Optimization of Toluidine blue for Rapid On-Site Evaluation (ROSE)

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Background

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 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 in ROSE.

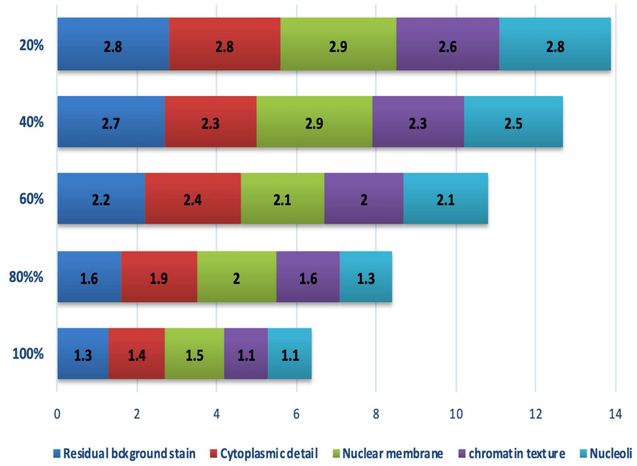
Methods

- Discarded, de-identified cytology body fluid specimens over 2-month period were screened for cellularity and storage time.
- Cellular specimen and specimen less than 2 weeks old were included in the study.
- Out of hundreds specimens, 10 cases were included.
- 5 slides smears were prepared from each case; all smears were fixed with alcohol.
- 5 TB concentrations (100%, 80%, 60%, 40%, and 20%) were prepared. Original (100%) concentration is based on the existing in-house TB formulation.
- Different TB concentrations are achieved by mixing volume percentage of TB with de-ionized water (DI).
- The resultant staining of 5 TB concentrations were compared using 5 criteria:
  1. Presence of residual background staining
  2. Cytoplasmic detail
  3. Nuclear membrane
  4. Chromatin texture
  5. Staining of nucleoli
- Stains were graded by a cytopathology fellow and a cytotechnologist student.
- The results were then tabulated and compared for each stain.

Results

CRITERIA	SCORES 3	2	1
1. Residual background stain	clean/absent	mild-moderate presence	dirty
2.Cytoplasmic detail	distinct	some detail	not distinct
3. Nuclear membrane	distinct	some detail	not distinct
4. Chromatin texture	distinct	some detail	not distinct
5. Nucleoli	distinct	some detail	absent/not distinct

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.



Graph 1. Average scores of different TB concentrations on 5 criteria. Each TB concentration (100%, 80%, 60%, 40%, and 20%) is graded using 5 criteria (residual background stain, cytoplasmic detail, nuclear membrane, chromatin texture, and nucleoli). As TB concentration becomes diluted to 80% and below, average scores on the 5 criteria improve. At 20% concentration, highest scores on the 5 criteria are recorded.

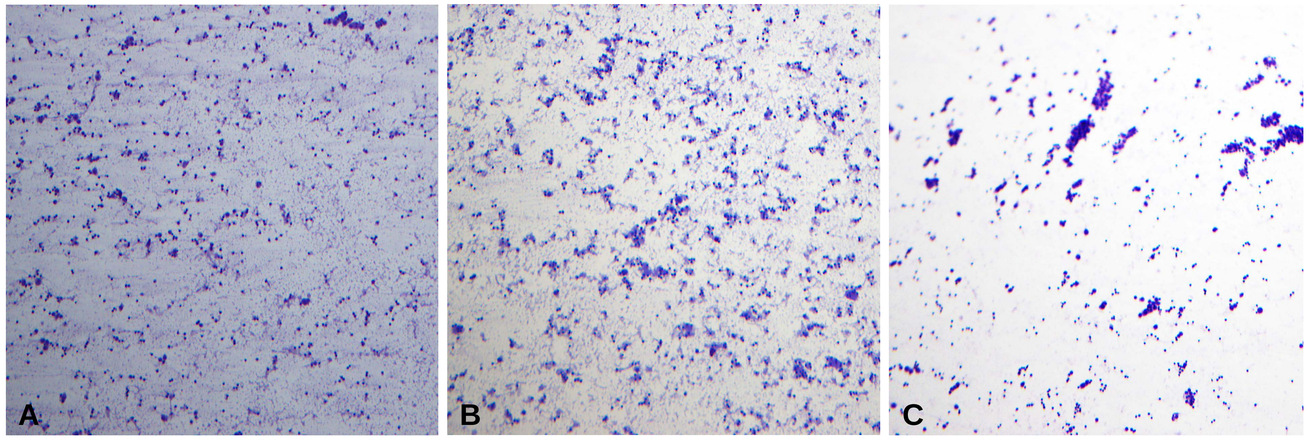


FIGURE 1. Pleural effusion at low power magnification using different TB concentrations. A. At 100% concentration, residual background stain is abundant. B. At 60% concentration, more spaces are clear of residual stains. C. At 20% concentration, background is clear and clean of residual stains.

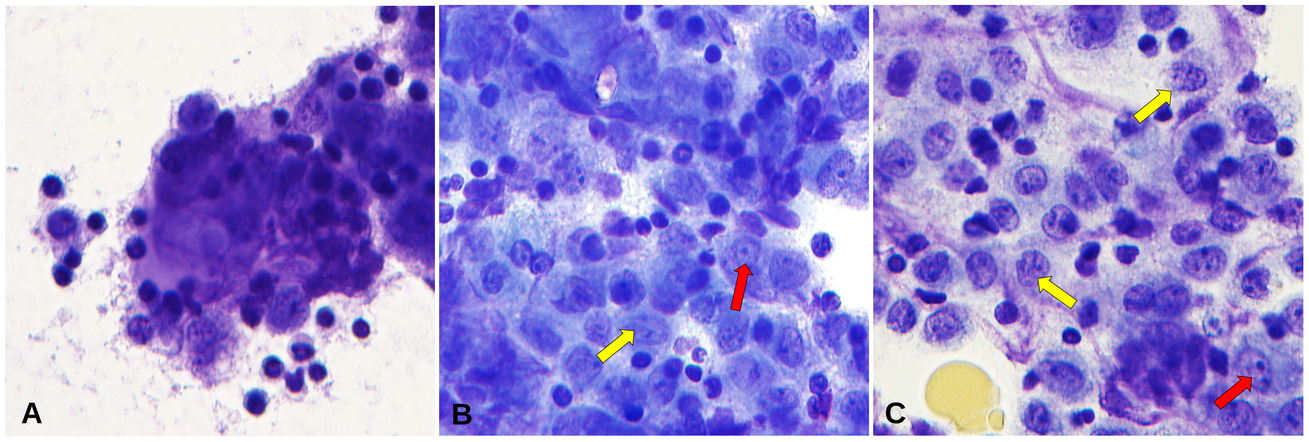


Figure 2. Pleural effusion at high power magnification using different TB concentrations. A. At 100% concentration, nuclear detail, chromatin, and nucleoli are indistinguishable. B. At 60% concentration, some nucleoli (red arrow) and nuclear membranes (yellow arrow) are starting to be visible. C. At 20% concentration, nucleoli (red arrow) and nuclear membranes (yellow arrow) are clearly visible.

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 less appreciated with 100% concentration. At 80% concentrations and below, cytoplasmic detail, nuclear membrane, chromatin texture, and nucleoli are more 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

- Diacon AH, Schuurmans MM, Theron J, et al. Utility of rapid on-site evaluation of transbronchial needle aspirates. Respiration. 2005;72:182-188.
- Diette GB, White P Jr, Terry P, Jenckes M, Rosenthal D, Rubin HR. Utility of on-site cytopathology assessment for bronchoscopic evaluation of lung masses and adenopathy. Chest. 2000;117:1186-1190.
- Klapman JB, Logrono R, Dye CE, Waxman I. Clinical impact of on-site cytopathology interpretation on endoscopic ultrasoundguided fine needle aspiration. Am J Gastroenterol. 2003;98:1289-1294.
- Pellise Urquiza M, Fernandez-Esparrach G, Sole M, et al. Endoscopic ultrasound-guided fine needle aspiration: predictive factors of accurate diagnosis and cost-minimization analysis of on-site pathologist. Gastroenterol Hepatol. 2007;30:319-324.
- Tambouret, et. Al., Cytopathology and More | FNA cytology: Rapid on-site evaluation—how practice varies. CAP TODAY, May 18 , 2016.
- Gosey LL, Howard RM, Witebsky FG, et al: Advantages of a modified Toluidine Blue stain and bronchoalveolar lavage for the diagnosis of Pneumocystis carinii pneumonia. J Clin Microbiol 1985; 22: 803–807.
- Canil M, Meir K, Jevon G, Sturby T, Moerike S, Gomez A. Toluidine Blue staining is superior to H&E staining for the identification of ganglion cells in frozen rectal biopsies. Histologic 2007; 40: 1–3.
- Layfield LJ, Bentz JS, Gopez EV. Immediate on-site interpretation of fine-needle aspiration smears: a cost and compensation analysis. Cancer (Cancer Cytopathol). 2001;93:319-322.
- Sridharan, G; Shankar, AA (2012). " Toluidine Blue: A review of its chemistry and clinical utility". J Oral Maxillofac Pathol. 16: 251–5. doi:10.4103/0973-029X.99081.