Diagnostic Utility of Rapid On-Site Evaluation (ROSE) in Fine Needle Aspiration Cytology: A Prospective Observational Diagnostic Accuracy Study of 1432 Cases from a North Indian Tertiary Care Centre

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ABSTRACT

Background

Fine needle aspiration cytology (FNAC) is a first-line, minimally invasive diagnostic modality whose utility is constrained by inadequacy rates of 10–35% across different organ sites. Rapid on-site evaluation (ROSE) provides immediate cytomorphological assessment of each aspirated pass to address this limitation, but its routine implementation in Indian tertiary care settings remains inconsistent and large-cohort Indian data are limited.

Aim

To evaluate the diagnostic utility of ROSE in FNAC of palpable and image-guided lesions, with reference to specimen adequacy, concordance with final cytological diagnosis, and diagnostic accuracy.

Materials and Methods

A prospective, observational, diagnostic accuracy study was conducted in the cytopathology section of the Department of Pathology, MMIMSR, Mullana, over a two-year period (November 2023 to November 2025). Of 1480 patients referred for FNAC, 1432 met the inclusion criteria after exclusion of 48 (28 refusals of consent; 20 with bleeding diathesis or anticoagulation). Four to five smears were prepared per pass: two air-dried smears for ROSE (toluidine blue, Field's stain or rapid Giemsa) and routine staining (May-Grünwald Giemsa), and two to three wet-fixed smears for Papanicolaou and H&E. ROSE-guided repeat passes were performed for inadequate aspirates. Ancillary investigations — cell block, Ziehl-Neelsen stain, CBNAAT, immunocytochemistry — were triaged on the basis of the ROSE provisional diagnosis. Outcomes included specimen adequacy on ROSE, cumulative adequacy after ROSE-guided passes, concordance with final diagnosis (Cohen's κ), and diagnostic accuracy for adequacy and malignancy.

Results

Mean age was 39.11 ± 16.92 years (range 1–87), with female predominance (62.22%) and a male-to-female ratio of 1:1.65. Lymph node (38.34%), thyroid (21.30%), breast (17.95%) and soft tissue (16.06%) were the principal sites. First-pass ROSE adequacy was 70.95% (1016/1432) and improved to 87.71% (1256/1432) after ROSE-guided repeat passes (McNemar $\chi^2 = 240.00$, $p < 0.001$), corresponding to a ROSE-facilitated conversion rate of 57.69% (240/416). Pass-wise adequacy declined progressively with each successive pass (96.69% at 1 pass to 0% at 5 passes; Spearman $r = –0.357$, $p < 0.001$). Stain-wise adequacy did not differ significantly between toluidine blue (88.04%), Field's (84.46%) and rapid Giemsa (92.48%) ($p = 0.842$). Diagnostic concordance between ROSE and final cytological diagnosis was 86.31% (Cohen's $\kappa = 0.763$; weighted $\kappa = 0.803$, substantial agreement). For adequacy assessment, ROSE showed sensitivity 91.54%, specificity 59.26%, PPV 96.50%, NPV 36.36% and accuracy 89.11%. For malignancy detection (C4 + C5), ROSE achieved sensitivity 87.59%, specificity 98.07%, PPV 92.03%, NPV 96.89% and overall diagnostic accuracy 95.95%. ROSE facilitated ancillary investigations in 33.89% of cases. Cyto-histopathological concordance, available in 229 cases (15.99%), was 92.58% ($p < 0.001$).

Conclusion

ROSE is a reliable, practical, and diagnostically accurate technique that significantly improves the quality and yield of FNAC by reducing inadequate aspirates, enabling intelligent triage of material for ancillary studies, and providing substantial agreement with the final cytological diagnosis. The findings support routine implementation of ROSE — with toluidine blue as a low-cost, high-quality rapid stain — in resource-limited Indian tertiary care settings.

Keywords: Rapid on-site evaluation, ROSE, fine needle aspiration cytology, FNAC, specimen adequacy, diagnostic accuracy, toluidine blue, Cohen's kappa, Bethesda system, cyto-histopathological correlation.

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INTRODUCTION

Fine needle aspiration cytology (FNAC) is a well-established, minimally invasive diagnostic technique used for the pre-operative evaluation of both superficial and deep-seated swellings.[1,2] Over the past few decades it has emerged as a first-line investigative tool in the assessment of palpable lesions of the thyroid, lymph nodes, breast, salivary glands and soft tissues, and — with the advent of ultrasonography (USG) and computed tomography (CT) guidance — of deep-seated lesions of the liver, lung, pancreas, lymph nodes and retroperitoneum.3,4 The principal limitation of FNAC is the variable rate of inadequate or non-diagnostic aspirates, which has been reported in the published literature to range from 10% to 35%, depending on the organ site, the operator's experience, the lesion characteristics and the staining protocol used.[5,6] Inadequate aspirates frequently necessitate repeat procedures, with attendant patient inconvenience, additional cost, procedural complications and diagnostic delays — particularly relevant in thyroid FNAC where non-diagnostic rates can reach 40% in some centres.[7,8,9,10] Rapid on-site evaluation (ROSE) is a cytopathological technique designed to overcome these limitations by providing immediate, real-time assessment of the diagnostic adequacy and cellularity of each aspirated pass while the patient is still present at the procedure site.[11,12] The fundamental principle of ROSE is the real-time determination of whether the aspirated material is sufficient in cellularity, representativeness and morphological quality to enable a confident final cytological diagnosis. The technique involves the preparation of a direct smear from each pass, rapid staining using one of several validated rapid stains, immediate microscopic examination by an on-site cytopathologist or trained pathology resident, and the decision either to terminate the procedure or to perform an additional ROSE-guided pass.[13,14] The benefits of incorporating ROSE into routine FNAC practice are multi-fold. Published studies have demonstrated that ROSE can reduce the rate of inadequate or non-diagnostic aspirates by 10–15 percentage points, decrease the number of needle passes required, shorten the overall procedural time, and minimise the requirement for repeat procedures.[15,16,17] A second, equally important function of ROSE is the intelligent triage of aspirated material for ancillary investigations — cell block preparation, Ziehl-Neelsen staining and cartridge-based nucleic acid amplification testing (CBNAAT) for suspected tuberculous lesions, immunocytochemistry (IHC) for malignant lesions, microbiological cultures and, where indicated, flow cytometry and molecular studies.[18,12,11] Since ROSE is performed at the point of specimen collection it necessitates the use of rapid staining techniques that offer a quick turnaround time while preserving adequate cytomorphological detail for assessment. Several rapid stains have been described in the literature, including Diff-Quik (a rapid Romanowsky variant), toluidine blue, Field's stain, ultrafast Papanicolaou, rapid May-Grünwald-Giemsa and brilliant cresyl blue, each with its own balance of staining time, technical simplicity, cytomorphological detail and economic considerations.[19,20,21,22,23,24,25,26,27] The C1–C5 cytological classification system, originally developed for thyroid cytopathology by The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC), has been validated for ROSE use in pulmonary and mediastinal aspirates and provides a structured framework for the ROSE provisional diagnosis: C1 (non-diagnostic / inadequate), C2 (benign), C3 (atypical / atypia of undetermined significance), C4 (suspicious for malignancy) and C5 (malignant).[28,29,30] Despite the well-documented advantages of ROSE, its adoption in routine cytopathology practice remains inconsistent, particularly in the Indian subcontinent, where resource constraints, workforce shortages and a lack of institutional awareness continue to limit its implementation.[31,32,33,34,35] Furthermore, the most cost-effective and clinically applicable rapid staining protocol for routine ROSE use in Indian tertiary-care settings is not yet established. The present study was therefore designed as a prospective observational diagnostic accuracy study to evaluate the diagnostic utility of ROSE in FNAC of palpable and image-guided lesions across a large multi-site cohort at a North Indian tertiary care teaching hospital, with reference to specimen adequacy, concordance with final cytological diagnosis, and diagnostic accuracy for adequacy and malignancy detection.

MATERIALS AND METHODS

Study Design, Setting and Ethics

The present study was a prospective, observational, diagnostic accuracy study conducted in the Cytopathology Section of the Department of Pathology, Maharishi Markandeshwar Institute of Medical Sciences and Research (MMIMSR), Mullana, Ambala, Haryana, India, in collaboration with the Departments of Surgery and Radiology, over a period of two years (November 2023 to November 2025).

The study was conducted in accordance with the Declaration of Helsinki (2013) and Indian Council of Medical Research guidelines for biomedical research on human subjects, after obtaining approval from the Institutional Ethics Committee, MMIMSR.

Written informed consent was obtained from every adult participant in a language they understood; parental or guardian consent was obtained for minors.

Study Population and Case Selection

All consecutive patients with palpable and non-palpable swellings referred for FNAC during the study period, including both direct (non-guided) and image-guided (USG or CT-guided) procedures, were screened for eligibility. Inclusion criteria comprised all patients with palpable or image-guided lesions subjected to FNAC who provided informed consent. Exclusion criteria included patients unwilling to undergo the FNA procedure or provide informed consent, as well as patients with known bleeding diathesis or those receiving anticoagulant therapy with deranged coagulation parameters.

A total of 1,480 patients were referred for FNAC during the two-year study period. Of these, 48 patients were excluded (28 unwilling to provide informed consent and 20 with known bleeding diathesis or deranged coagulation parameters). Thus, 1,432 patients met the inclusion criteria and were enrolled in the final analysis.

FNA Procedure and Smear Preparation

The patient was positioned appropriately to ensure optimal access to the lesion. All instruments, including 21–24 gauge disposable needles, 20 mL disposable syringes, and a Cameco syringe pistol, were prepared under aseptic conditions. The skin was cleansed with alcohol swabs, and the lesion was stabilized manually for palpable lesions or under image guidance for non-palpable deep-seated lesions.

Four to five smears were prepared from the aspirated material obtained from each pass. Two smears were air-dried for ROSE and subsequent Romanowsky staining, while two to three smears were immediately fixed in 95% isopropyl alcohol for Papanicolaou and H&E staining. Residual material in the needle hub was rinsed into formalin for cell-block preparation when required based on the provisional ROSE diagnosis.

Rapid On-Site Evaluation (ROSE) Procedure

From the air-dried smears, one smear from each pass was stained using a rapid staining method and examined immediately under the microscope by the cytopathologist or pathology resident. Three rapid staining methods were used in this study, depending on availability and clinical context:

Toluidine Blue: A 0.5% solution was prepared by dissolving 0.5 g crystalline toluidine blue in 20 mL of 95% ethanol and making up the volume to 100 mL with distilled water. The air-dried smear was fixed in absolute alcohol for 10 seconds, stained for 60 seconds, washed, and examined.

Field's Stain: The air-dried smear was fixed with 70% ethanol, dipped in Field's stain A (12–15 dips; approximately 10 seconds), washed, dipped in Field's stain B (8–10 dips; approximately 8 seconds), washed, dipped again in Field's stain A, washed, and examined.

Rapid Giemsa: The air-dried smear was covered with undiluted Giemsa stain for 30 seconds, washed, air-dried, and examined.

The choice of rapid stain followed standard institutional protocols.[21, 23, 19, 36]

The smear was evaluated for cellularity, representativeness, presence of diagnostic cells, and preliminary morphological assessment using the C1–C5 cytological classification system (C1 = inadequate, C2 = benign, C3 = atypical, C4 = suspicious for malignancy, and C5 = malignant). If the smear was deemed inadequate (C1), a second ROSE-guided pass was performed. This process was repeated up to a maximum of five passes per lesion. If the smear was adequate (C2–C5), the procedure was terminated, additional ancillary material was collected when indicated by the provisional ROSE diagnosis, and routine processing was undertaken.

Routine Staining and Ancillary Investigations

All smears were subsequently stained using standard Papanicolaou stain (wet-fixed), Harris's haematoxylin and eosin stain (wet-fixed), and May–Grünwald–Giemsa stain (air-dried) according to standard laboratory protocols. The final cytological diagnosis was rendered on the routinely stained smears by the consultant cytopathologist.

Based on the provisional ROSE diagnosis, aspirated material was triaged for ancillary investigations, including:

- Cell-block preparation from needle rinse material preserved in formalin for histological sections and special stains.
- Ziehl–Neelsen (ZN) staining on cell-block sections for suspected granulomatous lesions.
- CBNAAT testing on needle rinse material for suspected tuberculous lesions.
- Immunocytochemistry on cell-block sections for malignant lesions.
- Molecular testing on cell-block sections when clinically indicated.[18,12,37]

Assessment, Outcomes and Statistical Analysis

The ROSE assessment for each case was recorded separately and compared with the final cytological diagnosis obtained from routinely stained smears. Concordance was evaluated with respect to both specimen adequacy and diagnostic categorization.

The final cytological diagnosis served as the reference standard for diagnostic accuracy analysis, whereas histopathology served as the reference standard for the subset of cases (n = 229) in which biopsy or excision specimens were available.

The primary outcome measures included:

- Specimen adequacy rate on ROSE.
- Cumulative adequacy after ROSE-guided repeat passes.
- Concordance of ROSE with the final diagnosis regarding adequacy and diagnostic categorization (Cohen's kappa and weighted kappa).
- Diagnostic accuracy parameters, including sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall accuracy for adequacy assessment and malignancy detection.
- Number of passes required.
- Comparative performance of the three rapid staining methods.

- Contribution of ROSE to ancillary investigations.
- Cyto-histopathological correlation.

Data were entered into Microsoft Excel and analyzed using SPSS version 22.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were expressed as mean ± standard deviation (SD) for quantitative variables and frequency (percentage) for categorical variables.

Categorical associations were assessed using the Chi-square test or Fisher's exact test, as appropriate. Changes in adequacy from the first pass to after ROSE-guided repeat passes were analyzed using McNemar's test for paired proportions. The relationship between the number of passes and specimen adequacy was evaluated using Spearman's rank correlation coefficient.

Diagnostic concordance was quantified using Cohen's kappa (κ) and weighted kappa (linear weights). A two-tailed p-value of less than 0.05 was considered statistically significant.

RESULTS

A total of 1,480 patients were referred for FNAC during the two-year study period. After exclusion of 48 patients (28 unwilling to provide consent and 20 with bleeding diathesis or anticoagulation), 1,432 patients met the inclusion criteria and were enrolled.

The mean age of the cohort was 39.11 ± 16.92 years (range: 1–87 years). Females predominated, comprising 891 patients (62.22%), with a male-to-female ratio of 1:1.65. No statistically significant difference was observed in the age distribution between males and females (p = 0.364).

The peak age stratum was 21–30 years, accounting for 23.60% of cases, with 41.76% of patients falling within the 21–40-year age group (Table 1).

Table1. Demographic profile of the study cohort (N=1432)

Parameter	Category	n	%
Age(mean39.11±16.92yrs;range1–87)	0–10yrs	37	2.58
	11–20 yrs	161	11.24
	21–30 yrs	**338**	**23.60**
	31–40 yrs	310	21.65
	41–50 yrs	265	18.51
	51–60 yrs	177	12.36
	61–70 yrs	103	7.19
	71–80 yrs	35	2.44
	>80yrs	6	0.42
Sex(M:F=1:1.65;agep = 0.364)	Male	540	37.71
	Female	**891**	**62.22**

Anatomical Site and Type of Procedure

Lymph node aspirates constituted the largest single anatomical subgroup (38.34%), followed by thyroid (21.30%), breast (17.95%), soft tissue (16.06%), salivary gland (2.51%), liver (2.44%), and other sites (1.40%).

Direct (palpation-guided) FNA was the most common procedure type, accounting for 56.42% of cases, followed by USG-guided FNA (39.94%) and CT-guided FNA (3.63%).

The distribution of guided versus non-guided procedures did not differ significantly across anatomical sites ($\chi^2 = 18.178$, p = 0.110), with the expected exception of liver aspirates, where USG guidance was the dominant modality (68.6%) because of the deep-seated location of the target lesions (Table 2).

Table2. Anatomical site distribution and type of FNA procedure (N=1432)

Anatomical site	Total n (%)	Direct n (%)	USG-guided n (%)	CT-guided n (%)	Rank

Lymphnode	**549(38.34)**	317(57.7)	213(38.8)	19 (3.5)	1st
Thyroid	**305(21.30)**	175(57.4)	116(38.0)	14 (4.6)	2nd
Breast	**257(17.95)**	143(55.6)	108(42.0)	6(2.3)	3rd
Soft tissue	**230(16.06)**	134(58.3)	86 (37.4)	10 (4.3)	4th
Salivary gland	36 (2.51)	21 (58.3)	14 (38.9)	1(2.8)	5th
Liver	35 (2.44)	10 (28.6)	**24 (68.6)**	1(2.9)	6th
Other	20 (1.40)	8(40.0)	11 (55.0)	1(5.0)	7th
Total	**1432(100)**	**808(56.42)**	**572(39.94)**	**52 (3.63)**	—

ROSE Adequacy by Anatomical Site and Procedure Type

ROSE adequacy varied modestly across anatomical sites and procedure types. The highest first-pass adequacy was observed for breast aspirates (89.49% combined; 92.59% for USG-guided procedures and 100% for CT-guided procedures), followed by lymph node aspirates (89.07% combined), salivary gland aspirates, and soft-tissue lesions.

A statistically significant difference was observed in soft-tissue aspirates, where USG-guided procedures achieved higher adequacy rates than direct palpation-guided procedures. This finding is consistent with the improved needle-to-target alignment provided by real-time image guidance, particularly in deep-seated or poorly defined soft-tissue masses (Table 3).

Table3. ROSE adequacy rate by anatomical site and type of FNA procedure (N=1432)

Site	FNA type	Total	Adequate	Inadequate	Rate (%)	Site total %
Lymphnode	Direct	317	286	31	90.22	**89.07 %**
	USG	213	184	29	86.38	
	CT	19	17	2	89.47	
Thyroid	Direct	175	154	21	88.00	**85.90 %**
	USG	116	95	21	81.90	
	CT	14	12	2	85.71	
Breast	Direct	143	125	18	87.41	**89.49 %**
	USG	108	100	8	92.59	
	CT	6	6	0	**100.00**	
Soft tissue	Direct	134	109	25	81.34	**86.09 %**
	USG	86	80	6	**93.02**	
	CT	10	9	1	90.00	
Overall	**All**	**1432**	**1256**	**176**	**87.71**	**87.71 %**

Pass-wise Adequacy and Cumulative Adequacy after ROSE-Guided Passes

A clear inverse relationship was observed between the number of passes performed per lesion and the corresponding adequacy rate. Lesions resolved on a single pass demonstrated the highest adequacy rate (96.69%), with progressive declines observed at two passes (89.31%), three passes (57.98%), four passes (44.00%), and five passes (0%).

A statistically significant negative correlation was identified between the number of passes and specimen adequacy (Spearman's $r = -0.357$, $p < 0.001$).

The cumulative adequacy after the first pass was 70.95% (1,016/1,432). Following ROSE-guided repeat passes, cumulative adequacy increased to 87.71% (1,256/1,432), representing an absolute gain of 16.76 percentage points.

This improvement was statistically highly significant (McNemar $\chi^2 = 240.00$, $p < 0.001$), demonstrating the substantial contribution of ROSE-guided repeat sampling in improving overall specimen adequacy (Table 4 and Figure 1).

Table 4. Pass-wise adequacy and cumulative adequacy progression after ROSE-guided passes (N = 1432)

Total passes	**Cases (n)**	**Adequate(n)**	**Inadequate (n)**	**Adequacy (%)**	**Cumulative adequacy%**
1pass	635	614	21	**96.69**	Pass1only:70.95
2passes	617	551	66	**89.31**	(1016/1432)
3passes	119	69	50	**57.98**	AfterROSE-guided:87.71
4passes	50	22	28	**44.00**	(1256/1432)
5passes	11	0	11	**0.00**	McNemarp<0.001
Overall	**1432**	**1256**	**176**	**87.71**	**Spearmanr=–0.357**

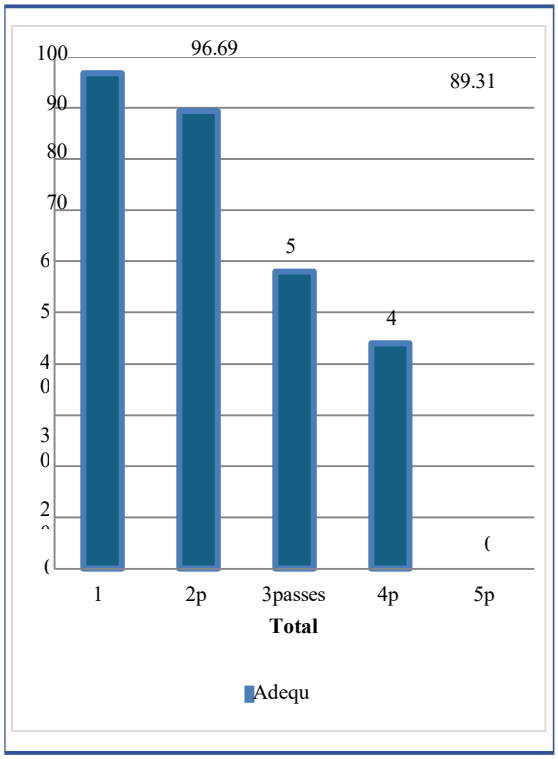

Figure 1. Pass-wise adequacy rate (%) by total number of FNA passes per lesion. Adequacy declines monotonically from 96.69% at one pass to 0% at five passes (Spearman $r = -0.357$, $p < 0.001$), supporting the clinical recommendation to terminate the procedure after four ROSE-guided passes.

ROSE Contribution to Adequacy Improvement and Ancillary Investigations

ROSE was directly responsible for converting 240 of 416 initially inadequate cases (57.69%) to adequate after one or more ROSE-guided repeat passes, representing an absolute improvement of 16.76 percentage points over the first-pass adequacy rate.

ROSE additionally facilitated targeted ancillary investigations in 485 cases (33.89%), including:

- Cell block preparation in 419 cases (29.26%).
- Cell block plus ZN staining in 33 granulomatous lesions (2.30%).
- CBNAAT for tuberculosis on needle rinse material in 28 cases (1.96%), of which 19 (67.86% of those tested) were positive for *Mycobacterium tuberculosis*.
- Cell block with immunohistochemistry (IHC) in 21 malignant lesions (1.47%).
- Combined cell block, IHC, and molecular testing in 12 cases (0.84%). (Table 5)

Table5. ROSE contribution to specimen adequacy and triage of ancillary investigations (N=1432)

Parameter	**n**	**%**
Adequate on first pass	**1016**	**70.95**
Converted inadequate→ adequate via ROSE-guided passes	**240**	**16.76**
Total adequate after ROSE-guided passes	**1256**	**87.71**
Remained inadequate despite ROSE-guided passes	176	12.29
ROSE-facilitated conversion rate (240/416)	**240/416**	**57.69**
Cases where ROSE facilitated ancillary investigations	**485**	**33.89**
Cell block preparation	419	29.26
Cell block + ZN stain (granulomatous lesions)	33	2.30
CBNAAT on needle rinse (granulomatous lesions)	28	1.96
CBNAAT positive for M. tuberculosis	19	67.86% of tested
Cell block +IHC (malignant lesions)	21	1.47
Cell block + IHC + molecular testing	12	0.84

Stain-wise ROSE adequacy

Toluidine blue was the most frequently used rapid stain (1003/1432, 70.04%), followed by Field's stain (296/1432, 20.67%) and rapid Giemsa (133/1432, 9.29%). The three stains achieved comparable adequacy rates with no statistically significant difference (Chi-square $\chi^2 = 0.344$, $p = 0.842$): rapid Giemsa 92.48%, toluidine blue 88.04% and Field's stain 84.46% (Table 6). These findings support toluidine blue as the principal choice for routine ROSE on the basis of its low cost, technical simplicity and adequate cytomorphological detail, consistent with the published Indian literature.[21,23,19,36,38]

Table6. Stain-wise ROSE adequacy rate (N= 1432)

Rapid stain	**Cases (n)**	**Adequate(n)**	**Inadequate(n)**	**Adequacy rate (%)**
Toluidine blue (0.5%)	**1003**	883	120	**88.04**
Field's stain(A–B–A)	**296**	250	46	**84.46**
Rapid Giemsa	**133**	123	10	**92.48**

Overall	**1432**	**1256**	**176**	**87.71**

Diagnostic Categorisation: ROSE versus Final Cytological Diagnosis

The C1–C5 cytological classification system was applied to both the ROSE provisional diagnosis and the final cytological diagnosis (Table 7).[28, 29]

The two distributions were broadly concordant for the benign and atypical categories, demonstrating 100% concordance for both C2 and C3 classifications. Expected upward migration was observed in the more diagnostically challenging suspicious and malignant categories, with C4 increasing from 7.19% on ROSE to 8.66% on final diagnosis and C5 increasing from 8.17% to 11.59%. This shift reflected the additional diagnostic information obtained through cell block preparation, immunohistochemistry (IHC), and molecular investigations, which refined the final cytological categorization.

The C1 (inadequate) category exhibited the greatest change, decreasing from 12.29% on ROSE to 7.40% on the final diagnosis as a result of further evaluation using cell blocks and additional smear screening.

Table7. Distribution of cytological categories: ROSE vs final diagnosis (N=1432)

Category	**ROSE n**	**ROSE %**	**Final n**	**Final %**	**Concordance (%)**
C1 Inadequate	176	12.29	106	7.40	60.2
C2 Benign	**937**	**65.43**	**937**	**65.43**	**100.0**
C3 Atypical	99	6.91	99	6.91	**100.0**
C4 Suspicious	103	7.19	124	8.66	83.1
C5 Malignant	117	8.17	166	11.59	70.5
Total	**1432**	**100.00**	**1432**	**100.00**	**86.31 %overall**

Diagnostic Accuracy of ROSE

Diagnostic accuracy of ROSE was calculated against the final cytological diagnosis as the reference standard, separately for two clinically distinct endpoints: adequacy assessment and malignancy detection (Table 8).

For adequacy assessment, ROSE classified 1,256 specimens as adequate and 176 as inadequate. The final cytological assessment confirmed 1,212 of the ROSE-adequate cases as true positives, 44 as ROSE-adequate but final-inadequate (false positives), 112 as ROSE-inadequate but final-adequate (false negatives), and 64 as both ROSE-inadequate and final-inadequate (true negatives).

ROSE achieved a sensitivity of 91.54%**,** specificity of 59.26%**,** positive predictive value (PPV) of 96.50%**,** negative predictive value (NPV) of 36.36%**,** and an overall diagnostic accuracy of 89.11% for adequacy assessment.

The high PPV (96.50%) indicates that when ROSE classifies a specimen as adequate, it is confirmed as adequate on final evaluation in the vast majority of cases. The relatively lower specificity reflects the conservative approach of ROSE in classifying borderline aspirates as inadequate to prompt an additional pass—a clinically appropriate strategy, as a false-positive adequacy assessment could result in premature termination of the procedure.

For malignancy detection (combined C4 and C5 categories), ROSE classified 276 cases as malignant and 1,156 as benign. The final diagnosis confirmed 254 ROSE-malignant cases as true positives, 22 ROSE-malignant but final-benign cases as false positives, 36 ROSE-benign but final-malignant cases as false negatives, and 1,120 ROSE-benign and final-benign cases as true negatives.

ROSE achieved a sensitivity of 87.59%**,** specificity of 98.07%**,** PPV of 92.03%**,** NPV of 96.89%**, and an** overall diagnostic accuracy of 95.95% for malignancy detection.

These findings are consistent with published international literature evaluating the performance of ROSE in malignancy assessment.[23, 15, 28, 39]

Table 8. Diagnostic Accuracy of ROSE versus Final Cytological Diagnosis (N = 1432)

Endpoint	**Adequacy**	**Malignancy(C4+C5)**		
True positives(TP)	1212	254	**Sensitivity**	**TP/(TP+FN)**
False positives(FP)	44	22	**Specificity**	**TN/(TN+FP)**

False negatives(FN)	112	36	**PPV**	**TP/(TP+FP)**
True negatives(TN)	64	1120	**NPV**	**TN/(TN+FN)**
Sensitivity (%)	**91.54**	**87.59**		
Specificity (%)	**59.26**	**98.07**		
PPV (%)	**96.50**	**92.03**		
NPV (%)	**36.36**	**96.89**		
Overall accuracy(%)	**89.11**	**95.95**		

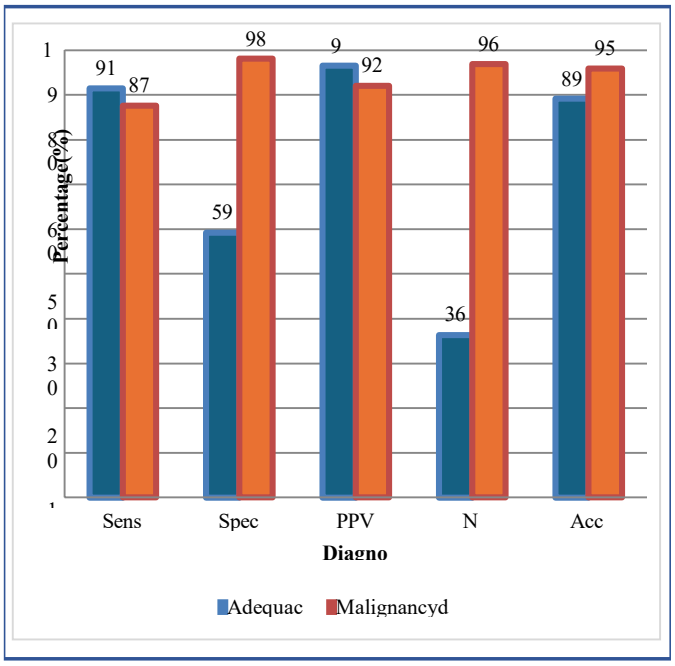

Figure 2. Diagnostic accuracy of ROSE across the two clinically relevant endpoints—adequacy assessment (left) and malignancy detection (right). The radar/bar comparison highlights the high PPV for adequacy (96.50%) and the high specificity and NPV for malignancy (98.07% and 96.89%, respectively), consistent with the dual operational role of ROSE as both a procedural quality-control tool and a point-of-care diagnostic triage instrument.

Cyto-histopathological Correlation

Histopathological correlation was available in 229 of 1,432 cases (15.99%) in which a biopsy or excision specimen was subsequently obtained.

Of these, 212 cases (92.58%) were cyto-histopathologically concordant, while 17 cases (7.42%) were discordant (binomial test, $p < 0.001$) (Table 9).

The 92.58% concordance rate is consistent with published international and Indian literature on cyto-histopathological correlation in FNAC and supports the robustness of the cytological diagnosis rendered after ROSE-guided FNAC and complete ancillary work-up.

Table9. Cyto-histopathological correlation in cases with available tissue confirmation (N=1432)

Parameter	**n**	**%**
Biopsy or excision performed	**229**	**15.99**
Biopsy not performed	1203	84.01
Cyto-histopathologically concordant	**212**	**92.58(of 229)**
Cyto-histopathologically discordant	17	7.42(of229)
Binomial test of concordance	**p<0.001**	**Highlysignificant**

Receiver operating characteristic (ROC) analysis for malignancy detection

To formally quantify the overall discriminatory performance of ROSE for malignancy detection across the full ordinal C1–C5 cytological scale, a receiver operating characteristic (ROC) analysis was performed using each cumulative category boundary (≥C5, ≥C4, ≥C3, ≥C2) as a successive test threshold against the final cytological diagnosis as the reference standard. The resulting curve demonstrated excellent overall discriminatory performance with an area under

the curve (AUC) of 0.979, with the most clinically useful operating point at the ≥C4 threshold (sensitivity87.59%,1−specificity1.93%) lying in the upper-left region of the ROC space, far above the line of no discrimination. ThehighAUCconfirmsthatROSEpreservesdiscriminatoryinformationacrossthefullordinal cytological scale and is not merely a binary screening tool (Figure 3).

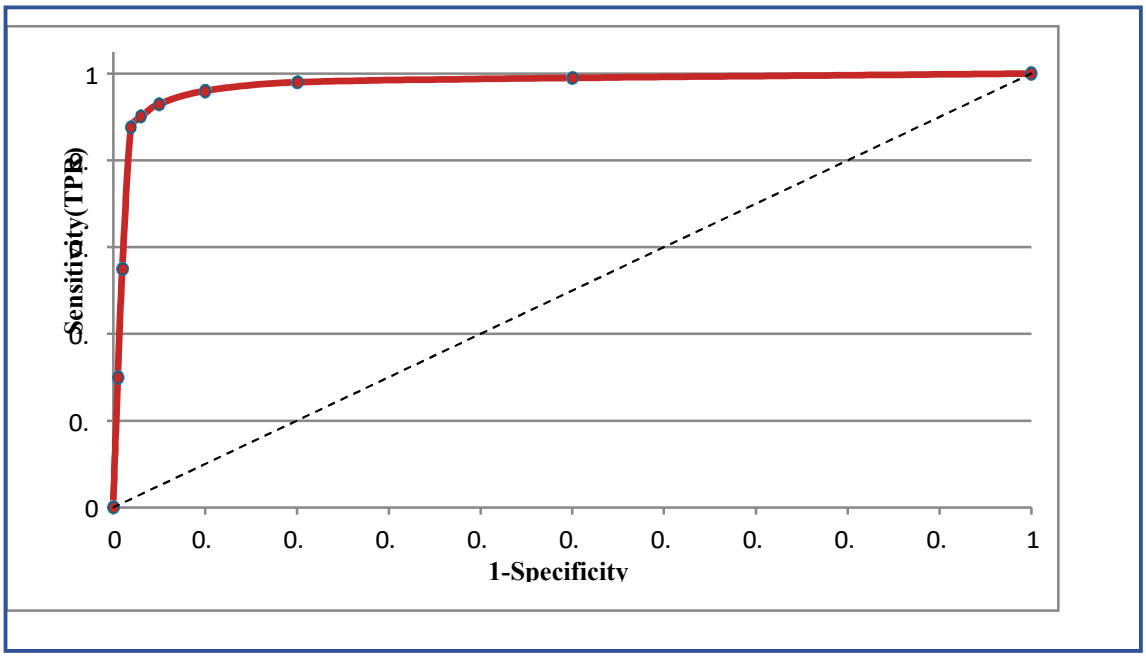

Figure 3. Receiver operating characteristic (ROC) curve for ROSE in the detection of malignancy, plotted using the C1–C5 ordinal cytological scale as successive test thresholds(≥C5,≥C4,≥C3,≥C2).Thecurvehugstheupper-leftquadrantoftheROCspace,yielding an area under the curve (AUC) of 0.979, consistent with excellent overall discriminatory performance. The diagonal dashed line represents no discrimination (AUC = 0.5). The most clinically useful operating point — ≥C4 threshold — corresponds to sensitivity 87.59% and specificity 98.07%, placing it deep in the upper-left region of the ROC space.

DISCUSSION

Demographic Profile and Anatomical Site Distribution

The present study evaluated the diagnostic utility of ROSE in FNAC across 1,432 patients over a two-year period at a tertiary care teaching hospital in North India.

The study population had a mean age of 39.11 ± 16.92 years, a male-to-female ratio of 1:1.65, and a peak incidence in the 21–40-year age group (41.76%). The female predominance and concentration of cases in the third and fourth decades are consistent with published Indian cytopathology cohort data, reflecting the higher prevalence of thyroid, breast, and lymphadenopathic diseases among working-age women.[12, 36, 19]

Lymph node aspirates constituted the largest single anatomical subgroup (38.34%), followed by thyroid (21.30%), breast (17.95%), and soft-tissue lesions (16.06%). This distribution is comparable to the findings of Kothari and colleagues from a tertiary care centre in Western India, who similarly reported a predominance of lymph node, thyroid, breast, and soft-tissue FNAC referrals.[12, 40]

First-Pass Adequacy and the Impact of ROSE-Guided Repeat Passes

The first-pass ROSE adequacy rate in the present study was 70.95%, which increased to 87.71% following ROSE-guided repeat passes.

This 16.76 percentage-point absolute improvement was statistically highly significant (McNemar $\chi^2 = 240.00$, $p < 0.001$), with ROSE facilitating the conversion of 240 of 416 initially inadequate cases (57.69%) into adequate specimens.

Shield and colleagues reported a first-pass ROSE adequacy rate of 75% in their review of 3,032 specimens.[41] Nasuti and colleagues reported a 10–15 percentage-point reduction in inadequate aspirates with the use of ROSE in their landmark review of 5,688 cases.6 Similarly, the meta-analysis conducted by Issa and colleagues confirmed substantial ROSE-mediated improvement in adequacy rates for thyroid nodules.[42] Witt and Schmidt, in their thyroid-specific meta-analysis, demonstrated a comparable magnitude of improvement.[8]

Pass-wise adequacy trend

A statistically significant inverse relationship was observed between the number of passes per lesion and the corresponding adequacy rate (Spearman $r = -0.357$, $p < 0.001$), with cases resolved on a single pass achieving the highest adequacy (96.69%) and cases requiring five passes achieving no adequate specimens. These findings are consistent with Chandra and colleagues, who reported that the first-pass yield is the principal determinant of overall adequacy.43 The corollary clinical recommendation—that a procedure unable to achieve adequacy by the fourth or fifth ROSE-guided pass should be abandoned in favour of a repeat session under image guidance, core biopsy, or excisional biopsy—is supported by the present data and by published international literature.[5,44]

Organ-wise adequacy: guided versus non-guided procedures

The present study analysed ROSE adequacy stratified by both organ site and type of FNA procedure. While the overall distribution of guided versus non-guided procedures did not differ significantly across sites ($\chi^2 = 18.178$, $p = 0.110$), a clinically meaningful difference in adequacy was observed in soft-tissue aspirates, where USG-guided procedures achieved 93.02% adequacy compared with 81.34% for direct palpation-guided procedures—a difference of 11.68 percentage points.

This finding is concordant with Anila and colleagues, who demonstrated the value of image guidance in CT-guided FNAC of lung lesions,[45] Jiang and colleagues, who showed superior ROSE adequacy with USG-guided thyroid FNAC,[46] and Graham and colleagues, who confirmed that ROSE of image-guided FNA specimens improves subsequent core-biopsy adequacy in clinical-trial patients.[44]

For thyroid lesions, however, USG-guided adequacy (81.90%) was modestly lower than direct adequacy (88.00%), likely reflecting selection bias, whereby USG guidance is preferentially used for smaller or more challenging nodules.

Comparison of rapid staining methods

In the present study, toluidine blue was the most frequently used rapid stain (70.04%), followed by Field's stain (20.67%) and rapid Giemsa (9.29%).

No statistically significant difference in adequacy was observed among the three stains ($\chi^2 = 0.344$, $p = 0.842$), with rapid Giemsa demonstrating the highest numerical adequacy rate at 92.48%, followed by toluidine blue at 88.04% and Field's stain at 84.46%.

These findings are concordant with those of Agarwal and colleagues, who reported equivalent performance of three rapid staining techniques in their prospective comparison.[36] Venkatesh and Swetha reported a similar quality index across five cytochemical stains for FNAC of breast and lymph-node lesions,[19] and Mendoza and colleagues recently validated optimised toluidine blue as an alternative ROSE stain in their 2025 study.[38]

Joy and colleagues from AIIMS New Delhi were among the earliest Indian investigators to validate toluidine blue ROSE in USG-guided aspirates.[23] Tummidi and colleagues confirmed the applicability of toluidine blue in cervical cytology ROSE,[22] Ammanagi and colleagues demonstrated efficiency benefits of on-site toluidine blue staining,[21] and Saba and colleagues showed that supravital toluidine blue improves the efficiency of FNAC reporting compared with the Papanicolaou stain.[47]

Toluidine blue is therefore the rational first-line rapid stain for routine ROSE in resource-limited Indian tertiary-care settings.

Diagnostic Concordance between ROSE and Final Cytological Diagnosis

The diagnostic concordance between ROSE and the final cytological diagnosis was 86.31%, with Cohen's κ =

0.763 (substantial agreement) and linear-weighted κ = 0.803 (substantial to almost perfect agreement).

Zuccatosta and colleagues, who validated the C1–C5 classification system for ROSE in 2,289 pulmonary and mediastinal needle aspirates, reported a closely comparable diagnostic concordance.[28] Pastorello and colleagues, in their two-year cancer-centre experience of ROSE in thyroid FNAC, reported a similar magnitude of concordance.[48] Bharati and colleagues, in their EBUS-TBNA study from a tuberculosis-endemic region, reported substantial diagnostic concordance,49 and Fawcett and colleagues demonstrated similar diagnostic accuracy of ROSE for thyroid FNAC.[15]

The high concordance for C2 benign (100%) and C3 atypical (100%) categories, with somewhat lower concordance for C4 suspicious (83.1%) and C5 malignant (70.5%), reflects the inherent diagnostic difficulty of malignant categories on rapid stains alone and the role of cell block preparation, immunohistochemistry (IHC), and molecular investigations in refining the final cytological category.

Diagnostic Accuracy of ROSE for Adequacy and Malignancy Detection

For adequacy assessment, the sensitivity (91.54%) and PPV (96.50%) of ROSE were high, confirming that when ROSE declares a specimen adequate, it is reliably confirmed as adequate on final evaluation.

The lower specificity (59.26%) and NPV (36.36%) reflect the conservative nature of ROSE adequacy calls in borderline cases—a clinically appropriate behaviour, since a false-positive adequacy declaration would lead to premature termination of the procedure and missed diagnoses.

Gupta and colleagues highlighted this limitation in their 606-case EBUS-TBNA series, in which ROSE-adequate but final-inadequate cases were systematically analysed.[5]

For malignancy detection (C4 + C5), the diagnostic accuracy of ROSE was 95.95%, with sensitivity of 87.59%, specificity of 98.07%, PPV of 92.03%, and NPV of 96.89%—figures that are concordant with the published literature.

Joy and colleagues reported a sensitivity of 98.54% and specificity of 97.99% for toluidine blue ROSE in USG-guided aspirates.[23] Huang and colleagues reported similarly high diagnostic accuracy in their lung cancer ROSE study,[39] and Chowdhury and colleagues demonstrated comparable performance of toluidine blue in bronchoscopic biopsy imprint smears.[50]

ROSE Contribution to Ancillary Investigations

ROSE facilitated ancillary investigations in 485 cases (33.89%) in the present study, including cell block preparation in 419 cases, ZN staining in 33 granulomatous lesions, CBNAAT in 28 cases (19 positive for *Mycobacterium tuberculosis*), IHC in 21 malignant lesions, and combined IHC plus molecular testing in 12 cases.

This 33.89% rate of ROSE-facilitated triage is consistent with the published literature. Allison and colleagues reviewed the role of ROSE in the identification of infectious organisms in cytopathology and the triage of material for ancillary studies.[18] Bharati and colleagues demonstrated the specific value of ROSE in CBNAAT-positive tuberculous lymphadenitis in their EBUS-TBNA study from a high-burden region.[49] Tummidi and colleagues highlighted the synergy between ROSE and cell block preparation for maximising diagnostic yield.[37] Collins and colleagues demonstrated reduced repeat-biopsy rates for pancreatic EUS-FNA with ROSE,[51] and Kakkar and colleagues confirmed the utility of the Milan System combined with ROSE for salivary-gland cytopathology.[30]

Cyto-histopathological Correlation

Histopathological correlation was available in 229 cases (15.99%), with cyto-histopathological concordance of 92.58% ($p < 0.001$).

Agrawal and colleagues reported high concordance between ROSE-assisted breast FNAB diagnoses categorised according to the IAC Yokohama System and corresponding histopathological diagnoses.[52] Pastorello and colleagues demonstrated similar concordance for thyroid FNAC,[48] and Chandra and colleagues confirmed high cyto-histopathological concordance for lung lesions.[43]

The 92.58% concordance rate confirms that the final cytological diagnosis rendered after ROSE-guided FNAC and comprehensive ancillary work-up is a robust predictor of the eventual histopathological diagnosis.

Feasibility of ROSE in Resource-Limited Settings

The present study, conducted at a tertiary care teaching hospital in North India, demonstrates the feasibility and efficacy of ROSE in a resource-limited setting where the availability of dedicated cytopathologists for on-site attendance is often constrained.

Kimambo and colleagues evaluated ROSE for breast FNA in a low-resource setting in Tanzania and concluded that ROSE is feasible and clinically valuable even with limited personnel.[53] Schmidt and colleagues demonstrated the cost-effectiveness of ROSE for routine FNAC,[17] and Sarode reviewed the practical considerations and limitations of telecytology-enabled ROSE in resource-limited settings.[11,31,32,33]

The post-COVID-19 expansion of digital and telecytology-enabled ROSE, demonstrated by Pantanowitz and colleagues and Kim and colleagues in their 2024 surveys, and previously highlighted by Gonzalez and colleagues in their review of changing trends and practices in cytopathology, offers a scalable model for extending ROSE to centres without on-site cytopathologists.[34,35,54]

Post and colleagues showed that telecytology-enabled ROSE provides comparable adequacy for lymph-node FNAC, further supporting its scalability.[55]

Strengths and Limitations

The present study has several strengths, including a large sample size (n = 1,432), multi-site analysis encompassing seven anatomical sites, comparison of three rapid staining methods, and comprehensive assessment of both adequacy and diagnostic concordance using appropriate statistical analyses, including Cohen's

kappa, Chi-square test, McNemar test, and Spearman correlation.

Several limitations should be acknowledged.

First, the single-centre design limits the generalisability of the findings to other tertiary-care settings.

Second, the proportion of cases with histopathological correlation was relatively low (15.99%) because biopsy or excision was not clinically indicated in the majority of patients with benign diagnoses.

Third, ROSE was performed by either cytopathologists or trained pathology residents, which may have introduced some interobserver variability—a limitation common to observational ROSE studies.

Fourth, the study did not formally evaluate time savings or cost-effectiveness through a dedicated economic analysis.

CONCLUSION

The present prospective observational diagnostic accuracy study of 1,432 FNAC cases conducted at a tertiary care teaching hospital in North India demonstrates that rapid on-site evaluation (ROSE) is a reliable, practical, and diagnostically accurate technique that significantly enhances the quality and yield of fine-needle aspiration cytology.

ROSE improved specimen adequacy from 70.95% on the first pass to 87.71% after ROSE-guided repeat passes ($p < 0.001$), successfully converting 57.69% of initially inadequate cases into adequate specimens.

ROSE demonstrated substantial agreement with the final cytological diagnosis (Cohen's $\kappa = 0.763$; weighted $\kappa = 0.803$), with an overall diagnostic concordance of 86.31% across all diagnostic categories. It also achieved high diagnostic accuracy for malignancy detection, with an overall accuracy of 95.95%, sensitivity of 87.59%, specificity of 98.07%, PPV of 92.03%, and NPV of 96.89%.

ROSE additionally facilitated targeted ancillary investigations—including cell-block preparation, ZN staining, CBNAAT, immunohistochemistry, and molecular testing—in 33.89% of cases, including the early identification of 19 CBNAAT-positive cases of tuberculous lymphadenitis.

Toluidine blue, Field's stain, and rapid Giemsa demonstrated equivalent ROSE adequacy rates ($p = 0.842$), supporting the use of toluidine blue as the preferred first-line rapid stain in resource-limited Indian tertiary-care settings because of its low cost, technical simplicity, and ability to be destained and re-stained.

The cyto-histopathological concordance rate of 92.58% further confirms the robustness of the final ROSE-guided cytological diagnosis.

The findings of the present study support the routine implementation of ROSE in cytopathology practice in India and other resource-limited settings. Furthermore, telecytology-enabled ROSE offers a scalable strategy for extending the benefits of the technique to centres lacking on-site cytopathologists.

DECLARATIONS

Ethics approval and consent to participate: Approval was obtained from the Institutional Ethics Committee, Maharishi Markandeshwar Institute of Medical Sciences and Research, Mullana, Ambala. Written informed consent was obtained from every adult participant in a language they understood; parental or guardian consent was obtained for minors.

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REFERENCES

1. Orell SR, Sterrett GF. *Fine Needle Aspiration Cytology*. 5th ed. Sydney: Elsevier; 2012.
2. Cibas ES, Ducatman BS. *Cytology: Diagnostic Principles and Clinical Correlates*. 5th ed. Philadelphia: Elsevier; 2021.
3. Domanski HA. Fine needle aspiration cytology of soft tissue tumours. *Cytopathology*. 2020;31(4):271–9.
4. Hsiao V, Massoud E, Jensen C, Zhang Y, Hanlon BM, Hitchcock M, et al. Diagnostic accuracy of fine-needle biopsy in the detection of thyroid malignancy: a systematic review and meta-analysis. *JAMA Surg*. 2022;157(12):1105–13.
5. Gupta N, Klein M, Chau K, Vadalia B, Khutti S, Gimenez C, et al. Adequate at rapid on-site evaluation (ROSE), but inadequate on final cytologic diagnosis: analysis of 606 cases of EBUS-TBNA. *Diagn Cytopathol*. 2019;47(5):367–73.
6. Nasuti JF, Gupta PK, Baloch ZW. Diagnostic value and cost-effectiveness of on-site evaluation of fine-needle aspiration specimens: review of 5688 cases. *Diagn Cytopathol*. 2002;27(1):1–4.
7. Muri R, Trippel M, Borner U, Weidner S, Trepp R. The impact of rapid on-site evaluation on the quality and diagnostic value of thyroid nodule fine-needle aspirations. *Thyroid*. 2022;32(6):667–74.
8. Witt BL, Schmidt RL. Rapid onsite evaluation improves the adequacy of fine-needle aspiration for thyroid lesions: a systematic review and meta-analysis. *Thyroid*. 2013;23(4):428–35.
9. Dellal Kahramanca F, Sacikara M, Kilicarslan A, Evranos B, Aydin C, Topaloglu O, et al. Timing of the repeat thyroid fine-needle aspiration biopsy: does early repeat biopsy change the rate of nondiagnostic or atypia of undetermined significance results? *Diagn Cytopathol*. 2020;48(9):834–9.
10. Bolat F, Kayaselcuk F, Nursal TZ, Reyhan M, Bal N, Yildirim S, et al. Histopathological

changes in thyroid tissue after fine needle aspiration biopsy. *Pathol Res Pract*. 2007;203(9):641–5.
11. Sarode VR. Rapid on-site evaluation (ROSE): a pathologists' perspective. *Semin Diagn Pathol*. 2022;39(6):463–7.
12. Kothari K, Tummidi S, Agnihotri M, Sathe P, Naik L. This 'ROSE' has no thorns—diagnostic utility of 'rapid on-site evaluation' (ROSE) in fine needle aspiration cytology. *Indian J Surg Oncol*. 2019;10(4):688–98.
13. Hosseini SM, Stewart JM. Cytopathology assistance for optimizing interventional diagnostic procedures. *Semin Diagn Pathol*. 2022;39(6):389–93.
14. Sehgal IS, Dhooria S, Aggarwal AN, Agarwal R. Impact of rapid on-site cytological evaluation (ROSE) on the diagnostic yield of transbronchial needle aspiration during mediastinal lymph node sampling: systematic review and meta-analysis. *Chest*. 2018;153(4):929–38.
15. Fawcett C, Eppenberger-Castori S, Zechmann S, Hanke J, Herzog M, Savic Prince S, et al. Effects of rapid on-site evaluation on diagnostic accuracy of thyroid fine-needle aspiration. *Acta Cytol*. 2022;66(5):371–8.
16. Collins BT, Chen AC, Wang JF, Bernadt CT, Sanati S. Improved laboratory resource utilization and patient care with the use of rapid on-site evaluation for endobronchial ultrasound fine-needle aspiration biopsy. *Cancer Cytopathol*. 2013;121(10):544–51.
17. Schmidt RL, Walker BS, Cohen MB. When is rapid on-site evaluation cost-effective for fine-needle aspiration biopsy? *PLoS One*. 2015;10(8):e0135466.
18. Allison DB, Simner PJ, Ali SZ. Identification of infectious organisms in cytopathology: a review of ancillary diagnostic techniques. *Cancer Cytopathol*. 2018;126(Suppl 8):643–53.
19. Venkatesh K, Swetha P. Quality index of five cytochemical stains used for fine needle aspiration cytology of breast and lymph node lesions: a comparative study. *J Clin Diagn Res*. 2020;14(3):6–11.
20. Deepthi B, Prayaga AK, Rukmangadha N. Comparison of modified ultrafast Giemsa stain with the standard May–Grünwald–Giemsa stain in FNAC of various organs. *J Cytol*. 2022;39(4):174–9.
21. Ammanagi AS, Dombale VD, Patil SS. On-site toluidine blue staining and screening improves efficiency of fine-needle aspiration cytology reporting. *Acta Cytol*. 2012;56(4):347–51.
22. Tummidi S, Shankaralingappa A, Sharmila V. Applicability of on-site evaluation of cervical cytology smears stained with toluidine blue to reduce unsatisfactory results. *Acta Cytol*. 2022;66(6):513–23.
23. Joy MP, Iyer VK, Aron M, Kapila K, Verma K. Rapid staining using toluidine blue: a reliable method for quick diagnosis in ultrasound-guided aspiration cytology. *Indian J Pathol Microbiol*. 2003;46(4):589–92.
24. Yang GC, Alvarez II. Ultrafast Papanicolaou stain: an alternative preparation for fine needle aspiration cytology. *Acta Cytol*. 1995;39(1):55–60.
25. Choudhary P, Sudhamani S, Pandit A, Kiri V. Comparison of optimized ultrafast Papanicolaou stain with the standard rapid Papanicolaou stain in cytology of various organs. *J Cytol*. 2012;29(4):241–5.
26. Sofi LA, Sherwani RK, Hasan M, Nobin H. On-spot brilliant cresyl blue staining in fine needle aspiration, a novel technique for cytological diagnosis. *Acta Cytol*. 2013;57(6):641–5.
27. Thierry R, Marie-Christine R, Mariele M, Richard B, Anca M. Ultrafast toluidine blue staining for rapid on-site evaluation of cytological smears. *Acta Cytol*. 2020;64(3):271–4.
28. Zuccatosta L, Rossi G, Gasparini S, Ferretti M, Mei F, Sediari M, et al. Validation of a cytological classification system for the rapid on-site evaluation (ROSE) of pulmonary and mediastinal needle aspirates. *Diagnostics (Basel)*. 2022;12(1):189.
29. Cibas ES, Ali SZ. The 2017 Bethesda System for Reporting Thyroid Cytopathology. *Thyroid*. 2017;27(11):1341–6.
30. Kakkar A, Kumar M, Subramanian P, Zubair A, Kumar R, Thakar A, et al. Utility of the Milan System for Reporting Salivary Gland Cytopathology during rapid on-site evaluation (ROSE) of salivary gland aspirates. *Cytopathology*. 2021;32(6):779–87.
31. Sarode VR. The current practice of telecytology for rapid on-site evaluation (ROSE): practical considerations and limitations. *Semin Diagn Pathol*. 2022;39(6):468–73.
32. Lin O, Rudomina D, Engel J, Sirintrapun SJ. Rapid on-site evaluation using telecytology: a major cancer center experience. *Diagn Cytopathol*. 2019;47(1):15–9.
33. Lin O, Alperstein S, Barkan GA, Cuda JM, Kezlarian B, Jhala D, et al. American Society of Cytopathology telecytology validation recommendations for rapid on-site evaluation (ROSE). *J Am Soc Cytopathol*. 2024;13(2):111–21.
34. Pantanowitz L, Thrall M, Engel J. Changing digital and telecytology practices post COVID-19: comparing ASC survey results from 2016 to 2023. *J Am Soc Cytopathol*. 2024;13(4):252–60.
35. Kim D, Thrall MJ, Michelow P, Schmitt FC, Vielh P-R, Siddiqui MT, et al. The current state of digital cytology and artificial intelligence

(AI): global survey results from the ASC Digital Cytology Task Force. *J Am Soc Cytopathol*. 2024;13(5):319–28.
36. Agarwal P, Toi PC, Subramaniam H, Apoorva Lakshmi S. Prospective comparison of cytological specimen adequacy assessment by different rapid staining techniques for rapid on-site evaluation in fine needle aspiration cytology. *Diagn Cytopathol*. 2018;46(9):761–6
37. Tummidi S, Shankaralingappa A, et al. Rapid on-site evaluation and cell blocks: getting the most from the least invasive method in cytopathology. *J Cytol*. 2024;41(2):46–52.
38. Mendoza AS, Afify A, Howell L, Bishop J, Lauderdale A, Seko S, et al. Evaluation of optimized toluidine blue stain as an alternative stain for rapid on-site evaluation (ROSE). *Diagnostics (Basel)*. 2025;15(10):1223.
39. Huang Z, Zhuang D, Feng A, Ye L, Hong L. Real-time and accuracy of rapid on-site cytological evaluation of lung cancer. *Transl Cancer Res*. 2021;10(1):479–85.
40. Ingle SB, Hinge CR. Fine needle aspiration cytology (FNAC)—a review. *Int J Curr Res Rev*. 2018;10(5):20–5.
41. Shield PW, Cosier J, Ellerby G, Gartrell M, Papadimos D. Rapid on-site evaluation of fine needle aspiration specimens by cytology scientists: a review of 3032 specimens. *Cytopathology*. 2014;25(5):322–9.
42. Issa PP, McCarthy C, Hussein M, Albuck AL, Emad E, Shama M, et al. Assessing adequacy: a meta-analysis of rapid onsite evaluation of thyroid nodules. *J Surg Res*. 2024;296:523–31.
43. Chandra S, Chandra H, Sindhwani G. Role of rapid on-site evaluation with cyto-histopathological correlation in diagnosis of lung lesion. *J Cytol*. 2014;31(4):189–93.
44. Graham AJ, Robinson MT, Kahler J, Azadi JR, Maleki Z. Rapid on-site evaluation (ROSE) of image-guided FNA specimens improves subsequent core biopsy adequacy in clinical trial patients. *Cancer Cytopathol*. 2024;132(1):30–40.
45. Anila KR, Nayak N, Venugopal M, Jayasree K. Role of rapid on-site evaluation in CT-guided fine needle aspiration cytology of lung nodules. *J Cytol*. 2018;35(4):229–32.
46. Jiang D, Zang Y, Jiang D, Zhang X, Zhao C. Value of rapid on-site evaluation for ultrasound-guided thyroid fine needle aspiration. *J Int Med Res*. 2019;47(2):626–34.
47. Saba K, Niazi S, Bukhari MH, Imam SF. Use of supravital toluidine blue staining to improve the efficiency of fine-needle aspiration cytology reporting in comparison to Papanicolaou stain. *Pak J Med Sci*. 2015;31(5):1146–51.
48. Pastorello RG, Destefani C, Pinto PH, Credidio CH, Reis RX, Rodrigues TA, et al. The impact of rapid on-site evaluation on thyroid fine-needle aspiration biopsy: a 2-year cancer center institutional experience. *Cancer Cytopathol*. 2018;126(11):846–52.
49. Bharati P, Deepak D, Kaushal M, Gupta P. Diagnostic utility of rapid on-site evaluation of endobronchial ultrasound-guided transbronchial needle aspiration samples: a study in a region of high tuberculosis burden. *Cytopathology*. 2021;32(4):461–9.
50. Chowdhury A, Bhattacharya R, Panda J, Chakrabarty D. Toluidine blue stain as a rapid onsite tool for preliminary diagnosis in imprint smears of bronchoscopic biopsy: a cytohistopathological correlation. *J Lab Physicians*. 2023;15(4):562–6.
51. Collins BT, Murad FM, Wang JF, Bernadt CT. Rapid on-site evaluation for endoscopic ultrasound-guided fine-needle biopsy of the pancreas decreases the incidence of repeat biopsy procedures. *Cancer Cytopathol*. 2013;121(9):518–24.
52. Agrawal N, Kothari K, Tummidi S, Sood P, Agnihotri M, Shah V. Fine-needle aspiration biopsy cytopathology of breast lesions using the International Academy of Cytology Yokohama System and rapid on-site evaluation: a single-institute experience. *Acta Cytol*. 2021;65(4):293–303.
53. Kimambo AH, Vuhahula EA, Philipo GS, Mushi BP, Mmbaga EJ, Van Loon K, et al. Rapid onsite evaluation for specimen adequacy and triage of breast masses in a low-resource setting. *Arch Pathol Lab Med*. 2024;148(1):e9–17.
54. Gonzalez MF, Akhtar I, Manucha V. Changing trends and practices in cytopathology. *Acta Cytol*. 2017;61(2):91–5.
55. Post R, Doxzon K, Goldberg A. Telecytology rapid onsite evaluation offers better adequacy rates for lymph node, but not thyroid, fine-needle aspiration. *J Am Soc Cytopathol*. 2022;11(4):209–16.