

Evaluating Field Stain for Rapid On-Site Evaluation in Fine Needle Aspiration Cytology of Breast Lesions: Diagnostic Utility with May-Grünwald Giemsa Staining

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Date of Submission: 10/03/2025

Date of Review: 15/03/2025

Date of Acceptance: 01/04/2025

ABSTRACT

Introduction: Fine Needle Aspiration (FNA) cytology is a well-established essential, basic diagnostic technique for investigating lumps and lesions at various anatomic sites. While performing FNA of the breast lumps a quick or rapid result is desirable to know the nature of the lump and allay the anxiety of the patient. Rapid On-Site Evaluation (ROSE) is useful to evaluate the cytological smears within a few minutes. This study aims to evaluate the utility of Field's stain in ROSE of breast lesions in arriving at a diagnosis and its comparison to routine MGG (May Grünwald Giemsa) stain. **Materials and methods:** This prospective observational study was conducted from July 2023 to December 2023 at a tertiary care center. A total of 89 cases with breast lumps were included. Previously diagnosed cases were excluded. All the cases were evaluated by ROSE using Field's stain for preliminary diagnosis and compared later with MGG stain for final diagnosis. The categorization of cases was done as per IAC Yokohama System and further management was advised accordingly. **Results:** After first and repeat aspiration 62 (69.6%) cases were categorized as benign, 12 (13.4%) cases were found to be malignant whereas 8 (8.9%), 5 (5.6%), 2 (2.24%) cases were included in the suspicious, atypical and insufficient categories respectively. Categorization of the breast lumps by ROSE using Field stain was in complete agreement with the MGG stained smears and the final FNA diagnosis and showed no discrepancy. **Conclusion:** The use of Field's stain for ROSE of breast lumps had comparable staining quality to MGG which resulted in accurate correlation between provisional and final cytologic diagnoses with the added

advantage of rapid turnaround time. Field stain was hence found to be reliable, cost effective and time saving when used for ROSE of breast lumps. Hence the use of this readily available and affordable stain for ROSE of FNA smears, especially of breast lumps, can prove to be highly beneficial for prompt categorization and triaging of patients.

KEYWORDS: Rapid on site evaluation, Breast, Field Stain, Prompt Categorization, Triage

INTRODUCTION

Fine needle aspiration cytology is a well-established essential, basic diagnostic technique for investigating lesions at various anatomic sites. It is a relatively safe, accurate procedure with a low complication rate. It has high sensitivity and specificity in the diagnosis of malignant lesions.^[1] Stains commonly used for staining FNA smears are Hematoxylin & Eosin (H&E), Papanicolaou, and Giemsa. The whole procedure takes 2-3 hours to complete. If the sample is adequate and representative it will lead to a correct diagnosis, but inadequate, non-representative will lead to repeat procedure causing delay in diagnosis, wastage of time and resources which increases the anxiety to patients.^[2] To minimize these complications, the demand for Rapid On-Site Evaluation (ROSE) has increased in cytopathology laboratories. Rapid on-site evaluation (ROSE) is a procedure that allows assessment of adequacy of cytological specimens generally obtained by needle aspiration techniques.^[3, 4] Using a quick staining method, it is possible to have a smeared cytological slide ready to be evaluated using light microscopy, within few minutes.^[5] It is an immediate, real-

time evaluation of fine-needle aspiration smears and biopsy touch imprints. It is highly advantageous adjunct to any routine diagnostic method using fine-needle aspiration (FNA), useful for assessment of lesions or masses from deep-seated organs. Samples obtained by ROSE can be used for diagnosis based on morphologic criteria alone and also for special ancillary studies, including molecular analysis. It is an effective way for morphological analysis of samples, ensuring sufficient quantities and quality of cells for complete diagnostic workup. The reported rate of non-diagnostic (ND) FNA is as high as 20%.^[6] Several studies concluded that ROSE resulted in a significant decrease in the number of non-diagnostic cases. ROSE has various advantages like reduced additional sampling and turnaround-time, cost-effective with improvement in diagnostic yield.^[7]

ROSE performing laboratories commonly use a variety of quick stains like Wright-Giemsa, Diff-Quick, Papanicolaou, which are costly. Stains like Toluidine Blue are cheaper but stains only the nucleus due to which cell morphology cannot be appreciated. Field stain is a Romanowsky's stain, which comprises Field stain A and B. Composition of Field stain A is methylene blue, azur, disodium dihydrogen phosphate anhydrous, potassium dihydrogen phosphate anhydrous and distilled water. Composition of field stain B is eosin powder, disodium dihydrogen phosphate anhydrous, potassium dihydrogen phosphate anhydrous and distilled water. Some studies have concluded that Field's stain has staining quality comparable to conventional Giemsa stain for both qualitative and quantitative analysis. This stain is used as a quick staining method for detection of malaria and filaria parasites in screening camps.^[8] The advantages of Field stain are rapid staining time, only 2 reagents with 70% alcohol as a fixative are required, inexpensive, high reproducibility, good correlation with histological diagnosis, helpful for detection of blood parasites and fungi.^[9] However, this stain has few limitations like not being ideal for thin smears, limited cellular details and potential for overstaining.^[8]

Aims and Objectives

- To evaluate the utility of Field Stain in ROSE in arriving at a diagnosis in comparison to routine stains (MGG).
- Screening for adequacy of FNA smears of breast lumps using Field's stain.

MATERIALS AND METHODS

Study Design: This was prospective observational study.

Source of data: Data from patients presented with breast lump in the cytology outpatient clinic was collected.

Study duration and location: This study was conducted between July 2023 to December 2023 in the Department of Pathology at tertiary care center.

Sample size: A total of 89 cases were included in this study.

Inclusion criteria: Patients presented with breast lump for the first time were included.

Exclusion criteria: Already diagnosed cases were excluded.

Procedure and Methodology: Detailed clinical history was taken along with findings of radiological and previous laboratory reports. Thorough physical examination of patients was done. Non-guided FNA and whenever required ultrasound-guided FNA was performed without local anesthesia using 23G needle, 10 ml syringe and aspiration gun. The aspirated material was spread on slides to make smears. One smear for ROSE was fixed in absolute alcohol or methanol and air dried rapidly. The fixed smear was then dipped in Field stain B 15 times and washed in water. Then the smear was dipped in Field stain A 20 times, washed in water and air dried. The Field stained smear was evaluated for ROSE to make a preliminary diagnosis. The other air dried smears were stained with MGG stain as per routine procedure for final diagnosis. The evaluation was not blinded as clinical examination and radiological investigations like ultrasonography were necessary for correlation. The multiple observers were involved with no interobserver variability in arriving at a preliminary diagnosis.

The reporting was carried out using IAC Yokohama System for Reporting Breast FNA Cytopathology to make a preliminary diagnosis. The criteria adopted for adequacy of smears was seven epithelial groups each consisting of 20 or more epithelial cells along with presence or absence of myoepithelial cells^[10]. If fewer than 7 epithelial groups found smears were labelled as scanty cellular while smears with more than 10 epithelial groups were labelled as highly cellular.

In those cases where initial FNA yielded scanty cellularity, repeat FNA was immediately done, and smear was stained using Field stain and ROSE reevaluation was again done. Smears stained with Field Stain were later compared with MGG stained smears. The features that were compared included cell morphology, background, stroma and any other additional features. Preliminary diagnosis on ROSE was also compared with final specific cytopathology diagnosis that was offered on MGG.

Statistical Methods: Data were analyzed using the SPSS software version 22.0. Descriptive statistics such as means and standard deviations were used to summarize continuous variables, and frequencies and percentages were used for categorical variables. Kappa statistics was used to measure the level of agreement. Sensitivity, specificity, positive predictive value, negative predictive values were calculated.

OBSERVATIONS AND RESULTS

A total of 89 cases presented with breast lump were included in this study. Majority of cases were in the age group of 11 to 20 years (26.96%) followed by 21 to 30 years age group (25.84%). The age range was 15 years to 86 years

and mean age (\pm SD) was 32.93 (\pm 8.48) years. There were 3 males while the remaining 86 were females. Table 1 shows age-wise distribution of cases.

Age Group (years)	Number of cases	Percentage
11-20	24	26.96%
21-30	23	25.84%
31-40	17	19.10%
41-50	12	13.48%
51-60	10	11.23%
>61	3	3.37%

Table 1: Age-wise distribution of cases with breast lumps

After the first attempt of FNA, all cases were evaluated and categorized as per Yokohama system. ROSE of Field stained smears revealed scant cellularity in 12 cases, while moderate cellularity and high cellularity were observed in 46 and 29 cases respectively. So, a total of 12 cases were categorized as Insufficient after ROSE of the first FNA. Repeat FNA was done immediately in these 12 cases. Of these 12 cases, ROSE revealed that repeat FNA yielded cellular smears in 10 cases which were re-categorized as per Yokohama system, while scant cellularity was again obtained in 2 cases that were included in the Insufficient category and biopsy was advised.

62 (69.6%) cases were categorized as benign that included 54 benign cases after first aspiration and 8 cases after immediate repeat aspiration that had initially been categorized as insufficient. 12 (13.4%) cases were found to be malignant after ROSE of which 11 cases were categorized as malignant after first aspiration and 1 case after repeat aspiration. 8 (8.9%) cases were included in the suspicious category which included 1 case after repeat aspiration and 5 (5.6%) cases were in atypical category.

Table 2 shows categorization of cases as per Yokohama system after ROSE.

Categorization of the breast lumps by ROSE using Field stain were in complete agreement with the MGG stained smears and the final FNA diagnosis and showed no discrepancy ($\text{Kappa}=1$ with 95% CI- 1.0 to 1.0). Overall sensitivity of this study was 97.75% (95% CI- 92.12% to 99.73%) while Positive predictive value was 100% (95% CI- 95.85% to 100%).

DISCUSSION

FNA is a multistep process, and many factors have the potential to affect the overall diagnostic yield. [11] Role of ROSE has been evaluated for assessing adequacy in various organs especially during advanced aspiration cytology techniques like CT guided FNA, endoscopic ultrasound-guided (EUS), transbronchial aspiration cytology. With the use of

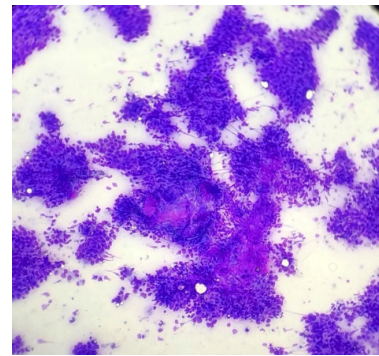


Figure 1: Highly cellular smear comprising benign epithelial cells in clusters, stromal fragments and dispersed bare bipolar nuclei in a clean background (Field stain) 10X

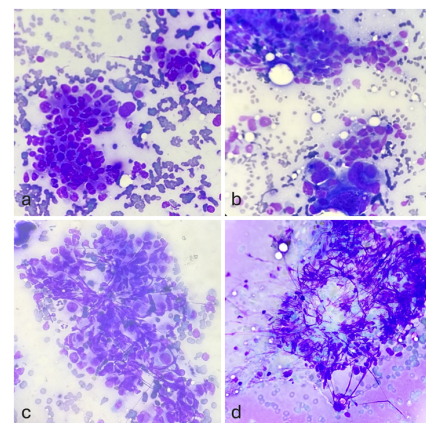


Figure 2: Duct carcinoma- Large cells with pleomorphic hyperchromatic nuclei and scant to moderate cytoplasm (a-Field, b-MGG 40X) with stromal infiltration and extensive fibrosis (c,d- Field 40X)

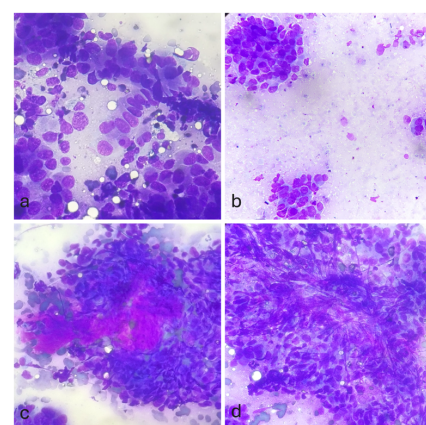


Figure 3: Elderly male with Duct Carcinoma (a columnar cell change in Field, b-MGG 40X), muscle infiltration and fibrin strands (c,d- Field 40X)

Categories	ROSE (Field stain)		Management (As per ROSE)	Final FNA Report (MGG)	Management (As per Yokohama System)
	1 st FNA	Repeat FNA*			
Insufficient	12 (13.48%)	2 (2.24%)	Repeat FNA (after 1 st) CNB (after repeat FNA)	2	Repeat FNA or Core Needle Biopsy
Benign	54 (60.67%)	62 (69.66%)	Reassurance & further management pre-planned	62	Follow up after 6 months/Surgical intervention
Atypical	5 (5.61%)	–	Follow up & wait for Final FNA report	5	Follow up with repeat FNA/ Advice for CNB
Suspicious	7 (7.86%)	8 (8.98%)	Wait for Final FNA report	8	Advice for CNB/Immediate surgical intervention
Malignant	11 (12.35%)	12 (13.48%)	Immediate surgical opinion	12	Advice for CNB/Immediate surgical intervention
Total		89		89	

(*Repeat FNA done immediately after ROSE yielded cellular smears based on which the 10 cases categorized as Insufficient could be re-categorized.)

Table 2: Categorization of cases as per Yokohama system after ROSE using Field's stain and Final FNA report using MGG stain

Categorization by ROSE using Field stain	MGG stained report	Final Specific Cytodiagnosis
Insufficient (2)	Insufficient (2)	-
Benign (62)	Benign (62)	Mastitis-4, Galactocoele-4, Gynaecomastia-2, Benign Breast Lesion (BBL) - 12, Fibroadenoma-40
Atypical (5)	Atypical (5)	Phyllodes- 2, Proliferative Breast Disease (PBD)- 3
Suspicious (8)	Suspicious (8)	PBD with Atypia-8
Malignant (12)	Malignant (12)	Ductal malignancy- 12

Table 3: Comparison of diagnostic categories between MGG-stained smears, Field-stained smears and Final cytodiagnosis

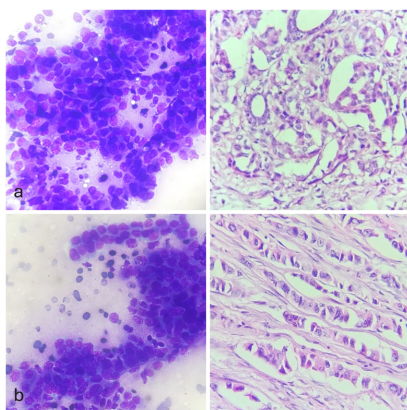


Figure 4: Duct Carcinoma. Comparability patterns on Field stain smear (crisp cellular morphology) and histopathology. (a- acinar pattern, b- trabecular pattern 40X)

ROSE many studies have shown that patients may spend less time in the hospital and require fewer diagnostic procedures and surgical interventions with minimum complications. [12] ROSE technique also allows for preliminary diagnosis so that additional material can be requested for ancillary studies such as flow cytometry, microbiology cultures, or molecular studies. [6] A recent meta-analysis of studies found an overall improvement of 12% in adequacy rates with ROSE but variations may be there according to the initial adequacy rate without ROSE. [13, 14] A recent study by Agrawal N. et al [15] integrated the use of ROSE along with the International Academy of Cytology (IAC) Yokohama System for Reporting Breast Fine Needle Aspiration Biopsy Cytopathology. The observation of the study was that there was improvement in the accuracy of diagnosis of breast lesions, decreased missed diagnoses by reducing number of insufficient cases, improved concordance between cytology and subsequent

biopsy reports along with a standardized reproducible system for the monitoring and auditing of breast pathology services and improved training at pathology centers.

A wide variety of cytologic stains (such as Haematoxylin and Eosin, Pap and May Grunwald Giemsa stain) are employed for staining of routine FNA smears across different laboratories. The average time required for fixation and staining of the smears varies for different stains, with air-dried smears stained with MGG stain requiring the least time that is around 20 minutes. For ROSE, stains like rapid H&E, ultra-fast PAP, toluidine blue, brilliant cresyl blue (BCB), and Diff-Quik have been used in various research articles.^[16,17] Some studies have used toluidine blue but there are disadvantages like high false positivity rate and low specificity in staining dysplasia.^[18]

In the present study ROSE of breast lesions was done using Field's stain. The main objective of this study was to evaluate the practicability of Field's stain in ROSE smears. Field stain was chosen because this stain is routinely used for examination of peripheral blood smears in various diagnostic camps held in remote Peripheral Health Care centers besides being cheap and readily available with good staining quality. So, it negates the need to carry any extra reagents or equipment.

It is a water based stain that provides good nucleocytoplasmic definition on air-dried alcohol fixed slides. Staining procedure by Field stain is extremely fast which takes less than a minute to be completed. Immediate screening of the smear can be done, and if the smears are found to be insufficient, repeat FNA can be performed immediately. Another advantage of using Field stain in ROSE is that the slide can be examined without mounting with DPX which further reduces time and expenditure. So, the overall turnaround time is rapid while being cost effective. To the best of our knowledge, none of the previous research articles have used this readily available and affordable Field stain in ROSE. Detailed cell morphology, arrangement of cells, stromal and muscle infiltration, and fibrosis if any can be very well appreciated in smears stained with Field stain. This was the major reason for total concordance of results between Field stain and MGG stain. And in few cases the cell arrangements observed on smears stained with Field stain were comparable to the subsequent histopathology sections.

Mean age in our study was 32.93 years which is comparable with studies done by Kanchan Kothari et.al.^[19] and Asteria H. Kimambo et.al.^[20] Age range in our study was 15 years to 86 years while it was wider in range in study done by Kanchan et.al.^[19] and in concordance with study done by Asteria H. Kimambo et.al. (18 to 75 years)^[20] and Yadav et.al. (18 years to 74 years)^[21]. Most of the patients in our study were females (86 of 89, 96.6%) as it was in the study by Asteria H. Kimambo et.al (48 of 50, 96%).^[20]

In the present study, the adequacy rate obtained after first aspiration was 84.2% which increased to 97.7% after

repeat aspiration was done in those cases that were categorized as insufficient after ROSE with the use of Field stain. The increase in adequacy has also been reported in other studies^[1]. In the study by Asteria H. Kimambo et.al^[20] among preliminary diagnoses 92% cases were benign while 8% cases were malignant and after final cytologic review 76% cases were benign and 20% cases were malignant. No difference was observed in the present study between preliminary diagnoses using Field stain and the final FNA diagnosis using MGG stain and also in terms of cell morphology, stroma, background staining and other features. In the present study, the maximum number of cases was benign (69.6%) who were reassured in the first visit itself and further management could be planned as per the patient's convenience. In 4 cases FNA proved to be therapeutic as it yielded milky aspirate, and the lumps regressed completely after aspiration. In these 4 cases, though the smears showed scant cellularity, lipo proteinaceous material was observed in the background with few foamy cells. Correlating the cytological features with history of lactation and with the ultrasound findings, these cases were categorized as Benign and interpreted as Galactocoele and repeat FNA was not required. 13.4% cases were categorized as malignant who were advised immediate surgical consultation that helped decrease the time period between final FNA report and further surgical management. The cases (14.5%) in the atypical and suspicious categories had to wait for the final FNA report but were informed about the provisional diagnosis and further possible management after ROSE. However, it was observed that there was total correlation of the provisional diagnoses and final diagnoses in all cases included in these two categories. Hence in these cases also ROSE with Field staining of smears can be used for prompt reports.

Sensitivity of this study was 97.75% with Positive predictive value of 100%. There was a very low false negative rate (2.24%) with no false positivity. This was in concordance with studies done by Kanchan et.al.^[19] and Joy et.al.^[2]

Breast lesions were included in this study as these are the most common lesions encountered in the cytology OPD of the Tertiary Care Center where this study was conducted. The incidence rate of breast cancer is increasing in relatively younger age groups (25-49 years) with an incidence of 32.89%.^[22] Though radiologic screening procedures like mammography are readily available in India, they are not easily accessible and affordable to patients in peripheral regions and in remote areas. Core Needle Biopsies too are best done under guidance at higher centers, and specifically in those cases where Duct Carcinoma is suspected. FNA of breast lumps is an established technique for diagnosing palpable breast lesions which is safe, effective, economical, and accurate. FNA along with staining of smears with Field stain and ROSE can prove to be invaluable as a screening technique in Peripheral Health Care centers and in diagnostic camps held in remote areas. The advantages of using Field stain for ROSE as already stated are that evaluation of breast

lumps or any other lesion can be done in a diagnostic camp at a remote center with no added financial burden and can be completed rapidly so that the patient can be issued a preliminary report within a few minutes. And most importantly it can help in the triage of patients into those who require immediate surgical referral to tertiary health care centers as against those who can plan their visit for a later date. This holds significance considering the underprivileged section of society living in remote areas that do not have easy and ready access to higher health care centers.

CONCLUSION

ROSE using Field stain is a quick and easy method in making provisional diagnosis in patients with breast lumps. In the present study it was observed that using Field stain for ROSE had comparable staining quality to MGG which resulted in accurate correlation between provisional and final diagnoses. Field stain is readily available and commonly used in diagnostic camps in Indian rural settings and hence can be easily used for screening breast or any other superficial lump patients in health camps without any added burden on budget. In government hospitals the majority of patients are poor who cannot afford multiple visits to higher centers. So, further line of management can be decided immediately in one visit, minimizing delay in diagnosis and repeated visits. Once validated, this would definitely help in patient care in settings where diagnostic services are limited. Thus, ROSE can definitely blossom in Field!.

DISCLOSURE

Funding: None

Conflict of Interest: None Declared

Author Contribution: All the authors involved in study have contributed equally at all stages of work

Acknowledgements: I would like to express my appreciation to all those who have supported and contributed to the completion of this project

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How to cite this article: Galat S, Nayak S, Tadvi F, Yedshikar V. Evaluating Field Stain for Rapid On-Site Evaluation in Fine Needle Aspiration Cytology of Breast Lesions: Diagnostic Utility with May-Grünwald Giemsa Staining. *Perspectives in Medical Research*. 2025;13(1):35-41
DOI: [10.47799/pimr.1301.08](https://doi.org/10.47799/pimr.1301.08)