1.0 **TITLE:** PROCEDURE FOR NONE-GYNE SPECIMEN

2.0 **PRECAUTIONS**
PPE should be worn at all times when handling potentially infectious body fluids in accordance with the KFHU Safety Policy. All preparatory procedures should be performed using a face shield. All infectious materials should be properly disposed of in bags identified for biological or infectious wastes for proper disposal.

3.0 **PROCEDURES FOR SPECIMEN FIXATION:**
All specimens processed in cytology should be fixed before staining.

   **Fixatives Used in Cytopathology**
   1. 80% isopropanol (800ml of absolute isopropanol + 200ml of water)
   2. Spraycyte fixative (Commercially prepared)
   3. Bouin’s fixative for cell block (prepared by Histopathology Section)

4.0 **PROCEDURE CENTRIFUGATION AND PROCESSING OF FLUID SPECIMENS**
Centrifugation can be done for any of the three purposes:
   1. To obtain a cell concentrate (pellet), from which to make cell spreads.
   2. To separate cells from their debris-laden solution, prior to filtration.
   3. To form a cell concentrate, or "button", for Cell block preparation

4.1 **Reagents, Instrumentation:** Vial with specimen. Centrifuge, tubes, Pasteur Pipettes.
4.2 **Step-by-step Description**
Specimen labels are checked against data on the Request Form for accuracy. All request forms are given a unique sequential number, and then logged in the computer. If a patient has a previous cytology or histology report the laboratory number and the result are noted.

   1. Note down the volume, color and consistency of each specimen at the upper right hand corner of the request form.
   2. Centrifuge the specimen in 15-25 cc centrifuges tubes (depending on the volume of the specimen) and centrifuge at the 1800- 2500 revolutions per minute (RMP) for 10 minutes.
   3. Remove the supernatant with the help of a Pasteur pipette.
   4. Place one or two drops from the sediment or the buffy coat on the pre-labeled frosted end glass slides and spread evenly with the help of another clean glass slides.
5. Fix them immediately with spraycyte or dip in 80% isopropanol/95% ethanol.
6. Stain them with Papanicolaou technique and mount.

4.3 Effect of centrifugation on Cells
Although of little observable significance in exfoliative cytology, several effects of centrifugation on the quality and quantity of cells have been described. Reduction in numbers of cells always occurs. It may be caused by one of the following factors:

4.3.1 Rupture of delicate, senescent, or degenerate cells by shear stresses.
4.3.2 Impaction along the wall of the centrifuge tube, or "walled effect".
4.3.3 Inadequate centrifugal force, thereby causing some cells to suspend in the supernatant.
4.3.4 Damage to the cellular morphology by centrifugal force is seen primarily in the cytoplasm of the cells. Cells thus damaged can exhibit ragged cytoplasmic borders. In turn, these borders can cause cells to clump secondarily. The presence of protein in some specimens largely protects against these effects.

4.4 Report of Results: Specimen is screened by senior cytotechnologist and submitted to the consultant cytopathologist for reporting.

4.5 Maintaining of Centrifuges/Cytospin
4.5.1 Daily Cleaning Cleaning outer surfaces and if there is ugly spillage, immediately clean with hospicide spray/10% chlorox. Dry bucket before use. Switch off centrifuge before cleaning.
4.5.2 Weekly Cleaning and monthly Remove buckets – clean the following: bowl, carriages, inside cover, external surfaces with hospicide spray/10% chlorox – rinse ē water and dry before use.
4.5.3 Changing Carbon Brush if applicable. Maintained by BioMed Department

<table>
<thead>
<tr>
<th>Speed Check</th>
<th>Speed</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Centrifuge machine (Heraeus)</td>
<td>2000 rpm</td>
<td>10 minutes</td>
</tr>
<tr>
<td>Centrifuge machine (Hettich)</td>
<td>2000 rpm</td>
<td>10 minutes</td>
</tr>
<tr>
<td>Cytospin Shandon</td>
<td>800 rpm</td>
<td>3 minutes</td>
</tr>
</tbody>
</table>

· Precautions: Close the lid before start. Never use with open lid.

5.0 PROCEDURE CYTOSPIN PREPARATIONS:
5.1 Material and Equipment
Biohazard hood
Gloves (Nitrite)
Cytocentrifuge
Pencil
5.2 Step by step procedure for smear preparation

5.2.1 The volume and description of the specimen is noted in the corresponding numbered request form. This number should be indicated in the specially-made slides for the Cytospin i.e., slides with white rings. It should be noted that the slides are cleaned with alcohol prior to use.

5.2.2 Switch the Cytospin on, open the lid, and lift the head assembly out of the instrument before loading the samples. The head assembly can then be opened by pulling up the central button. The slide clips are then removed from the head assembly and loaded with the glass slide-filter paper-sample chamber combination and seeing to it that the outlet port coincides with the filter card holes and the pre-drawn circle on the slide. Once checked, this slide assembly is then securely held by the retaining hooks of the slide clips. This assembly should be made in duplicate and place in opposite slots of the head assembly to balance.

5.2.3 Three to five drops of the sample are placed inside in each of the sample chambers and the head assembly being closed securely. The head containing the samples is then loaded in the center cone of the machine. With the safety cover in position, and locked; the correct speed program is entered, and centrifugation is started. A built-in timer and actual speed indicator enables the operator to monitor the centrifugation process, and alarms to indicate speed-drop or cycle completion.

5.2.4 The slide assembly is separated from each other with the filter card detached from the slide in a slow peeling motion to prevent smudge in other areas of the slide other than inside the drawn circle.

5.2.5 The prepared slides are immediately sprayed with Spraycyte to fix. These are then allowed to dry for 5 minutes and ready to stain. The Cytospin 4 is a special instrument which concentrates small number of cells in minute volume. It uses centrifugal force to spin cell suspensions and sediments unto a glass slide with the medium being absorbed by a filter card. The result is a monolayer of cells spread within a 6.0 mm. area on the slide.

6.0 PROCEDURE SPECIMEN WITH BRUSHES

6.1 Specimen Required
Smears are made from Esophageal, gastric and bronchial brush specimens in the clinics. Alternatively these can be sent fresh, in small amounts of saline or RPMI solution, just enough to cover the brush.

All specimens are sent fresh to the cytology laboratory

6.3 Step-by-step Description
6.3.1 Transfer the specimen to an appropriate centrifuge tube, preferably (15-25 ml).
6.3.2 Agitate the sample (vortex) with the brush on it.
6.3.3 Centrifuge at 2,000 rpm for 10 minutes.
6.3.4 Remove the brush from the centrifuge tube with forceps. Discard the brush in the biohazard container.
6.3.5 Pour off supernatant.
6.3.6 Agitate the cell pellet (vortex).
6.3.7 Proceed with the appropriate smear preparation.

7.0 PROCEDURE TO ELIMINATE RED BLOOD CELLS FROM CYTOLOGIC SPECIMENS BY HEMOLYZING FIXATIVE (CYTORICH RED):
- Mix/shake the sample well.
- Pour a representative amount into a properly labeled 50-ml centrifuge tube.
- Centrifuge the sample for 10 minutes at 2000 rpm.
- Decant the supernatant fluid and add 25 ml of cytorich red fluid.
- Cap the tube and vortex for 10-15 seconds.
- Allow the sample to fix and lyse for at least 15 minutes.
- Process specimen according to lab preparation technique.

Precautions
- Use PPE.
- Follow biohazard precautions when handling samples.

8.0 PROCEDURE FOR CELL BLOCK
This procedure utilizes histologic procedures for processing cytologic material, thus allowing multiple sections of the same material for use with routine as well as special stain application. This technique can be applied to all cytologic specimens but is especially useful for fluids and aspirates or whenever tissue fragments are incidentally obtained during cytologic procedure. Cell blocking procedure should be prepared whenever it is needed especially in a specimen with clot and substantial amount of cell pellet after the smear preparation.

Precautions
8.1 Solution: Bouin’s solution

8.2 Materials
- Cassette
- Spatula/applicator stick
- Pipette/forceps
- 25-50 mL centrifuge tube/universal container
- Lens paper/filter paper

8.3 Step by step procedure
1. Sediments and tissue fragments are placed in Bouin’s fixative. It should be noted that fibrin clots on the side of the fibrin container are gently twisted and removed by applicator stick with fluid wrung out with the trapped cells in the mentioned fixative. Allow to stand for at least two (2) hours to fix (optional).
2. Centrifuge at 1,800-2,500 RPM for 10 minutes.
3. Pour off supernatant or remove supernatant by draining the tube on the absorbent paper leaving the cell button or sediment.
4. Carefully remove the sediment from the centrifuge tube using a spatula/applicator stick and wrap in lens paper/filter paper. The wrapped sediments are then placed in tissue cassette with their corresponding cytology accession number. (Use pencil only).
5. Take specimen to histopathology for processing.

SPECIAL PROCEDURE FOR CERTAIN SAMPLES

9.0 PROCEDURE FOR RESPIRATORY TRACT SPECIMEN (sputum, brush, lavage):

9.1 Materials
1. Biohazard hood
2. PPE
3. Frosted end microscopic slides
4. Pencil
9.2 Reagents:
   1. Spraycyte
   2. 80% isopropanol
   3. Papanicolaou stain

9.3 Samples (Sputum, Bronchial brush, Bronchial lavage)

9.4 Procedures: (Processing)
Procedure for Preparation & fixation of slides will be different for each type of specimens - Details are given below:

9.4.1 Bronchial Brush:
Smears are prepared and fixed in bronchoscopy room and sent to the cytology lab- for processing and reporting.

9.4.2 Bronchial Lavage:
Specimen is centrifuged at 2,000 RPM for 10 minutes and smears prepared from the sediment as from fluids & fixed immediately with spraycyte or 80% isopropanol.

9.4.3 Sputum:
Sputum is a mixture of saliva and secretions form the lower respiratory tract. In addition, nasopharyngeal mucus may be admixed with the specimen. Therefore, some specimens designated as “sputum” may in fact be only nasopharyngeal material. The tracheobronchial tree presents a very large area for sampling, and mucus from different locations in the patient’s lower respiratory tract may possess different gross physical characteristics within a given specimen. For this reason, material from sputum smears should be selected by picking out small portions of the material with different color and texture. These small samples are pooled on one slide and smeared together. Such a selective sampling procedure is not, of course, necessary for specimens that appear grossly homogeneous.
The following steps are to be used in the preparation of sputum specimens for microscopic examination:

1. Specimen must be fresh or prefixed sputum.
2. Label two slides with the patient’s name, accession number and source on the frosted end of the slide.
3. Select suspicious particles from the sample.
4. Transfer particle to the slide.
5. Gently crush sample between 2 slides, distributing material thinly and evenly over surface of the slide. (See diagram).

FIGURE 33-4. Diagrammatic representation of steps in the preparation of smears from sputum.
Note:
Thick, slippery of tenacious specimens are difficult to smear into the proper finished product and must be manipulated for some length of time before the material begins to spread properly and adhere to the slide evenly. Specimens that are thin or watery, on the other hand require only a minimum of time to smear.

6. Spray fix or fix slide(s) immediately in 80% isopropanol for a minimum of 10 minutes.

Note:
Some specimens do not adhere to the slide when placed in fixative and consequently the specimen will float away. When this happens, additional smears should be prepared if possible and spray fix and leave to dry before staining.

10.0 PROCEDURE VOIED URINE, CATHETERIZED URINE OR BLADDER WASH.
1. Note the general appearance of the sample and indicate the description in the request form.
2. Pour the specimen into 15-50 ml. centrifuge tubes and spin for ten (10) minutes at recommended speed of 1,800-2,500 rate per minute. While doing the centrifugation, two superfrost plus slides are prepared and labeled accordingly.
3. Pour of the supernatant fluid back into the original container, keeping the remaining fluid (with the sediment) at the bottom of the centrifuge tube for the next step.
4. Mix the sediment by slightly tapping the tube or for thicker fluid by the use of the vortex mixer.
5. A drop or two is placed on one of the slides and, using the “pull-apart” method, the sediment is spread evenly on the surface of
both slides. Remains of the sediment in the centrifuge tubes are stored in the refrigerator until the diagnosis has been released.

6. The smears are fixed immediately by Cytospray or immersed in 80%/isopropanol/95% ethyl alcohol. The slides are allowed to stand for at least 10-15 minutes before staining.

11.0 PROCEDURE CEREBRO SPINAL FLUID (CSF)

11.1 Sample:
Cerebrospinal fluid is obtained through lumbar tap or ventricular tap as a clinical procedure in the clinics and immediately sent to the lab without any delay. Because any delay in processing more than an hour will affect the cells negatively.

11.2 Regent: Spraycyte/80% isopropanol, MGG stain

11.3 Procedure for Smear Preparation
1. The volume and description of the specimen is noted in the corresponding numbered request form. This number should be indicated in the specially-made slides for the Cytospin i.e., slides with white rings. It should be noted that the slides are cleaned with alcohol prior to use.
2. Switch the Cytospin on, open the lid, and lift the head assembly out of the instrument before loading the samples. The head assembly can then be opened by pulling up the central button. The slide clips are then removed from the head assembly and loaded with the glass slide-filter paper-sample chamber combination and seeing to it that the outlet port coincides with the filter card holes and the pre-drawn circle on the slide. Once checked, this slide assembly is then securely held by the retaining hooks of the slide clips. This assembly should be made in duplicate and place in opposite slots of the head assembly to balance.
3. Three to five drops of the sample (CSF) are placed inside in each of the sample chambers and the head assembly being closed securely. The head containing the samples is then loaded in the center cone of the machine. With the safety cover in position, and locked; the correct speed program is entered, and centrifugation is started. A built-in timer and actual speed indicator enables the operator to monitor the centrifugation process, and alarms to indicate speed-drop or cycle completion.
4. The slide assembly is separated from each other with the filter card detached from the slide in a slow peeling motion to prevent smudge in other areas of the slide other than inside the drawn circle.
5. The prepared slides are immediately sprayed with Spraycyte to fix. These are then allowed to dry for 5 minutes and ready to stain. The Cytospin4 is a special instrument which concentrates small number of cells in minute volume e.g., CSF. It uses centrifugal force to spin cell suspensions and sediments unto a glass slide with the medium being absorbed by a filter card. The result is a monolayer of cells spread within a 6.0 mm. area on the slide.
6. Smears then are given to Histo section for Giemsa stain.

12.0 PROCEDURE TZANCK SMEAR PROCEDURE:
The Tzanck smear procedure is used to identify certain characteristic cells (multinucleated Syncytial giant cells) or cell inclusions, usually viral.

Material: Frosted end microscope glass slide. 80% isopropanol in Coplin jars or spray fixative.

13.0 PROCEDURE PNEUMOCYSTIS CARINII PROCEDURE
Pneumocystis carinii pneumonia (PCP), as the condition is commonly termed (although the causative organism has been renamed Pneumocystis jiroveci) is the most common opportunistic infection in persons with HIV infection. Pneumocystis is a genus of unicellular fungi found in respiratory tract of mammals and humans. It is sometimes found concurrent with the other opportunistic infections such as aspergillosis or cryptococcosis.

Although latent infection exists in animals and man, the disease is almost restricted to premature and debilitated infants, older individual on calorie-deficient diets, and particularly children and adults under treatment with cytotoxic drugs (for leukemia and lymphoma) or other immunologically compromised hosts.

13.1 Equipment & Material
- Equipment: (Microscope, Centrifuge)
- Materials:
  - Glass Slides, cover slips
  - Centrifuge tube
  - Staining dish, Coplin jars and basin
  - Slide holder and tray
  - Mountant
  - Distilled water
  - Spraycyte
  - Tissue paper (Kimwipes)

- Reagents: Grocott’s methenamine silver stain, Pap stain

13.2 Step by Step Procedure for Smear Preparation:
Some of the specimens received in slides, however if a mucoid specimen received; the preparation is the same as in sputum. For fluid specimen, the same as in the processing of effusion fluid.

**Microscopic screening**
The screening of slides is usually performed starting from the low power objective (x10), to the high power objective(x40). The high power objective is utilized for close inspection of the Pneumocystis carinii organisms. The cysts resembles crushed ping-pong balls and are present in aggregates of 2 to 8 (not to confused with Histoplasma or Cryptococcus which typically do not form aggregates of spores or cells). Organisms are often seen in foamy alveolar casts (rounded masses of organisms). Casts stain eosinophilic to basophilic in Papanicolaou stain. In Grocott’s methenamine stain, the cell wall of the cyst stains black, often with a central dark dot.

**14.0 Responsibility:**
- Applies to clinicians or laboratory staff responsible for handling the specimens.

**15.0 Attachment:**
- Pap Staining Monitoring Form 01

**16.0 Distributions:**
- LMD Administration
- Cytology Laboratory
- All Clinical Departments

**17.0 References:**
- Cytology- Diagnostic Principles and Clinical Correlates Third Edition. By Edmund S. Cibas and Barbara S. Ducatman