Rapid on-site evaluation (ROSE) has been shown to improve fine needle aspiration (FNA) sensitivity and diagnostic yield. The method of staining used for ROSE is important in influencing the speed of the procedure and the quality of the microscopic image. There are two methods commonly used to rapidly stain smeared slides during ROSE: Toluidine blue (TB) and Diff-Quik (DQ). The cytopathology laboratory at UC Davis prefers TB for its in-house ROSE. For over 30 years, this has been the preferred method of staining. TB is a metachromatic cationic (basic) thiazine dye that has a high affinity to acidic tissue components and turns nucleic acid blue and polysaccharides purple. The use of TB in ROSE is efficient, cost-effective, and practical. TB use saves time as it does not require air-drying of the smeared specimen and involves only one stain solution in the process. A major advantage is that the slide can be re-stained later with the Pap stain providing a better nuclear detail for final diagnosis. A drawback is that TB does not provide the tissue components with a two-tone eosinophilic-basophilic contrast. It provides a monochromic appearance of the fluid sample, thus requiring some degree of training and experience in interpretation. TB may not sufficiently penetrate the areas of the smear with obscuring artifacts such as blood and thick mucus, limiting evaluation during ROSE. At UC Davis, we investigated whether optimizing TB concentration will improve staining outcome during ROSE.

**Results**

<table>
<thead>
<tr>
<th>CRITERIA</th>
<th>SCORES</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Residual background</td>
<td>3</td>
</tr>
<tr>
<td>2. Cytoplasmic detail</td>
<td>2</td>
</tr>
<tr>
<td>3. Nuclear membrane</td>
<td>2</td>
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<tr>
<td>4. Chromatin texture</td>
<td>2</td>
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<tr>
<td>5. Nucleoli</td>
<td>2</td>
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</tbody>
</table>

Table 1. Criteria for grading. Each 5 criteria is graded from 1 to 3. The higher the score is, the better is the staining outcome. For instance, absence of residual background stain is given a score of 3. Conversely, presence of abundant residual stain in the background is given a score of 1.

**Conclusion**

- Different concentrations of TB show different staining results.
- Presence of background staining is more pronounced at 100% concentration. Mucus and necrotic tissue retain TB at 100%. At 80% concentrations and below, residual background stain is less. At 20% concentration, background is clear of residual stains.
- At 100% concentration cytoplasm and nuclei appear dark blue. Cytoplasmic detail, nuclear membrane, chromatin texture, and nucleoli are better appreciated with 100% concentration. At 80% concentrations and below, cytoplasmic detail, nuclear membrane, chromatin texture, and nucleoli are less vivid. At 20% concentration, cytologic details appear the best.
- A less concentrated TB, ideally 80% concentration and below, results to a better ROSE staining.
- 20% concentration provides the best staining for fluid specimens.
- TB is an efficient and cost-effective ROSE stain and optimization results to a better microscopic image.

**References**

- Tambouret, et al., Cytology and Pathology | FNA cytology: Rapid on-site evaluation—how practice varies. CAP TODAY, May 18, 2016.

**Figure 1**

A. At 100% concentration, residual background stain is abundant. B. At 50% concentration, more spaces are clear of residual stains. C. At 20% concentration, background is clear and clear of residual stains.

**Figure 2**

A. At 100% concentration, nuclear detail, chromatin, and nucleoli are indistinguishable. B. At 60% concentration, some nucleoli (red arrow) and nuclear membranes (yellow arrow) are starting to be visible. C. At 20% concentration, nucleoli (red arrow) and nuclear membranes (yellow arrow) are clearly visible.