

INSTRUCTIONAL MANUAL
CAT. #26750 Series
EMS Rapid Pro Processing System

Protocol: Cytology

Electron Microscopy Sciences

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Cytology Protocol

Cytologic studies include:

- Papanicolaou smear (Pap test, GYN cytology)
- Fluid cytology
- Sputum cytology
- Fine needle biopsy

Cytologic samples are cellular suspensions, which are spread, smeared or centrifuged on to glass microscope slides. These are immediately fixed with ERPP solution either ERPP-1 or ERPP-3 and subsequently stained for morphologic evaluation. The optimal sample is a preparation consisting of a monolayer of viable cells with immediate (rapid) fixation. Specimens, which are routinely processed for cytology include: cervical and endocervical smears of the gynecologic tract (Pap smears); fine needle biopsies of all body sites; cerebrospinal fluids; pleural effusions; peritoneal effusions; peritoneal washes; urines; and scrapings or brushing from either skin, respiratory or gastrointestinal sites. Classically cytologic samples are divided into those that are exfoliative cytology and aspiration cytology.

EMS Rapid Pro Processing Solutions Used in the Following Protocols

EMS Catalog Number	Solutions Used
26750-01	ERPPS-1 (10-12% formalin)
26750-03	ERPPS-3 (95% ethanol)

Papanicolaou Smear

Purpose

The purpose of obtaining a Pap test (a scraping of cells from the uterine cervix) is to detect cervical cancer and its precursors. Inflammation, reparative changes and other abnormalities of the reproductive tract may also be detected during the examination of the specimen.

Patient Preparation

Obtaining the specimen for Pap smear is usually done during routine or diagnostic pelvic examinations. Any individual preparing for medical examination may experience anxiety over the possibility of an abnormal finding.

Obtaining the Specimen

All materials used to obtain the Papanicolaou test should be ready prior to the collection of the sample.

Supplies to Include

- Cervical spatula
- Endocervical brush
- Glass slide or pap vial with collection fluid
- Spray fixative
- Slide holder

The glass slide or vial should be labelled with the patient's name and date before obtaining the specimen. The vaginal speculum should be placed using only water for lubrication (jellies can obscure the cytologic test). If a woman is over 40 years old, a vaginal pool specimen should be obtained with the blunt end of the spatula and handed to the assistant. In all women, the ectocervical sample should be obtained by firmly rotating the spatula 360 degrees against the visible portion of the cervix. The mucus plug should be removed from the endocervical canal prior to sampling with the endocervical brush. The endocervical brush should be inserted into the endocervical canal and rotated 180 degrees.

Preparing the Sample

There are two acceptable methods for preparing a Pap test. The goal is to prepare the sample as quickly as possible. If making a smear, an even distribution of cellular material should be attempted and fix the specimen immediately.

Method A: Conventional

1. Obtain the endocervical brush specimen.
2. Cover one-half of the glass slide with the paper towel or a piece of paper.
3. Smear the brush over the uncovered area evenly.
4. Immediately spray fix the smeared sample keeping one-half of the glass slide covered, holding the spray fixative 10 inches from the slide.
5. Uncover the remainder of the slide. Smear the ectocervical sample on the unfixed portion (avoiding the frosted end).
6. Immediately spray the remainder of the slide.

Method B: Conventional

1. Obtain cervical brush specimen.
2. Either hand the brush to an assistant or place the brush on the glass slide near (not on) the frosted end. Do not smear.
3. Obtain the ectocervical sample.
4. Spread the specimen evenly toward the end of the slide.
5. Spray fix immediately holding the spray fixative 10 inches from the slide.

Slide Fixation

Fixatives are agents used to prevent cell distortion and to maintain cellular detail. Improper fixation usually renders a smear uninterruptible and accurate evaluations of cell populations are precluded.

1. Fixation should be done immediately after the specimen has been spread on to the slide.
2. DO NOT WAIT until after the examination is complete. Spray fixatives are ERPP solutions, either ERPPS-1 (formalin) or ERPPS-3 (95% alcohol), which cover the surface of the prepared smears. Actual fixation occurs when the specimen is processed in the laboratory.
3. When using a spray fixative, the nozzle of pump spray should be held 10 - 12 inches from the slide.

NOTE: Holding the spray fixative too close may blow cells off the slide or cause cellular artifacts rendering the slides less useful for diagnosis.

NOTE: Holding the spray fixative too far from the slide may result in inadequate fixation with air-drying artifact.
4. The specimen should be allowed to dry prior to placement into mailing packages. If the slide does not dry, it will adhere to the cardboard or plastic holders in transit.
5. Use either ERPPS-1 or ERPPS-3.

NOTE: Both are ideal cellular fixative for all cytologic smears.
6. The freshly prepared smear is immediately immersed into either ERPPS-1 or ERPPS-3.
7. Fixation occurs within 1-2 minutes. Immersing the smear for a longer period of time will not injure the cells.

Non-Gynecologic Cytology

Non-gynecologic cytology is the morphologic evaluation of fine needle biopsies, body cavity fluids, washings, scrapings, brushings, and urine specimens. The primary goal of non-gynecologic cytology examination is to detect malignancy. The non-gynecologic requisition form must be filled out entirely. Absence of information will limit the ability of the cytologist to fully evaluate the specimen.

Sputum Cytology Sampling Conditions

- Early morning deep cough specimens are the most cellular and most representative of the respiratory regions.
- The oral cavity should be rinsed thoroughly prior to collection of the specimen.
- A preservative agent should be added immediately, if a fresh specimen cannot be taken to the Cytology Laboratory.
- An adequate sputum evaluation for cancer consists of three consecutive early morning samples. A single post-bronchoscopy deep cough specimen may be of great diagnostic value.

Breast Secretions

Nipple discharge or breast secretions can be obtained to evaluate the presence or absence of malignant diseases of the breast.

Supplies to Include

- Ten frosted slides labeled with the patient's name and hospital number should be available.
- ERPPS-1 or ERPPS-3 solution

Patient Preparation

The nipple should be cleansed with an alcohol sponge. The breast should be stripped and the initial breast secretions discarded. Lactiferous sinuses (near the nipple) often hold secretions for a long period of time and contain degenerated cells.

NOTE: Initial expressions from the nipple should always be discarded prior to making smears.

Obtaining Specimen

1. After the initial secretions are discarded, allow a drop to accumulate.
2. Support the areola and nipple with one hand, and with the other hand place a slide upon the nipple, pause to allow material to spread laterally, and then draw the slide quickly across the nipple.
3. Immediately drop the slide in a solution of either ERPPS-1 or ERPPS-3. *Do not air dry.*
4. The procedure is repeated using ten slides.

NOTE: It is important to make this many slides because when carcinoma is present, the last slides in the series are usually diagnostic.

Surface Scraping Cytology (Skin or Mucosal Scrapings)

Cytologic samples of these slides are taken to rule out the possibility of malignancy or infection when biopsy of the area is not desirable.

Supplies to Include

- Glass slides with frosted ends labeled with the patient's name and hospital number.
- ERPPS-1 or ERPPS-3 solution
- Wooden spatula, or endocervical brush
- Glove with gauze

Sampling

1. A moistened gauze square of single thickness over gloved finger or endocervical brush or a moistened wooden spatula should be used for sampling (moistening the spatula is essential to avoid air drying).

NOTE: Endocervical brushes may be most useful for mucosal lesions. Keratotic lesions should be abraded to remove much of the surface keratin. Stop abrading if small bleeding points appear.

2. Using fresh gauze, brush or spatula to sample, smear the specimen on the slide. Vesicular lesions should be ruptured and the base sampled (vesicle fluid is not acceptable due to degeneration). "Red" lesions are sampled without special preparations.
3. The specimen is immediately smeared on the glass slide using a single stroke.
4. Then, immediately plunge slide into ERPPS-1 or ERPPS-3 solution.

Protocol for Conditions and Collecting Various Types of Specimens

Brushing and Washing Specimens

These specimens are obtained by washing cells from surfaces or abrading surfaces with small brushes. Classically, this involves the gastrointestinal and respiratory tracts and peritoneal cavity.

- In general, all samples must be processed using balanced salt solution; washings are generally attained after brushing.
- All samples should be transported rapidly to the laboratory.
- Brushes should be immediately submerged in 10 to 16 milliliters of balanced salt solution in a sterile container. The brush should be agitated briskly in the fluid and clipped off into the container. (Leave two inches of cable in the brush.)
- All specimens must be labelled as to the specific site, especially if several areas are sampled.

Respiratory Tracts Brushing and Washings

- These specimens are usually acquired via fiber optic bronchoscopy.
- The brush must be removed through the scope with retraction into the protective sheath.
- The brush should be submitted in a solution of either ERPPS-1) or ERPPS-3, leaving at least one inch of cable above the brush.
- Brushing specimens should be obtained prior to cutting (forceps) biopsies and followed by saline wash.
- These should be submitted fresh to the laboratory as soon as possible.

Gastrointestinal Tract

- Gastric and esophageal brushes and washes are the most common type of gastrointestinal tract specimen.
- Both are acquired by endoscopy, however, washings may sometimes be obtained by nasogastric tube.
- Any washing from the esophagus or gastric area must be placed on ice and delivered immediately to the laboratory to prevent degeneration, which occurs due to the natural acidity of gastric contents.
- Esophageal and gastric brushing should be placed in a solution of either ERPPS-1 or ERPPS-3 and sent to the laboratory.
- Colonic and intestinal samples must be preceded by meticulous colon preparation.

Peritoneal Washings

- These samples are usually used for staging of abdominal neoplastic disease, especially of gynecologic origin.
- After washing with a balanced salt solution or saline, the specimen should be submitted immediately to the cytology laboratory for processing.

Urinary Bladder

- Urinary bladder washings or arbitrage are recommended for morphologic evaluation in suspected cases of transitional cell carcinoma.
- Because degenerative changes may occur, such washings should be submitted immediately to the cytology laboratory.

Fine Needle Biopsy

The fine needle aspiration biopsy is a rapid, inexpensive, simple cytologic technique that can be done on an outpatient basis.

Supplies to Include

- Several 10 ml disposable plastic syringes,
- 4-6 23 or 25 gauge needles
- An FN handle
- Alcohol swabs
- Either solution: ERPPS-1 or ERPPS-3
- 8 - 12 clean glass microscope slides
- Local anesthesia (1 % solution of Xylocaine, Lidocaine or other anesthesia) is used for skin infiltration.
- Physiologic saline solutions or a small vial of fixative may be used for needle rinses.

Obtaining a Palpable Fine Needle Aspirate

1. The skin is cleansed using an alcohol swab.
2. A small amount of local anesthetic is infiltrated into the skin and subcutaneous tissue.
3. There are two accepted methods for obtaining fine needle cytologic specimens: the "aspirate" technique and the "no-aspirate" technique.

Performing an Aspiration

1. Insert a syringe, with a 25-gauge needle, into a lesion.
2. Apply negative pressure and move the needle back and forth within the lesion, with a slight redirection of the needle during each motion.
3. Release needle pressure prior to removing needle from the skin.
4. Withdraw needle.
5. Hand the syringe and needle to the cytotechnologist, who then expresses the cells and smears them between two slides.
6. A single slide is air-dried and the other is immediately plunged into ERPPS-1 or ERPPS-3.
7. Needles may be rinsed with a saline solution or fixative for cell block preparation.
8. This is repeated four to six times until the lesion is well sampled.

Performing a "No Aspirate" Technique

A "no aspirates" may also be performed if preferred by the operator.

1. A 25-gauge needle without a syringe is placed within the lesion after patient preparation and anesthesia.
2. Without covering the hub, the needle is moved back and forth sharply, redirecting the tip with each movement.
3. The needle is withdrawn and the contents expelled on to a slide and processed in a similar manner as an "aspirate" technique above.
4. Four to six needle punctures are performed until the lesion is well sampled.

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