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Comparative Cytomorphologic Performance of Rehydrated Air-Dried and Conventional Papanicolaou Staining in Cervical Smears in a Prospective Paired Study

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Abstract

Background: Immediate alcohol fixation is central to conventional Papanicolaou staining (CPAP) but can be difficult to maintain in high-volume and peripheral settings. Controlled air drying followed by saline rehydration may reduce erythrocyte-related obscuration while preserving epithelial morphology.

Objective: To compare the paired cytomorphologic performance of rehydrated air-dried Papanicolaou staining (RADPS) with CPAP in cervical smears.

Methods: This prospective, single-center study analyzed 100 women with complete paired CPAP and RADPS preparations. Coded assessments compared adequacy, background, cellularity, cytoplasmic staining, cell borders, nuclear borders, chromatin, and six-month preservation. Exact McNemar and Stuart-Maxwell tests, matched odds ratios, bootstrap confidence intervals (CIs), agreement statistics, and Holm multiplicity correction were used.

Results: Satisfactory smears increased from 90 (90.0%) with CPAP to 99 (99.0%) with RADPS, a paired increase of 9.0 percentage points (95% bias-corrected and accelerated (BCa) CI: 4.0-16.0; exact $p = 0.004$). Clear backgrounds increased from 38 (38.0%) to 95 (95.0%), and distinct cell borders increased from 74 (74.0%) to 98 (98.0%) (Holm-adjusted $p < 0.001$ for both). The remaining secondary cytomorphologic outcomes were not statistically significant after adjustment. Broad cytologic-category agreement was 88 (88.0%) (Cohen kappa: 0.664; Gwet agreement coefficient 1 (AC1): 0.864). The exploratory seven-item technical-quality score increased by 0.98 criteria (95% BCa CI: 0.77-1.23; $p < 0.001$).

Conclusion: In this 100-participant paired analysis, RADPS was associated with higher smear adequacy, cleaner backgrounds, and better cell-border definition. The findings support RADPS as a technical alternative when immediate wet fixation is difficult, while diagnostic superiority remains unproven.

Categories: Obstetrics/Gynecology, Pathology, Preventive Medicine

Keywords: cervical cytology, cytomorphology, papanicolaou stain, rehydrated air-dried smears, saline rehydration technique

Introduction

Cervical cancer remains a major global health concern [1]. International prevention strategies emphasize screening and timely treatment of precancerous lesions [2,3]. Cervical cytology remains relevant where access to human papillomavirus (HPV) testing is limited. Conventional Papanicolaou staining (CPAP) provides nuclear detail and polychromatic cytoplasmic differentiation, but its quality depends on prompt, adequate fixation [4,5]. Delayed or incomplete fixation can produce air-drying artifact, while blood, mucus, and inflammatory material may obscure epithelial cells.

Intentional air drying followed by saline rehydration and alcohol post-fixation provides a controlled alternative [6]. Air drying causes greater physical membrane damage to erythrocytes than to epithelial cells; brief rehydration in isotonic 0.9% saline therefore promotes rupture and clearance of the damaged erythrocytes while retaining epithelial cells. Rehydration also restores water to the dehydrated epithelial cells before alcohol post-fixation, helping preserve nuclear borders and chromatin detail [6,7]. Previous cervical-smear studies have examined background clearance, adequacy, and morphologic preservation with this approach [7-11]. Additional comparisons have used differing fixation and rehydration protocols, including prolonged air drying [12-17]. The magnitude and consistency of paired technical effects therefore warrant assessment with confidence intervals and appropriate control for multiple comparisons.

This study compared rehydrated air-dried Papanicolaou staining (RADPS) with CPAP in paired cervical smears. Smear adequacy was the principal endpoint. Secondary endpoints were background clarity,

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cellularity, cytoplasmic staining, cell-border definition, nuclear-border definition, chromatin clarity, and six-month preservation. Cytologic-category agreement, organism detection, a composite technical-quality score, and age-related patterns were exploratory.

Materials And Methods

Study design and setting

A prospective, single-center, within-participant paired comparison was conducted in the pathology and gynecology services of Gandhi Medical College, Bhopal, India. Data collection began in May 2024 and ended in November 2024, while follow-up continued through May 2025. Reporting was guided by the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement [18].

Participants and study size

Women undergoing cervical cytologic evaluation were eligible when paired CPAP and RADPS slides and adequate clinical documentation were available. Broken, lost, or unpaired preparations were excluded. At the manuscript data cutoff, 100 complete participant pairs were available, and all were included in this focused analysis.

The parent three-preparation study estimated the required sample using the single-proportion formula shown below, with satisfactory smear adequacy as the planning parameter.

$$n = \frac{Z_{1-\alpha/2}^2 P(1-P)}{d^2}$$

Here, $Z(1-\alpha/2) = 1.96$ for a 95% confidence level, $P = 0.90$, $1-P = 0.10$, and the absolute precision $d = 0.05$.

$$n = \frac{(1.96)^2 (0.90)(0.10)}{(0.05)^2} = 138.30 \approx 139$$

The minimum was rounded to 139 participants, and the parent-study recruitment target was set at 150 to allow for incomplete or non-evaluable preparations. The present article reports the 100 complete CPAP-RADPS pairs available at the manuscript data cutoff and is therefore an available-case analysis of the paired data.

Smear collection and processing

After placement of a Cusco speculum, the transformation zone was sampled with an Ayre spatula, followed by an endocervical brush. The study generated CPAP and RADPS slides at the same visit. Random allocation of preparation order could not be established, and possible order effects were considered a limitation.

Staining procedures

For CPAP, the freshly prepared smear was immediately immersed in 95% ethanol for 30 minutes and then processed through hematoxylin, differentiation, bluing, Orange G, Eosin Azure, graded alcohol, xylene, and mounting. For RADPS, the slide was intentionally air-dried at 25-27 °C for 30 minutes, rehydrated in 0.9% saline for 30 seconds, post-fixed in 95% ethanol for 30 minutes, and then stained using the same sequence. Protocol-level processing time was 60 minutes for CPAP and 90 minutes for RADPS.

Cytomorphologic assessment

A trained pathologist evaluated coded slides using a standardized proforma. Cellularity was categorized as high (>5 clusters per 10× field), intermediate (2-5 clusters), or low (<2 clusters). Favorable binary criteria were a clear background; optimal cytoplasmic staining; distinct cell and nuclear borders; distinct chromatin; excellent six-month preservation; and Bethesda-defined satisfactory adequacy [19]. A clear background lacked red blood cells, debris, or contaminants. Cytologic interpretations were standardized into broad categories for agreement analysis.

Reference-standard restriction

The final clinical interpretation in the source dataset was based on the slide providing the best cytologic picture, most often RADPS. It was therefore not independent and was not used to calculate sensitivity, specificity, predictive values, or receiver operating characteristic curves.

Statistical analysis

All comparisons preserved within-participant pairing. Binary outcomes were analyzed with exact two-sided

McNemar tests. Paired absolute differences were summarized with 20,000-sample bias-corrected and accelerated (BCa) bootstrap 95% confidence intervals (CIs). These bootstrap intervals were used as estimation summaries, while exact McNemar p-values governed binary hypothesis testing. Matched odds ratios with exact conditional CIs were reported where estimable. Three-category cellularity and four-category cytologic distributions were examined with Stuart-Maxwell marginal-homogeneity tests. Observed agreement, Cohen kappa, linear-weighted kappa, and Gwet agreement coefficient 1 (AC1) were calculated as appropriate, with paired-bootstrap CIs for agreement coefficients [20]. Holm correction was applied across seven secondary cytomorphologic comparisons and separately across exploratory organism analyses. Statistical analyses were performed using Statistical Product and Service Solutions (SPSS, version 25.0; IBM SPSS Statistics for Windows, Armonk, NY). Independent statistical cross-checks and figure generation used Python 3.12 (Python Software Foundation, Wilmington, DE) and Matplotlib 3.10.8.

Exploratory analyses

A technical-quality score counted the seven favorable binary criteria for each slide (range: 0-7). CPAP and RADPS scores were compared with the paired Wilcoxon signed-rank test; mean paired change received a participant-level bootstrap CI, and rank-biserial correlation summarized effect size. Spearman correlation assessed age against the net number of favorable criteria gained with RADPS. A sensitivity analysis restricted broad cytologic agreement to pairs satisfactory by both methods. These analyses were exploratory and were not used to redefine the principal conclusion.

Ethics

The Institutional Ethics Committee of Gandhi Medical College and Associated Hamidia Hospital, Bhopal, India, approved the study under protocol 189/IEC/2024 on March 27, 2024. Written informed consent was obtained from all participants before inclusion. No additional diagnostic charges were imposed.

Results

Participants

All 100 participants available at the manuscript data cutoff contributed a complete CPAP-RADPS pair. Mean age was 36.1 years (standard deviation (SD): 9.5), median age was 35.0 years (interquartile range: 29.0-41.2), and the range was 22-68 years. The largest age groups were 30-39 years: 38 (38.0%), 20-29 years: 27 (27.0%), and 40-49 years: 26 (26.0%) (Table 1). Figure 1 summarizes the analyzed population.

Characteristic	Value
Mean (SD), years	36.1 (9.5)
Median (IQR), years	35.0 (29.0-41.2)
Range, years	22-68
Age group 20-29, N (%)	27 (27.0%)
Age group 30-39, N (%)	38 (38.0%)
Age group 40-49, N (%)	26 (26.0%)
Age group 50-59, N (%)	7 (7.0%)
Age group 60-69, N (%)	2 (2.0%)

TABLE 1: Participant age characteristics (n = 100)
Data are presented as mean ± standard deviation (SD), median with interquartile range (IQR), range, or number (percentage) as applicable.

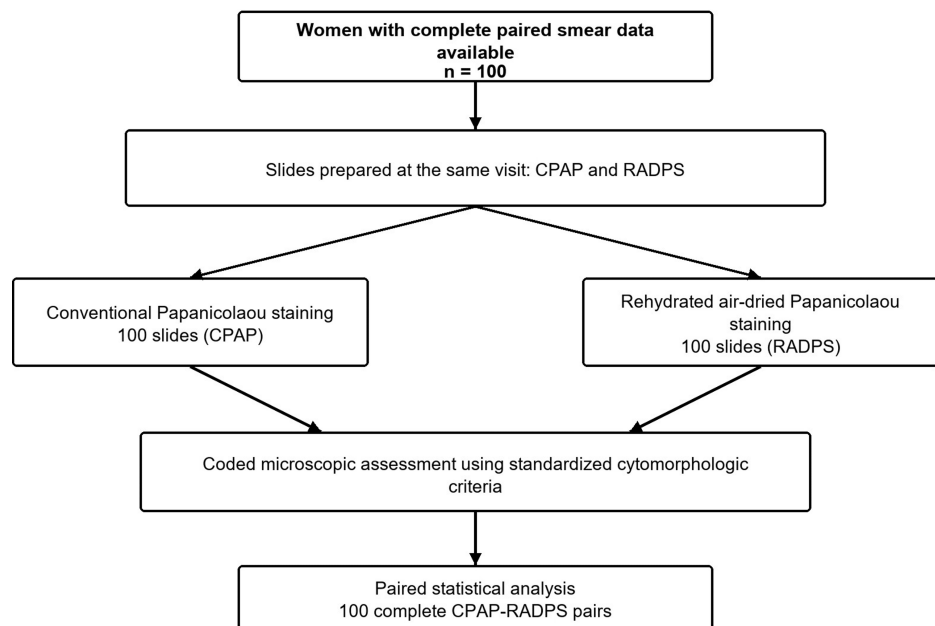


FIGURE 1: Study flow and analysis population

Flow diagram showing participant selection and analysis pathway. A total of 100 women with complete paired cervical smear data were included. Two slides were prepared during the same visit (conventional Papanicolaou staining (CPAP) and rehydrated air-dried Papanicolaou staining (RADPS)). Coded microscopic assessment was performed using standardized cytomorphic criteria, followed by paired statistical analysis of 100 complete CPAP-RADPS pairs.

Primary and secondary cytomorphic outcomes

Satisfactory adequacy was observed in 90 (90.0%) CPAP smears and 99 (99.0%) RADPS smears. Nine pairs improved with RADPS, and none worsened, giving a 9.0-percentage-point paired increase (95% BCa CI: 4.0-16.0; exact McNemar $p = 0.004$). Clear backgrounds increased from 38 (38.0%) to 95 (95.0%), a 57.0-point increase (95% BCa CI: 47.0-67.0; Holm-adjusted $p < 0.001$). Distinct cell borders increased from 74 (74.0%) to 98 (98.0%), a 24.0-point increase (95% BCa CI: 16.0-34.0; adjusted $p < 0.001$). Cytoplasmic staining, nuclear borders, chromatin, preservation, and cellularity did not meet the Holm-adjusted significance threshold (Figures 2, 3; Table 2).

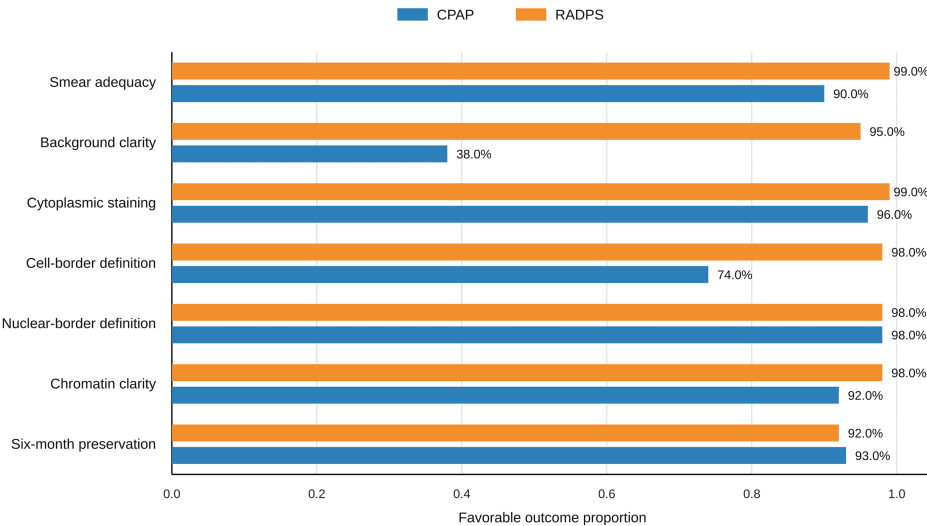


FIGURE 2: Favorable cytomorphologic outcome proportions by staining method

Bars show marginal proportions from paired observations and should not be interpreted as independent groups. Data labels show exact counts and percentages in N (%) format.

CPAP, conventional Papanicolaou staining; RADPS, rehydrated air-dried Papanicolaou staining

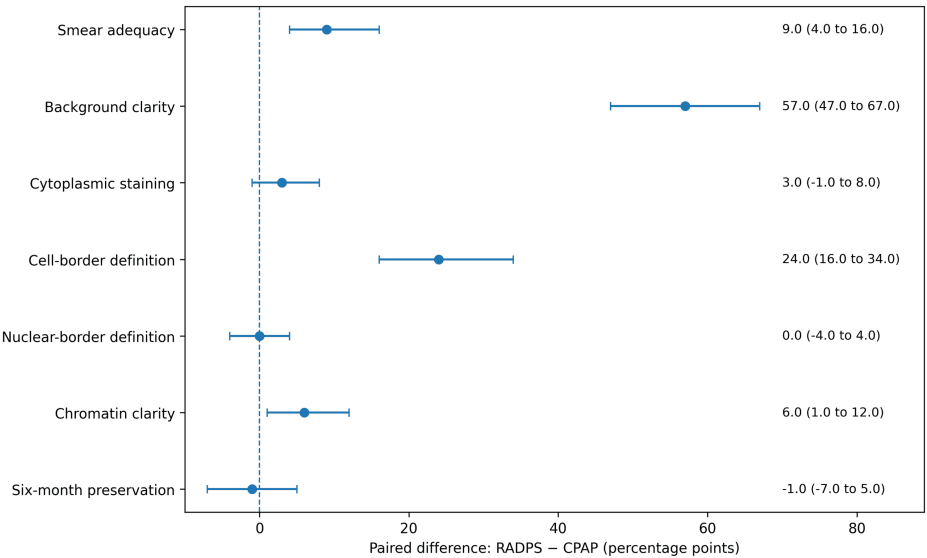


FIGURE 3: Paired absolute differences in favorable cytomorphologic outcomes

Forest plot showing paired absolute differences in favorable cytomorphologic outcomes between rehydrated air-dried Papanicolaou staining (RADPS) and conventional Papanicolaou staining (CPAP). Points represent the paired difference (RADPS - CPAP) in percentage points, and horizontal lines indicate 95% bias-corrected and accelerated (BCa) bootstrap confidence intervals. Values greater than zero indicate improvement with RADPS. The dashed vertical line represents no difference between methods. Outcomes assessed included smear adequacy, background clarity, cytoplasmic staining, cell-border definition, nuclear-border definition, chromatin clarity, and six-month preservation.

Outcome	CPAP N (%)	RADPS N (%)	Improved/worsened	Difference, pp (95% BCa CI)	Exact p	Holm p	Matched OR (95% CI)
Smear adequacy	90 (90.0%)	99 (99.0%)	9/0	9.0 (4.0 to 16.0)	0.004	-	Inf (1.97-Inf)
Background clarity	38 (38.0%)	95 (95.0%)	57/0	57.0 (47.0 to 67.0)	<0.001	<0.001	Inf (14.96-Inf)
Cytoplasmic staining	96 (96.0%)	99 (99.0%)	4/1	3.0 (-1.0 to 8.0)	0.375	1	4.00 (0.40-196.99)
Cell-border definition	74 (74.0%)	98 (98.0%)	25/1	24.0 (16.0 to 34.0)	<0.001	<0.001	25.00 (4.09-1026.45)
Nuclear-border definition	98 (98.0%)	98 (98.0%)	2/2	0.0 (-4.0 to 4.0)	1	1	1.00 (0.07-13.80)
Chromatin clarity	92 (92.0%)	98 (98.0%)	7/1	6.0 (1.0 to 12.0)	0.07	0.281	7.00 (0.90-315.48)
Six-month preservation	93 (93.0%)	92 (92.0%)	4/5	-1.0 (-7.0 to 5.0)	1	1	0.80 (0.16-3.72)

TABLE 2: Paired binary cytomorphologic outcomes by staining method

Comparison of paired binary cytomorphologic outcomes between conventional Papanicolaou staining (CPAP) and rehydrated air-dried Papanicolaou staining (RADPS) in 100 cervical smear pairs. Values are presented as number (percentage). Improved/worsened indicates the number of participants who changed from an unfavorable to favorable outcome or from a favorable to unfavorable outcome, respectively. Differences are expressed as percentage-point (pp) changes with 95% bias-corrected and accelerated (BCa) bootstrap confidence intervals. Exact McNemar p values were used for paired binary comparisons, with Holm correction applied to secondary outcomes. Matched odds ratios (ORs) with 95% confidence intervals (CIs) are reported where estimable.

Note: Smear adequacy was the principal endpoint and was not multiplicity-adjusted. Holm adjustment covered seven secondary comparisons, including cellularity. BCa intervals summarize estimation; exact McNemar tests governed binary hypothesis decisions.

pp, percentage points; OR, odds ratio, Inf, infinity

Cellularity and agreement

High cellularity increased descriptively from 72 (72.0%) with CPAP to 79 (79.0%) with RADPS. The Stuart-Maxwell test did not reach the conventional significance threshold (chi-square = 5.769, df = 2, p = 0.056), and the Holm-adjusted p-value was 0.279. Exact cellularity agreement was 85 (85.0%) among 100 pairs, with linear-weighted kappa 0.666 (95% bootstrap CI: 0.488-0.809). Broad cytologic-category agreement was 88 (88.0%) among 100 pairs, Cohen kappa 0.664 (95% CI: 0.483-0.821), and Gwet AC1 0.864 (95% CI: 0.784-0.933). Marginal broad-category distributions differed (Stuart-Maxwell chi-square = 10.976, df = 2, p = 0.004), largely because nine CPAP-unsatisfactory preparations became classifiable with RADPS. Among 90 pairs satisfactory by both methods, broad-category agreement was 87 (96.7%) and kappa was 0.880 (95% bootstrap CI: 0.715-1.000) (Figure 4, Table 3).

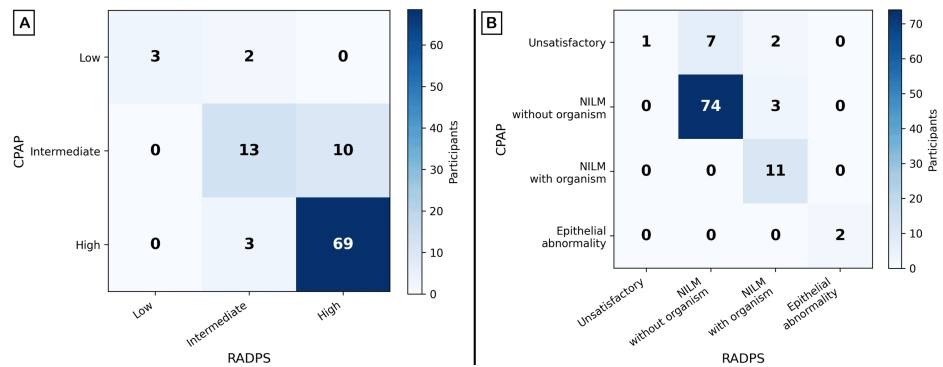


FIGURE 4: Within-participant transition matrices for cellularity and broad cytologic-category agreement

Within-participant transition matrices comparing conventional Papanicolaou staining (CPAP) and rehydrated air-dried Papanicolaou staining (RADPS). Cell entries represent the number of participants in each transition category. Rows represent CPAP classifications and columns represent RADPS classifications. Panel A shows transitions in cellularity categories (low, intermediate, and high). Panel B shows agreement across broad cytologic categories: unsatisfactory, negative for intraepithelial lesion or malignancy (NILM) without organism, NILM with organism, and epithelial abnormality.

Statistic	Smear adequacy	Cellularity	Broad cytologic category	Broad category: both satisfactory
Analysis n	100	100	100	90
Observed agreement, N	91	85	88	87
Observed agreement, %	91	85	88	96.7
Kappa type	Unweighted	Linear-weighted	Unweighted	Unweighted
Kappa estimate	0.167	0.666	0.664	0.88
Kappa 95% CI: lower	0	0.488	0.483	0.715
Kappa 95% CI: upper	0.485	0.809	0.821	1
Gwet AC1 estimate	0.9	-	0.864	-
Gwet AC1 95% CI: lower	0.822	-	0.784	-
Gwet AC1 95% CI: upper	0.958	-	0.933	-
Statistical test	Exact McNemar	Stuart-Maxwell	Stuart-Maxwell	Sensitivity analysis
Chi-square statistic	-	5.769	10.976	-
Degrees of freedom	-	2	2	-
Unadjusted p-value	0.004	0.056	0.004	-
Holm-adjusted p-value	-	0.279	-	-

TABLE 3: Agreement metrics for paired cytologic assessments

Observed agreement counts (N) and percentages (%) are reported in separate rows; the denominator is 100 pairs except for the both-satisfactory sensitivity analysis (n = 90). Kappa is unweighted except for cellularity, which uses linear weights. Agreement coefficient CIs are paired-bootstrap intervals. The cellularity test has two degrees of freedom and an unadjusted p value of 0.056. Dashes indicate analyses not reported.

CI, confidence interval; AC1, agreement coefficient 1; df, degrees of freedom

Exploratory technical-quality score and age correlation

The mean seven-item technical-quality score increased from 5.81 (SD: 1.20) with CPAP to 6.79 (SD: 0.48) with RADPS. The mean paired increase was 0.98 favorable criteria (95% BCa CI: 0.77-1.23; Wilcoxon p <

0.001; rank-biserial correlation: 0.980). Older age showed a weak positive association with the net number of favorable criteria gained with RADPS (Spearman rho = 0.214; 95% bootstrap CI: 0.017-0.400; p = 0.032). This association was exploratory and does not establish age as an effect modifier (Figure 5, Table 4).

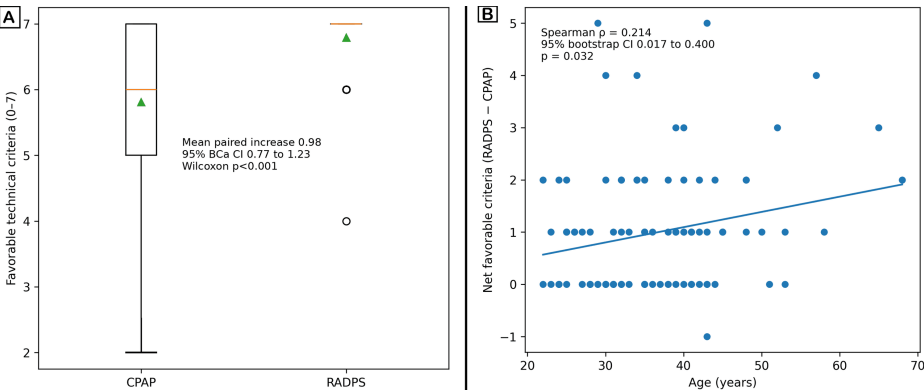


FIGURE 5: Exploratory participant-level analyses of technical score improvement and age association

(A) Favorable binary criteria per slide. Boxes show the interquartile range, horizontal lines the medians, green triangles the means, and circles points beyond the whiskers. (B) Age and the net number of favorable criteria gained with RADPS; overlapping points may represent multiple participants. The fitted line is descriptive; the reported association is Spearman correlation. Panel A displays the observed score range (2-7); the theoretical scale range was 0-7.

CPAP, conventional Papanicolaou staining; RADPS, rehydrated air-dried Papanicolaou staining; BCa, bias-corrected and accelerated; CI, confidence interval

Statistic	Technical-quality score (0-7)	Age vs. net paired improvement
CPAP mean	5.81	-
CPAP standard deviation	1.2	-
RADPS mean	6.79	-
RADPS standard deviation	0.48	-
Mean paired increase	0.98	-
Mean increase 95% BCa CI: lower	0.77	-
Mean increase 95% BCa CI: upper	1.23	-
Wilcoxon p-value	<0.001	-
Rank-biserial correlation	0.98	-
Spearman rho	-	0.214
Spearman rho 95% CI: lower	-	0.017
Spearman rho 95% CI: upper	-	0.4
Spearman p-value	-	0.032

TABLE 4: Exploratory analyses of technical-quality improvement and age-related association

Exploratory analyses comparing conventional Papanicolaou staining (CPAP) and rehydrated air-dried Papanicolaou staining (RADPS). Technical-quality scores represent the number of favorable technical criteria met (range: 0-7). Paired change estimates represent RADPS minus CPAP differences with bootstrap confidence intervals (BCa). The Wilcoxon signed-rank test assessed paired score differences. The association between age and net paired improvement was evaluated using Spearman correlation and interpreted as exploratory.

Cytologic interpretations and organisms

Inflammatory smears negative for intraepithelial lesion or malignancy (NILM) were the most frequent interpretation under both methods. Unsatisfactory interpretations decreased from 10 (10.0%) with CPAP to 1 (1.0%) with RADPS. Bacterial vaginosis was recorded in nine CPAP and 12 RADPS preparations; the three discordant pairs favored RADPS, but the exploratory paired comparison was not statistically significant after Holm correction. Trichomonas vaginalis was detected in one CPAP and three RADPS preparations, while Candida/fungal organisms were detected in one preparation by each method. These results reflect cytologic detection yield rather than diagnostic accuracy (Table 5).

Category	CPAP, N (%)	RADPS, N (%)
Unsatisfactory	10 (10.0%)	1 (1.0%)
NILM/within normal limits	20 (20.0%)	22 (22.0%)
Inflammatory NILM	56 (56.0%)	58 (58.0%)
Atrophic inflammatory/NILM	1 (1.0%)	1 (1.0%)
Bacterial vaginosis	9 (9.0%)	12 (12.0%)
Trichomonas vaginalis	1 (1.0%)	3 (3.0%)
Candida/fungal organisms	1 (1.0%)	1 (1.0%)
Atypia/other abnormality	1 (1.0%)	1 (1.0%)
LSIL	1 (1.0%)	1 (1.0%)

TABLE 5: Distribution of cytologic categories by staining method

Distribution of cytologic categories identified in paired conventional Papanicolaou staining (CPAP) and rehydrated air-dried Papanicolaou staining (RADPS) preparations. Data are presented as number of cases and percentage of the total sample (n = 100).

NILM, negative for intraepithelial lesion or malignancy; LSIL, low-grade squamous intraepithelial lesion

Exploratory organism tests: bacterial vaginosis exact p = 0.250, Holm p = 0.750; Trichomonas vaginalis exact p = 0.500, Holm p = 1.000; Candida/fungal organisms p = 1.000. These are detection-yield comparisons, not diagnostic-accuracy estimates. Paired cervical-smear photomicrographs are shown in Figures 6A-6D.

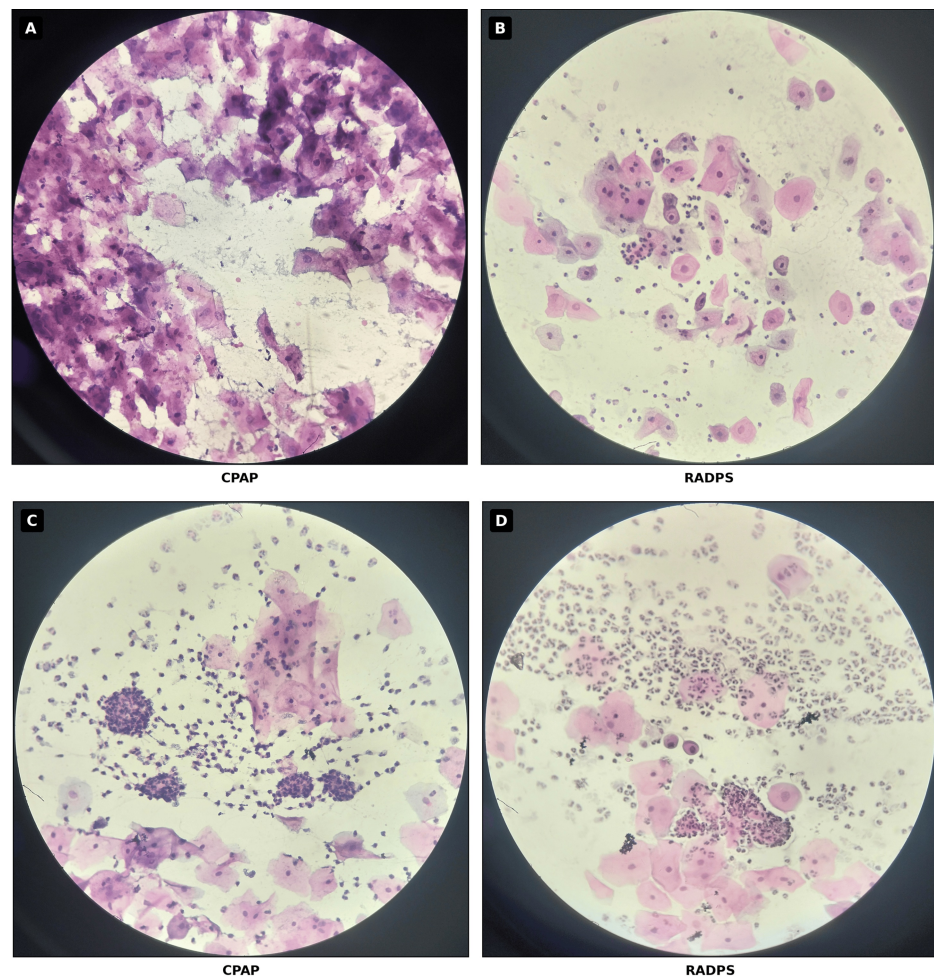


FIGURE 6: Cytomorphologic comparison between conventional and rehydrated air-dried Papanicolaou preparations

(A, B) First participant pair. (C, D) Second participant pair. Left panels (A, C) show conventional Papanicolaou staining (CPAP); right panels (B, D) show rehydrated air-dried Papanicolaou staining (RADPS). All panels are Papanicolaou-stained cervical smears from the analyzed population. The images illustrate morphology and do not provide independent validation of diagnostic accuracy. All photomicrographs were acquired using a 10× objective at 100× total magnification.

Discussion

Principal findings

In the 100 paired participants available at the manuscript data cutoff, RADPS was associated with higher smear adequacy, markedly clearer backgrounds, and better cell-border definition. Background clearance was the largest paired effect, consistent with rehydration-induced rupture and clearance of erythrocytes whose membranes had been damaged by air drying [6,7]. Nuclear-border quality, chromatin, cytoplasmic staining, preservation, and cellularity did not remain statistically significant after multiplicity correction. The exploratory technical-quality score summarized the same overall pattern at participant level but should not be interpreted as a validated scale.

Comparison with previous evidence

The adequacy findings are directionally consistent with previous cervical-smear comparisons reporting fewer unsatisfactory rehydrated preparations and improved background quality [9–11]. However, preservation is not uniformly superior: Jaiwong et al. reported slightly inferior nuclear morphology in rehydrated smears despite adequate interpretation [13], while another comparison found no statistically significant overall difference in morphologic preservation [14]. Work in non-gynecologic cytology has also supported the technical feasibility of saline rehydration in selected settings [21]. The present study estimates paired technical effects and agreement; it does not establish diagnostic accuracy or equivalence.

Agreement and exploratory analyses

The low adequacy kappa despite high raw agreement illustrates the prevalence sensitivity of kappa when one category is uncommon; Gwet AC1 tracked the observed agreement more closely. Broad-category agreement improved when analysis was restricted to slides satisfactory by both methods, indicating that much of the disagreement arose from CPAP slides that were unclassifiable rather than from discordant epithelial-lesion interpretation. The age correlation was weak and exploratory, with a confidence interval close to the null, and should not be interpreted as evidence of effect modification.

Operational implications

Protocol-level processing time was 90 minutes for RADPS and 60 minutes for CPAP. Thus, RADPS increased the minimum processing time by 30 minutes (50%) and offered no processing-time advantage under this protocol. Although the additional interval primarily reflects passive air drying rather than hands-on work, it could delay slide availability or reduce batch throughput in high-volume laboratories unless drying and staining are scheduled in parallel. Its potential value lies in reducing dependence on immediate alcohol fixation and improving the readability of bloody preparations. Implementation should therefore balance these benefits against the longer turnaround time. Transport convenience, repeat-smear avoidance, hands-on staff time, batch capacity, and cost-effectiveness were not measured and require workflow-specific evaluation.

Strengths and limitations

Strengths include complete paired observations in the analyzed data cutoff, coded assessment, exact paired inference, confidence intervals, multiplicity control, matched odds ratios, agreement sensitivity analysis, and six-month preservation assessment. Important limitations remain. The source documents available during manuscript preparation were inconsistent regarding slide-preparation order, so the analysis cannot exclude order-related effects and does not assume randomization. A single observer assessed slides, and interobserver or intraobserver reproducibility was not measured. The source documentation did not fully specify field-selection rules, reading sequence, washout interval, or storage conditions for the preservation assessment. Although the magnification is reported in Figure 6, image-calibration metadata were unavailable, preventing the reliable addition of scale bars and limiting the reproducibility of the visual comparison. The available documentation did not provide the total number screened, excluded counts, or the exact manuscript data cutoff date. The parent-study target was 150 participants, but this focused analysis includes 100 complete CPAP-RADPS pairs available at the data cutoff; precision may therefore be lower than originally planned. Histology, HPV testing, and microbiologic confirmation were unavailable as concurrent independent reference standards. Only two preparations per method were classified as epithelial abnormalities, limiting inference for abnormal lesions. RADPS also required 90 minutes compared with 60 minutes for CPAP; this 50% longer protocol may constrain turnaround time and throughput in high-volume settings, although hands-on staff time and batch capacity were not measured. Although the individual cytomorphologic criteria were predefined, the binary scoring rubric and seven-item technical-quality score were study-specific and not externally validated, which may limit reproducibility and cross-study comparability. The composite score and age analysis should therefore be interpreted as exploratory.

Conclusions

RADPS produced more satisfactory cervical smears, cleaner backgrounds, and more distinct cell borders than CPAP in this paired dataset. Other assessed cytomorphologic outcomes did not show robust differences after multiplicity correction, and the study does not establish diagnostic superiority.

RADPS is therefore a credible technical alternative when immediate wet fixation is difficult to maintain. Further work using clearly documented sampling order, multiple observers, and independent reference standards is needed before broader diagnostic or service-level claims are made.

Additional Information

Author Contributions

All authors have reviewed the final version to be published and agreed to be accountable for all aspects of the work.

Concept and design: Shilpa Dudhande, Rajni Choudhary

Acquisition, analysis, or interpretation of data: Shilpa Dudhande, Varsha Rampuri, Kajol Pradhan, Maneesh Sulya

Drafting of the manuscript: Shilpa Dudhande, Rajni Choudhary, Varsha Rampuri, Kajol Pradhan

Critical review of the manuscript for important intellectual content: Shilpa Dudhande, Varsha Rampuri, Kajol Pradhan, Maneesh Sulya

Supervision: Rajni Choudhary, Varsha Rampuri, Maneesh Sulya

Disclosures

Human subjects: Informed consent for treatment and open access publication was obtained or waived by all participants in this study. Institutional Ethics Committee, Gandhi Medical College and Associated Hamidia Hospital, Bhopal, Madhya Pradesh, India issued approval 189/IEC/2024. This study was approved by the Institutional Ethics Committee of Gandhi Medical College and Associated Hamidia Hospital, Bhopal, Madhya Pradesh, India, under protocol number 189/IEC/2024 (approved on March 27, 2024). Written informed consent was obtained from all participants prior to inclusion in the study. The study was conducted in accordance with institutional ethical guidelines. **Animal subjects:** All authors have confirmed that this study did not involve animal subjects or tissue. **Conflicts of interest:** In compliance with the ICMJE uniform disclosure form, all authors declare the following: **Payment/services info:** All authors have declared that no financial support was received from any organization for the submitted work. **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. **Other relationships:** All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

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