

GUIDELINES FOR OBTAINING AN ADEQUATE GYN CYTOLOGY SAMPLE (PAP TEST)

The following guidelines are based on the NCCLS Document GP15-A: Papanicolaou Technique; Approved Guideline. Permission to excerpt portions of GP15-A has been granted by NCCLS.

INTRODUCTION:

Every person possesses her own pattern or distribution of mucosal epithelium of the cervix. At birth the location of the junction between the native squamous epithelium that covers the portio vaginalis and the native columnar epithelium that lines the portio endocervicalis is not at the same level in all individuals. Pregnancy, hormonal changes after puberty, vaginal pH, menopause, and prior therapy, in addition to individual anatomy, are factors that induce gradual change or “transformation” of the exposed endocervical “zone”. The original thin, translucent single layer of mucinous columnar cells covering the native endocervical zone is gradually transformed by a process of squamous metaplasia to one of stratified squamous epithelium, e.g. the transformation zone.

As the islands of squamous metaplasia coalesce and mature, the new squamo-columnar junction gradually advances upward toward the external os. By the time of menopause the mature metaplastic zone has usually reached the area of the external os. The additional factors of the ‘indrawing’ and other postmenopausal structural changes in the cervix enhance the tendency for little or no endocervical mucosa to remain exposed.

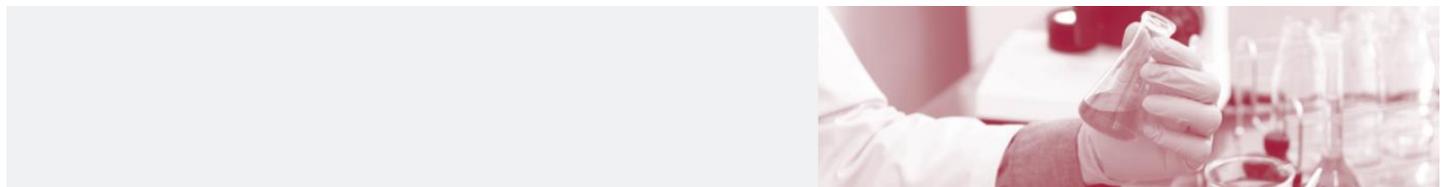
ADEQUATE SAMPLE:

The majority of all squamous epithelial abnormalities (cancerous and precancerous) of the cervix arise in the transformation zone. It is the transformation zone (not the endocervix above it) that is the primary target for representative sampling of the cervix by the Pap technique. The presence of squamous metaplasia on the Pap is the most useful indicator that the original endocervical zone or transformation zone has been sampled. The presence of intact endocervical cells indicates that the upper end of the zone has been sampled, not necessarily the target area or transformation zone.

Squamous metaplasia usually progresses to a mature benign state. Atypia may arise within this zone as reflected by the minor abnormality “atypical squamous cells of undetermined significance” (ASC-US) or progress on to “low grade squamous intraepithelial lesion” (LGSIL) or “high grade squamous intraepithelial lesion” (HGSIL).

The type of cervix being examined directly affects the adequacy of the technique utilized. The specific sampling instruments and the sampling technique used should be based on individual patient anatomy, paying particular attention to the location and configuration of the transformation zone.

Sampling instruments include the cervical spatula, endocervical brush, and broom (papette).

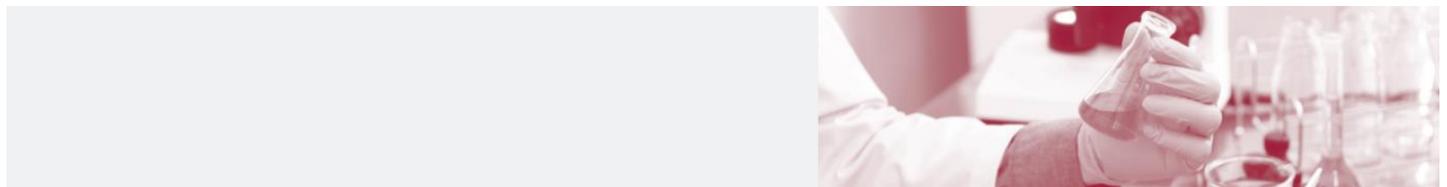


THINPREP PAP TEST COLLECTION GUIDELINES

Proper sample collection is very important, especially proper rinsing of the devices in the ThinPrep vial. Also, do NOT leave the collection device in the vial.

Physicians Laboratory provides handouts detailing proper collection techniques for the endocervical brush/spatula collection devices, the broom-like device, and the Rovers Cervex-Brush Combi device. These handouts are located in the Client Services section of our website (www.plpath.com), under Collection Instructions and may be downloaded and printed there.

1. Ideally, the Pap test should be taken two weeks after the first day of the menstrual period. It should not be performed during menstruation. If it must be taken then, first carefully clean the cervix of any excess blood with a clean cotton swab.
2. Label all materials with the patient's name and DOB. This includes the vial or slides if doing a conventional smear. The name should be written on the frosted end of the slide with a pencil and the sample should be spread on the same side.
3. Universal precautions should be observed while performing a Pap test. Cytological specimens are considered infectious until fixed with a germicidal fixative.
4. The patient is placed in the dorsolithotomy position.
5. The speculum should be warmed to body temperature using warm running water. Saline may be used to moisten the instrument. Lubricant interferes with the adherence of cellular material to the slide and also with proper staining of the cells. It should be avoided as much as possible. If it must be used, avoid using those containing ingredients known as "carbomers" or "carbopol polymers" which are known to interfere with the test.
Also, apply the lubricant sparingly on the outer portion of the speculum taking great care to avoid the tip.
Lubricants approved by Hologic are Surgilube, Surgel, Pap Test Lubricating Jelly (PP024) and KY Jelly.
6. The cervix should be visually inspected for abnormalities. Identify the transformation zone, if visible, and direct sampling to this area. If an elevated, ulcerated, necrotic or exudate-covered lesion is observed biopsy should follow the cytology sample.
7. The spatula with the contoured end best conforming to the anatomy of the cervix should be used. Rotate the spatula 360 degrees around the circumference of the cervical os and ectocervix, while maintaining firm contact with the epithelial surface. A clockwise rotation beginning and ending at 9 o'clock, or a counterclockwise rotation at the 3 o'clock position, will retain collected material on the upper horizontal surface as the instrument is removed.



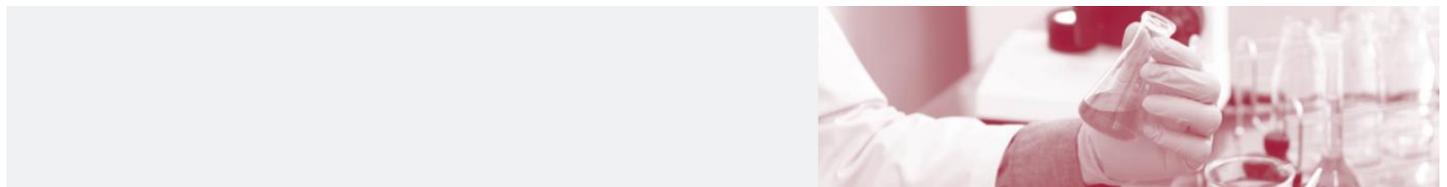
8. *ThinPrep collection:* Rinse the spatula as quickly as possible in the ThinPrep vial by swirling it vigorously 10 times. Discard the spatula.
Conventional Pap collection: Hold the spatula between the fingers of the non-sampling hand with the specimen face-up, while the cervical brush material is collected without delay.
9. The cervical brush is inserted into the os with gentle pressure until only the bottom-most fibers are exposed. Slowly rotate 1/4 to 1/2 turn in one direction. **DO NOT OVER-ROTATE.** If using a broom 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 5 times. The combi-brush should be rotated in a clockwise direction 2 times.
10. *ThinPrep Collection:* Rinse the brush/broom as quickly as possible in the Thin Prep vial by rotating the device in the solution 10 times while pushing it against the vial wall. Swirl it vigorously to further release material. Discard the device.
Conventional Pap Collection: The material from the spatula should be spread evenly over the slide with a single smooth stroke motion. Roll the brush across the slide by twirling the handle. Transfer the material from both sampling instruments to the slide within a few seconds and fix **IMMEDIATELY** in order to avoid air-drying artifact.

Note: It is best to collect the endocervical cell sample last as these cells are the most fragile and susceptible to air-drying.

It is important to have a uniform spread of the sample as the fixative will most easily penetrate and fix the cells, the staining will be more uniform, and 100% of the sample is viewable for assessment.
11. Tighten the vial cap and place all materials (with any slides in plastic slide holders) in a biohazard bag along with a properly filled out Gyn requisition.

FIXATION FOR CONVENTIONAL SMEARS

Smears should be fixed immediately with either a pump spray fixative or 95% alcohol. Air-drying for even a few seconds should be avoided prior to fixation. If using a spray hold it 15-25cm from the slide. Allow it to dry 10 minutes after spraying before placing in plastic slide holders. Smears may also be fixed in 95% alcohol for 20-30 minutes and then removed, dried, and placed in plastic slide holders.



THINPREP IMAGING SYSTEM

Physicians Laboratory cytotechnologists screen slides with the assistance of the Hologic ThinPrep Imaging System. This system uses a dual screening approach by combining expert human review with the power of an interactive Imaging system that scans every cell and cell cluster.

The system consists of an Image Processor and Review Scopes. Computer imaging technology is used to assist cytotechnologists in primary cervical cancer screening of ThinPrep Pap specimens. The system increases sensitivity and improves specificity to increase the likelihood that abnormalities will be found.

ONE VIAL, MULTIPLE TESTS

HR HPV TESTING

Physicians Laboratory uses Hologic's FDA-approved Aptima methodology for High Risk HPV detection and 16 and 18/45 genotyping. This test can be performed from the same vial used to collect the Pap. The Aptima test detects the 14 most common high risk HPV types, including specific testing for the presence of types 16 or 18/45 if requested. Reports are sent when all testing is completed.

Specimens for Aptima HPV testing must be cervical/vaginal specimens collected in a ThinPrep vial. Please call Physicians Laboratory Client Services if you are unsure about any other specimen types or testing requirements.

TESTING OPTIONS

Physicians Laboratory endorses the latest screening guidelines published in the American Journal of Clinical Pathology. We offer several different options on our requisition for ordering HPV testing. The following combinations are available:

- If ASC-US do HPV testing. (If the Pap result ends up being ASC-US then we will perform the testing)
- Perform HPV co-testing (HPV + Pap)

The option to reflex any positive result to 16 and 18/45 genotyping is also given.

HPV testing may also be ordered following the Pap report. Orders must be received within three weeks of specimen collection. An order form is provided on our website under Client Services, and can be faxed to 1-605-322-7053. A signature on the form is required to perform the testing. An amended report with results will be sent.





CHLAMYDIA TRACHOMATIS AND NEISSERIA GONORRHEA TESTING

Physicians Laboratory uses the Aptima Combo 2 assay for Chlamydia and Gonorrhea testing.

Orders for Chlamydia and Gonorrhea (CT/NG) testing **MUST** be made at the time of the ThinPrep Pap specimen collection to avoid any possibility of contamination during processing. If the tests are desired and the order was missed the specimen will need to be re-collected. PL will do solely the CT/NG testing if desired (no Pap screening). Be sure to check the order box if either or both tests are needed. Results will be included in the Pap report.

TRICHOMONAS VAGINALIS TESTING

Orders for Trichomonas testing **MUST** be made at the time of the ThinPrep Pap specimen collection to avoid any possibility of contamination during processing. PL will do solely the TV testing if desired (no Pap screening). Results will be included in the Pap report.

