CYTOLOGY

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PRINCIPLE:

The purpose of cytologic examination of body fluids, lavages, aspirations, and scrapings is to determine the nature of the cells present within the specimen for diagnostic and potential therapeutic treatment. This examination is facilitated by using direct smear and concentration techniques to optimize recovery of material. A multitude of stains and ancillary tests may also be performed on this material to assist in diagnosis. A variety of quality control measures are in place to assure continuity of reliable diagnoses.

REAGENTS:

1) Frosted-end glass slides.
2) Cardboard carrier.
3) Spray fixative (Baxter Cat.#S7741-2).
4) Collin jars with 95% alcohol.
5) Plasmalyte, isolyte, RPMI or sterile saline.
6) Thromboplastin C and normal plasma.
7) Cytospin funnels with filters.
8) Thin Prep Preservcyte Vials.

INSTRUMENTATION:

1) Cytospin centrifuge.
2) IEC centrifuge.
3) Germ-free fume hood.
4) Cytyc Thin Prep 2000.
5) Vortex.

SPECIMEN COLLECTION AND SMEAR PREP:

A. GYNECOLOGIC – CONVENTIONAL:

1. Prepare patient and collect specimen in the usual and accepted procedure by appropriate and qualified medical personnel.
2. Smear the specimen from collection utensil in appropriate fashion (i.e., Brush-roll onto slide; Spatula-smear onto slide; Broom-one pass vertically down the slide).
3. Immediately spray slides 10-12 inches away with spray fixative or place immediately in 95% alcohol.
4. Label each slide with the patient's name and place in cardboard carrier. Label the carrier with proper patient and special identification.
5. Fill out requisition properly with the patient's name, physician, date of birth, last menstrual period and the date the specimen was collected.
6. Deliver the specimen to the laboratory with the proper cytology requisition.
7. Log in and assign a Cytology-GYN (P) accession number.
8. Stain all the slides with Papanicolaou separately from non-gyn slides on the TissueTek DRS.

B. GYNECOLOGIC – THIN PREP:

1. Prepare the patient and collect the specimen using the spatula, brush or broom in the usual and accepted procedure by appropriate and qualified medical personnel.
2. Transfer the specimen to the pink Thin Prep Preservcyte sample vial. Swish the collection device several times to dislodge the sample.
3. Label the vial with the patient's name, doctor, and the date.
4. Fill out the requisition slip properly with the patient's name, physician, date of birth, last menstrual period and the date the specimen was collected.
5. Deliver the specimen to the laboratory with the proper cytology requisition slip.
6. Log in and assign a Cytology-GYN (T) accession number.
7. Process the sample according to the Thin Prep protocol.
8. Stain all the slides (conventional and Thin Prep) on the Tissue-Tek DRS according to the correct specimen type.
9. Coverslip and place the Thin Prep slides on the Cytyc Imager.

C. SPUTUM – CONVENTIONAL: (See Thin Prep protocol for detailed instructions on alternate procedure).

1. Collect the specimen in a sterile expectorate container.
2. Collect prior to breakfast and after washing the mouth with water.
3. Have the patient produce an early morning deep cough directly into expectorate container.
4. Label the container with the proper patient and specimen identification.
5. Keep the specimen refrigerated until delivery to the laboratory.
6. Log in and assign Cytology Non-GYN accession number.
7. Record volume and appearance on the requisition slip.
8. Add Cytolyte solution and vortex for 10 minutes.
9. Centrifuge at 2600 RPM for 5 minutes and discard supernatant.
10. Re-suspend the button and place one drop on a labeled glass slide.

11. Place another clean slide over the drop and gently spread over the labeled slide yielding an even layer. Allow this slide to completely air dry and stain with DiffQuick.
12. Proceed with Thin Prep protocol for additional slide to stain with Papanicolaou.

D. BODY CAVITY: (thoracentesis, pericardial, paracentesis, cul-de-sac, etc. Conventional (see Thin Prep protocol for detailed instructions on alternate procedure).

1. Prepare the patient and collect the specimen in the usual and accepted procedure by appropriate qualified medical personnel.
2. Label the container (bag) with the proper patient and specimen identification.
3. Keep the specimen refrigerated until delivery to the laboratory.
4. Log in and assign a Cytology Non-GYN accession number.
5. Record total volume and general appearance.
6. Mix the specimen well by gentle inversion and transfer representative volume (100 mls.) into sterile conical tubes for centrifugation. Label with accession number. NOTE: If specimen has a low cellularity, additional conical tubes will need to be centrifuged.
7. Centrifuge for 5-10 minutes at 2600 RPM and decant supernatant.
8. Label 1 glass slide with the Cytology accession number.
9. Re-suspend button and place a drop on a labeled slide.
10. Place another slide over the drop and gently spread over the labeled slide yielding an even layer. Allow to completely air dry.
11. Proceed with Thin Prep protocol for additional slide to stain with Papanicolaou.
12. Continue with “cell block” preparation on remaining sediment.
13. Stain the alcohol fixed Thin Prep slide with Papanicolaou and the air-dried slides with Diff Quick.

E. BRONCHIAL WASHINGS/LAVAGES: (See Thin Prep protocol for detailed instructions immediately following Cytology section).

1. Prepare patient and collect specimen in the usual and accepted procedure by appropriate qualified medical personnel.
2. Label the container with proper patient and specimen identification.
3. Keep the specimen refrigerated until delivery to the laboratory.
4. Log in and assign a Cytology Non-GYN accession number.
5. Record total volume and general appearance.
6. Transfer representative or total volume aseptically to a sterile conical tube for centrifugation. Label with accession number.
7. Centrifuge for 5-10 minutes at 2600 RPM and decant supernatant.
8. Observe sediment. If blood is grossly visible, follow with Cytolyte solution and vortex treatment.
9. Label 1 glass slide with the Cytology accession number.
10. Re-suspend button and place a drop on a labeled slide.
11. Place another slide over the drop and gently spread over the labeled slide yielding an even layer. Allow to completely air dry.
12. Prepare Thin Prep slide and place in 95% alcohol.
13. Continue with “cell block” preparation on remaining sediment.

14. Stain the alcohol fixed Thin Prep slide with Papanicolaou and the air-dried slides with Diff Quick.

NOTE: Smears for Pneumocysitis carinii (PCP) are allowed to air dry, then fixed with 95% alcohol prior to staining with GMS and AFB procedure.

F. BRUSHINGS: (esophageal, bronchial, gastric, renal, etc.)

1. Prepare patient and collect specimen in the usual and accepted procedure by appropriate qualified medical personnel.
2. Cut the disposable brush and place directly into vial of plasmalyte, isolyte, sterile saline, or RPMI.
3. Label the vial with proper patient and specimen identification.
4. Deliver the specimen with proper requisition to the laboratory ASAP.
5. Log in and assign a Cytology Non-GYN accession number.
6. Shake the vial with brush vigorously to detach the cellular material. Discard brush in sharps container. Teasing with a fresh scalpel blade may be necessary to dislodge tissue.
7. Prepare Thin Prep slide and place in 95% alcohol for Papanicolaou staining and one Cytospin slide air dried for DiffQuick.

G. CYST FLUIDS:

1. Aspirate cyst fluids aseptically. Remove needle and replace with syringe hub.
2. Label the syringe with proper patient and specimen identification
3. Deliver the specimen with proper requisition to the laboratory ASAP.
4. Log in and assign a Cytology Non-GYN accession number.
5. Record total volume and appearance.
6. Transfer specimen to sterile conical tubes for centrifugation and label with accession number.
7. Centrifuge for 5-10 minutes at 2600 RPM and decant supernatant.
8. Prepare Thin Prep slide and place in 95% alcohol.
9. Stain the alcohol fixed smears with Papanicolaou.

H. CEBEBRAL SPINAL FLUID (CSF):

1. Prepare patient and collect specimen in the usual and accepted procedure by appropriate qualified medical personnel.
2. Place specimen in sterile conical tubes.
3. Label the tubes with proper patient and specimen identification.
4. Deliver the specimen with proper requisition to the laboratory ASAP.
5. Log in and assign a Cytology Non-GYN accession number.
6. Record total volume and appearance.
7. Prepare Thin Prep slide and place in 95% alcohol.
8. Stain the alcohol fixed smears with Papanicolaou.

I. URINE, BLADDER, RENAL PELVIC AND URETERAL WASHINGS:

1. Collect *voided* urine as follows:
   a. Hydrate the patient by having him/her drink as much water as possible for 1½ to 2 hours.
   a. Have the patient void the urine and discard the urine at will during the period of hydration.
   b. Have the patient void the urine and discard it at the end of the hydration period.
   c. Collect the next voided urine (about ½ hour later), in a sterile specimen container. Collect the specimen fresh and unfixed.
   d. Collect 50-100 mls. of urine.
   e. Label the container with proper patient and specimen identification.
   f. Deliver the specimen with proper requisition to the laboratory ASAP and place in the refrigerator or keep it refrigerated until it can be delivered.
   g. Repeat for three successive days if clinically indicated.

NOTE: If the patient cannot be hydrated, send random voided urines. Never send 24-hour urine or first morning urine:

2. Collect *catheterized* urine as follows:
   a. Hydrate the patient with several glasses of water and exercise the patient if possible.
   b. Collect the specimen from the catheter directly into a sterile specimen container.
   c. Collect specimen after a good urine flow is established.
   d. Do not submit urine obtained at the time of initial catheterization since it has been in the bladder for sometime and contains only degenerated cells.
   e. Collect 50-100 mls. of fresh, unfixed specimen.
   f. Label the proper container with specimen and patient identification.
   g. Deliver the specimen with proper requisition to the laboratory ASAP and place in the refrigerator or keep it refrigerated until it can be delivered.
3. Collect *bladder washings*, *cystoscopic urine, renal pelvic washings*, and ureteral washings as follows:
   a. Have the patient void the urine.
   b. Inject electrolyte solution into the bladder and retrieve.
   c. Collect 50-100 mls.of fresh, unfixed specimen in a sterile container.
   d. Label the proper container with specimen and patient identification.
   e. Deliver the specimen with proper requisition to the laboratory ASAP or keep it refrigerated until it can be delivered.
4. Log in and assign Cytology Non-GYN accession number.
5. Record total volume and appearance.
6. Transfer representative volume (100 mls.) into sterile conical tubes for centrifugation. Label the accession number.
7. Centrifuge 5-10 minutes at 2600 RPM and decant supernatant.
8. Prepare a Cytospin slide and allow to air dry.
9. Prepare a Thin Prep slide and place in 95% alcohol.

10. Stain the air-dried slide with DiffQuik and the alcohol fixed slide with Papanicolaou.

J. BREAST SECRETIONS:

1. Label glass slides with patient and specimen identification.
2. Have the patient hold a coplin jar with 95% alcohol below breast.
3. Have the patient gently stroke sub-areolar area and nipple using thumb and forefinger. Allow only a drop, the size of a pea, to accumulate on the apex of the nipple.
4. Place a slide upon the nipple touching the drop of secretion and allowing it to spread a little laterally. Then draw the slide quickly across the nipple.
5. Immediately place the slide in 95% alcohol coplin jar or spray fix.
6. Repeat procedure and allow a slide to air-dry and place in cardboard carrier.
7. Continue above steps until all the secretions obtainable are utilized.
8. Deliver the specimen with proper Cytology requisition to the laboratory ASAP.
9. Log in and assign Cytology Non-GYN accession number.
10. Stain the alcohol fixed smears with Papanicolaou and the air-dried smear with DiffQuick.

K. FNA: Refer to procedures for pathologist and radiology guided FNA's.

The Pathology Laboratory

I have performed the annual review of this Procedure

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Signature_____ Date_____