Quantitative Assessment of Spray vs Immersion Fixation for Thyroid Fine-Needle Aspiration Specimens

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Key Words: Thyroid; Biopsy; Fine-needle aspiration; Fixation; Spray fix; Immersion; Adequacy

Abstract

Whether spray or immersion fixation results in higher adequacy rates for thyroid fine-needle aspiration is not known. I compared 1-year adequacy rates, number of cells, and atypia rates for immersion fixation with split spray fixation and immersion. Adequacy rates increased from 71.9% (289/402) for complete immersion to 78.1% (332/425) with spray fixation of half of the slides (P = .02). The spray-fixed slides had an average ± SD of 49.7 ± 47.5 cells compared with their paired immersed slides, with 25.4 ± 36.8 cells (96% more cells for spray fix; P < .001). Of the 402 immersion cases, 10 (2.5%) were diagnosed as atypical related to preparation artifact compared with 14 (3.3%) of cases with split preparation (P = .48). Spray fixation results in 96% more follicular cells in scant thyroid aspirates than immersion fixation and a significant increase in adequate aspirates without a significant increase in the atypia rate related to preparation.

Fine-needle aspiration (FNA) of the thyroid is the method of choice to evaluate most thyroid nodules. Although air-dried and alcohol fixation are acceptable methods of slide preparation, many cytologists prefer alcohol-fixed slides. These can be prepared by spray fixation, immersion in alcohol, or with liquid-based cytology.1,2 Although some authors have noted that “some cellular material appears to fall off the slides” with immersion,1 the degree to which this is a problem is not known. To evaluate this, I compared the cellularity, adequacy, and atypia rates of a large series of thyroid FNAs with immersion and spray fixation.

Materials and Methods

Until June 1, 2006, all thyroid FNAs performed at Baptist Hospital, Miami, FL, were entirely fixed by immersion in 95% ethyl alcohol. All of the pathologists at this institution prefer alcohol-fixed slides, and air-dried slides were not used. Multiple efforts were made before the study to improve the yield of these cases, including reducing the time between preparation of the slides and fixation, the thickness of the smears that were prepared, and the use of extremely clean slides. It was the impression of the pathologists that the immersion fixation could not be improved further at the start of the study (June 1, 2005). Starting June 1, 2006, between 1 and 4 passes from each patient were split, and 1 slide was fixed by immersion and 1 slide was spray fixed. The slide that was immersed was placed in ethanol directly after smearing, and the cytotechnologist then sprayed the second slide (Spray-Cyte, Clay Adams Brand, Becton Dickinson, Sparks, MD) as quickly thereafter as possible, following
of more than 20 years’ experience and the second more than 5 years’ experience with this preparation technique. Immediate interpretation was not provided. Comparisons were made between 1 year of cases that were entirely spray fixed (June 1, 2005-June 1, 2006) and 1 year of split immersion and spray fix (June 1, 2007-June 1, 2008).

Adequacy was assessed using the criteria outlined by Goellner and Goellner et al and required 6 groups of at least 10 epithelial cells in the aspirate.

Results

Adequacy rates increased from 71.9% (289/402) for immersion of all slides to 78.1% (332/425) with spray fixation of half of the slides ($P = .02$). Of 100 cases reviewed (56 nondiagnostic and 44 scant but adequate), 19 had no epithelial cells on any slides. Of the remaining 81 cases, there was an average of 2.4 spray-fixed slides with matching immersed slides. The spray-fixed slides had an average ± SD of $49.7 ± 47.5$ cells compared with their paired immersed slides, with $25.4 ± 36.8$ cells (96% more cells for spray fix; $P < .001$).

Of the 56 nondiagnostic cases, 37 had at least some epithelial cells (mean, 29.7 cells; range, 0-50 cells). If the number of cells on only the immersed slides were increased 96%, 10 of 37 cases would be adequate (60 cells total). Overall, the nondiagnostic cases would be expected to decrease an additional 10 of 56 (18%) if all slides were spray fixed, for an approximate overall adequacy rate of 82%.

During the first year of complete immersion there were 10 cases (10/402 [2.5%]) in which the diagnosis was atypical or “air drying,” and the atypia was thought to be related to “air drying.”

Discussion

I undertook this study to determine if spray fixation could improve the yield in thyroid FNAs. I hoped to improve overall adequacy in our department because the adequacy rate in this study is lower than in many other studies. Although the pathologists in our department and I have examined many possibilities for this low adequacy rate, we believe the major reason for the low rate is patient selection. Many of the patients referred for biopsy are referred because an aspiration performed elsewhere was not satisfactory. Thus, the nodules that are aspirated in our laboratory represent a selected group of particularly difficult nodules to aspirate. Technical issues such as excessively bloody smears do not seem to have a significant role, as demonstrated by the relatively low rate of atypia related to this. Nevertheless, I wanted to determine if the method of fixation could also have a part in this problem.

Traditionally, our laboratory has used immersion fixation, and some pathologists in the laboratory strongly believe the preservation of cytologic detail is superior with immersion fixation compared with spray fixation. However, like others, we had noted that some of the aspirated material failed to adhere to the slide with immersion fixation, although our direct observation suggested that although RBCs often failed to adhere to the slide, epithelial cells appeared to adhere preferentially. Nevertheless, we were not sure to what degree immersion fixation affected the overall yield, and we were unable to find any data in the literature to clarify this.

Initially in the performance of this study, review of individual cases failed to reveal whether spray fixation yielded more cells. In some cases, more cells were present on the spray-fixed slides, but in other cases, more cells were present on the immersion-fixed slides. By the midpoint of the study, it was tempting to stop the study because some of the pathologists strongly preferred the fixation that resulted from immersion. However, the study continued, and the surprising results showed that spray fixation can result in a nearly 100% increase in cellularity, at least with scant aspirates. Taken together, the results suggest that there is a great deal of variation between individual passes and individual slides made from the same pass with thyroid FNA. This observation of marked variation is supported by the very high standard deviation reported. This marked variation in yield can easily mask differences in overall yield that may come from differences in fixation method.

Table 1

<table>
<thead>
<tr>
<th>No. of Epithelial Cells</th>
<th>Spray-Fixed</th>
<th>Immersion-Fixed</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>25</td>
<td>42</td>
</tr>
<tr>
<td>0-10</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>11-20</td>
<td>9</td>
<td>18</td>
</tr>
<tr>
<td>21-30</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>31-40</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>41-50</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>51-60</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>&gt;60</td>
<td>19</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

* Data are given as number of cases.
The most important benefit of spray fixation is the increased yield and lower inadequacy rate. In this study, simply spray fixing half of the slides resulted in a significant increase in overall adequacy rates—projections suggest that spray fixing all of the slides should also result in another increase in the adequacy rate. Although there may be differences in cytologic detail with the 2 different methods, and there were pathologists in the laboratory who were quite certain of this, all pathologists in the laboratory were able to interpret spray-fixed specimens, and the study was not able to demonstrate a significant difference in cases diagnosed as atypical for reasons that may be related to the manner in which the slides were prepared. It was also noted that in spray-fixed cases, there seemed to be more blood on the slides, and, occasionally, the epithelial cells were obscured by this blood, but no significant difference could be demonstrated in the related atypia rate.

This study shows that spray fixation results in 96% more follicular cells in scant thyroid aspirates than immersion fixation and a significant increase in adequate aspirates without a significant increase in the atypia rate related to the manner of preparation of the slides.

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References