TPM Vol. 32, No. S1, 2025
ISSN: 1972-6325
https://www.tpmap.org/

DIFF-QUIK EXPEDITED DIFFERENTIAL STAINING VS. ROUTINE TRADITIONAL LEISHMANSTAINING PROTOCOLS: A COMPARATIVE ANALYSIS"

DR.SATHYA.P[1], DR.YOGALAKSHMI[2],
DR. R. SANKAR NARAYANAN[3]
[1]MBBS* POSTGRADUATE ,DEPARTMENT OF PATHOLOGY, SAVEETHA MEDICAL COLLEGE
[2]MBBS, DCP, TUTOR, DEPARTMENT OF PATHOLOGY, SAVEETHA MEDICAL COLLEGE.
[3]READER, DEPARTMENT OF ORAL MEDICINE & RADIOLOGY, SREE BALAJI DENTAL COLLEGE & HOSPITAL, CHENNAI, INDIA

ABSTRACT:

INTRODUCTION:

Peripheral blood smear staining is a pivotal technique that offers critical insights into blood cell morphology and its composition. It remains an indispensable tool for diagnosing and characterizing a wide range of clinical conditions that reflects the overall state of the human body. The usual protocol followed is, on making high-quality smears and studying the details under the microscope, the diagnosis is concluded with confirmation from patient's clinical profile. Traditionally, manual examination of blood smears was standard practice, but advancements in automated digital systems now aid in streamlining this process. This study aims to compare the effectiveness and efficiency of Diff-Quik staining versus the conventional Leishman stain in peripheral blood smear analysis.

MATERIALS AND METHODS:

This investigation was conducted at a tertiary care academic hospital from January to March 2024, it evaluated the staining efficacy of Diff-Quik and conventional Leishman stain in peripheral blood smear. A total of 100 consecutive samples were included, irrespective of age, sex, or cell count, with haemolysed samples excluded. Each sample yielded two smears—one stained with Diff-Quik and the other with Leishman stain. Smear grading was performed using a standardized scoring system ranging from 1 to 2, based on smear preparation quality, overall smear integrity, and the ability to assess blood cell composition.

RESULTS:

Among the 100 samples analyzed, 58 were male and 42 female, with ages ranging from 6 to 78 years. Diff-Quik staining demonstrated superior differentiation of blood cell types and significantly faster staining times compared to the conventional Leishman method. Morphologically, RBCs and WBCs exhibited enhanced clarity with Diff-Quik, whereas platelet morphology and background staining were more distinct with Leishman stain.

Key words: Diff-Quik, Leishman stain, Peripheral Smear, smear integrity, staining efficacy

INTRODUCTION

Peripheral blood smear evaluation is a cornerstone of haematological diagnostics, providing critical insights into blood cell morphology and pathology. This technique involves spreading uniformly peripheral blood cells on a slide for cytological examination [1]. Despite its simplicity and intricacies, a high-quality blood smear is indispensable for diagnosing a variety of medical conditions. Clinicians with haematologists must be aware and vigilant to appropriately implement the results to make well informed patient management decisions [2].

The choice of staining technique significantly influences the clarity, accuracy, and efficiency of smear interpretation. Among the widely used stains, Diff-Quik, a rapid Romanowsky stain, and Leishman stain, a conventional hematological stain, serve distinct roles in cytological evaluation [3] [4].

Diff-Quik is valued for its expedited staining process, enabling rapid assessment of blood cell components with minimal preparation time. Its widespread use in emergency and resource-limited settings stems from its ability to produce quick and reliable results, particularly in point-of-care applications [5]. In contrast, Leishman stain, a

traditional Romanowsky-based stain, is extensively employed in routine hematological investigations due to its meticulous staining quality, offering well-defined cytological detail and improved background clarity [6] [7]. However, its longer staining duration poses a challenge in time-sensitive diagnostic environments.
This investigation systematically evaluates the effectiveness, efficiency, and cytological detail of Diff-Quik versus Leishman stain in peripheral blood smear analysisand to compare the efficacy and efficiency of Diff-Quik staining with traditional staining methods for peripheral blood smears

MATERIALS AND METHODS

Written informed consent was obtained from all participants, and ethical clearance was secured from the institutional ethics committee for the use of human volunteers. The study was carried out at a tertiary academic hospital from January to March 2024.
One hundred consecutive (n-100) blood samples were included in the study, with no exclusions based on age, sex, or cell count. However, samples that were haemolysed or obtained from individuals who did not provide consent were excluded. For each participant, two separate smears were prepared under strictly standardized protocols: one smear was stained using Diff-Quik, and the other was processed with the Leishman stain.

Staining Procedures

- *Diff-Quik Protocol:*
  The procedure commenced with complete air drying of the smear, followed by fixation for a minimum of 30 seconds. The slide was then sequentially dipped—approximately 5 to 10 dips, with each dip lasting about one second—in red and blue staining solutions. After a gentle rinse with water, the slide was allowed to dry in an upright position. Final imaging was performed by applying immersion oil and mounting a coverslip [8].
- *Leishman Staining Protocol:*
  A thin, tongue-shaped smear was first prepared using blood collected in an EDTA tube. Leishman stain was then applied directly to the smear, which was incubated for 8 minutes. Once the stain was removed, an amount of distilled water double that of the stain was added, and the preparation was allowed to stand for an additional 2 minutes before the slide was air dried [9].

Evaluation Criteria
The stained smears were meticulously analyzed based on staining quality, the ease with which different blood cell types could be differentiated, and the overall time required for both staining and subsequent interpretation. Morphological assessments—including evaluations of red blood cells, white blood cells, platelets, and background elements—were conducted using a standardized scoring system ranging from 0 (poor) to 2 (excellent).

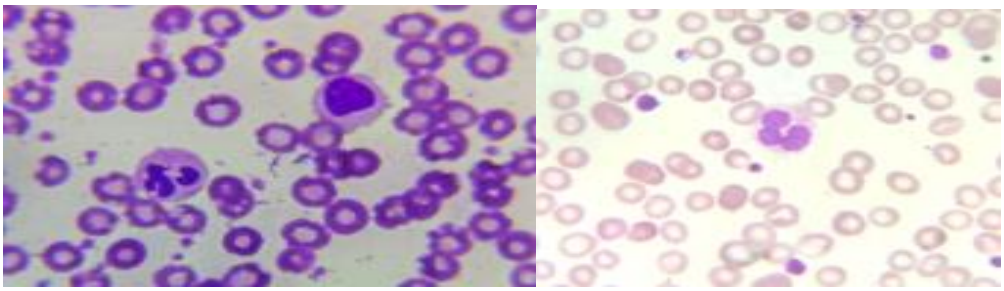


FIGURE1:-DIFF-QUIK AND LEISHMAN STAINED PERIPHERAL SMEAR

RESULT

Out Of 100 effusion samples taken into the study, 58are male and 42female. The age of participants ranged from 6 years to 78 years,divided into 8 categories.the frequency and percentages for each group are as follows:1-10 years 15 members,11-20 years-197members,21-30 years 19members,31-40 years 11members,41-50 years 13 members,51-60 years 16 members,61-70 years-5 members,71-80 years 4 members (Table 1a,b).

Table 1a- Gender Distribution

Gender	*Frequency*	*Percentage (%)*
Male	58	58%
Female	42	42%
Total	100	100%

Table 1b- Age Distribution

Age Group (years)	*Frequency*	*Percentage (%)*
1–10	15	15%
11–20	17	17%
21–30	19	19%
31–40	11	11%
41–50	13	13%
51–60	16	16%
61–70	5	5%
71–80	4	4%
Total	100	100%

The comparison indicates a clear trade-off between speed and detail. Diff-Quik offers an exceptionally fast turnaround (1–2 minutes) and high ease of use, making it ideal for rapid assessment in emergency settings. Although it provides very good nuclear detail and overall acceptable cytoplasmic and platelet visualization, it falls slightly short in these areas compared to the comprehensive morphological detail achieved by traditional stains (Giemsa/Wright), which require a longer staining time (15–30 minutes) and more precise handling. In summary, while Diff-Quik is superior for quick preliminary evaluations, traditional stains are preferred when detailed morphological analysis is crucial for diagnosis (Table 2).

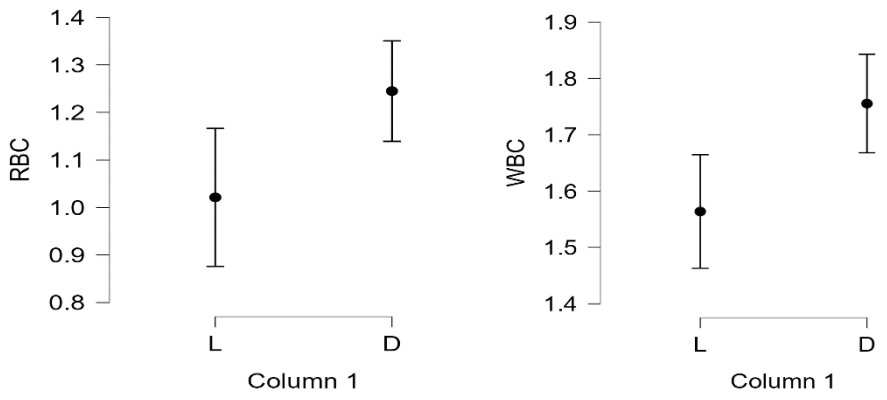
Table 2: Comparative Parameters for Diff-Quik and Traditional Staining

CRITERIA	DIFF-QUIK	TRADITIONAL STAIN (LEISHMANN STAIN
STAINING TIME	1–2 minutes	15–30 minutes
EASE OF USE	High	Moderate (requires precise timing and handling)
NUCLEAR DETAIL	Very Good	Excellent (optimal when protocol is strictly followed)
CYTOPLASMIC DETAIL	Good	Excellent
PLATELET VISUALIZATION	Good	Very Good

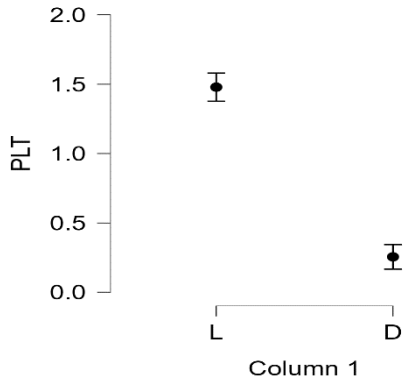
Diff-Quik-stained smears showed superior differentiation of blood cell types, offering a more pronounced nucleus-cytoplasm contrast and dramatically shorter processing times. In contrast, while Leishman staining provided enhanced platelet morphology and a cleaner background, its overall turnaround was slower. According to the comparative graph:

A. RBC Morphology: Better with Diff-Quik
B. WBC Morphology: Better with Diff-Quik
C. Platelet Morphology: Better with Leishman
D. Background Quality: Better with Leishman

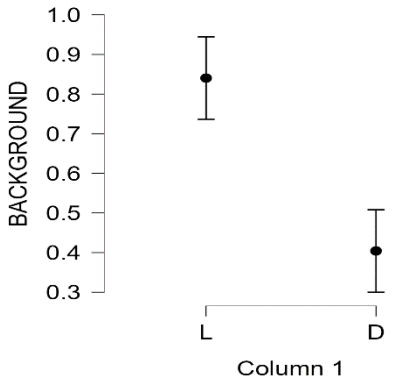
Graph 1 : Ability of differentiation of Components of Blood in a peripheral smear



A.RBC B.WBC



C.PLATELETS



D.BACKGROUND

The paired t-test comparison clearly indicates that the two staining methods yield statistically significant differences in all evaluated parameters. Diff-Quik exhibits superior performance in highlighting red and white blood cell morphology, as demonstrated by the significantly lower p-values for RBC (0.016) and WBC (0.005); these findings suggest that its rapid staining technique creates a more distinct contrast between nuclear and cytoplasmic elements. Conversely, Leishman stain excels in visualizing platelets and providing a cleaner background, with p-values of <0.001 for both parameters. This implies that while Diff-Quik is favored for rapid preliminary assessments due to its enhanced cellular detail and quick turnaround, Leishman stain is preferable for comprehensive morphological analysis where platelet detail and minimized background interference are of greater diagnostic value. (Table 3)

Table 3: Comparative Parameters of Diff-Quik vs. Leishman Stains

PARAMETER	DIFF-QUIK	LEISHMAN STAIN	P-VALUE
RBC	Superior differentiation with a clear nucleus-cytoplasm contrast	Relatively lower contrast in differentiating RBCs	0.016
WBC	Better nuclear and cytoplasmic detail, allowing for clear cell identification	Inferior detail compared to Diff-Quik	0.005
PLATELET	Suboptimal visualization of platelets	Enhanced platelet morphology and better delineation	<0.001
BACKGROUND	Less clean, with more background staining artifacts	Cleaner background that facilitates interpretation	<0.001

Aggregated operator ratings across key diagnostic parameters provide an overall assessment of each method.Diff-Quik stains significantly reduce processing time (62 ± 5 s vs. 660 ± 80 s; $p < 0.001$) and score higher in rapid turnaround and overall composite operator ratings (8.8 ± 0.5 vs. 7.6 ± 0.6; $p = 0.02$). Although traditional Giemsa staining shows superior diagnostic accuracy ($p < 0.05$) and reproducibility ($p = 0.001$), Diff-Quik's benefits in speed, ease of use, and operator satisfaction make it more suitable for rapid assessments. (Table 4)

Table 4: Overall Diagnostic Utility Scores

Parameter	*Diff-Quik Score*	*Traditional (Giemsa) Score*	*P value*
Rapid Turnaround	10	5	NS
Ease of Use	9	7	NS
Diagnostic Accuracy (Morphology)	8	9	<0.05
Reproducibility	8	9	0.001
Operator Satisfaction	9	8	NS
Composite Score (Average)	8.8±0.5	7.6±0.6	0.02
Staining Time (s)	62±5	660±80	<0.001

DISCUSSION

Peripheral blood examination is a fundamental tool in hematology, pivotal for diagnosing anemia, thrombocytopenia, leukemic abnormalities, and parasitic infections. The reliability of its diagnostic insights hinges on the strict implementation of standardized staining protocols and operating procedures [10].

Adherence to these protocols minimizes errors—such as distorted red blood cells, artificial gaps, or overly thin cell margins—that can cause misleading diagnosis. Inadequate staining may lead to the loss of critical details, such as polychromasia, spherocytosis, and rouleaux formation, which are key indicators in clinical evaluations [11].

When performing Leishman staining, it is essential to adhere to specific guidelines to achieve optimal results. One important practice is to avoid pipetting directly from the stock solution, as this minimizes exposure to moisture that can degrade the stain [12].

In the Leishman protocol, the differential uptake of dyes is key: the DNA and acidic protein groups in cell nuclei and the primitive cytoplasm attract the basic dye Azure B, while the basic groups on hemoglobin attract the acidic dye Eosin Y. This interaction produces a brilliant violet coloration in the nuclei and neutrophil granules, enhancing the clarity of differential counts. Consequently, Leishman stain provides superior contrast between the cytoplasm and the nucleus compared to simpler methylene blue and eosin-based stains (table 5).[13]

Cell Type	Nucleus	Cytoplasm	Granules
Red Blood Cells (RBCs)	No nucleus	Pink to red, depending on age and hemoglobin content	None
Neutrophils	Deep blue, bilobed	Pale blue to lilac-pink	Fine, pink to purple
Basophils	Deep blue, round or oval	Deep blue to purple	Large, dark purple to black
Eosinophils	Deep blue, bilobed	Pale pink to orange	Large, orange-red
Monocytes	Deep blue, round or indented	Pale blue to gray	Fine, faint pink
Lymphocytes	Deep blue, round or indented	Pale blue to clear	None
Platelets	No nucleus	Pale purple to pink	None

Table 5 shows morphology of blood and its components using Leishmann staining technique

Diff-Quik staining employs two dyes to rapidly differentiate cellular structures. The eosinophilic component (eosin Y) binds to acidic cytoplasmic elements—rendering erythrocytes a pinkish hue due to hemoglobin interaction, and highlighting leukocyte granules—while the basophilic component (methylene blue) binds to basic structures like nuclei and certain granules, imparting a blue tint. This contrast facilitates quick and detailed identification of various blood cell types (table 6). [14]

Structure	*Colour*
Erythrocytes	Pink/yellowish red
Platelets	Violet/purple granules
Neutrophils	Blue nucleus, pink cytoplasm, violet granules
Eosinophils	Blue nucleus, blue cytoplasm, red granules
Basophils	Purple/dark blue nucleus, violet granules
Monocyte	Violet nucleus, light blue cytoplasm
Bacteria and fungi	Dark blue

Table 6 shows morphology of blood and its components under

On directly comparing the performance of Diff-Quik and Leishman stains on peripheral blood smears, Diff-Quik offers an exceptionally fast turnaround time, typically 1–2 minutes, compared to the 15–30 minutes required for traditional stains like Leishmann.

Also, Diff-Quik showed superior differentiation of red blood cell (RBC) and white blood cell (WBC) morphology, including a more pronounced nucleus-cytoplasm contrast. However, another source, comparing Papanicolaou (Pap) stain and Leishman stain on peripheral blood smears, noted that Pap caused lysis of cells and changes in morphology, making differentiation difficult, while Leishman maintained proper morphology [15]. The bat blood smear source, comparing various stains including Romanowsky-Giemsa and Pappenheim,

found these two were highly efficient for cell identification and showed easily differentiable cytoplasmic and nuclear structures[16].

Diff-Quik provided "Very Good" nuclear detail and "Good" cytoplasmic detail. Another study by Balaji V et al noted that for cytologic samples, Papanicolaou stain is considered best for the visualization of fine nuclear detailswhile Diff-Quik is better for cytoplasmic features [17].

Regarding platelets,Leishman stain excelled in visualizing platelets and provided enhanced platelet morphology compared to Diff-Quik, with a statistically significant difference ($p<0.001$). This aligns with the observation in another source that in a Leishman's stained peripheral blood preparation, platelets appear as distinct purple bodies with irregular borders[18]. In contrast, the study by Niveditha G et al comparing Pap and Leishman on peripheral smears found that platelet morphology was well defined with Leishman, but platelets were not at all visible with Pap stain [15]. The unstained peripheral smear study notes that verifying abnormal platelet counts by manual examination of Leishman's stained smears is a standard procedure [19].

Taking into account, background quality,Leishman stain provided a cleaner background, which facilitates interpretation, whereas Diff-Quik resulted in a less clean background with more staining artifacts ($p<0.001$). This finding is partially supported by the effusion fluid source, which mentioned that alcohol-fixed smears (used for Pap and H&E) had minimal background specks compared to air-dried smears (used for Diff-Quik and Leishman) . However, the effusion fluid source also ranked H&E as best for background features, followed by Pap and Leishman, with Diff-Quik scoring lowest among the four stains tested [18].

CONCLUSION

The optimal choice between Diff-Quik and traditional stains like Wright-Giemsa depends on the clinical context and specific diagnostic needs. Diff-Quik is particularly beneficial where rapid turnaround and ease of use are essential—for example, in emergency departments or resource-limited settings. Its fast staining time allows for quick initial assessments that can guide urgent clinical decisions [20] [21].
Conversely, traditional stains such as Wright-Giemsa excel in delivering detailed morphological insights and higher diagnostic accuracy. This method is invaluable for comprehensive evaluations where nuanced cellular features need to be discerned. Although it requires more processing time and precise handling, its ability to reveal subtle diagnostic details makes it indispensable in complex cases.
In summary, while each technique has unique advantages, their application should be tailored to the clinical scenario. Employing a hybrid approach—using Diff-Quik for rapid preliminary screening and following up with Wright-Giemsa staining for in-depth analysis when needed—can leverage the benefits of both methods and yield optimal diagnostic outcomes.

REFERENCES

1) Prasad V, Sharma D, Verma SL. Peripheral Blood Smear Examination: An Overview. Webology. 2022 Apr 1;19(2).
2) Mishra SB, editor. Critical Care Hematology. Springer Nature; 2024.
3) Neuber A, Nuttall T. Diagnostic techniques in veterinary dermatology. John Wiley & Sons; 2017 Apr 20.
4) Campbell TW, Ellis CK. Avian and exotic animal hematology and cytology. John Wiley & Sons; 2013 Jul 3.
5) Muniraj F. Diff-Quik: A reliable stain for rapid onsite evaluation and differentiation of round cells in semen. CytoJournal. 2022 Dec 1;19.
6) Stain DQ. Rapid H&E Stain. Atlas of Touch Preparation Cytopathology. 2017 Oct 28:21.
7) Gajendra S, Jha B, Goel S, Sahni T, Sharma R, Shariq M, Jaiswal S, Sachdev R. Leishman and G iemsa stain: a new reliable staining technique for blood/bone marrow smears. International Journal of Laboratory Hematology. 2015 Dec;37(6):774-82.
8) Hamasaki M, Chang KH, Nabeshima K, Tauchi-Nishi PS. Intraoperative squash and touch preparation cytology of brain lesions stained with H+ E and Diff-Quik™: a 20-year retrospective analysis and comparative literature review. Acta Cytologica. 2018 Jan 4;62(1):44-53.
9) Fasakin KA, Okogun GR, Omisakin CT, Adeyemi AA, Esan AJ. Modified Leishman stain: the mystery unfolds.
10) Han Z, He J, Xie X, Fang J, Li Y. Investigation and analysis on the application of peripheral blood specimens for routine blood testing by laboratory physicians. Annals of Palliative Medicine. 2021 Sep;10(9):9516522-9522.

11) Suganya Devi K, Arutperumjothi G, Srinivasan P. Diagnosis evaluation and interpretation of qualitative abnormalities in peripheral blood smear images—a review. Health informatics: a computational perspective in healthcare. 2021:341-65.
12) Mondal H, Mondal S, Pal A. Optimum volume of water to be added to the leishman stain while preparing a blood smear. Int J Clin Exp Physiol. 2017 Oct 1;4(4):198-201.
13) KUHLMANN WD. Dyes, stains, and special probes in histology.
14) Jimenez-Heffernan JA, Alvarez F, Muñoz-Hernández P, Bárcena C, Azorin D, Bernal I, Pérez-Campos A. Cytologic features of ventricular tumors of the central nervous system: a review with emphasis on Diff-Quik stained smears. Acta Cytologica. 2021 Mar 18;65(2):111-22.
15) Nivedhita G, Brundha MP. Analysis of Papanicolaou stain on peripheral smear compared to Leishman's stain: A prospective study. International Journal of Clinicopathological Correlation. 2020 Jul 1;4(2):40-.
16) Ghazaryan A, Adamyan S, Hayrapetyan T, Papov G, Hakobyan L, Abroyan L, Bayramyan N, Hakobyan S, Poghosyan A, Torossian H, Karalyan Z. Comparative staining of Rhinolophus spp. white blood cells in blood smears. Veterinary World. 2022 Mar 29;15(3):750.
17) Balaji V, Yamini PM, Kumaravel A, Sonti S. Chromatic Chronicles—A Comparative Journey through Cell Morphology in Effusion Fluids. InRecent Developments in Microbiology, Biotechnology and Pharmaceutical Sciences 2025 May 6 (pp. 182-188). CRC Press.
18) Umashankar T, Thomas BM, Sahana P. Estimation of platelet count in unstained peripheral blood smears in comparison with stained smears and evaluation of its efficacy. The Malaysian Journal of Pathology. 2014 Dec 1;36(3):195.
19) Sharkey LC. In-hospital hematology: how to save a life with a blood smear.
20) Piaton E, Fabre M, Goubin-Versini I, Bretz-Grenier MF, Courtade-Saïdi M, Vincent S, Belleannée G, Thivolet F, Boutonnat J, Debaque H, Fleury-Feith J. Guidelines for May-Grünwald–Giemsa staining in haematology and non-gynaecological cytopathology: recommendations of the French Society of Clinical Cytology (SFCC) and of the French Association for Quality Assurance in Anatomic and Cytologic Pathology (AFAQAP). Cytopathology. 2016 Oct;27(5):359-68.
21) He J, Gong Y. Cytology Techniques. InPractical Cytopathology: Frequently Asked Questions 2019 Nov 1 (pp. 1-18). Cham: Springer International Publishing.

REFERENCES

1.Adewoyin AS, Nwogoh B. Peripheral blood film - a review. Ann Ib Postgrad Med. 2014 Dec;12(2):71-9. PMID: 25960697; PMCID: PMC4415389.
2. Lynch EC. Peripheral blood smear. Clinical Methods: The History, Physical, and Laboratory Examinations. 3rd Edition. 1990.
3.Hegde RB, Prasad K, Hebbar H, Sandhya I. Peripheral blood smear analysis using image processing approach for diagnostic purposes: A review. Biocybernetics and Biomedical Engineering. 2018 Jan 1;38(3):467-80.
4. Sareen R, Kapil M, Gupta GN. Incubation and its effect on Leishman stain. J Lab Physicians. 2018 Jul-Sep;10(3):357-361. doi: 10.4103/JLP.JLP_154_17. PMID: 30078977; PMCID: PMC6052813.
5.Muniraj F. Diff-Quik: A reliable stain for rapid onsite evaluation and differentiation of round cells in semen. CytoJournal 2022;19:59.
5.Fasakin KA, Okogun GR, Omisakin CT, Adeyemi AA, Esan AJ. Modified Leishman stain: The mystery unfolds. Br J Med Med Res. 2014;4:4591–606. [Google Scholar]
6.Shute PG, Maryon M. An Improved Method of Preparing Romanowsky Stains (with some hints on staining) 1965. [Last accessed on 2018 Mar 21]. Available from: http://www.whqlibdoc.who.int/malaria/WHO_Mal_478.64.pdf .
*****