Ultrafast Papanicolaou Stain Is Not Limited to Rapid Assessments: Application to Permanent Fine-Needle Aspiration Smears

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Ultrafast Papanicolaou (Pap) stain, a 90-second preparation originally designed for the immediate assessment of fine-needle aspiration (FNA) smears (Yang and Alvarez, Acta Cytol 1995;39:55-60), can also be adapted for permanent FNA smears. It involves the addition of three simple steps prior to the conventional Pap procedure: the first step is to make the cells appear larger, thus increasing the resolution for analysis of cellular details; the second step is to hemolyse the background blood, thus unmasking tumor cells; and the third step is to bring out the vibrant colors in the cells and the nucleoli, which stain red. Diagn Cytopathol 1995; 13:160-162. © 1995 Wiley-Liss, Inc.

Key Words: Ultrafast Papanicolaou stain; FNA smears; Air-dried preparation

Introduction

Recently, a 90-second preparation named the “ultrafast” Papanicolaou (Pap) stain, has been developed for the immediate assessment of fine-needle aspiration (FNA) smears. The objective of this article is to point out that it is also applicable to the permanent FNA smears with much higher resolution in cellular details.

Materials and Methods

The modifications involve the addition of three simple steps to the conventional Pap protocol. Before fixing in 95% ethanol, the wet smears are first allowed to air-dry (step 1), followed by dipping in normal saline for 30 sec (step 2). After proper fixation in 95% ethanol, and prior to the beginning of the standard Pap stain, the smears are dipped in previously described alcoholic formalin for 10 sec (step 3). The smears can then be processed as usual with any Pap staining desired.

Results

After the three modifications, the blood hemolysed, the cells appeared larger, various colors in the Pap stain appeared more vibrant, and the nucleoli stained red. The high resolution, wide field, and vibrant colors of endometrial adenocarcinoma with squamous differentiation in a direct smear, processed by the ultrafast Pap protocol, is illustrated in Figure C-1. The hemolysis of background blood is the result of rehydration of air-dried smears by normal saline technique developed by Chan and Kung in 1988 (Fig. C-2). Polychromasia of ultrafast Pap stain is illustrated by the keratinizing squamous cell carcinoma of the lung in Figure C-3.

Discussion

Pap stain is the favorite stain for the majority of American cytopathologists because of its transparency, allowing the study of nuclear details. Smears are fixed, while wet, by 95% ethanol or Cytospray, and processed through Harris hematoxylin, orange G, and EA. It was developed by Papanicolaou in 1942 for the study of squamous cells in cervicovaginal smears. It then applied to FNA smears without modification. However, FNA smears differ from cervicovaginal smears in two very important ways: 1) the former is always bloody and the latter is most often not; 2) most cells obtained by cervicovaginal scrapes are flat, squamous cells and most of the deeper cells targeted and aspirated by FNA are tri-dimensional cells. If the cervicovaginal smears are as bloody as the FNA smears, we qualify our interpretations by saying, “the interpretation is limited by obscuring blood.” However, up to the present, we have either tolerated the enormous amount of blood in direct FNA smears or switched to filter preparation or Thin-Prep®, where the blood had been hemolysed (a few cytopathology laboratories use Carnoy’s fixative to hemolysed the blood aspirated by fine needle). Wet-fixation is
Figs. C-1–C-3. Fig. C-1. Direct smear of endometrial adenocarcinoma with squamous differentiation. Arrows point to squamous cells, one orange and the other bluish yellow. The tissue fragment to the right side of the orange cell shows numerous neutrophils within the cytoplasm of the adenocarcinoma. Notice the flatness of the image over a wide field (Ultrafast Papanicolaou stain, original magnification, ×200). Fig. C-2: Routine Papanicolaou stain (right) vs. ultrafast Papanicolaou stain (left). The bloody half was fixed immediately in alcohol while wet, the clear half was air-dried, followed by dipping it in 0.9% NaCl for 30 sec. An FNA smear of recurrent colonic adenocarcinoma (Magnification, ×100). Fig. C-3: Squamous carcinoma showing keratinizing squamous cells in blue to orange colors (Ultrafast Papanicolaou stain, original magnification, ×200).
excellent for the study of squamous cells since they are flat to begin with, but it is less than optimal for the study of glandular cells since they are tri-dimensional (3-D), thus on widely variable focal planes. In addition, the cells appear much smaller in wet-fixture than in air-dried preparation due to the fact that the cells are 3-D in the former and 2-D in the latter. The rationale for modifications in the ultrafast Pap stain are as follows: air-drying is to make the cells appear larger, thus increasing the resolution for analysis of cellular details; normal saline is to rehydrate the cells so that transparency is regained in addition to the hemolysis of the background blood; and alcoholic formalin (pH 5) is to bring out the vibrant colors in the cells and the nucleoli, which stain red. The ultrafast Pap protocol combines the advantages of air-dried cytopreparation and the conventional Pap stain, and allows the study of nuclear and cytoplasmic details at a high resolution, in vibrant colors, and with a clean background.

References