



CYTOLOGY SPECIMEN COLLECTION MANUAL

The primary purpose of the CLH/PPPI Cytology Collection Manual is to provide standardized cytopathologic specimen collection and processing techniques. When followed properly, these techniques will help to enhance results by minimizing unwanted artifacts and allow for well-preserved and well-stained cells. These guidelines place an emphasis on technique effectiveness and quality and in combination with our “quality controlled” laboratory processing techniques, will allow the highest potential for cell preservation, cytologic detail, and diagnostic accuracy¹.

STANDARD PRECAUTIONS

Please handle all human blood specimens and body substances according to “Standard Precaution” guidelines, available from the U.S. Centers for Disease Control and Prevention (Guideline for Isolation Precautions in Hospitals, Infection Control and Hospital Epidemiology, CDC, Vol. 17; 1:53-80). Standard Precaution guidelines combine major features of “universal precautions and body substance isolation practices”¹.

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GENERAL INFORMATION

<u>Locations</u>	<u>Contact</u>	<u>Phone</u>	<u>Fax</u>	<u>Hours</u>
Oahu 99-193 Aiea Heights Dr. Aiea., HI 96701	Pathology Lab Cytology Lab	(808) 834 5120 (808) 834 5125	(808) 834 5147 (808) 834 5148	M-F 0800 - 1700 Sat 0800 - 1130
Hilo 33 Lanihuli St. Hilo, HI 96720	Account Service Rep	(808) 936 8339	(808) 969 4984	M-F 0800 – 1700
Kona 75-5905 Walua Rd. Kailua-Kona, HI 96740	PSC Manager Kona Hos. Manager	(808) 329 2205 (808) 322-9366	(808) 329 5298 (808) 324-1892	M-F 0600 - 1700 M-F 0730 - 1700 Sun 0800 - 1400
Maui	Account Service Rep MMMC	(808) 269 2304 (808) 242-2064	(808) 242-6137 (808) 244-7246	
Kauai	Lab manager	(808) 245 1078	(808) 241-1325	

I. SERVICES PROVIDED

A. Preparation/Processing of Gynecological and Non-gynecological Specimens

1. Gynecological specimens:

- a. Conventional pap smears are processed by trained cytology assistants.
- b. ThinPrep pap smears are prepared using the Hologic (Cytoc) instrument, by trained cytology assistants.

Preparation/processing to include the following requests:

- (i) ThinPrep Pap test only
- (ii) ThinPrep with reflex to HPV testing if ASCUS
- (iii) ThinPrep and HPV testing
- (iv) HPV Primary Screening Protocol
- (v) Additional testing from the ThinPrep vial: GC & Chlamydia or Chlamydia only (by DNA-PCR technology)
- c. HPV testing only. (please submit ThinPrep)
- d. GC & CT only (by DNA-PCR technology)

Note: HPV testing is done by Roche® (real-time PCR utilizing hydrolysis probes with the Roche Cobas 4800 system). Roche's test reporting format automatically includes status on genotypes 16 and 18 if the HPV-hr is positive.

2. Non-gynecological specimens:

- a. All types and sources of non-gynecological specimens are prepared by cytology assistants, under the direction of a Cytotechnologist/Cytopathologist.



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B. Microscopic Examination for:



1. Malignant cells
2. Precursors of carcinoma (i.e. dysplasia, hyperplasia)
3. Benign atypia due to inflammation, drug Rx, radiation Rx, etc.
4. Microbiology (parasites, fungi, viral changes, bacteria)
5. Other incidental findings, e.g. asbestos bodies, crystals, psammoma bodies, casts, etc.
6. Maturation index (when requested)

C. Interpretation and Reporting of Gynecological and Non-gynecological Specimens

1. Gynecologic specimens:

- a. ThinPrep pap smears are processed using the ThinPrep Imaging System, and interpreted by board certified Cytotechnologists and Cytopathologist/Pathologists.
- b. Under the direction of a Cytopathologist, cytologic findings are reported using The 2001 Bethesda System terminology.

Reports contain a statement of adequacy, a diagnosis, and comments or recommendations for follow-up (when applicable).

- c. Reports will be available within 1 week from receipt of specimen.
 - (i) Reports without **HPV testing** will be available within 1 to 3 business days from the date of collection.
 - (ii) Reports with **HPV testing** will be available within 5 to 8 business days from the date of collection.
 - (iii) Reports for **HPV testing only** will be available within 1 week from the date of DNA testing.
- d. Cytopathologists/Cytotechnologists are available to address any questions/problems during normal laboratory hours. Contact Cytology (Aiea) at phone:

(808) 834-5170 – Oahu

2. Non-gynecological specimens:

- a. All smears and cell blocks are interpreted by board certified Cytotechnologists and Cytopathologists/Pathologists.

Interpretation of smears to include:

- (i) Special stain requests:
 - GMS (Silver stain), AFB, PAS and Oil-red-O stains
- (ii) Interpretation of Tzanck smears for herpetic/viral bodies

Interpretation of a cell block and tissue fragments to include:

- (i) Immunohistochemistry studies (performed as needed)



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- b. Under the direction of a Cytopathologist/Pathologist, cytologic findings are reported using descriptive terminology.
Reports contain descriptive terminology, final diagnosis, and comments or recommendations for follow-up (when applicable).
- c. Reports will be available within 2 days from receipt of specimen.
- d. Cytopathologists/Cytotechnologists are available to address any questions/problems during normal laboratory hours at phone:

(808) 834-5170 – Oahu
(808) 974 6898 – Hilo
(808) 329 8550 or (808) 322 9366 – Kona
(808) 269 2304 or (808) 242-2064 – Maui
(808) 245-1078 or (808) 245-7775 – Kauai

D. **STAT Processing of Gynecological and Non-gynecological Specimens (when requested)**

1. Gynecologic specimens:
 - a. A preliminary or final report will be made by phone within one hour from receipt of specimen.
2. Non-gynecological specimens:
 - a. A Pathologist will provide an on-site, “STAT” reading(s), during Radiology and Ultrasound-guided, Fine Needle Aspiration procedures.

Special arrangements should be made with site-specific hospital locations or by contacting the Laboratory Manager at phone:

(808) 834-5170 – Oahu
(808) 974-6898 – Hilo
(808) 329 8550 or (808) 322-9366 – Kona
(808) 242-2064 – Maui (MMMC)
(808) 245-1078 or (808) 245-7775 – Kauai

- b. A preliminary or final report will be made by phone, for all other specimen types, within two hours from receipt of specimen.

E. **Specimen Pick-up and Delivery of Reports/Supplies**

Contact Dispatch at phone: (808) 677-7998 – Oahu
(808) 935-4814 – Hilo
(808) 322-9366 – Kona
(808) 244-5567 – Maui
(808) 245-7775 – Kauai

1. Routine specimen pick-up and delivery of reports/supplies is provided daily by CLH-Courier Services from pre-designated locations.

NOTE: A list of delivery schedules and designated pick-up sites is available upon request

2. **STAT**/Special pick-up and delivery is available upon request.



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F. Patient's Slide/Report Send-out Requests

1. Slide requests:

- a. Requests may initially be verbal.
- b. Requests must be made at least 48 hours in advance.
- c. A signed "**Authorization for Release of Information**" **MUST BE COMPLETED** before slides are released.

Forms are furnished by the patient's primary care physician's office.

- (i) Forms should be filled out by either an RN, Medical Records Dept. or qualified office personnel, and then verified and signed by patient.
- (ii) Forms must include:
 - Name and phone number of the person responsible for receiving the slides (e.g. Pathologist assistant).
 - Name and complete address of the institution the slides are being sent to.
 - Institution's courier account number where the slides are being sent to. (We are required by law to be able to readily retrieve the slides).
- (iii) The signed form should then be faxed to phone:

(808) 834-5148 – Oahu
(808) 935-6928 – Hilo
(808) 324-1892 – Kona
(808) 986-0583 – Maui (MMMC)
(808) 245-1063 – Kauai

Then, the original should be filed in the patient's chart.

2. Copies of Reports:

- a. For copies of reports on Gynecological and Non-gynecological cases, contact Client Services at phone: (808) 677-7998 – Oahu
- b. For outside physicians, a verbal request must be followed immediately by a signed "**Authorization for Release of Information**" form.

Forms are furnished by the patient's primary care physician's office.

- (i) Forms should be filled out by either an RN, Medical Records Dept. or qualified office personnel, and then verified and signed by patient.
- (ii) The signed form should then be faxed to Cytology (Aiea) at phone:

(808) 834-5148 – Oahu

Then, the original should be filed in the patient's chart.



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G. Slide Review/Consultation Requests

1. Pathologist's are always available to review/consult on problem cases.

Requests should be made by contacting the Pathology/Cytology Department at phone:

(808) 834-5170 -Oahu
(808) 836 9336 - Hilo
(808) 329 8550 - Kona
(808) 242-2064 - Maui (MMMC)
(808) 245-1078 - Kauai

H. Other Services

1. For other services not listed above, please contact the Laboratory Manager at phone:

(808) 834-5170 -Oahu
(808) 836 9336 - Hilo
(808) 329 8550 - Kona
(808) 242-2064 - Maui (MMMC)
(808) 245-1078 - Kauai

II. SUPPLIES

Contact Supplies at phone: (808) 677-7999 – Oahu
(808) 935-4814 or Fax (808) 961-9614 – Hilo
(808) 322-4126 or Fax (808) 324-1892 – Kona
(808) 242-2064 – Maui
(808) 245-7775 – Kauai

A. Requisitions

1. Laboratory Test Order Form (Cytology) is used for all cytology requests.
(see next page)

BARCODE HERE



PAN PACIFIC PATHOLOGISTS, LLC

PHYSICIAN

Island Research

Group BLANK

Clnt Pt Id:

BILL TO PT INSURANCE OR GUARANTOR

BILL TO GROUP ACCT #

SPECIAL INSTRUCTIONS

PATIENT NAME (LAST, FIRST, M.I.)

DATE OF BIRTH

SEX

M F

MAILING ADDRESS

CITY

STATE

ZIP

HOME PHONE #

WORK PHONE #

PRIMARY INS

MEMBERSHIP #

CC SUBSCRIBER'S NAME

RESPONSIBLE PARTY

SECONDARY INS

MEMBERSHIP #

CC SUBSCRIBER'S NAME

RESPONSIBLE PARTY

DATE OF INJURY OR PREGNANCY (LMP)

ACCIDENT

NO-FAULT

EMPLOYER CAUSE OF INJURY

WORK

OTHER

STAT

CALL

Name/Institution: _____

PRE-OP

FAX

Phone # _____

Fax # _____

SEND COPY TO:

NON-GYN CYTOLOGY

Urine, voided Urine, instrumented
 w/reflex to UroVysion if abnormal

UroVysion test

Sputum

Nipple Discharge

Fine Needle Aspiration Biopsy

Site: _____

Other Source: _____

COLLECTION: _____ am

Date: ___/___/___ TIME: ___:___ pm

CLINICAL DIAGNOSIS/ICD 10 CODE(S):

GYN CYTOLOGY - PAP TEST PROTOCOL OPTIONS

ThinPrep* Pap test only *(Additional charges may be applied if abnormal or reflex testing is triggered)

ThinPrep* w/Reflex to HPV if ASCUS and LSIL

ThinPrep* and HPV test (Co-testing), (30 - 65 years)

HPV Primary Screening Protocol **(reflex to Pap if HPV+, 16/18-, [25 years & older])

**Interim Clinical Guidelines: *Obstet Gynecol.* 2015 Feb; 125(2):330-7.

HPV Test only, other than Primary Screening protocol (please submit in ThinPrep vial only)

CONVENTIONAL SMEAR

SPECIMEN SOURCE:

Cervical Endocervical Vaginal _____

Other: _____

COLLECTION:

DATE:

TIME:

PATIENT HISTORY: LMP: ___/___/___

Previous Cytology _____

(Lab accession number if possible)

HPV Test Result/Date

CHECK ALL THAT APPLY FOR PAP TEST:

Hormonal Therapy

Oral Contraceptives

Hysterectomy

Radiation Therapy

Nuva Vaginal Ring

Ortho Patch

Hysterectomy, Partial

Chemotherapy

IUD

Implant Contraceptives

Depo Provera

Carcinoma

Abnormal Bleeding

Pap Test CLINICAL DIAGNOSIS/ICD-10 CODES:

Z12.4 Screening for malignant neoplasm of cervix

Z77.9 High Risk

Z12.72 Screening for malignant neoplasm of vagina

Z11.51 Special screen exam for HPV

_____ Pregnancy (Z34.90 - Z34.93 based on trimester)

N95.0 Postmenopausal Bleeding

Z39.2 Postpartum

_____ Vulvitis/Vaginitis (N76.0 - N76.3)

N95.2 Atrophic Vaginitis

R87.610 ASC-US

N72 Cervicitis

R87.611 ASC-H

N92.0 Frequent Menstruation

R87.612 Low Grade SIL

N92.6 Irregular Bleeding

R87.613 High Grade SIL

N89.8 Leukorrhea/Vaginal Discharge

R87.810 Positive HPV Test

N95.1 Menopausal Syndrome

N87.9 Dysplasia NOS

Z72.89 Other Problems related to lifestyle

N87.0 Mild Dysplasia

N87.1 Moderate Dysplasia

Other ICD-10 Code/Diagnosis:

GC AND CHLAMYDIA TESTS

SPECIMEN SOURCE:

GC and Chlamydia (CLH # 0187)

CLINICAL DIAGNOSIS/ICD10 CODES:

Chlamydia (CLH # 0184)

Z11.8 Screening Exam for Unspecified Chlamydial Diseases

Z11.3 Screening Exam for Venereal Disease N89.8 Leukorrhea/Vaginal Discharge

Z72.89 Other problem related to lifestyle

_____ Pregnancy (Z34.00 - Z34.93)

Other ICD-10 Code/Diagnosis:

Form 5124 Rev.12/04/2017

IF PAP TEST ALLOWED FREQUENCY IS EXCEEDED, PLEASE HAVE THE PATIENT SIGN THE ABN IN THE BACK OF THE REQUISITION.

MEDICARE Patients - Please complete all sections highlighted in yellow

Notifier: Pan Pacific Pathologists, LLC, 33 Lanihuli Street, Hilo, HI 96720 Billing: 1-800-820-4341 www.clinicallabs.com

A. Patient Name:

C. Identification Number:

ADVANCED BENEFICIARY NOTICE OF NONCOVERAGE (ABN)

NOTE: If Medicare doesn't pay for **D.Laboratory test(s)** below, you may have to pay.

Medicare does not pay for everything, even some care that you or your health care provider have good reason to think you need. We expect Medicare may not pay for the **D.Laboratory test(s)** below.

D. Laboratory Test(s)	E. Reason Medicare May Not Pay:	F. Estimated Cost
Thinprep Liquid Based Pap Smear	Medicare pays for this service once every 24 (twenty four) months	\$55.00 - \$175.00 (See "H" below)

WHAT YOU NEED TO DO NOW:

- * Read this notice, so you can make an informed decision about your care.
- * Ask us any questions that you may have after you finish reading.
- * Choose an option below about whether to receive the **D.Laboratory test(s)** listed above.
Note: If you choose Option 1 or 2, we may help you to use any other insurance that you might have, but Medicare cannot require us to do this.

G. OPTIONS: Check only one box. We cannot choose a box for you.

- OPTION 1.** I want the **D.Laboratory test(s)** listed above. You may ask to be paid now, but I also want Medicare billed for an official decision on payment, which is sent to me on a Medicare Summary Notice (MSN). I understand that if Medicare doesn't pay, I am responsible for payment, but I can appeal to Medicare by following the directions on the MSN. If Medicare does pay, you will refund any payments I made to you, less co-pays or deductibles.
- OPTION 2.** I want the **D.Laboratory test(s)** listed above, but do not bill Medicare. You may ask to be paid now as I am responsible for payment. I cannot appeal if Medicare is not billed.
- OPTION 3.** I don't want the **D.Laboratory test(s)** listed above. I understand with this choice I am not responsible for payment, and I cannot appeal to see if Medicare would pay.

H. Additional Information: Additional charges may be necessary if an interpretation is required or if additional reflex testing is triggered. For more information, please contact Pan Pacific Pathologist Billing Department: 33 Lanihuli Street, Hilo, HI 96720, or call toll free 1(800) 820-4341, or visit us at www.clinicallabs.com.

This notice gives our opinion, not an official Medicare decision. If you have other questions on this notice or Medicare billing, call 1-800-MEDICARE (1-800-633-4227/TTY: 1-877-486-2048).
Signing below means that you have received and understand this notice. You also receive a copy.

I. Signature:	J. Date:
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B. Collection Supplies and Fixatives:

1. Gynecological Specimens (refer to **Figure 1, below**):
 - a. ThinPrep collection supplies (ThinPrep pap test physician education video, spatula and cytobrush, cytobroom or Rovers[®] Cervex-Brush[®]- *not shown below*)
 - b. ThinPrep collection vials prefilled with alcohol fixative for pap smear and HPV test collection.
 - c. Complete PAP kits (includes: 1 slide, cytobrush, spatula, fixative, and slide holder)
 - d. Cytology spray-fixative in a bottle (pump = Protocol or aerosol = Spraycyte) for immediate fixation of smears.
 - e. APTIMA[®] Specimen Collection Kit (for Chlamydia and GC only- *not shown below*)
 - f. Digene *hc₂* DNA Collection Kit (for Anal HPV test only)
 - g. Plastic, Biohazard specimen bag (refer to **Figure 2, pg.11**)

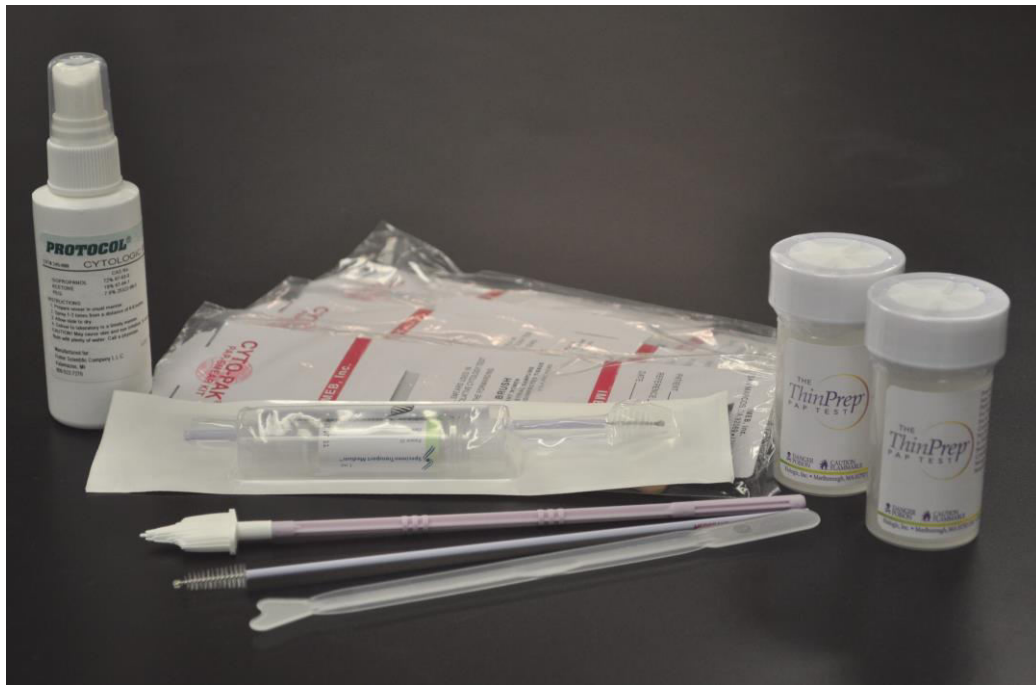


Figure 1: (from left to right, front to back) Plastic spatula, Cytobrush, Cytobroom, Digene *hc₂* DNA Collection Kit [MSDS-p.61-64], ThinPrep Collection Vials with fixative (Preservcyt) [MSDS-p.65-68], Cytology Spray-fixative (pump = Protocol [MSDS-p.34-40]), Cyto-Pak[®] Pap Smear Kits.



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2. Non-gynecological Specimens (refer to **Figure 2, below**):

- a. Frosted-end glass slides for smears
- b. Sterile, 50 cc plastic containers for specimen collection
- c. Cardboard/plastic slide holders for transporting smears
- d. Cytolyt fixative (provided by Cytology - Aiea) for transport of various specimen types refer to site-specific collection procedures in **section VI-pgs.17-30**.

Alternative (for Neighbor Island sites)

Plastic specimen container prefilled with Non-gyn collection fluid (Shandon).

NOTE: Non-gyn collection fluid = Cytolyt fixative

- e. Plastic, leak-proof slide containers prefilled with alcohol fixative for immediate fixation and transport of smears (e.g. Bronchial brushings, Fine needle aspirations (FNA's)).

Alternative (for Neighbor Island sites):

Cytology spray-fixative in a bottle (pump = Protocol or aerosol = Spraycyte).

- f. 16cc plastic vials/tubes prefilled with 2% Carbowax fixative (provided by Cytology-Aiea) for transport of Cystic-FNA's and/or FNA-Needle Rinse Material/Fluid.
- g. Plastic, Biohazard specimen bag



Figure 2: (from left to right, front to back) Frosted-end Glass Slides, Cardboard Slide Holders, Biohazard Specimen Bag, Cytology Spray-fixative (pump = Protocol [MSDS-p.34-40], 50cc Sterile, Plastic Specimen Container, 16cc Plastic tube with Cytolyt Fixative (In-house/Shandon) [MSDS-p.41-46],. Plastic Slide Holder Containers with Alcohol Fixative [MSDS-p.47-53].



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III. LABELING INFORMATION

A. Labeling of Slides:



1. Slides should be clearly labeled to ensure correct identification **BEFORE** the sample is applied.
2. Labeling should be done with a **#2 lead pencil** on the frosted end of the slide, using one of the slide labeling options below:

DO NOT USE: Ball point pen, permanent marker, label or wax pencil to label, as it will wash-off/disappear during processing.

Option 1:

Full patient's name
Date of birth

Smith, Casey 4/4/61

Option 2:

Patient's last name, name
Medical Record No.

Smith, CAsey #123789

IMPORTANT NOTE: The sample should be placed on the **labeled** side of the slide without encroaching on the frosted area.

B. Labeling of Specimens:



1. All specimens should be clearly labeled to ensure correct identification **BEFORE** being sent to the laboratory for testing.
2. Labeling should be done, using one of the options listed below:
 - a. Affix patient addressograph label directly on the specimen container. (e.g. bottle, vial, sterile bag, syringe, slide holder container, etc.)
 - b. If no addressograph label is available, write the patient's full name and date of birth directly onto the specimen container. (e.g. bottle, vial, sterile bag, syringe, etc.)

NOTE: Special care and detail in labeling should be made on specimens that consist of:

- Material or fluid collected from multiple sites (e.g. bronchial brushing and washing, FNA Breast @ 1 o'clock and 6 o'clock, etc.)
- Material or fluid collected from the same site and submitted in multiple containers.

Each container must be labeled individually to ensure proper identification and processing.



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C. Completing Laboratory Test Order Form:



1. All specimens must be submitted for testing with a completed requisition form. Please provide all pertinent information in a **legible format**.
2. Completed requisition form should provide the following information:
 - a. Full name of patient
 - b. Patient identification number / medical record number
 - c. Date of birth and age
 - d. Date and time of specimen collection
 - e. Relevant clinical history and diagnosis code(s) (ICD-10 Code)
 - f. Specimen source (e.g. cervix, endocervix, breast, neck, etc.)
 - g. Collection method (e.g. Fine Needle Aspiration, Bronchial Brushing or Washing, 24-hour Sputum, Voided Urine, etc.)
 - h. Number and type of specimen(s) being submitted
 - i. Type of fixative added to specimen (if applicable)
 - j. Type of testing requested (to include requests for reflex HPV testing, GC & CT, special stains for microorganisms, etc.)
 - k. Full name and phone number of the ordering physician

IV. SPECIMEN REJECTION

- A. Following documented efforts to contact the clinician/office and obtain information, specimens will not be accepted for processing based on the following criteria:
 1. No patient identification on slide or specimen container
 2. No patient identification on requisition
 3. Slide/requisition that has been separated and neither are properly identified
 4. No client/physician name or number on requisition
 5. Slides received broken beyond reasonable repair
 6. Gynecological specimen that is collected in less than 6 weeks after a prior pap smear
 7. Specimen submitted by an unauthorized source

Note: Specimens may be accepted from the following sources:

- a. M.D.'s
- b. D.O.'s
- c. P.A.'s



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- d. N.P's
 - e. A.P.R.N.s
 - f. Podiatrist
 - g. Veterinarians
 - h. Dentists (D.D.S.)
 - i. Clinical Pharmacist
 - j. Chiropractor
 - k. Therapeutically Certified Optometrists
8. Improper preservation of specimen
 9. Improper fixation of specimen

V. COLLECTION AND PREPARATION OF GYNECOLOGIC SPECIMENS

A. ThinPrep® pap smear:

Note: Rapid repeat of a pap smear (less than a 6 week interval) will not be accepted.

1. Materials needed for collection and preparation:
 - a. Laboratory Test Order Form (Cytology)
 - b. ThinPrep collection vial prefilled with alcohol fixative (*PreservCyt*® Solution)
 - c. Plastic Spatula (i.e. Ayre's)
 - d. Cytobrush, Cytobroom or Cervex-Brush®
2. Specimen collection and transport:

(for further instructions, refer to the ThinPrep Pap Test Physician Education Video²)

 - a. *Special Patient Information Guidelines*^{6,7}:
 - (i) The patient **should not** be tested during menses.

*Although ThinPrep Pap Test reduces obscuring blood, clinical studies have demonstrated that excessive amounts of blood may still compromise the test and possibly lead to an **unsatisfactory** result^{7,8}.*

Testing should be done 2 weeks after the 1st day of patient's last menses.
 - (ii) The patient **should not** use vaginal medication, vaginal contraceptives, or douches during the 48 hours before the exam.
 - b. Fill out the Laboratory Test Order Form completely (refer to **section III-C, pg.13**).
 - c. Label the collection vial with both the patient and physician's full name **before** the procedure begins (refer to **section III-B, pg.12**).



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d. *Special Collection Guidelines*^{6,8}:

- (i) Use lukewarm water to warm/lubricate the speculum, if desired, use approved water-soluble gel lubricant sparingly applied to the posterior blade of the speculum. Excessive amounts of gel lubricant **should not** be used as gels may compromise the test and possibly lead to an **unsatisfactory** result.
- (ii) Excess cervical mucus or other discharge **should be** gently removed **before** taking the sample; using ring forceps holding a folded gauze pad.
The cervix **should not** be cleaned, by washing with saline.
Application of saline to the cervix may result in a relatively acellular smear.
- (iii) Excess inflammatory exudate from the cervical canal **should be** removed **before** taking the sample, by placing a dry 2x2" piece of gauze over the cervix and peeling it away after the exudate is absorbed or by using a dry proctoswab or scopette.

e. Collection using:

Spatula and cytobrush combination²:

- (i) Using the spatula, collect an adequate amount of material by rotating 360° over the circumference of the cervical os and ectocervix.
- (ii) Rinse the spatula in the collection vial by swirling vigorously to release cells.
- (iii) Discard the spatula after rinsing.
- (iv) Next, insert the cytobrush with gentle pressure into the endocervical canal until only the bottom bristles are exposed.
- (v) Fully rotate the brush in one direction. Do not over rotate.
Over-rotation may damage some cells, and often induces capillary bleeding.
- (vi) Rinse the brush in the same vial used to rinse the spatula.
- (vii) Push the brush against the wall of the vial while stirring the solution, 10x.
Then, swirl the brush vigorously to further release cells.
- (viii) Discard the brush after rinsing.
- (ix) Cap the vial, making sure that the torque line on the cap passes the line on the vial.

Cytobroom, only²:

- (i) Gently insert the central bristles of the broom into the endocervical canal until the shorter bristles contact the ectocervix.
- (ii) With gentle pressure, rotate the broom 5x in one direction.



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- (iii) Rinse the device in the collection vial by pushing broom against the bottom of the vial, forcing the bristles to bend apart, 10x. Then, swirl the brush vigorously between the thumb and forefinger to further release cells.
- (iv) Inspect the device to ensure no material remains attached and then discard.
- (v) Cap the vial, making sure that the torque line on the cap passes the line on the vial.

Note: *Rovers® Cervex-Brush®* collection instructions available on video.

- f. Place properly sealed ThinPrep collection vial(s) into to a Biohazard specimen bag and include the completed Laboratory Test Order Form(s) in the outside pocket of the bag.
- g. Place specimen bag(s) in the designated drop-off area for courier pick-up and delivery.

NOTE: If specimen requires **Special pick-up**, please contact Dispatch, phone #'s on **pg.5**.

B. Conventional pap smear:

Note: Rapid repeat of a pap smear (less than a 6 week interval) will not be accepted.

1. Materials needed for collection and preparation:
 - a. Laboratory Test Order Form (Cytology)
 - b. Frosted-end glass slides
 - c. Cytology spray-fixative in a bottle (pump or aerosol)
 - d. Plastic/cardboard slide holder OR Plastic slide box for transport of smears
 - e. Plastic Spatula (i.e. Ayre's)
 - f. Cytobrush
2. Specimen collection and transport:
 - a. *Special Patient Information Guidelines*^{6,7} (refer to **section V-A, pg.14**).
 - b. Fill out the Laboratory Test Order Form completely (refer to **section III-C, pg.13**).
 - c. Label frosted end of the glass slide using a pencil **before** the procedure begins (refer to **section III-A, pg.12**).





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- d. *Special Collection Guidelines*^{6,8} (refer to **section V-A, pg.15**).
- e. Visually inspect the cervix for abnormalities.
- f. Identify the transformation zone, if visible and direct sampling efforts to encompass this area.

COMMENT: If an elevated, ulcerated, friable, necrotic, or exudate covered lesion is observed, a biopsy following cytology sampling may be warranted.

- g. Choose the contoured end of the spatula which best conforms to the anatomy of the cervix and the transformation zone.
- h. Rotate the spatula 360° over the circumference of the cervical os and ectocervix while maintaining firm contact with the epithelial surface.

A 360° rotation ending in the horizontal position, 3 and 9 o'clock, will retain the sample on the upper surface of the spatula as the instrument is removed.

Most wooden samplers allow good adherence of the sample to the device for transfer to the glass slide.

- i. **DO NOT** smear the sample at this time.
- j. Insert the cytobrush into the os with gentle pressure and rotate only 90-180° in one direction.

Over-rotation may damage some cells, and often induces capillary bleeding.

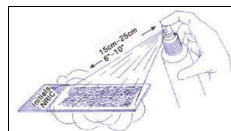
- k. Working carefully, but quickly, spread the material collected on the spatula in a thin, even layer over the length of the glass slide with a single smooth stroke.

Next, spread the material collected on the cytobrush in a thin, even layer over the length of the glass slide by twirling the handle of the brush.

Thin out large clumps as much as possible.

Avoid excessive manipulation, which can damage cells.

- l. **FIX IMMEDIATELY** by applying Cytology spray-fixative evenly over the smear.
Do not allow the specimen to air-dry without fixative.



Note: The use of hairspray is NOT recommended due to variation in composition; its use may result in very poor specimen preservation.

- m. Allow the fixed smear to dry completely before packing for transport.



CYTOLOGY SPECIMEN COLLECTION MANUAL

- n. Place dry, spray-fixed smear(s) into plastic/cardboard slide holder for transport.
 - (i) Secure cardboard slide holder(s) with scotch tape or rubber bands to prevent opening during transport.
- o. Place the holder/container(s) into a Biohazard specimen bag and include the completed Laboratory Test Order Form(s) in the outside pocket of the bag.
- p. Place specimen bag(s) in the designated drop-off area for courier pick-up and delivery.

NOTE: If specimen requires **Special pick-up**, please contact Dispatch, phone #'s on **pg.5**.

C. Cervical sample for HPV testing only (by Roche Real Time PCR – method):

- 1. Materials needed for collection and preparation:
 - a. Laboratory Test Order Form (Cytology)
 - b. ThinPrep collection vial prefilled with alcohol fixative (*PreservCyt*[®] Solution).
- 2. Specimen collection and transport:
 - a. Fill out the Laboratory Test Order Form completely (refer to **section III-C, pg.13**).
 - b. Label the collection vial with both the patient and physician's full name **before** the procedure begins (refer to **section III-B, pg.12**).
 - c. Follow the collection/preparation instructions included in the collection kit.
 - d. Place properly sealed Digene Collection Tube into to a Biohazard specimen bag and include the completed Laboratory Test Order Form(s) in the outside pocket of the bag.
 - e. Place specimen bag(s) in the designated drop-off area for courier pick-up and delivery. **NOTE:** If specimen requires **Special pick-up**, please contact Dispatch, phone #'s on **pg.5**.

D. Cervical Sample for GC & Chlamydia testing only (by DNA/PCR –method)

- 1. Materials needed for collection and preparation:
 - a. Laboratory Test Order Form (Cytology)
 - b. *APTIMA*[®] *Specimen Collection Kit*.
- 2. Specimen collection and transport:
 - a. Fill out the Laboratory Test Order Form completely (refer to **section III-C, pg.13**).
 - b. Label the collection tube with both the patient and physician's full name **before** the procedure begins (refer to **section III-B, pg.12**).
 - c. Follow the collection/preparation instructions included in the collection kit.
 - d. Place properly capped *APTIMA*[®] specimen tube into to a Biohazard specimen bag, include the completed Laboratory Test Order Form(s) in the outside pocket of the bag, keep at room temperature.



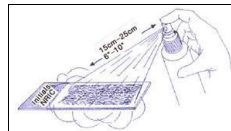
CYTOLOGY SPECIMEN COLLECTION MANUAL

- d. Place specimen bag(s) in the designated drop-off area for courier pick-up and delivery.

NOTE: If specimen requires **Special pick-up**, please contact Dispatch, phone #'s on **pg.5**.

D. Vaginal Pool Sample:

1. Materials needed for collection and preparation:
 - a. Laboratory Test Order Form (Cytology)
 - b. Frosted-end glass slide
 - c. Cytology spray-fixative in a bottle (pump or aerosol)
 - d. Plastic/cardboard slide holder OR Plastic slide box for transport of smears
 - e. Wooden Spatula (i.e. Ayre's)
2. Specimen collection and transport:
 - a. Fill out the Laboratory Test Order Form completely (refer to **section III-C, pg.13**).
 - b. Label frosted end of the glass slide using a pencil **before** the procedure begins (refer to **section III-A, pg.12**).
 - c. Use the blunt end of the spatula to collect mucus from the posterior vaginal fornix.
 - d. Working carefully, but quickly, spread the material in a thin, even layer over the length of the glass slide.
 - e. **FIX IMMEDIATELY** by applying Cytology spray-fixative evenly over the smear.
Do not allow the specimen to air-dry without fixative.



Note: The use of hairspray is NOT recommended due to variation in composition; its use may result in very poor specimen preservation.

- f. Allow the fixed smear to dry completely before packing for transport.
- g. Place dry, spray-fixed smear(s) into plastic/cardboard slide holder for transport.
 - (i) Secure cardboard slide holder(s) with scotch tape or rubber bands to prevent opening during transport.
- h. Place the holder/container(s) into a Biohazard specimen bag and include the completed Laboratory Test Order Form(s) in the outside pocket of the bag.
- i. Place specimen bag(s) in the designated drop-off area for courier pick-up and delivery.

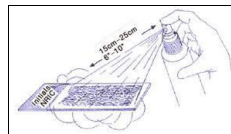
NOTE: If specimen requires **Special pick-up**, please contact Dispatch, phone #'s on **pg.5**.



CYTOLOGY SPECIMEN COLLECTION MANUAL

F. Lesions of the Vagina or Vulva:

1. Materials needed for collection and preparation:
 - a. Laboratory Test Order Form (Cytology)
 - b. Frosted-end glass slide
 - c. Cytology spray-fixative in a bottle (pump or aerosol)
 - d. Plastic/cardboard slide holder OR Plastic slide box for transport of smears
 - e. Wooden Spatula (i.e. Ayre's)
2. Specimen collection and transport:
 - a. Fill out the Laboratory Test Order Form completely (refer to **section III-C, pg.13**).
 - b. Label frosted end of the glass slide using a pencil **before** the procedure begins (refer to **section III-A, pg.12**).
 - c. Use the spatula to scrape the lesion.
 - d. Working carefully, but quickly, spread the material in a thin, even layer over the length of the glass slide.
 - e. **FIX IMMEDIATELY** by applying Cytology spray-fixative evenly over the smear.
Do not allow the specimen to air-dry without fixative.



Note: The use of hairspray is NOT recommended due to variation in composition; its use may result in very poor specimen preservation.

- f. Allow the fixed smear to dry completely before packing for transport.
- g. Place dry, spray-fixed smear(s) into plastic/cardboard slide holder for transport.
 - (i) Secure cardboard slide holder(s) with scotch tape or rubber bands to prevent opening during transport.
- h. Place the holder/container(s) into a Biohazard specimen bag and include the completed Laboratory Test Order Form(s) in the outside pocket of the bag.
- i. Place specimen bag(s) in the designated drop-off area for courier pick-up and delivery.

NOTE: If specimen requires **Special pick-up**, please contact Dispatch, phone #'s on **pg.5**.



CYTOLOGY SPECIMEN COLLECTION MANUAL

VI. COLLECTION AND PREPARATION OF NON-GYNECOLOGIC SPECIMENS

A. Body Fluids (Ascitic/Peritoneal, Pleural, Pericardial, Synovial, Vitreous, Cyst-except Breast)

1. Materials needed for collection and preparation:
 - a. Laboratory Test Order Form (Cytology)
 - b. Sterile, leak-proof container or sterile vacuum bottle
 - c. Sterile heparin
2. Specimen collection and transport:
 - a. Fill out the Laboratory Test Order Form completely (refer to **section III-C, p.13**).
 - b. Label the collection container **before** the procedure begins (refer to **section III-B, p.12**).
 - c. Collect body fluid in the labeled, sterile container or sterile vacuum bottle.
 - d. **PLEASE READ CAREFULLY**

Add 2000 units of heparin per 100 ml of fluid (mixing well) to the following specimens **ONLY**:

- (i) Ascitic/Peritoneal fluid
- (ii) Pleural fluid
- (iii) Pericardial fluid

NOTE: Clotted specimens will be rejected!

- e. The specimen may then be handled by either method listed below:

Preferred method – Submit fresh, unfixed specimen:

DO NOT ADD ANY FIXATIVE TO THE SPECIMEN

Alternative method – Submit specimen fixed in CytoLyt or Non-gyn collection fluid:

- (i) Add a volume of fixative to the specimen such that the ratio of fixative to specimen is 2:1; 2 fixative to 1 specimen.
 - (ii) On the container and requisition, notate the type of fixative that has been added to the specimen.
- f. Seal the container well, to prevent leaking.
 - g. Place the labeled container into a Biohazard specimen bag and include the completed Laboratory Test Order Form in the outside pocket of the bag.
 - h. Send the entire specimen to the laboratory, ASAP.

*****REFRIGERATE FRESH/UNFIXED SPECIMEN IF IMMEDIATE DELIVERY TO LAB/CYTOLOGY IS NOT POSSIBLE*****

NOTE: If specimen requires **Special pick-up**, please contact Dispatch, phone #'s on **pg.5**.



CYTOLOGY SPECIMEN COLLECTION MANUAL

B. Pelvic/Peritoneal Washings

1. Materials needed for collection and preparation:
 - a. Laboratory Test Order Form (Cytology)
 - b. Sterile, normal saline
 - c. Sterile, leak-proof container
2. Specimen collection and transport:
 - a. Fill out the Laboratory Test Order Form completely (refer to **section III-C, pg.13**).
 - b. Label the collection container **before** the procedure begins (refer to **section III-B, pg.12**).
 - c. Collect the saline washing in the labeled, sterile container.
 - d. **The specimen may then be handled by either method listed below:**
Preferred method – Submit fresh, unfixed specimen:
DO NOT ADD ANY FIXATIVE TO THE SPECIMEN
Alternative method – Submit specimen fixed in Cytolyt or Non-gyn collection fluid:
 - (i) Add a volume of fixative to the specimen such that the ratio of fixative to specimen is 2:1; 2 fixative to 1 specimen.
 - (ii) On the container and requisition, notate the type of fixative that has been added to the specimen.
 - e. Seal the container well, to prevent leaking.
 - f. Place the labeled container into a Biohazard specimen bag and include the completed Laboratory Test Order Form in the outside pocket of the bag.
 - g. Send the entire specimen to the laboratory, ASAP.

*****REFRIGERATE FRESH/UNFIXED SPECIMEN IF IMMEDIATE DELIVERY TO LAB/CYTOLOGY IS NOT POSSIBLE*****

NOTE: If specimen requires **Special pick-up**, please contact Dispatch, phone #'s on **pg.5**.



CYTOLOGY SPECIMEN COLLECTION MANUAL

C. Urine, Bladder cancer profile by FISH (UroVysion) and Bladder/Ureter/Kidney Washings and Ductal Lavages

1. Materials needed for collection and preparation:
 - a. Laboratory Test Order Form (Cytology)
 - b. Sterile, normal saline
 - c. Sterile, leak-proof container
2. Specimen collection and transport:
 - a. Fill out the Laboratory Test Order Form completely (refer to **section III-C, pg.13**).
 - b. Label the collection container **before** the procedure begins (refer to **section III-B, pg.12**).
 - c. Collect the fluid in the labeled, sterile container.

NOTE, for URINE COLLECTION:

Collect **freshly** voided, **SECOND-MORNING CLEAN CATCH** urine.

Due to rapid cellular degeneration (within an hour)¹, first morning urine **is not suitable** for Cytologic examination. Discard the first morning urine and collect a second-morning clean catch specimen.

*NOTE, for urine cytology with **Bladder cancer profile by FISH**, please submit a minimum of 60 mL of urine, refrigerated within 24 hours.*

- d. The specimen may then be handled by either method listed below:

*Preferred method – Submit fresh, refrigerated, unfixed specimen immediately:
DO NOT ADD ANY FIXATIVE TO THE SPECIMEN*

Alternative method – Submit specimen fixed in PreserveCyt or 2% Carbowax collection fluid:

- (i) Add a volume of fixative to the specimen such that the ratio is 2:1; 2 urine to 1 fixative.
 - (ii) On the container and requisition, notate the type of fixative that has been added to the specimen.
- e. Seal the container well, to prevent leaking.
- f. Place the labeled container into a Biohazard specimen bag and include the completed Laboratory Test Order Form in the outside pocket of the bag.
- g. Send the entire specimen to the laboratory, ASAP.

*****REFRIGERATE FRESH/UNFIXED SPECIMEN IF IMMEDIATE DELIVERY TO LAB/CYTOLOGY IS NOT POSSIBLE*****

NOTE: If specimen requires **Special pick-up**, please contact Dispatch, phone #'s on **pg.5**.



CYTOLOGY SPECIMEN COLLECTION MANUAL

D. Bronchial, Esophageal, Gastric, and Colonic Brushings and Washings

Brushing Specimen:

1. Materials needed for collection and preparation:
 - a. Laboratory Test Order Form (Cytology)
 - b. Sterile, leak-proof container
 - c. Sterile, normal saline
 - d. Wire cutter/scissors for cutting fiber optic brush
 - e. *For Alternative collection method (for Neighbor Island sites):*
 - (i) **2** Frosted-end slides
 - (ii) Plastic, slide holder container prefilled with alcohol fixative
OR
Cytology spray-fixative in a bottle and cardboard slide holder.
2. Specimen collection and transport:
 - a. Fill out the Laboratory Test Order Form completely (refer to **section III-C, pg.13**).
 - b. Label the collection container **before** the procedure begins (refer to **section III-B, pg.12**).

Then, fill the container with approximately 20cc of sterile, normal saline before the procedure begins.

For ALTERNATIVE collection method:

Label 2 frosted-end slides and the plastic, slide holder container **before** the procedure begins.
 - c. **After sampling, the specimen may be handled by either method listed below:**

Preferred method – Submit fresh, unfixed specimen:

 - (i) Retract the fiber optic brush back into the plastic sheath and cut the brush approximately 3 inches long.

Immediately immerse the brush into the labeled container of saline to prevent drying of the specimen. If necessary, add more sterile saline to the container.

DO NOT ADD ANY FIXATIVE TO THE SPECIMEN



CYTOLOGY SPECIMEN COLLECTION MANUAL

Alternative method – *Submit Physician prepared, fixed smears along with the remaining brush specimen (for cell block preparation):*

- (i) Immediately immerse the brush into the labeled container of saline.
- (ii) *Use the sterile saline to keep the brush moist at all times.*

Working on one slide at a time, gently roll the fiber optic brush across the slide (covering at least $\frac{3}{4}$ of the slide surface) spreading the specimen in a **THIN**, even layer.



- (iii) **FIX IMMEDIATELY** by immersing the slide into the plastic slide holder container filled with alcohol **OR** by applying Cytology spray –fixative evenly over the smear. *Do not allow the specimen to air-dry without fixative*

Label the spray-fixed smear with “**Fixed**”, on the frosted end.

- (iv) Once the smears are complete, retract the brush back into the plastic sheath and cut the brush approximately 3 inches long.

Immediately immerse the brush into the labeled container of saline to prevent drying. If necessary, add more sterile saline to the container.

DO NOT ADD ANY FIXATIVE TO THE SPECIMEN

- d. Seal the specimen container and slide holder (if applicable) well, to prevent leaking.
- e. Place the labeled container and slide holder (if applicable) into a Biohazard specimen bag and the completed Laboratory Test Order Form in the outside pocket of the bag.
- f. Send the entire specimen to the laboratory, ASAP.

*****REFRIGERATE FRESH/UNFIXED SPECIMEN IF IMMEDIATE DELIVERY TO LAB/CYTOLOGY IS NOT POSSIBLE*****

NOTE: If specimen requires **Special pick-up**, please contact Dispatch, phone #'s on **pg.5**.

Washing Specimen:

1. Materials needed for collection and preparation:
 - a. Laboratory Test Order Form (Cytology)
 - b. Sterile, leak-proof container
 - c. Sterile, normal saline.
 - d. *For Alternative collection method (for Neighbor Island sites):*
 - (i) CytoLyt fixative or Non-gyn collection fluid



CYTOLOGY SPECIMEN COLLECTION MANUAL

2. Specimen collection and transport:

- a. Fill out the Laboratory Test Order Form completely (refer to **section III-C, pg.13**).
- b. Label the collection container **before** the procedure begins (refer to **section III-B, pg.12**).
- c. Collect the saline washing in the labeled, sterile container.
- d. **The specimen may then be handled by either method listed below:**

Preferred method – Submit fresh, unfixed specimen:

DO NOT ADD ANY FIXATIVE TO THE SPECIMEN

Alternative method – Submit specimen fixed in CytoLyt or Non-gyn collection fluid:

- (i) Add a volume of fixative to the specimen such that the ratio of fixative to specimen is 2:1; 2 fixative to 1 specimen.
 - (ii) On the container and requisition, notate the type of fixative that has been added to the specimen.
- e. Seal the container well, to prevent leaking.
 - f. Place the labeled container into a Biohazard specimen bag and include the completed Laboratory Test Order Form in the outside pocket of the bag.
 - g. Send the entire specimen to the laboratory, ASAP.

*****REFRIGERATE FRESH/UNFIXED SPECIMEN IF IMMEDIATE DELIVERY TO LAB/CYTOLOGY IS NOT POSSIBLE*****

NOTE: If specimen requires **Special pick-up**, please contact Dispatch, phone #'s on **pg.5**.

E. Cerebrospinal (CSF) and Ventricular Fluids

1. Materials needed for collection and preparation:

- a. Laboratory Test Order Form (Cytology)
- b. Sterile, leak-proof container or sterile collection tube

2. Specimen collection and transport:

- a. Fill out the Laboratory Test Order Form completely (refer to **section III-C, pg.13**).
- b. Label the collection container **before** the procedure begins (refer to **section III-B, pg.12**).
- c. Collect a minimum of 1.0 ml of fluid in the labeled, sterile container.
DO NOT ADD ANY FIXATIVE TO THE SPECIMEN
- d. Seal the container well, to prevent leaking.



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- e. Place the labeled container into a Biohazard specimen bag and include the completed Laboratory Test Order Form in the outside pocket of the bag.
- f. Send the entire specimen to the laboratory, **STAT**.
The specimen must be processed immediately to assure well-preserved cells for evaluation.

*****REFRIGERATE FRESH/UNFIXED SPECIMEN IF IMMEDIATE DELIVERY TO LAB/CYTOLOGY IS NOT POSSIBLE*****

NOTE: If specimen requires **Special pick-up**, please contact Dispatch, phone #'s on **pg.5**.



F. Fine Needle Aspirations

1. Materials needed for collection and preparation:
 - a. Laboratory Test Order Form (Cytology)
 - b. Frosted-end glass slides
 - c. Plastic, leak-proof slide container with alcohol fixative or Cytology spray-fixative
 - d. Cardboard slide holder
 - e. 16cc plastic vial/tube prefilled with CytoLyt fixative or Non-gyn collection fluid.
 - f. 22 gauge-1 ½” or smaller needle (e.g. 23 gauge-1”, 25) and syringe.
2. Specimen collection and transport:
 - a. Fill out the Laboratory Test Order Form completely (refer to **section III-C, pg.13**).
 - b. Label a **minimum of 4** frosted-end glass slides (**maximum of 12**) and a 16cc plastic tube with fixative **before** the procedure begins (refer to **sections III-A,B, pg.12**).

NOTE: Prepare a minimum of 2 “mirror” image sets of smears for each FNA site (i.e. 2 fixed and 2 air-dried smears).

 - c. Perform the puncture.

Cystic Masses

- (i) If a cyst is encountered during aspiration, evacuate all fluid from the cyst.
- (ii) Remove the needle from the mass.

IMPORTANT: The needle should never be removed while any negative pressure is applied to the syringe.



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(iii) The specimen may then be handled by either method listed below:

Preferred method – Submit fresh, unfixed specimen:

(a) Carefully detach the needle from the syringe and place a sterile cap on the syringe.

DO NOT ADD ANY FIXATIVE TO THE SPECIMEN

Alternative method – Submit specimen fixed in CytoLyt or Non-gyn collection fluid:

(a) Express the evacuated fluid into the labeled tube containing fixative.

If the volume of fixative to specimen is less than 2:1, transfer the specimen to a larger sterile container and add more fixative.

(b) Then, aspirate ~2cc of fixative into the syringe, through the needle, to “rinse” the needle and syringe of any remaining specimen. Express into the collection tube, 2x.

(c) On the tube and requisition, notate the type of fixative that has been added to the specimen.

NOTE for performing multiple “passes”:

Any additional cystic fluid that is aspirated from the same specimen site may be:

- added to the original collection container
- aspirated into a new syringe and labeled appropriately
(refer to III-B, pg. 12)

However, if fluid is aspirated from “new” cystic sites (other than the original location), use a new needle, syringe, and collection tube.

(iv) Perform a second aspiration on any residual mass, using a new needle, syringe, and collection tube.

Follow the collection and transport procedure listed below for “Solid Masses”.

(v) Seal the tube/container well, to prevent leaking.



CYTOLOGY SPECIMEN COLLECTION MANUAL

Solid Masses

- (i) Remove the needle from the mass.

IMPORTANT: The needle should never be removed while any negative pressure is applied to the syringe.

- (ii) The specimen may then be handled by either method listed below:

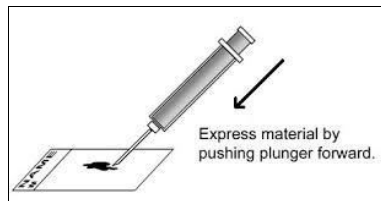
Preferred method – Submit Physician prepared, fixed and air-dried smears with needle rinse material/fluid (for cell block preparation):

- (a) After the needle is removed from the mass, **carefully** detach it from the syringe.
- (b) Pull back on the plunger, drawing air into the syringe, then reattach the needle.

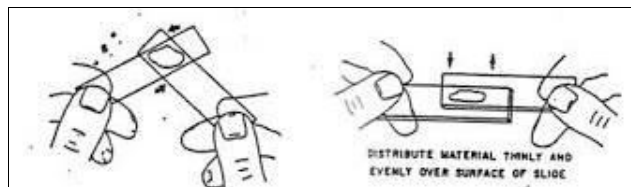
Be careful not to draw any existing specimen up into the syringe.

- (c) Place the beveled edge of the needle against the surface of the glass slide.
- (d) Push gently on the plunger and express a small amount of specimen (no larger than the size of a grain of rice) onto the center of the slide. If too much material is expressed onto the slide, slightly withdraw the syringe plunger to re-aspirate the extra material.

Expressing too much material will result in a suboptimal preparation; the smear will be excessively thick.



- (e) Immediately place a 2nd glass slide, face down, on top of the specimen on the 1st slide. Exert gentle pressure to spread the specimen, then quickly lift the top slide up & off—creating a set of “mirror-imaged” slides.





CYTOLOGY SPECIMEN COLLECTION MANUAL

- (f) **FIX IMMEDIATELY** – place **ONE** of the **two smears** into the plastic slide holder container with alcohol fixative **OR** by apply Cytology spray-fixative evenly over the smear.

Do not allow the specimen to air-dry without fixative.



OR



- (g) Label the fixed smear with "**Fixed**", on the frosted end.
- (h) Allow the remaining "mirror-imaged" slide to "air-dry" completely. Label the "air-dried" smear with "**AD**", on the frosted end before placing it in the cardboard slide holder for transport.
- (h) Repeat steps (c) thru (h) for a 2nd set of smears.

NOTE: If smears from the 1st slide set appear to have scant material, **DO NOT MAKE** an additional sets of smears.

- (j) Then, aspirate ~2cc of fixative into the syringe, through the needle, to "rinse" the needle and syringe of any remaining specimen. Express into the collection tube; 2x.



If the volume of fixative to specimen is less than 2:1, transfer the specimen to a larger container and add more fixative.

- (k) On the tube and requisition, notate the type of fixative that has been added to the specimen.

Alternative method – *Submit fresh, unfixed specimen in a syringe (with the needle attached):*

- (a) Carefully recap the needle, leaving it attached to the syringe.
DO NOT ADD ANY FIXATIVE TO THE SPECIMEN
- d. Seal all containers, syringes, and slide holders well, to prevent leaking.
- e. Place all labeled containers/syringes/slide holders into a Biohazard specimen bag and include the completed Laboratory Test Order Form in the outside pocket of the bag.



CYTOLOGY SPECIMEN COLLECTION MANUAL

- f. Send the entire specimen to the laboratory, **STAT**.

*****REFRIGERATE FRESH/UNFIXED SPECIMEN IF IMMEDIATE DELIVERY TO LAB/CYTOLOGY IS NOT POSSIBLE*****

NOTE: If specimen requires **Special pick-up**, please contact Dispatch, phone #'s on **pg.5**.

G. Breast/Nipple Secretions

1. Materials needed for collection and preparation:

- a. Frosted-end glass slide(s)
- b. Cytology spray-fixative in a bottle (pump or aerosol)
- c. Cardboard slide holder

2. Specimen collection and transport:

- a. Fill out the Laboratory Test Order Form completely (refer to **section III-C, pg.13**).
- b. Label frosted-end of the glass slide using a pencil **before** the procedure begins (refer to **section III-A, pg.12**).

NOTE: The number of smears will depend on the amount of secretion available for examination.

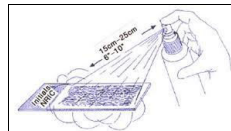
- c. Using the thumb and forefinger, gently express the nipple and subareolar area. Allow only a small drop (“pea-size” or smaller) to collect on the nipple¹.

NOTE: If no secretion appears, do not manipulate further.

- d. Immobilize the breast and use the nipple to smear the material in a thin, even layer over the length of the glass slide¹.

- e. **FIX IMMEDIATELY** by applying Cytology spray-fixative evenly over the smear.

Do not allow the specimen to air-dry without fixative.



Note: *The use of hairspray is NOT recommended due to variation in composition; its use may result in very poor specimen preservation.*

- f. Label the fixed smear with “**Fixed**” on the frosted end.
- g. Allow the spray-fixed smear to dry completely before packing for transport.
- h. Place dry, spray-fixed smear(s) into plastic/cardboard slide holder for transport.
 - (i) Secure cardboard slide holder(s) with scotch tape or rubber bands to prevent opening during transport.



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- i. Place the holder/container(s) into a Biohazard specimen bag and include the completed Laboratory Test Order Form(s) in the outside pocket of the bag.
- j. Place specimen bag(s) in the designated drop-off area for courier pick-up and delivery.

NOTE: If specimen requires **Special pick-up**, please contact Dispatch, phone #'s on **pg.5**.

H. Touch Preparations/Imprints¹

Touch preparations/imprints may be obtained from the following sources:

- Surgical specimens
- Core-needle biopsy specimens
- Lymph nodes
- Exposed body surfaces

1. Materials needed for collection and preparation:

- a. **2** Frosted-end glass slides
- b. Cytology spray-fixative in a bottle (pump or aerosol) or Plastic, leak-proof slide container with alcohol fixative
- c. Cardboard slide holder

2. Specimen collection and transport:

- a. Fill out the Laboratory Test Order Form completely (refer to **section III-C, pg.13**).
- b. Label frosted-end of the glass slide using a pencil **before** the procedure begins (refer to **section III-A, pg.12**).
- c. Obtain wet tissue sample for touch preparation/imprint.
- d. Gently touch the wet tissue sample to the surface of the 1st labeled glass slide. Place slide aside and allow to air dry.
- e. Then, gently touch the wet tissue sample to the surface of the 2nd labeled glass slide.

FIX IMMEDIATELY by applying Cytology spray-fixative evenly over the smear or by immersing the slide into the plastic slide holder container with alcohol fixative.

Do not allow the specimen to air-dry without fixative.



OR



Note: *The use of hairspray is NOT recommended due to variation in composition; its use may result in very poor specimen preservation.*



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- f. Label the air dried slide with “**AD**”, on the frosted end.
Label the 2nd, fixed slide with “**Fixed**”, on the frosted end.
- g. Let air-dried and spray-fixed smears dry completely before packing for transport.
- h. Place dry smear(s) into plastic/cardboard slide holder for transport.
 - (i) Secure cardboard slide holder(s) with scotch tape or rubber bands to prevent opening during transport.
- i. Place the holder/container(s) into a Biohazard specimen bag and include the completed Laboratory Test Order Form(s) in the outside pocket of the bag.
- j. Place specimen bag(s) in the designated drop-off area for courier pick-up and delivery.

NOTE: If specimen requires **Special pick-up**, please contact Dispatch, phone #'s on **pg.5**.

I. Sputum (Early AM or 24-hour)

1. Materials needed for collection and preparation:
 - a. Laboratory Test Order Form (Cytology)
 - b. Sterile, plastic leak proof container (without fixative)
 - c. Collection instructions (for Early AM or 24-hr)

NOTE: Hospital floors, Laboratory sites, and Clinic offices should maintain an on-site, accessible supply of sputum collection material and instructions.

Please contact **CLH-Supplies** at phone: (808) 677-7999.

2. Specimen collection and transport:
 - a. Fill out the Laboratory Test Order Form completely (refer to **section III-C, pg.13**).
 - b. Label the collection container **before** the procedure begins (refer to **section III-B, pg.12**).
 - c. Instruct the patient to collect the sputum specimen according to the following:
 - (i) Upon awakening, clear your throat of any material, which may have accumulated overnight, and discard it.
 - (a) Perform oral hygiene duties (i.e. brush teeth, etc.).
 - (b) Gargle and rinse your mouth with warm water several times.
 - (ii) **DO NOT EAT BREAKFAST** (food particles will distort test results).
 - (iii) Cough deeply several times during the first hour that you are awake.

This is best accomplished by **sitting up straight, breathing deeply, and coughing forcefully**.

REMEMBER: FOR BETTER RESULTS, YOU MUST PROVIDE SPUTUM FROM YOUR LUNGS, NOT SALIVA FROM YOUR MOUTH.



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- (iv) Expectorate (spit) all material (ideally 4-5 tablespoons) into the specimen collection cup (up to the first hour that you are awake).

SPECIAL NOTE:

- Patients who cannot produce sputum spontaneously should be referred to the Respiratory Therapy Department for an induced sputum collection.
- d. When collection is complete, secure the cover on the specimen container, to prevent leaking.
- e. Place the labeled container into a Biohazard specimen bag and include the completed Laboratory Test order Form in the outside pocket of the bag.
- f. Send the entire specimen to the laboratory, **ASAP**.

*****REFRIGERATE FRESH/UNFIXED SPECIMEN IF IMMEDIATE DELIVERY TO LAB/CYTOLOGY IS NOT POSSIBLE*****

NOTE: If specimen requires **Special pick-up**, please contact Dispatch, phone #'s on **pg.5**.

J. Tzanck Smears (for Viral Inclusions)

1. Material needed for collection and preparation:
 - a. Laboratory Test Order Form (Cytology)
 - b. Frosted-end glass slide
 - c. Cytology spray-fixative in a bottle (pump or aerosol)
 - d. Plastic/cardboard slide holder
2. Specimen collection and transport:
 - a. Fill out the Laboratory Test Order Form completely (refer to **section III-C, pg.13**).
 - b. Label the collection container **before** the procedure begins (refer to **section III-B, pg.12**).
 - c. Pre-moisten the suspect lesion with saline. If possible, select a fresh vesicle that has not ruptured and crusted^{1,5}.
 - d. Using a disposable needle, carefully expose a fresh vesicle or crusted lesion^{1,5}.
 - e. Using the edge of a spatula, scalpel blade, or glass slide, carefully scrape the lesion along its margin (the edges will yield cells with the best morphologically recognizable inclusions)^{1,5}.

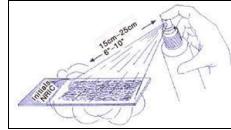
Do Not Use a cotton swab or similar material to sample because diagnostic cells may become trapped within the fiber matrix.
 - f. Working carefully, but quickly, spread the material in a thin, even layer over the length of the glass slide.



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- g. **FIX IMMEDIATELY** by applying Cytology spray-fixative over the length of the glass slide.

Do not allow the specimen to air-dry without fixative.



Note: The use of hairspray is NOT recommended due to variation in composition; its use may result in very poor specimen preservation.

- h. Allow the fixed smear to dry completely before packing for transport.
- i. Place dry, spray-fixed smear(s) into plastic/cardboard slide holder for transport.
- (i) Secure cardboard slide holder(s) with scotch tape or rubber bands to prevent opening during transport.
- h. Place the holder/container(s) into a Biohazard specimen bag and include the completed Laboratory Test Order Form(s) in the outside pocket of the bag.
- i. Place specimen bag(s) in the designated drop-off area for courier pick-up and delivery.

NOTE: If specimen requires **Special pick-up**, please contact Dispatch, phone #'s on **pg.5**.

K. Anal Pap Test and Anal HPV Test

ThinPrep® Anal Pap Test:

- Materials needed for collection and preparation:
 - Laboratory Test Order Form (Cytology)
 - ThinPrep collection vial prefilled with alcohol fixative (*PreservCyt*® Solution).
 - Dacron™ Swab or Cytobrush
- Specimen collection and transport:
 - No special preparation is needed for the patient, though the patient may be advised to refrain from receptive anal intercourse or the use of intra-anal preparations before collection.
 - Fill out the Laboratory Test Order Form completely (refer to **section III-C, pg.13**).
 - Label the collection container **before** the procedure begins (refer to **section III-B, pg.12**).
 - Collect the sample with the patient in either the lateral recumbent or dorsal lithotomy position



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- e. Insert a tap water-moistened Dacron™ swab about 5-6 cm into the anal canal, past the anal verge, into the rectal vault. This is done without direct visualization of the anal canal
- f. Apply firm lateral pressure to the swab handle as it is rotated and slowly withdrawn from the anal canal, inscribing a cone-shaped arc.
Care should be taken to ensure that the transition zone is sampled.
- g. Push the swab/brush against the wall of the vial while stirring the solution 10 times, then, swirl the swab/brush vigorously to further release cells.
- h. Discard the broom after rinsing.
- i. Cap the vial, making sure that the torque line on the cap passes the matching line on the vial.
- j. Place the properly sealed ThinPrep collection vial(s) into a Biohazard specimen bag and include the completed Laboratory Test Order Form(s) in the outside pocket of the bag.
- k. Place the specimen bag(s) in the designated drop-off area for courier pick-up and delivery.

NOTE: If specimen requires **Special pick-up**, please contact Dispatch, phone #'s on **pg.5**.

Note: A swab or smear of the peri-anal skin is an unsatisfactory sample for Anal Pap and HPV tests. Avoid using cotton swabs on a wooden stick because the handle may break and splinter during collection.

Digene® Anal HPV Test:

1. Materials needed for collection and preparation:
 - a. Laboratory Test Order Form (Cytology)
 - b. Digene *hc₂* DNA Collection Kit
2. Specimen collection and transport:
 - a. Fill out the Laboratory Test Order Form completely (refer to **section III-C, pg.13**).
 - b. Label the collection vial with both the patient and physician's full name **before** the procedure begins (refer to **section III-B, pg.12**).
 - c. Follow the collection/preparation instructions included in the collection kit.
 - d. Place properly sealed Digene Collection Tube into to a Biohazard specimen bag and include the completed Laboratory Test Order Form(s) in the outside pocket of the bag.
 - e. Place specimen bag(s) in the designated drop-off area for courier pick-up and delivery.

NOTE: If specimen requires **Special pick-up**, please contact Dispatch, phone #'s on **pg.5**.



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L. **Other Specimens (not listed above)**

1. Please contact CLH-Cytology Department at phone: (808) 834 5125 for instructions before attempting to collect specimens that are not listed above.

VII. **REFERENCES**

1. NCCLS. *Nongynecologic Cytologic Specimens: Collection and Cytopreparatory Techniques; Approved Guideline*. NCCLS document GP23-A [ISBN 1-56238-380-9]. NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 1999.
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4. Keebler CM, Somrak TM. *The Manual of Cytotechnology*. 7th ed. Chicago, IL: ASCP Press. 1993.
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6. NCCLS. *Papanicolaou Technique Approved Guidelines*. NCCLS document GP15-A. NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA.
7. Lee et al. *Comparison of Conventional Papanicolaou Smears and a Fluid-Based, Thin-Layer System for Cervical Cancer Screening*. *Ob Gyn* 1997; 90:278-284.
8. *Cytyc Corporation ThinPrep Pap Test Training Bulletin*. © 2000, Cytyc Corporation. Part no. 85662-001, Rev. A. 85 Swanson Road, Boxborough, MA 01719.

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