

Abstract

Background: Telecytology in rapid on-site evaluation (ROSE) during a fine needle aspiration (FNA) biopsy is an emerging trend. Remote evaluation of FNA aspirate through imaging is a possible solution to obstacles (e.g. manpower needs and lack of proper billing) that hinder the implementation of ROSE. However, the ideal staining method suitable for ROSE imaging has not yet been fully studied. We investigated the rapid staining methods most commonly used for ROSE at UC Davis, in particular, whether Toluidine blue (TB) or Diff-Quik (DQ) is better for ROSE telecytology. **Design:** Eleven cases with adequate cellularity were included in the study. Three slide smears were prepared from each case; two smears were fixed with alcohol for Pap stain and TB; one smear was air-dried for DQ. Pap stain was used as a reference in the study. DQ and TB were compared using 3 parameters: image quality, efficiency, and cost-effectiveness. Image quality was graded according to five criteria: presence of residual background staining, cytoplasmic detail, nuclear membrane, chromatin texture, and staining of nucleoli. Each image quality criterion is given a score of 1 to 3. For efficiency, we compared the total time to perform each staining method. For cost-effectiveness, we compared the total direct cost of each rapid stain. The results were tabulated and compared. **Results:** DQ results in better cytoplasmic and nuclear detail and leaves a cleaner background. The average cost to perform TB is \$12.00 per slide versus \$27.00 for DQ. The average time to complete one slide for TB is 10 seconds versus 120 seconds for DQ. **Conclusion:** DQ provides a good image quality but is not efficient and cost-effective. TB is efficient and cost-effective but does not provide an excellent image quality. A different rapid stain that is efficient, cost-effective, and provides excellent image quality should be explored.

Background

Successful pathologic diagnosis after image-guided fine needle aspiration (FNA) depends on a number of factors including nature of the lesion, aspirator skill, and availability of rapid on-site evaluation (ROSE)¹. ROSE has proven to improve the sensitivity and diagnostic yield of FNA. There is currently a growing trend in the ordering of molecular biomarkers on FNA specimens. ROSE is no longer performed solely for material adequacy. ROSE helps maximize the material recovered from FNA for molecular analysis. There are, however, several obstacles that hinder the implementation of ROSE during a fine needle aspiration procedure, that may limit the availability of ROSE services. The need for experienced on-site professionals and lack of proper billing of the ROSE procedure^{9,20} are just two of these obstacles. With the increasing demand to procure specimens for biomarkers, there is now a great need to innovate ROSE. Remote evaluation of FNA aspirate through imaging is a possible solution. Telecytology in ROSE is an emerging trend. However, the ideal staining method suitable for ROSE imaging has not yet been fully studied. The type of rapid stain for ROSE is the rate-limiting factor for achieving ideal ROSE images. We investigated the rapid staining methods most commonly used for ROSE at UC Davis, in particular, whether Toluidine blue (TB) or Diff-Quik (DQ) is better for ROSE telecytology. Using three parameters (image quality, efficiency, and cost-effectiveness), we compared DQ and TB. For the image quality parameter, we used five criteria in grading: residual background stain, cytoplasmic detail, nuclear membrane, texture of chromatin, and nucleoli stain.

Results

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

Table 1. Image quality criteria scoring for each criteria. A score of 1 to 3 may be assigned to each criteria, depending on the quality. Lowest score is 1 and the highest score is 3 for each criteria.

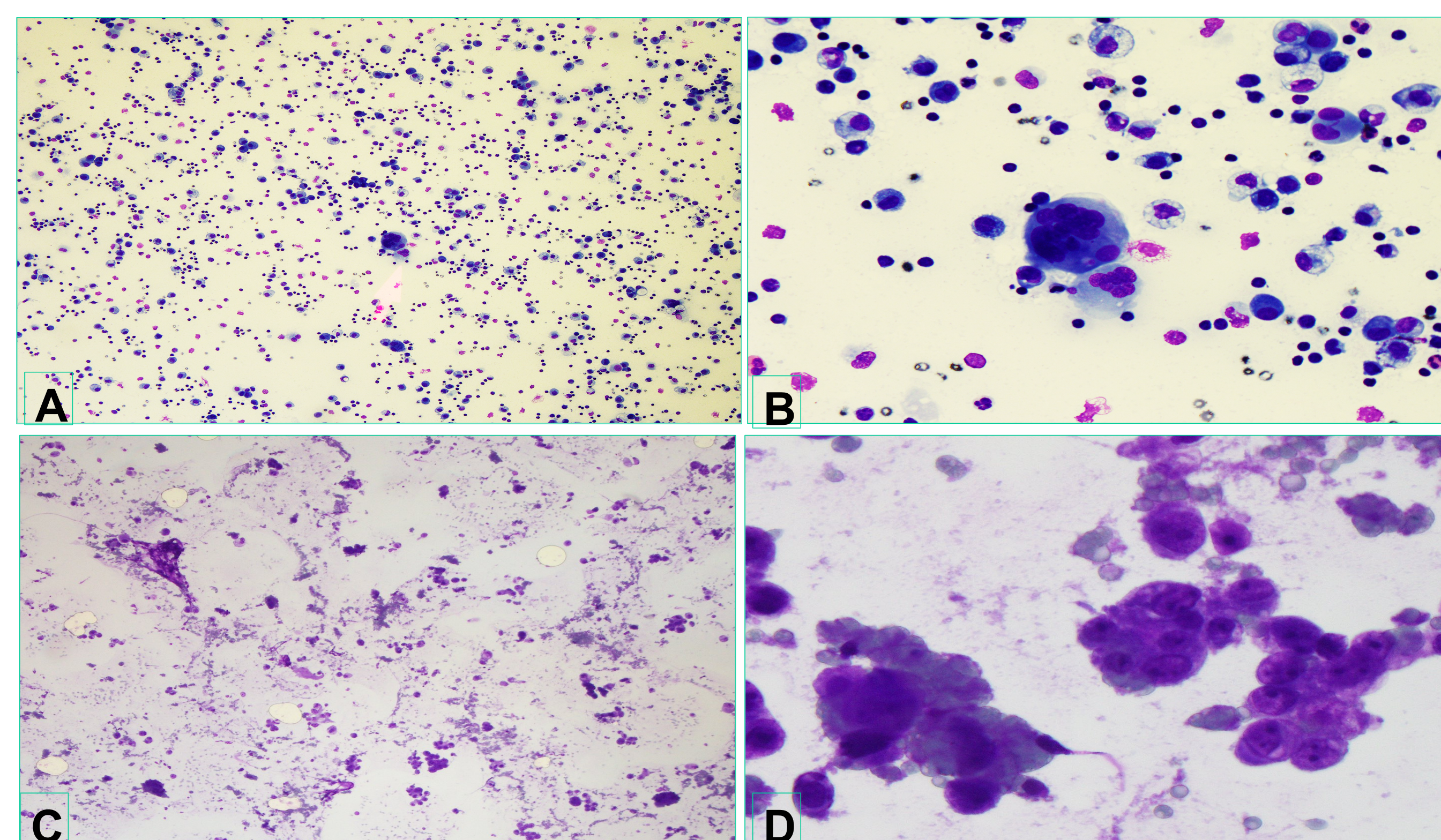


Figure 1. A. Diff-Quik at low power magnification (40x). B. Diff-Quik, at high power magnification showing good cytoplasmic and nuclear membrane detail (400x). C. Toluidine blue at low power magnification showing residual background staining (40x). D. Toluidine blue at high power magnification showing nuclear membrane and nucleoli details (400x). Also notice the residual stain in the background.

	Nuclear Membrane	Chromatin Texture	Nucleoli	Cytoplasmic detail	Residual Background Staining
DQ	2.45	1.45	1.73	2.30	2.55
TB	2.09	1.64	1.79	1.67	1.12
T-Test	0.0015	0.08311	0.6245	0.00028	< 0.0001

Table 2. Mean and p values of the paired T-test in all the 5 image quality criteria. P<0.05 in nuclear membrane, cytoplasmic detail, and residual background staining criteria.

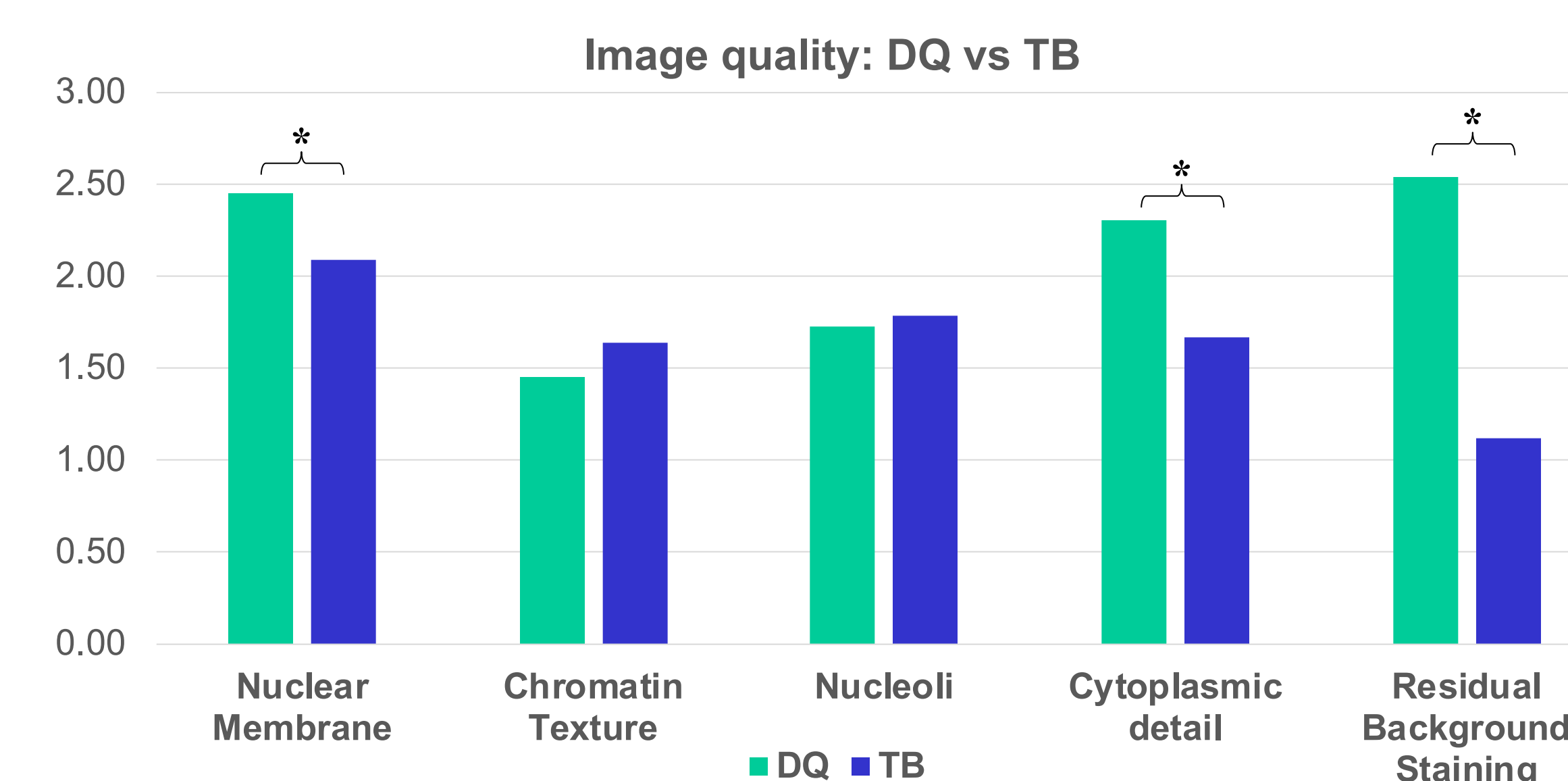


Figure 2. Bar graph showing mean scores of Diff-Quik and Toluidine blue. Diff-Quik is significantly better in nuclear membrane, cytoplasmic detail, and residual background staining criteria. Diff-Quik has a better image quality than Toluidine blue. * P<0.05

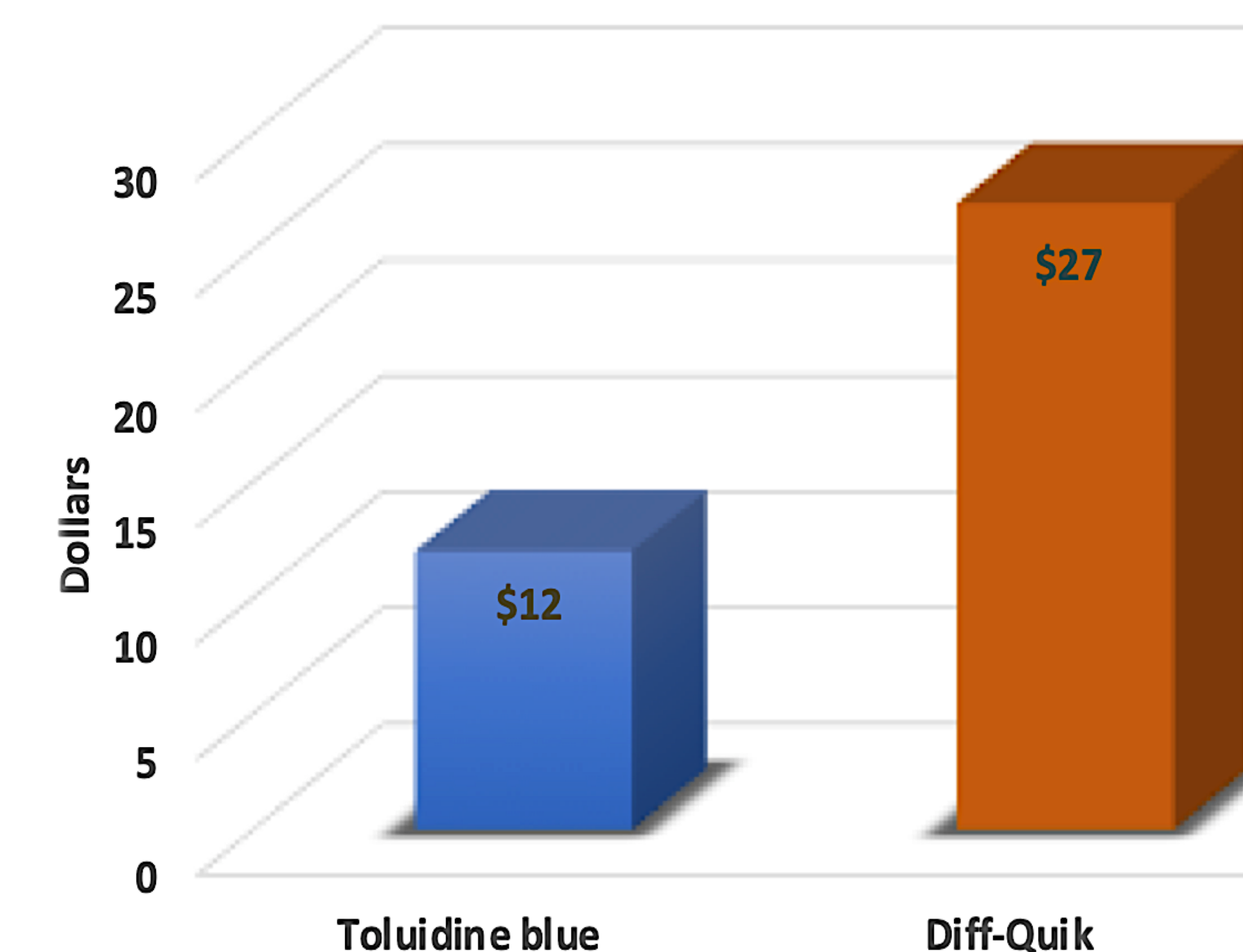


Figure 3. Bar graph showing the average cost to perform each stain. Toluidine blue costs \$12 average to perform versus \$27 for Diff-Quik. Toluidine blue is more cost-effective than Diff-Quik.

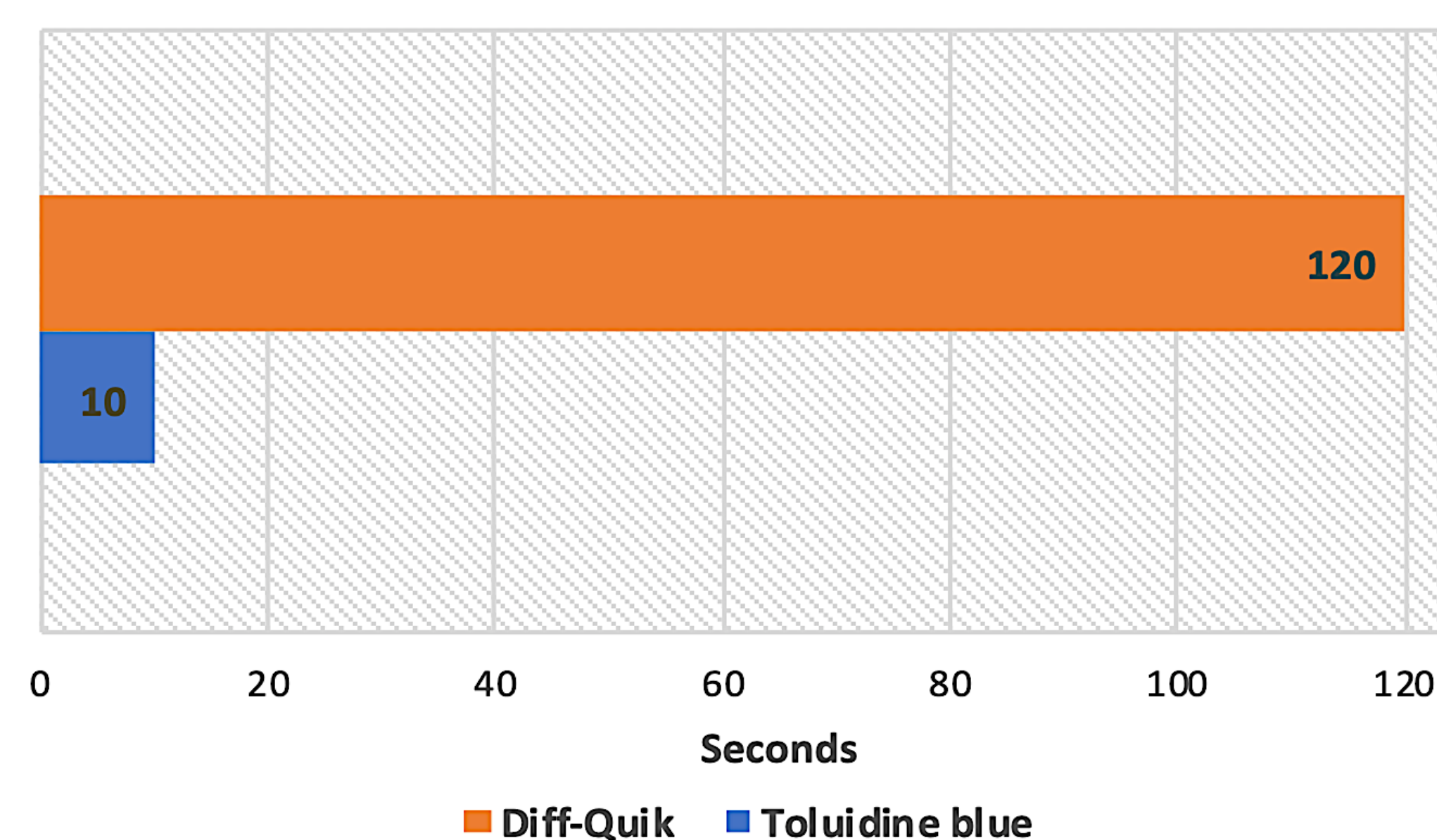


Figure 4. Bar graph showing the average time to perform each stain. Toluidine blue takes an average of 10 seconds to perform versus 120 seconds for Diff-Quik. Toluidine blue is more efficient than Diff-Quik.

Methods

- This is a retrospective study in which discarded, de-identified cytology body fluid specimens were selected based on specimen cellularity and sample preservation.
- 11 cases were included in the study.
- 4 slide smears were prepared from each case; 3 smears were fixed with alcohol for Pap stain and Toluidine blue stains; 1 smear was air-dried for Diff-Quik.
- Pap stain was used as the reference/gold standard in this study.
- Diff-Quik and Toluidine Blue were compared using 3 parameters: image quality, efficiency, and cost effectiveness.
- **Image quality:** was graded according to 5 criteria:
 1. Presence of residual background staining
 2. Cytoplasmic detail
 3. Nuclear membrane (Distinct border and irregularities).
 4. Chromatin texture (fine vs coarse)
 5. Staining of nucleoli (picked up by the stain or not)
- For image quality, each criteria was graded from 1 to 3 (1 being poorest and 3 being highest quality) by 2 cytopathologists and a cytotechnologist.
- **Efficiency:** we measured the total time to perform each staining method
- **Cost-effectiveness:** we calculated the total direct cost of each rapid stain.
- The results were then tabulated and compared for each stain.

Conclusions

- **Image quality:** DQ has a better image quality overall. DQ results in better cytoplasmic and nuclear detail and leaves a cleaner background compared to TB.
- **Efficiency:** TB is more efficient. It takes an average of 10 seconds to perform a single slide using TB and an average of 120 seconds using DQ.
- **Cost effectiveness:** TB is more cost effective. TB costs \$12.00 to perform per slide versus \$27 to perform DQ.
- The ideal rapid stain for ROSE telecytology should be efficient, cost-effective, and above all, provide an excellent image quality.
- Current existing rapid stains, TB and DQ, do not have all the qualities ideal for ROSE imaging.
- A different rapid stain that is efficient, cost-effective, and provides an excellent image quality should be explored. Optimization of Toluidine blue to improve image quality or exploring a new rapid stain are possible options.

References

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