Rehydration of Air-Dried Smears with Normal Saline

In fine-needle aspiration cytologic examination, nuclear features are often better assessed in hematoxylin and eosin (H and E) or Papanicolaou- (Pap) stained than Romanowsky-stained smears. However, both H and E and Pap stains require the use of immediately wet-fixed smears for cytomorphologic preservation. Some degree of air drying is usually inevitable. Placing air-dried smears in normal saline for 30 seconds before fixation in 95% alcohol is found to be a simple means of rehydrating the cells. The quality of the rehydrated smears is superior or identical to that of the immediately wet-fixed smears, provided that the period of drying does not exceed 30 minutes. (Key words: Aspiration cytology; Drying artifacts; Rehydration; Normal saline) Am J Clin Pathol 1988; 89: 30-34

FINE-NEEDLE ASPIRATION cytologic examination is gaining popularity in the preoperative diagnosis of tumors and tumor-like conditions.8,12 There has been a lot of controversy as to whether wet-fixed smears stained with hematoxylin and eosin (H and E) or Papanicolaou’s (Pap) stain or air-dried smears stained with Romanowsky’s stain are better. In fact, both are complementary, but H and E and Pap staining permit better assessment of nuclear features and are preferred by many histopathologists.9 However, staining with H and E and Pap becomes highly unsatisfactory once air drying has occurred.6,7 This is particularly so if the amount aspirated is small. Drying artifacts are sometimes difficult to avoid, because it takes time, no matter how minimal, to spread the aspirated material and transfer the slide to the fixative. A well-spread smear is often thin and therefore even more subjected to the effect of drying. Some authors prefer Romanowky’s stain simply because “the alternative of wet fixation is less practicable as the smears dry too quickly.”11 Rehydration of air-dried smears has seldom been studied.2,10 We endeavor to investigate and describe this technic for application in aspiration cytology.

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Materials and Methods

For ethical reasons, the study was conducted in two phases. In the pilot study, the optimal condition for rehydration of air-dried smears was determined. In the main study, this technic was applied in aspiration cytologic examination.

Pilot Study

To test the various methods of rehydration, imprint smears made from fresh tissue, including one papillary carcinoma of thyroid, two follicular adenomas of thyroid, two metastatic carcinomas in lymph nodes, and one reactive lymph node, were used.

Choice of Rehydrating Solution. The air-dried smears were placed for 5 seconds or 30 seconds in tap water, normal saline (NS), 3/4 NS, 1/2 NS, 1/4 NS, or 50% aqueous glycerin2 before fixation in 95% ethanol and staining with H and E. 1 NS was made by dissolving 9 g (154 mmol) of sodium chloride in 1 L of distilled water. Because NS was found to be most satisfactory, it was the only solution used in subsequent tests.

Optimal Time for Rehydration of Air-Dried Smears. The air-dried smears were placed for 5 seconds or 30 seconds in tap water, normal saline (NS), 3/4 NS, 1/2 NS, 1/4 NS, or 50% aqueous glycerin2 before fixation in 95% ethanol and staining with H and E and Pap. NS was made by dissolving 9 g (154 mmol) of sodium chloride in 1 L of distilled water. Because NS was found to be most satisfactory, it was the only solution used in subsequent tests.

Optimal Time of Air Drying. To determine whether prolonged air drying had any deleterious effects on subsequent rehydration, the air-dried smears were left at room temperature for varying periods (5 minutes, 10 minutes, 15 minutes, 30 minutes, one hour, two hours, three hours, four hours, 6 hours, one day, two days, three days) before hydration (for 30 seconds, 1 minute, 2 minutes), fixation, and staining with H and E.

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Application in Aspiration Cytologic Examination

Based on the results of the pilot study, the rehydration technic was applied to 80 consecutive cases of fine-needle aspiration cytologic examination. A direct smear was made from the aspirated material and wet-fixed immediately in 95% ethanol. Another direct smear was quickly air dried and then rehydrated for 30 seconds in NS before fixation and staining with H and E. The quality of the wet-fixed and rehydrated smears was compared.

Results

Pilot study

Choice of Rehydrating Solution. In air-dried smears stained with H and E or Pap, all the nuclei appeared large, poorly stained, and smudged, and the chromatin pattern could not be assessed (Figs. 1 and 2). On rehydration of air-dried smears with the various solutions, NS was found to give the best results: the nuclear chromatin and nucleoli were as crisp as those in the wet-fixed smears, and the cytoplasmic outlines remained distinct (Fig. 1). Another significant finding was lysis of most of the red blood cells, so that the background appeared much cleaner (Fig. 2). When hypotonic saline, tap water, or aqueous glycerin was used, a proportion of nucleated cells were lyzed, appearing as naked nuclei. Rehydration failed if the air-dried smears had been fixed in alcohol.

Optimal Time for Rehydration of Air-Dried Smears in Normal Saline. Hydration periods ranging from 5 seconds to 5 minutes gave equally satisfactory results. If the slide was left in NS for less than 5 seconds, some of the cells on the smears still showed drying artifacts. If the slide was left in NS for longer than 5 minutes, the cytoplasmic outline of the cells appeared blurred and the nuclei were wrinkled, particularly in the lymphoid cells. Optimal Time of Air Drying. Best results were obtained if air drying did not exceed 30 minutes. For longer periods of air drying up to one day, although most cells could still be rehydrated and well stained, the chromatin pattern appeared less crisp and the nucleoli appeared less distinct. The results were highly unsatisfactory if the slides had been dried for more than one day.

Application in Aspiration Cytologic Examination

The rehydration technic (30 seconds in NS) had been applied to 80 consecutive cases of fine-needle aspiration cytologic examination, including 63 thyroid aspirates (49 colloid nodules, 4 follicular neoplasms, 1 anaplastic carcinoma, 1 thyroiditis, 8 inadequate); 7 breast aspirates (6 benign, 1 tuberculosis); 8 lymph node aspirates (5 metastatic carcinomas, 1 reactive, 1 tuberculosis, 1 lymphoma); 1 liver aspirate (metastatic carcinoma); and 1 parotid gland aspirate (normal). We adopted 30 seconds as the hydration period because this was found to be the optimal time for "cleaning up" of the background. This rendered search for the diagnostic cells less tedious and avoided the problem of overlapping red blood cells obscuring cellular details (Fig. 2).

The nuclear staining in rehydrated smears was superior or comparable to that of the wet-fixed smears (Fig. 3). The cells appeared slightly larger than those in wet-fixed smears because the individual cells were spread over larger areas. As a result, the cells appeared less three dimensional, and many nuclei in a group of cells could often be brought into focus at one plane (Figs. 2 and 4).

Discussion

H and E or Pap are generally regarded as the best stains for assessment of chromatin pattern in cytologic smears and ensure maximum resemblance with the corresponding cells in tissue sections. However, a prerequisite is immediate fixation, usually in 95% ethanol. Air drying has deleterious effects on the nuclear and cytoplasmic staining. In the 1950s and 1960s, there were some studies describing methods of rehydration of air-dried smears. Lencioni and colleagues used sequential tap-water and acetic acid–alcohol solutions for rehydration. Bonime recommended the use of 50% aqueous glycerin. Nieburgs' method of rehydrating with hydroxypropyl methylcellulose ether in water–ether alcohol required prior coating with hydroxypropyl methylcellulose ether. Rehydration technics, however, have not made a strong impact in exfoliative cytologic examination because of availability of the much simpler spray fixatives. In the recent cytology textbooks, rehydration is not mentioned at all or is only mentioned very briefly. Naib mentioned only in passing, "if the smear is accidentally allowed to air dry, it can be rehydrated by placing it in tap water for a few minutes before fixation."

In exfoliative cytologic examination, the material is often wet because of the admixture with mucus, and drying is not often a serious problem. However, in aspiration cytologic examination smears, some degree of drying is inevitable, particularly at the edges of the smears, where the diagnostic cells are often concentrated. This drying artifact is particularly noticeable if the amount of aspirate is small and relatively dry. Therefore, it is highly desirable to develop methods to rehydrate these dried-up cells. In this study, we have shown that tap water, aqueous glycerin, and hypotonic saline are not very satisfactory, because of lysis of a proportion of nucleated cells. Instead, we have found NS a satisfactory and readily available solution to rehydrate the
FIG. 1 (upper). Metastatic carcinoma in lymph node. A (left). Wet-fixed imprint smear. B (center). Air-dried smear stained with hematoxylin and eosin, in which the cells appear much larger and the nuclear chromatin is smudged. C (right). Rehydrated smear, in which the cells appear slightly larger than those in wet-fixed smear. The chromatin pattern is as crisp. Hematoxylin and eosin (x750).

FIG. 2 (lower). Follicular adenoma of thyroid. A (left). Wet-fixed smear. A three-dimensional cluster of follicular cells lies in a heavily blood-stained background. B (center). Air-dried smear, in which the chromatin details are lost. C (right). Rehydrated smear, in which the background is much cleaner. The nuclei within the group of cells are more readily brought into focus at one plane. The nucleoli show up well. Hematoxylin and eosin (x300).
FIG. 3 (upper). Apocrine epithelium from a breast mass aspirate. A (left). Wet-fixed smear. B (right). Rehydrated smear. The nuclear and cytoplasmic features are comparable to those of wet-fixed smear, except that the nuclei appear "flatter." Hematoxylin and eosin (×750).

FIG. 4 (lower). Benign breast tubule from a breast mass aspirate in a rehydrated smear. Both the epithelial cells with large pale nuclei (large arrows) and the myoepithelial cells with smaller dark nuclei (small arrows) can be brought into focus at one plane. Hematoxylin and eosin (×400).
smears, producing results superior or comparable to immediately wet-fixed smears. The time that the smears can be left in the NS is not very critical: there is no appreciable difference whether it is 5 seconds or 5 minutes, although it takes about 30 seconds for lysis of the red blood cells in the background to occur. Longer immersion in NS is detrimental because of nuclear wrinkling, particularly in the lymphoid cells. With regard to the duration of air drying that will not compromise subsequent rehydration, the limit is 30 minutes, but smears air dried for up to one day may also be salvaged by rehydration, although less satisfactorily. The less satisfactory rehydration and staining after prolonged air drying may result from autolytic changes in these unfixed cells. It is also because of the detrimental effects of prolonged air drying that we do not recommend submitting the routine gynecologic smears to the laboratory as air-dried smears. However, if the gynecologist feels that there may be some drying in cases with a low yield on cervical scraping, the smear can be briefly rehydrated in NS before spraying with fixative.

This method of rehydration offers several advantages over the conventional wet fixation. First, the smears can be spread more thinly and leisurely; smears made hastily for fear of air drying are usually not very satisfactory. Second, the problem of air drying in the edges of the smear can be avoided. Third, when a wet smear is placed in 95% ethanol, the larger particles or thicker portions of the smear may fall off.5 If the smear has been fully air dried, the cells adhere better to the slide and do not fall off as easily on rehydration and fixation. Fourth, lysis of most of the red blood cells creates a much less distracting background to permit better cytolologic assessment. Fifth, the cells appear flatter and the depth of focus on the nuclei is much more shallow. We have found this a great advantage on taking photomicrographs. For example, in breast aspirates, we have found it practically impossible to bring the epithelial and myoepithelial cells of the tubules into focus simultaneously at one plane for photomicrography without using the rehydration technique (Fig. 4). Sixth, to make best use of all materials available, the slide used for spreading the smears may also be salvaged by rehydration for cytologic examination.

We therefore propose adding a further simple step to the procedure of smear preparation in fine-needle aspiration cytologic examination as follows:

1. Spread the aspirated material on clean or gelatinized glass slide.
2. Allow the slide to air dry completely and quickly.
3. Place the slide in normal saline for 30 seconds for rehydration.
4. Transfer the wet slide to 95% ethanol for fixation.
5. Stain with H and E or Pap as usual.

Because of the potential of cross-contamination of the different cases, we recommend changing the NS solution for every case. Depending on individual preference, additional air-dried smears may be made for Romanowsky’s staining, particularly for lymph node aspirates.

References