Quick Reference Guide

## Endocervical Brush/Spatula Protocol



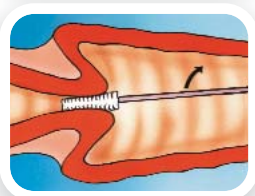
### Obtain...

an adequate sampling from the ectocervix using a plastic spatula. If desired, use lukewarm water to warm and lubricate the speculum. Water-soluble gel lubricant sparingly applied to the posterior blade of the speculum can be used if necessary.<sup>1</sup> Select contoured end of plastic spatula and rotate it 360 degrees around the entire exocervix while maintaining tight contact with exocervical surface.



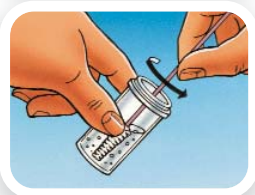
### Rinse...

the spatula as quickly as possible into the PreservCyt® Solution vial by swirling the spatula vigorously in the vial 10 times. Discard the spatula.



### Obtain...

an adequate sampling from the endocervix using an endocervical brush device. Insert the brush into the cervix until only the bottom-most fibers are exposed. Slowly rotate 1/4 or 1/2 turn in one direction. DO NOT OVER-ROTATE.



### Rinse...

the brush as quickly as possible in the PreservCyt Solution by rotating the device in the solution 10 times while pushing against the PreservCyt vial wall. Swirl the brush vigorously to further release material. Discard the brush.



### Tighten...

the cap so that the torque line on the cap passes the torque line on the vial.



### Record...

the patient's name and ID number on the vial.

### Record...

the patient information and medical history on the cytology requisition form.

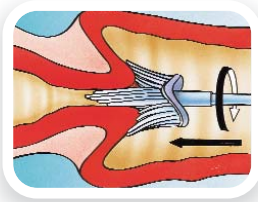


### Place...

the vial and requisition in a specimen bag for transport to the laboratory.

# Quick Reference Guide

## Broom-Like Device Protocol



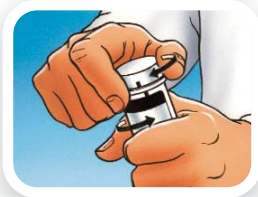
### Obtain...

an adequate sampling from the cervix using a broom-like device. If desired, use lukewarm water to warm and lubricate the speculum. Water-soluble gel lubricant sparingly applied to the posterior blade of the speculum can be used if necessary.<sup>1</sup> 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.



### Rinse...

the broom as quickly as possible into the PreservCyt® Solution vial by pushing the broom into the bottom of the vial 10 times, forcing the bristles apart. As a final step, swirl the broom vigorously to further release material. Discard the collection device.



### Tighten...

the cap so that the torque line on the cap passes the torque line on the vial.



### Record...

the patient's name and ID number on the vial.

### Record...

the patient information and medical history on the cytology requisition form.



### Place...

the vial and requisition in a specimen bag for transport to the laboratory.

### [www.thinprep.com](http://www.thinprep.com)

1. Cervicovaginal Cytology Based on the Papanicolaou Technique; Approved Guideline – Third Edition (Clinical and Laboratory Standards Institute GP15-A3).

#### United States / Latin America

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Marlborough, MA 01752 USA  
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[www.hologic.com](http://www.hologic.com)

## HIGH RISK HPV TESTING

The Hybrid Capture 2 HPV Test, manufactured by Qiagen Corporation is an *in vitro* diagnostic test for high risk HPV subtypes. The test has been FDA approved for the detection of high-risk HPV types to aid in diagnosis of sexually transmitted HPV; to screen patients with ASCUS Pap Results to determine need for colposcopy; and to assist the physicians in the management of women with Low-Grade Squamous Intraepithelial Lesions (LSIL). As an adjunct to the ThinPrep Pap Test, the hc2 HPV Test aids in early detection of more disease and more efficiently directs additional cytological follow-up procedures and colposcopy. (Please refer to the tables on the following pages for treatment guidelines.)

### Suggested HPV Triage for Patients with ASCUS Results

Knowing the HPV status of a patient with an ASCUS Pap result can be of benefit in deciding the most appropriate case strategy for the patient. High-risk HPV types have been shown to play a casual role in the development of cervical disease and cancer. Their presence in a patient with an ASCUS Pap test result indicates she is at increased risk for disease and could benefit from immediate colposcopy.

If a patient has an ASCUS Pap result on a ThinPrep Pap Test, you can request an HPV test be done from the same liquid cytology sample. **PLEASE NOTE: HPV Testing CANNOT be performed on a conventional Pap smear.** A request for HPV Testing can be made at any time by calling the Cytology Department of PSIP or by marking your request on the patient's requisition to perform reflexive testing if the diagnosis is ASCUS. This will inform PSIP that if the Pap test should result in an ASCUS diagnosis, an HPV Test will be done on the residual cytology specimen. If your practice is interested in providing HPV Testing for your patients, please contact PSIP for more information. (Standing orders for reflexive HPV Testing are also available to PSIP's clients.)

### HPV with Pap (Regardless of Diagnosis)

HPV testing with Pap on all patients **over 30 years of age** (regardless of diagnosis) is recommended to help manage these patients for future screening. Patients over 30 years of age with negative Pap results and negative HPV results can be screened every three years. Please mark your request for ThinPrep Pap Testing *and* HPV Testing on the patient's requisition. (Refer to the tables on the following pages for recommended follow-up.)

**All testing must be performed within six weeks from the day the sample is collected.** PSIP will store all liquid cytology specimens for six weeks. Physicians can contact PSIP and request an HPV Test at any time within the six-week window stated above.



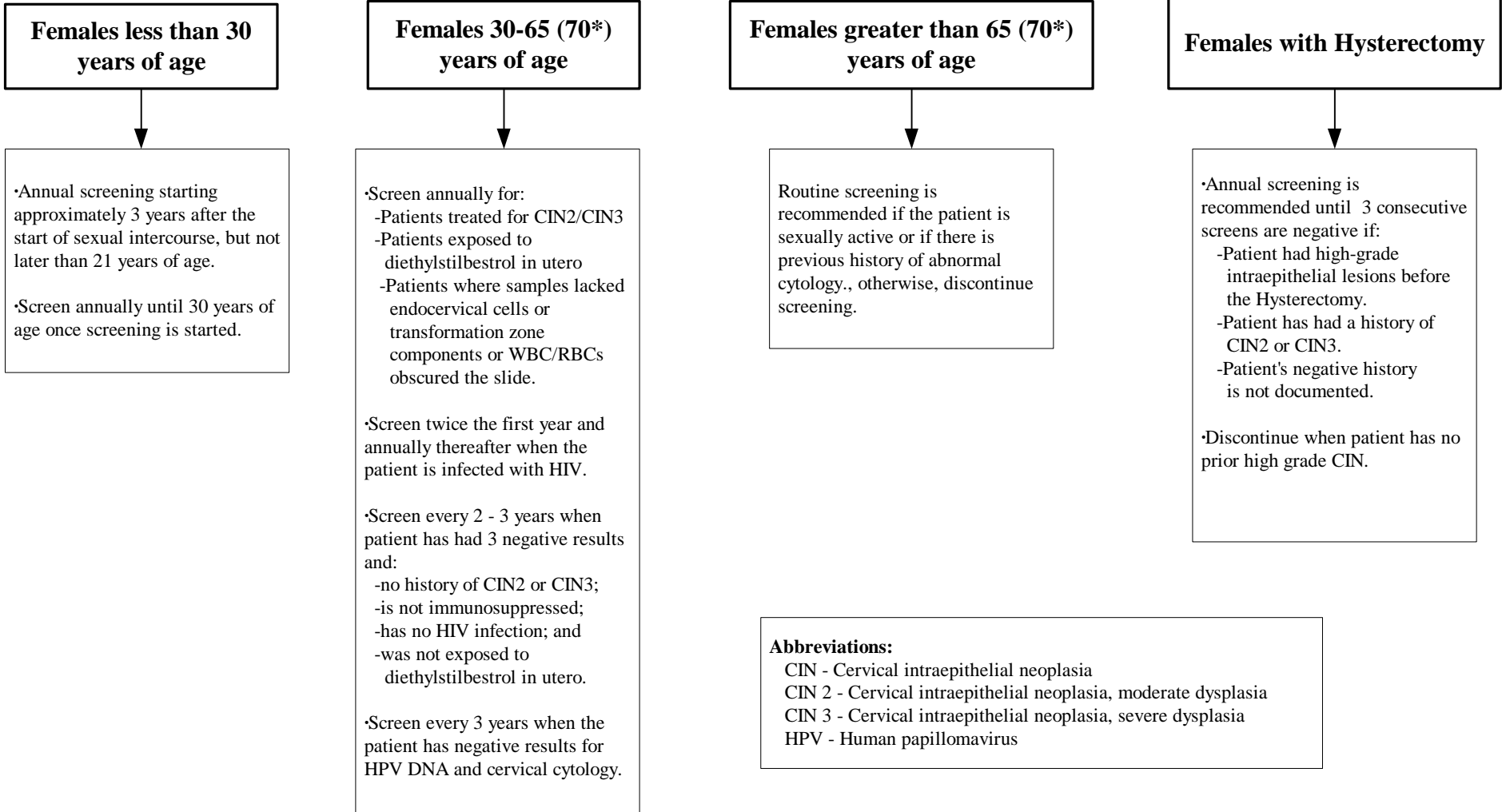
## TABLE OF GYN CYTOLOGIC DIAGNOSES AND RECOMMENDED FOLLOW-UP

BETHESDA SYSTEM	NEGATIVE FOR INTRAEPITHELIAL LESION OR MALIGNANCY	ATYPICAL SQUAMOUS CELLS			SQUAMOUS INTRAEPITHELIAL LESION / CARCINOMA	ENDOCERVICAL GLANDULAR LESIONS	ENDOMETRIAL GLANDULAR LESIONS	UNSATISFACTORY
<b>DESCRIPTIVE DIAGNOSIS</b>	ACTINOMYCES (REMOVE IUD) BACTERIAL VAGINITIS YEAST TRICHOMONAS ENDOMETRIAL CELLS PRESENT IN A WOMAN 40 YEARS OR OLDER (ENDOMETRIAL BIOPSY ONLY IF SYMPTOMATIC) HERPES HYPERKERATOSIS TYPICAL REPAIR RADIATION EFFECT REACTIVE CELLULAR CHANGES	ASC-US - CELLS OF UNDETERMINED SIGNIFICANCE  *ASC-H - CANNOT EXCLUDE HIGH GRADE DYSPLASIA  <b>*HPV TESTING IS STRONGLY RECOMMENDED</b>			LOW GRADE SQUAMOUS INTRAEPITHELIAL LESION (LSIL) Mild Dysplasia / HPV / CIN 1  HIGH GRADE SQUAMOUS INTRAEPITHELIAL LESION (HSIL) Moderate and Severe Dysplasia: CIS/CIN2/CIN3  SQUAMOUS CELL CARCINOMA	AGUS ENDOCERVICAL FAVOR NEOPLASM  ENDOCERVICAL ADENO-CARCINOMA IN SITU  ENDOCERVICAL ADENO-CARCINOMA	ENDOMETRIALS PRESENT, POST MENOPAUSAL  ATYPICAL ENDOMETRIAL CELLS  ENDOMETRIAL ADENO-CARCINOMA	UNSATISFACTORY
		HPV TEST NEGATIVE	HPV TEST POSITIVE	HPV TEST UNKNOWN	COLPOSCOPY BIOPSY AND ECC  ENDOMETRIAL BIOPSY  REPEAT SMEAR			
<b>RECOMMENDED PATIENT FOLLOW-UP</b>	TREAT INFECTIONS AS CLINICALLY APPROPRIATE  ROUTINE PAP SMEAR SCHEDULE AS CLINICALLY INDICATED	ROUTINE SCREENING	COLPOSCOPY / BIOPSY	REPEAT IN 3 MONTHS OR COLPOSCOPY/ BIOPSY AS CLINICALLY INDICATED				
		COLPOSCOPY RECOMMENDED FOR ASC-H IN ABSENCE OF HPV TESTING.  *ASC-H -r/o HSIL - ACOG Recommends Colposcopy Regardless of HPV Status						

# Cervical Cytology Screening (PAP Screening) Guideline

Washington State Clinical Laboratory Advisory Council (CLAC)  
January 2005

**FOR EDUCATIONAL PURPOSES ONLY**  
The individual clinician is in the best position to determine which tests are most appropriate for a particular patient.



References:  
1. ACOG Practice Bulletin, Clinical Management of Guidelines for Obstetrician-Gynecologists Number 45, August 2003

\*The American Cancer Society uses age 70; the US Preventive Services Task Force has set age at 65.

## **VAGINAL SMEARS IN DES EXPOSED WOMEN**

In order to substantiate the diagnosis of vaginal adenosis in a patient with a history of intrauterine DES exposure, it is necessary to demonstrate, either by cytology or histology, the presence of glandular epithelium of Mullerian origin in the vagina.

These areas of adenosis are most often submucosal and therefore do not shed cells through the intact vaginal mucosa. However, when glands open onto the surface, cells can be shed directly into the vaginal lumen. These surface abnormalities are visible to the eye especially following full strength iodine staining of the mucosa (unstained areas), or by colposcopy, and are frequently associated with unusual vaginal folds.

If cytology is used for diagnosis, it is first necessary to remove from the vaginal wall all contaminating glandular cells shed from the endocervix and endometrium, by gently swabbing the entire vaginal surface with a cotton ball. To avoid endocervical contamination, the vaginal scrapes must be done prior to any cervical scrape or aspiration. Endometrial contamination is avoided by performing the examination during the latter half of the menstrual cycle. To cover the entire area at risk, four quadrant vaginal scrapes are recommended.

In order to make our screening easier and more efficient, we suggest that two divided, labeled slides be submitted, one containing scrapes from the anterior and posterior walls and the second containing scrapes from the right and left lateral walls. In addition, the routine cervical-endocervical scrape should be performed following the vaginal exam and submitted on a third slide. All three slides are requisitioned and charged as a single case.

If these precautions are taken, the presence of glandular cells in the vaginal scrapes will become significant and a diagnosis of vaginal adenosis will be rendered more secure.

## **PAP SMEAR QUALITY ASSURANCE PROGRAM**

The Pap smear was originally intended as a screening test to be applied to the general population to identify those women with cancerous and precancerous changes of the cervix. It is not meant to be a diagnostic test. Any abnormal result should be followed by additional tests as indicated clinically or as recommended in the "PSIP Table of Comparable Diagnoses and Recommended Follow-up." In addition, it should be noted that the Pap smear is not 100% sensitive. The reported sensitivity of Pap smears varies widely in literature. However, it is safe to estimate that it is no better than 80 to 90% . False negatives probably occur most frequently due to the absence of diagnostic cells on smears. Therefore, every effort should be made to obtain a representative, well-preserved Pap smear.

To assure an adequate Pap smear, it should be taken at the optimal time and under optimal conditions. It is suggested that it be done in the mid portion of the menstrual cycle in the absence of discharge of excessive inflammation. It is also recommended that smears not be taken following recent sexual activity, use of vaginal douches, medications, foams, jellies or lubricants.

A thorough scraping of the ectocervix and endocervix must be performed to ensure that the entire area at risk has been adequately sampled on the smear. Cytobrushes are available to assist in endocervical sampling. Instructions for taking optimal smears are included on the inside cover of each Pap Pak kit as well as in the PSIP service manual. The importance of immediate fixation of the smear is stressed, as air-drying occurs in a matter of seconds and may cause artifactual distortion of cells.

If a slide is received broken, inadequately stained or poorly coverslipped, abnormal cells may be missed, overlooked or misinterpreted. If the slide is misidentified or the data is improperly entered into the computer, the patient may receive an erroneous report. The laboratory is responsible for assuring proper processing. PSIP will inform you if the slide is broken beyond repair and request a repeat smear. The personnel processing the smears are appropriately trained. Stains are monitored daily and adjustments are made as needed to provide optimal color differentiation and clarity. Slides are identified by name and accession number and matched with the corresponding requisition. We require that you write the patient's name in pencil on the frosted end of the slide to ensure positive identification.

PSIP is inspected and accredited by the College of American Pathologists and the State of Washington, and meets or exceeds all standards outlined in the 1988 Federal Clinical Laboratory Improvement Act. Optimal screening is assured by hiring only registered cytotechnologists who have undergone specialized college level training, by providing a quiet work environment with state-of-the-art microscopes, and by monitoring volume to ensure that there is ample time for thorough examination of each smear. In an average workday, 70 to 75 slides are reviewed by each Cytotechnologist, which is far fewer than the limit set by the federal and state guidelines.

Ten percent of all normal smears are randomly screened by a second cytotechnologist. An additional 10-15% of normal smears are rescreened based on high risk criteria defined by CLIA '88.

All abnormal smears are reviewed by a pathologist, who determines the final diagnosis. Significant cytotechnologist/pathologist discrepancies are reviewed by the cytology supervisor and discussed with the cytotechnologist to ensure uniformity of diagnosis within the lab. Every effort will be made to correlate smears with follow-up biopsies. A search and review of previous smears are done on any patient found to have a high grade squamous epithelial lesion.

PSIP puts strong emphasis on continuing education. Two hours of continuing medical education is provided for each of the cytotechnologists on a monthly basis. Attendance at local, regional and national cytology meetings is also encouraged and subsidized.

Monthly statistical summaries of each cytotechnologist's and pathologist's work are reviewed. Reports of cytology/histology correlation are also reviewed on a regular basis. These reports identify any significant deviations from the laboratory mean of performance and allow for early intervention. We also request biopsy follow-up by questionnaire for all significant abnormal smears for which we do not receive tissue.

Monthly patient summaries are sent to all clients. Recall lists are also sent to clients monthly.

## **COLLECTION AND FIXATION OF NON-GYNECOLOGIC CYTOLOGY SPECIMENS**

Puget Sound Institute of Pathology will supply appropriate containers and fixative for collection of patient specimens. A Pathology Requisition form should be filled out completely for each specimen submitted, specifying it is for cytology.

### **INSTRUCTIONS FOR COLLECTION OF FLUID SPECIMENS**

Pleural fluid	Synovial fluid
Pericardial fluid	Breast cyst fluid
Abdominal fluid	Bronchial washings
Culdocentesis fluid	

Immediately add the fluid specimen to a plastic pre-filled container of CytoLyt fixative. If the total volume is large (i.e. effusions, washes, urines) immediately secure a well mixed 100 ml aliquot and add to an equal volume of CytoLyt in a plastic container.

If CytoLyt is not available, specimens may be added to an equal volume of 70% isopropyl (rubbing) alcohol in a clean container.

### **INSTRUCTIONS FOR COLLECTION OF SPUTUM SPECIMENS**

The plastic containers with CytoLyt can be sent home with the patient for collection of sputum specimens. Please instruct the patient as follows:

**Sputum** is material from the lungs and is obtained by deep coughing.

**Saliva** is fluid in the mouth and is of no value in this test.

**Post-nasal drainage** comes from the nose and settles in the throat, especially at night. This thick material, which is not sputum, must be cleared from the throat before collecting the sputum sample.

#### **Directions**

1. Label the container with name and date.
2. Upon awakening in the morning and before breakfast, clear throat if necessary and discard this material. Rinse mouth with water and discard.
3. Breathe deeply 8 to 10 times and cough deeply to bring up the sputum from deep in the chest.
4. Spit into the container, seal the lid and shake briskly for a few seconds to break up mucous.
5. With each addition of sputum, shake briskly.
6. Sputum may be collected for 24 hours and added to the same container.
7. Bring the specimen with requisition slip to the laboratory or the doctor's office for pick up.

Copies of these instructions will be supplied on request to give to the patients.

## **INSTRUCTIONS FOR COLLECTION OF URINE SPECIMENS**

A catheterized specimen is highly recommended for all female patients to avoid vaginal-perineal contamination and is preferable for male patients. However, voided urine from the male is usually adequate. When a lesion in the kidney or ureter is suspected, ureteral specimens are desirable. The specimen must be immediately mixed with an equal amount of CytoLyt. If the total volume is large, immediately secure a well-mixed 100 ml aliquot and add an equal volume of fixative. First morning urines are to be avoided since exfoliated cells have set in the bladder for many hours and are often degenerated.

## **INSTRUCTIONS FOR COLLECTION OF CEREBROSPINAL FLUID SPECIMENS**

After obtaining the specimen and apportioning for other required tests, add all remaining fluid (at least 1 ml) to equal amounts of CytoLyt. 10 ml tubes containing 2 ml of fixative are provided for collection. It is important to keep the amount of spinal fluid and fixative equal in order to prevent dilution of specimen.

## **INSTRUCTIONS FOR HANDLING BRUSHINGS FOR CYTOLOGY**

Write the patient's name in pencil on the frosted end of a glass slide.

Wet the brush in saline prior to obtaining the specimen. Wooden tongue blades are too blunt and do not yield deep cells.

Set out several frosted end glass slides and label with the patient's name.

Scrape the inside of the cheek for a buccal smear, or scrape the oral or skin lesion firmly. Moistening skin lesions with sterile saline prior to scraping may help loosen the cells.

Rapidly spread the material on the slide and immediately immerse in a bottle containing 95% Alcohol Fixative for Cytology Smears (green label). Any air-drying will result in an unsatisfactory smear. Alternatively, the smears may be immediately flooded with the cytology fixative from the Pap-Pak kit.

## **INSTRUCTIONS FOR HANDLING SCRAPINGS FOR CYTOLOGY – BUCCAL SMEARS, ORAL LESIONS, SKIN LESIONS**

A stainless steel spatula is ideal for obtaining the specimen. Wooden tongue blades are too blunt and do not yield deep cells.

Set out several frosted end glass slides and label with the patient's name.

Scrape the inside of the cheek for a buccal smear, or scrape the oral or skin lesion firmly. Moistening skin lesions with sterile saline prior to scraping may help loosen the cells.

Rapidly spread the material on the slide and immediately immerse in a bottle containing 95% Alcohol Fixative for Cytology Smears (green label). Any air-drying will result in an unsatisfactory smear.

Alternatively, the smear may be immediately flooded with the cytology fixative from a Pap-Pak kit.

### **INSTRUCTIONS FOR COLLECTION OF SMEARS FROM NIPPLE**

Gently press subareolar area and nipple with thumb and forefinger. Do not massage the breast. If secretion occurs allow only a drop the size of a pea to accumulate on the apex of the nipple.

Support areola and nipple with one hand. With the other hand, place slide immediately on nipple, touching drop to slide, then draw slide quickly across nipple, and IMMEDIATELY drop the slide into bottle of 95% Alcohol Fixative for Cytology (green label) or immediately flood slide with the cytology fixative from the Pap-Pak kit.

Repeat procedure until all secretion is utilized. If clinically indicated, repeat procedure with other breast.

If there is nipple erosion or ulceration without nipple secretion, physiologic saline can be gently mixed with lesion to exfoliate cells. Smears are made as above.

## **FINE NEEDLE ASPIRATION TECHNIQUES/SPECIMEN PREPARATION**

### **Equipment:**

- 20 ml. disposable plastic syringe
- 22 gauge needle (1 ½ or 3 ½ inches long)
- Alcohol prep sponges

FNA kit for cytology available from PSIP on request. Includes:

- 4 frosted end glass slides
- 2 bottles of 95% alcohol fixative for cytology (green label)
- 1 plastic container of CytoLyt Transport Medium
- 1 bottle of 10% formalin for tissue specimens
- Sheet of instructions for handling fine needle aspirations for cytology

### **Preparation of Smear:**

Place the bevel of the needle against the top portion of a glass slide, and about one-half inch below the frosted portion. Gently express a small drop onto the slide without splattering. Do this quickly on two to four slides, then immediately make smears as follows:

Place a glass slide on the drop and wait for approximately a second for the drop to spread between the slides, then quickly but smoothly pull the top and bottom slides apart in a parallel direction. Immediately place the slides back to back and place into a bottle of 95% alcohol fixative for cytology. After four slides are prepared, place the rest of the material in the syringe in the plastic container of CytoLyt by drawing the fixative up into the syringe and expelling it back into the container. If a lymphoid malignancy is suspected, RPMI should also be used so that flow cytometric immunophenotyping can be performed.

**NOTE: REMOVE NEEDLE FROM SYRINGE.** Specimens submitted with needle attached to syringe will be subject to rejection.

## **ROUTINE TISSUE HANDLING**

All tissue specimens should be placed in 10% formalin, the volume being five times that of the specimen. Formalin and containers are supplied by our laboratory. Small biopsy bottles containing 10% *neutral* buffered formalin are also available.

All specimens should be accompanied by a completed requisition form including birth date, patient name, social security number, clinical history, specimen type, physician and billing information including ICD9 codes. The container should also bear the patient name and specimen information. When available, gummed labels should be removed from the requisition and placed on the specimen container for identification confirmation. Specimen may be sent by courier or by mail. Special mailing tubes are available from PSIP.

Turnaround time on routine cases is 24 hours.

All malignancies are reviewed within the department by a second pathologist. Significant disagreements are resolved through consultation outside the department by appropriate experts. The results of extradepartmental consultations are forwarded to referring clinicians. Corrected or amended reports are issued when appropriate and significant discrepancies are always telephoned to the referring clinician as soon as possible. Frozen section diagnosis are correlated with the final diagnosis and reported monthly. Statistics are also kept on in-house quality review agreement and on outside consultation agreement. All pathologists participate in quarterly Pathologist Improvement Programs provided by the College of American Pathology. Pathologists are also encouraged to attend periodic society meetings.

## **SPECIMENS FOR IMMUNOFLUORESCENCE**

Immunofluorescence is used to detect the deposition of immunoglobulins or immune complexes in tissue. The predominant sites of clinical importance are skin and kidney. Immunofluorescence is useful in confirming a diagnosis of discoid lupus erythematosus, pemphigus, dermatitis herpetiformis and pemphigoid to mention the most common entities.

Biopsy specimens should be placed in Transport media or Zeus's media as soon as possible and transported by courier. The media is not a fixative, but rather a holding balanced salt solution. Specimens are adequately preserved in this solution for only a few days. The mail should not be used for transport of these specimens.

When skin is submitted for immunofluorescence, it is optimal to send one specimen in Zeus's media and a second of similar quality in formalin for routine histology. A large punch biopsy or skin ellipse may be divided or two individual small punch biopsies should be representative of the lesion. If the specimen is sufficient for only one study, formalin fixation for routine histology is the more useful study unless the specimen is submitted to confirm a well established and limited differential diagnosis.

## **IMMUNOHISTOCHEMISTRY**

Immunohistochemistry refers to a broad class of staining techniques including the subset commonly referred to as immunoperoxidase stains. The basis of all these stains is the use of a specific antigen/antibody reaction and its elucidation via a color-producing reaction. The technique may be used to demonstrate the presence, location and even the quantity of the antigens present in tissue sections. In theory, the technique is simple, but in practice is fraught with technical and interpretive pitfalls. At PSIP, immunohistochemistry is employed only as an adjunct to good clinical information, and sound gross and microscopic interpretation.

Immunohistochemical stains done at PSIP are designed to be performed on formalin fixed tissue. The peroxidase-antiperoxidase method is used.

All stains are produced by histotechnologists experienced with the technique. Both a negative and positive control are performed with each antibody. Stains of dubious quality or results are repeated.

The stock of antibodies retained at PSIP is constantly changing to reflect the state-of-the-art.

Whenever an unusual neoplasm or malignant lymphoma is suspected, it is optimal for the pathologist to receive the tissue fresh, thus a frozen section or touch preparation may be performed as needed, and any special handling can be accomplished by the pathologist. However, tissue should be left unfixed only in those cases in which delivery to the pathologist will be immediate (less than an hour). Tissue left without fixative or refrigeration for a prolonged period will quickly become useless, even for routine processing. The primary consideration in any case is adequate and proper fixation. When in doubt, place in 10% neutral buffered formalin, which is available from PSIP. Adequate formalin should be used – roughly five to ten times the volume of tissue. Encapsulated nodules or organs and biopsies larger than 1.0 cm in least dimension should be sectioned.

## **SPECIAL BREAST CANCER STUDIES**

The treatment of breast cancer patients is based on the stage of the tumor at diagnosis, number of axillary lymph nodes with metastatic disease, and the status of hormone (estrogen and progesterone) receptors. A major goal of breast cancer research is the identification of biomarkers that would identify patients who might benefit for adjuvant therapy. In addition to hormone receptors, promising markers include HER-2/neu and ki-67 determinations. We employ immunohistochemical assays to identify these biomarkers. The assays are performed on routine formalin fixed paraffin embedded tissue. It is important to fix the tissue in formalin immediately. This immunohistochemical method can be performed on current or on archived tumors as long as paraffin blocks are available.

## **Estrogen and Progesterone Receptor Immunohistochemistry**

Approximately 55-65% of primary breast carcinomas and 45-55% of the metastases from the breast carcinomas have been found to be estrogen receptor positive (ER+). Studies have shown that 55-60% of women with ER+ tumors respond to additive or ablative hormone therapy compared to about 8% of women with ER- tumors. ER- carcinomas show a better response to cytotoxic chemotherapy. Tumors that are better differentiated are more likely to be ER+, and ER+ carcinomas have a relatively better prognosis.

Approximately 45-60% of primary and metastatic breast cancers contain progesterone receptors (PR). The presence of both ER and PR in the tumor increases the likelihood of response to endocrine therapy from 55% (ER+ only) to 75-80%. The loss of PR by tumor cells is associated with a worse prognosis.

Hormone receptor analysis is also occasionally useful in evaluating endometrial and ovarian carcinomas.

## **HER-2/neu (c-erbB-2, HER2) Oncogene Immunohistochemistry**

A semi-quantitative immunohistochemical assay is used to determine HER-2/neu overexpression in breast carcinoma. HER-2/neu (also known as c-erbB-2 and HER2) is an oncogene which encodes a transmembrane glycoprotein with tyrosine kinase activity. The protein is a normal component expressed by a number of epithelial cell types, when the gene is amplified. In breast cancer the gene is overexpressed with multiple copies appearing in the nucleus.

Amplification of HER-2 is seen in almost all cases of comedo-type duct carcinoma in situ, in 10-40% of invasive duct carcinomas and in only a few cases of invasive lobular carcinoma. The expression of this protein has been associated with poor histologic grade, spread to axillary nodes, and number of nodes involved. A negative association between HER-2 expression and ER and PR has been noted. No significant association has been found between HER-2 expression and ploidy. In axillary node-positive patients, there is significant correlation between amplification of HER-2 and shorter disease-free and overall survival. It has also been claimed that patients with overexpression of HER-2 show a better response to Adriamycin-based chemotherapy and less likely to respond to hormonal therapy (even if ER+). Interest in HER-2 status has increased with the arrival of FDA approved Herceptin (Trastuzumab), a monoclonal antibody of HER-2. The antibody binds to the HER-2 membrane protein inhibiting the proliferation of tumor cells that overexpress HER-2. Herceptin is reported to help prevent the spread of metastasis from breast cancer.

If the immunohistochemical assay for HER-2/neu is inconclusive, fluorescence in situ hybridization (FISH) is available on deparaffinized formalin fixed material. This method directly visualizes the amplification of the HER-2/neu gene in tumor nuclei.

## **Ki-67**

Ki-67 is a monoclonal antibody that reacts with an undefined nuclear antigen present in all non-G0 cells of the cell cycle, identifying proliferating cells within a tumor. Since cells from any phase of the cycle can enter the G0 phase regardless of their DNA content, determination of the Ki-67 fraction could reflect more significant information regarding the proliferating cell component of a tumor compared with the DNA content. Using immunohistochemistry, the Ki-67 fraction "growth fraction" reflects the percentage of positively stained cells in a tumor. The higher the Ki-67 growth fraction, the more aggressive the tumor.

## **FLOW CYTOMETRY**

The flow cytometer is an instrument capable of rapid quantitative multiparametric analysis of heterogeneous cell populations on a cell-by-cell basis.

### Immunophenotyping Panels of Leukemias and Lymphomas:

The Flow Cytometer allows us to immunophenotype and monitor therapy of patients with leukemia and lymphoma.

Specimen requirements are as follows:

1. Peripheral blood should be submitted in AOD, heparin or EDTA and held at room temperature. The specimen cannot be clotted.
2. Bone marrow aspiration material should be submitted in ACD or heparin and held at room temperature. Again, clotting should be avoided.
3. Tissue or fine needle aspiration material should be held in RPMI and refrigerated (2-8 degrees Celsius).

## **DIAGNOSTIC CYTOGENETICS**

Cytogenetics is used for determining the karyotype of vital, unfixed cellular material, usually bone marrow, viable fetal tissue or rare tumors. This can be helpful in further classifying tumors, myelodysplastic syndromes, leukemias or determining fetal cytogenetic abnormalities.

**Specimen requirements are as follows:**

## **PERIPHERAL BLOOD**

**Peripheral blood:** 10-15 ml of blood in a preservative free sodium-heparin (green top) tube. Invert tube to mix. Prometaphase analysis will be performed on all specimens unless otherwise specified.

**Newborn & PUBS:** Minimum of 1 ml peripheral blood in a preservative free sodium-heparin (green top) tube. Invert tube to mix well.

## **SOLID TISSUE**

All solid tissue sampled should be collected aseptically and transported in tissue culture media or Hank's balanced salt solution. Do NOT put in water, fixative, formalin or saline. Please keep sample at room temperature.

**Products of Conception/Fetal Tissue:** Large chorionic villi sample (approximately 3 cm) and a fetal tissue sample such as skin, lung or pericardium. Please send multiple tissue types if possible. Label tube with tissue type or origin.

**Skin Biopsy/Solid tissue:** 1-3 mm or more tissue. Label tube with tissue type or origin.

## **NEOPLASIA**

**Bone Marrow:** Aspirate 1-2 ml bone marrow into a sterile syringe containing 0.1 ml preservative free sodium heparin, invert syringe to mix and transfer to a 3 ml preservative free sodium-heparin (green top) Vacutainer tube.

**Leukemic Peripheral Blood:** Patient should have a WBC of 15,000 or higher with approximately 10% circulating immature myeloid or lymphoid blast cells. Collect 5 ml of peripheral blood in a preservative free sodium-heparin (green top) Vacutainer tube.

**Solid Tumor Tissue:** >5 mm representative tumor tissue collected under aseptic conditions and transported in sterile tissue culture media.

**Lymph Node Biopsy:** >5 mm tumor biopsy collected under aseptic conditions and transported in sterile tissue culture media.

## **MOLECULAR ANALYSIS/DNA TESTING**

**Peripheral Blood:** 5-10 ml blood in EDTA (lavender top) tube for molecular testing and 5-10 ml of blood in preservative free sodium-heparin (green top) tube for cytogenetic studies. (Molecular studies will be forwarded to an outside laboratory).

**Prenatal:** 15-20 ml of amniotic fluid in 2 sterile tubes. Cytogenetic analysis will be performed and amniocytes will be cultured to send to an outside laboratory for molecular studies.

## **FLUORESCENCE IN SITU HYBRIDIZATION (FISH)**

FISH studies are indicated as an adjunct to classic Cytogenetics. Specimen collection is described previously for the tissue to be studied. These studies are performed in house at PSIP.

## **PATHOLOGISTS' BIOGRAPHIES**

### **Shane Anderson, M.D., Ph.D.**

*Puget Sound Institute of Pathology; Medical Director, Auburn Regional Medical Center*

**Residency:** University of Washington Medical Center - Anatomic and Clinical Pathology

**Fellowship:** New York Presbyterian Hospital, Cornell-Weill Medical College –  
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**Board Certifications:** Anatomic Pathology, Clinical Pathology, Dermatopathology

**Sub-Specialty Interest(s):** Immunohistochemistry, Genitourinary Pathology, Breast Pathology, Gastrointestinal Pathology, Digital Pathology

### **Peter Benda, M.D.**

**Residency:** University of Washington Medical Center – Anatomic and Clinical Pathology

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### **Peter Bertozzi, M.D.**

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### **Linda Burkhardt, M.D.**

*Medical Director, Franciscan Health Systems Department of Pathology and Laboratory Services*

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### **Brian Folz, M.D.**

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### **Douglas Hansen, M.D.**

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**Michael Kalnoski, M.D.**

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**Aashiyana Koreishi, M.D**

**Residency:** Massachusetts General Hospital – Anatomic and Clinical Pathology

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**Sub-Specialty Interest(s):** Hematopathology

**Kenneth Meckler, M.D.**

*Managing Partner*

**Residency:** University of Washington Medical Center – Anatomic and Clinical  
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**Board Certifications:** Anatomic Pathology, Clinical Pathology

**Sub-Specialty Interest(s):** Gastrointestinal and Liver Pathology

**Angie Pham, M.D.**

**Residency:** Loma Linda University - Anatomic and Clinical Pathology

**Fellowship:** City of Hope - Hematopathology

**Board Certifications:** Anatomic Pathology, Clinical Pathology

**Sub-Specialty Interest(s):** Hematopathology

**Sandra White, M.D.**

**Residency:** Oregon Health Sciences University – Anatomic and Clinical Pathology

**Fellowships:** Oregon Health Sciences University – Surgical Pathology and  
Cytopathology

**Board Certifications:** Anatomic Pathology, Clinical Pathology, Cytopathology

**Sub-Specialty Interest(s):** Cytology including fine needle aspirations

**Katie Wilkinson, M.D.**

*Medical Director, Transfusion and Tissue Services, Franciscan Health System*

**Residency:** Loma Linda University Medical Center – Anatomic and Clinical Pathology;  
Los Angeles County/University of Southern California Medical Center – Anatomic and  
Clinical Pathology

**Fellowship:** Puget Sound Blood Center – Blood Banking/Transfusion Medicine

**Board Certification:** Anatomic Pathology, Clinical Pathology, Blood  
Banking/Transfusion Medicine

**Subspecialty Interest(s):** Blood utilization, massive transfusion