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Advancing Pap Smear Cytology: Effects of the Wash Technique on Sample Adequacy and Diagnostic Reliability

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ABSTRACT

Cervical cancer is one of the most preventable cancers, yet many women still face delayed or missed diagnoses because of something as simple as an "unsatisfactory" Pap smear result. When a sample is reported as inadequate, often due to gel, blood, or inflammation, the patient may need to return for another test, creating anxiety, extra costs, and possible delays in detecting disease. To overcome this problem, laboratories have started using a simple but promising approach known as the *wash technique*. In this method, inadequate samples are gently rinsed in a mild acetic acid solution and reprocessed, allowing hidden cells to be recovered and reducing background debris. We retrospectively reviewed 645 Thin Prep Pap smears that underwent the wash protocol at King Fahad Armed Forces Hospital between 2020 and 2024. After reprocessing, 69.77% of cases were satisfactory, with 83.56% reported as NILM and 16.44% showing epithelial abnormalities, including ASC-US, LSIL, HSIL, and AGC. No squamous cell carcinoma or adenocarcinoma were detected. We conclude that the wash technique substantially improves Pap smear adequacy, salvaging the majority of initially unsatisfactory cases and enabling the detection of clinically relevant abnormalities. Unsatisfactory rates increased with advancing age, highlighting the need for age-aware approaches in cytology practice. Incorporating wash protocols into routine cytology practice may reduce repeat testing, minimize diagnostic delays, and strengthen the effectiveness of cervical cancer screening programs.

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Introduction

Cervical cancer screening is one of the most effective public health interventions for cancer prevention, but its accuracy relies heavily on the quality of cytological specimens. The Bethesda System (2014 update) provides standardized criteria for specimen adequacy and diagnostic categories [1,2]. Liquid-based cytology (LBC), including ThinPrep, was developed to improve specimen quality and reduce background artifacts. Large randomized trials and meta-analyses show mixed results for sensitivity and specificity compared with conventional cytology, but consistently report lower unsatisfactory rates with LBC [3-6].

Despite these improvements, unsatisfactory results remain a challenge. Major causes include blood, inflammation, and more recently, carbomer-containing lubricants used during gynecological examinations [7-9]. Laboratory advisories and clinical studies demonstrate that such lubricants can significantly reduce specimen adequacy, leading to higher rates of unsatisfactory Pap smears [10-12].

To overcome these limitations, laboratories have adopted an acetic-acid wash technique, in which inadequate samples are centrifuged and rinsed in a 10% acetic acid solution with CytoLyt before the second slide preparation. This process helps lyse red blood cells, reduce mucus and lubricant contamination, and recover diagnostically useful epithelial cells [13-15]. Manufacturer manuals also include acetic acid steps as part of routine ThinPrep processing [16-18].

Several studies have specifically investigated reprocessing unsatisfactory ThinPrep smears. Early prospective and retrospective analyses showed that a substantial proportion of previously unsatisfactory cases could be converted into satisfactory specimens, often yielding final diagnoses ranging from NILM to epithelial abnormalities [19-21]. Later work confirmed these findings, reporting reduced unsatisfactory rates after reprocessing and highlighting the clinical importance of salvaging diagnostically relevant information from inadequate samples [22-24].

Population-level evaluations further reveal variability in unsatisfactory rates across different institutions and platforms (ThinPrep vs SurePath), emphasizing the need for local audits and quality-improvement interventions [25,26]. More recent research continues to examine lubricant effects and adequacy in ThinPrep, reflecting the ongoing relevance of this issue [27,28].

Methods

Study Setting: The study was carried out in the Anatomical Pathology Division, Department of Medical Laboratory, King Fahad Armed Forces Hospital (KFAFH), Jeddah, Saudi Arabia. The laboratory receives cervico-vaginal cytology samples from hospital clinics.

Specimen Collection and Processing: Cervical samples were obtained using a cytobrush or spatula and preserved in ThinPrep solution. Smears initially classified as unsatisfactory because of obscuring factors such as blood, mucus, inflammation, or lubricating gel were subjected to the wash technique. This procedure consisted

of centrifugation, rinsing of the sediment in 10% acetic acid prepared in CytoLyt, and reprocessing through the ThinPrep system. All slides were stained by the Papanicolaou method according to standard operating protocols.

Cytological Evaluation: Slides were screened by experienced cytotechnologist and verified by a cytopathologist. Reporting followed the Bethesda System (2014), including the following diagnostic categories: Negative for Intraepithelial Lesion or Malignancy (NILM), Atypical Squamous Cells of Undetermined Significance (ASC-US), Atypical Squamous Cells, cannot exclude HSIL (ASC-H), Low-Grade Squamous Intraepithelial Lesion (LSIL), High-Grade Squamous Intraepithelial Lesion (HSIL), Squamous Cell Carcinoma (SQCCA), Atypical Glandular Cells (AGC) and Adenocarcinoma (ADCA).

Data Collection: For each case, the following variables were retrieved from the Laboratory Information System: adequacy status after wash (satisfactory/unsatisfactory), final Bethesda category, and year of reporting.

Quality Assurance: Data were collected and analyzed and each entry was independently double-checked for accuracy. Cytology results were validated through the laboratory's routine double-reading and sign-out policy.

Ethical Approval: The study was reviewed and approved by the Research Ethics Committee of KFAFH (Approval No. REC 850).

Results

A total of 645 ThinPrep Pap smears that underwent the wash protocol between January 2020 and December 2024 were analyzed. Results are presented in Table 1. **Adequacy outcomes:** Of the reprocessed cases, 195 (30.23%) remained unsatisfactory, while 450 (69.77%) were satisfactory and yielded reportable diagnoses: **Bethesda Diagnostic Distribution:** Among the 450 satisfactory smears, the majority were reported as Negative for Intraepithelial Lesion or Malignancy (NILM) (376 cases; 83.56%). Abnormal cytological findings were detected in 74 cases (16.44%), including: ASC-US (51; 11.33%), ASC-H (1; 0.22%), LSIL (5; 1.11%), HSIL (2; 0.44%), and Atypical Glandular Cells (15; 3.33%), no squamous cell carcinoma or Adenocarcinoma were detected. **Age-Group Distribution:** Unsatisfactory rates increased progressively with age. The lowest rate was observed in women aged 21–30 years (19.44%), while higher proportions were recorded in the 41–50 years (32.37%) and 51–60 years (32.39%) groups, reaching the highest level in women aged 61–70 years (53.19%).

Table 1: Distribution of ThinPrep Pap smears processed with the wash technique (2020–2024) by age group, adequacy status, and Bethesda System diagnostic category

Distribution of ThinPrep Pap smears processed with the wash technique (2020–2024) by age group, adequacy status, and Bethesda System diagnostic category																			
Age group	**UNSAT**	**%**	**NILM**	**%**	**ASCUS**	**%**	**ASC-H**	**%**	**LSIL**	**%**	**HSIL**	**%**	**SQCCA**	**%**	**AGC**	**%**	**ADCA**	**%**	**Total**
21-30	7	19.44%	23	79.31%	6	20.69%	-	-	-	-	-	-	-	-	-	-	-	-	36
31-40	33	19.76%	112	83.58%	14	10.45%	-	-	4	2.99%	-	-	-	-	4	2.99%	-	-	167
41-50	78	32.37%	136	83.44%	19	11.66%	1	0.61%	1	0.61%	-	-	-	-	6	3.68%	-	-	241
51-60	46	32.39%	79	82.29%	12	12.50%	-	-	-	-	1	1.04%	-	-	4	4.17%	-	-	142
61-70	25	53.19%	21	95.45%	-	-	-	-	-	-	-	-	-	-	1	4.55%	-	-	47
71+	6	50.00%	5	83.33%	-	-	-	-	-	-	1	16.67%	-	-	-	-	-	-	12
Grand Total	195	30.23%	376	83.56%	51	11.33%	1	0.22%	5	1.11%	2	0.44%	-	-	15	3.33%	-	-	645

Percentages in the UNSAT column are calculated from the total number of cases in each age group. Percentages for Bethesda categories are calculated from satisfactory smears in that age group. Grand-total Bethesda percentages use the total number of satisfactory smears (n=450)

Discussion

This study evaluated the impact of the wash technique on ThinPrep Pap smears over a five-year period and demonstrated that reprocessing substantially reduced the proportion of unsatisfactory smears while enabling recovery of clinically meaningful diagnoses. Of 645 samples analyzed, nearly 70% yielded satisfactory results after washing, a finding consistent with prior reports of reprocessing efficacy [24-28]. In our series, the vast majority of recovered smears were categorized as NILM, but 16.44% revealed epithelial abnormalities, including ASC-US, LSIL, and HSIL. This underscores the clinical relevance of salvaging inadequate cases, as failure to reprocess would have left such abnormalities undetected. Comparable findings have been reported in previous investigations, where acetic acid wash protocols not only improved specimen adequacy but also facilitated detection of epithelial lesions [24-25]. The observation that unsatisfactory rates increased with advancing age is noteworthy. Similar age-related patterns have been described in population-based studies, where atrophic changes and chronic inflammation contributed to higher inadequacy rates among older women [29-30]. Institutional variability in unsatisfactory rates has also been documented, reflecting both biological and procedural influences on adequacy outcomes from a quality-assurance perspective, the high salvage rate observed here supports the incorporation of wash protocols into routine laboratory practice. Previous systematic reviews comparing liquid-based cytology to conventional methods have consistently shown lower unsatisfactory rates with ThinPrep, though variability persists across centers [4,31,32]. By adopting adjunctive reprocessing methods, laboratories can further minimize inadequacy, reduce patient recall, and optimize cervical cancer screening efficiency. In conclusion: The wash technique effectively improved the adequacy of ThinPrep Pap smears, converting the majority of initially unsatisfactory samples into satisfactory ones and revealing epithelial abnormalities that would otherwise have been missed. Unsatisfactory rates increased with advancing age, highlighting the need for age-aware approaches in cytology practice. Incorporating wash protocols into routine laboratory workflows can enhance diagnostic yield, reduce repeat testing, and strengthen the overall efficiency of cervical cancer screening programs.

References

1. Nayar R, Wilbur DC (2015) The Bethesda System for Reporting Cervical Cytology. 3rd ed. Springer https://link.springer.com/book/10.1007/978-3-319-11074-5.
2. Williams ARW, McCluggage WG (2016) The Bethesda System and the 2014 WHO classification of female genital tumors: interrelated frameworks for reporting cervical cytology and histology. Pathology 48: 21-26.
3. Ronco G, Cuzick J, Pierotti P, Cariaggi MP, Palma PD, et al. (2007) Accuracy of liquid-based cytology versus conventional cytology: systematic review. BMJ 335: 28.
4. Arbyn M, Bergeron C, Klinkhamer P, Hirsch PM, Siebers AG, et al. (2008) Liquid-based vs conventional cytology in cervical screening: a systematic review and meta-analysis. BMJ 337: 167-177.
5. Siebers AG, Klinkhamer PJJM, Arbyn M, Johanna MG, Angelique BB, et al. (2009) Comparison of liquid-based cytology with conventional cytology for cervical precancer detection: randomized controlled trial. JAMA 302: 1757-1764.
6. Davey E, d'Assuncao J, Irwig L, Macaskill P, Chan SF, et al. (2007) Accuracy of ThinPrep liquid-based cytology: a systematic review. Cytopathology 18: 137-144.
7. Khunamornpong S, Settakorn J, Sukpan K, Siriaunkgul S (2006) Comparison of conventional Pap smear and liquid-based cytology: an experience from northern Thailand. Asian Pac J Cancer Prev 7: 395-398.
8. Okecha N, Osman A, Hassan M (2020) Evaluation of sample adequacy and diagnostic utility of ThinPrep Pap smears in a tertiary center. J Cytol 37: 137-142.
9. Kalinicheva T, Frisch N, Giorgadze T, Madan S, Shidham A, et al. (2015) Etiologic factors in unsatisfactory ThinPrep Pap tests. Acta Cytol 59: 123-128.
10. Lin S, Taylor J, Alperstein S, Hoda R, Holcomb K (2014) Carbomer-containing lubricants reduce adequacy of ThinPrep Pap tests. Cancer Cytopathol 122: 754-761.
11. Lin S (2013) Effect of lubricants on ThinPrep Pap test adequacy. J Low Genit Tract Dis 17: 160-165.
12. (2018) ThinPrep 2000 Operator's Manual. Marlborough, MA: Hologic. Hologic Inc.
13. (2019) ThinPrep 5000 Operator's Manual. Marlborough, MA: Hologic. Hologic Inc.
14. (2019) PreservCyt and CytoLyt Solution Instructions for Use. Marlborough, MA: Hologic. Hologic Inc.
15. Munson E, Chateau BK, Nelson BE, Griep J, Czarnecka J, et al. (2012) Effect of glacial acetic acid treatment on liquid-based cytology preparations. J Clin Microbiol 50: 1394-1396.
16. Frisch NK, Ahmed Y, Sethi S, Neill D, Kalinicheva T, et al. (2015) Effectiveness of acetic acid wash protocols in Pap smear processing. J Pathol Inform 6: 23.
17. Bentz JS (2002) Prospective evaluation of reprocessing unsatisfactory ThinPrep smears. Arch Pathol Lab Med 126: 122-127.
18. Agoff SN (2002) Efficacy of reprocessing unsatisfactory liquid-based cytology samples. Am J Clin Pathol 117: 719-725.
19. Islam S (2004) Reprocessing unsatisfactory ThinPrep samples reduces inadequate rates. Cancer Cytopathol 102: 262-267.
20. Fontaine D (2012) Variability in unsatisfactory Pap test rates across institutions. BMJ Open e000730.
21. Jeong H (2017) Causes of unsatisfactory Pap tests across institutions. J Pathol Transl Med 51: 433-439.
22. Yaraghi M, Gharavi S, Najafian A, Hazari V (2024) Impact of lubricants on ThinPrep adequacy: study protocol. J Pathol Inform 15: 12.
23. Lander ME, Feldman K, Perlman B, Greenberg P, Heller DS, et al. (2024) Carbomer vs non-carbomer lubricants and Pap adequacy. J Low Genit Tract Dis 28: 45-52.
24. Munson E, Du Chateau BK, Nelson BE, Griep J, Czarnecka J, et al. (2012) Effect of glacial acetic acid treatment on liquid-based cytology preparations. J Clin Microbiol 50: 1394-1396.
25. Frisch NK, Ahmed Y, Sethi S, Neill D, Kalinicheva T, et al. (2015) Effectiveness of acetic acid wash protocols in Pap smear processing. J Pathol Inform 6: 23.
26. Bentz JS (2002) Prospective evaluation of reprocessing unsatisfactory ThinPrep smears. Arch Pathol Lab Med 126: 122-127.
27. Agoff SN (2002) Efficacy of reprocessing unsatisfactory liquid-based cytology samples. Am J Clin Pathol 117: 719-725.
28. Islam S, Marie AW, Saboorian MH, Ashfaq R (2004) Reprocessing unsatisfactory ThinPrep samples reduces inadequate rates. Cancer Cytopathol 102: 262-267.
29. Fontaine D (2012) Variability in unsatisfactory Pap test rates across institutions. BMJ Open 2: e000730.
30. Jeong H, Hong SR, Chae SW, Young JS, Yoon HK, et al. (2017) Causes of unsatisfactory Pap tests across institutions. J Pathol Transl Med 51: 433-439.
31. Davey E, d'Assuncao J, Irwig L, Macaskill P, Chan SF, et al. (2007) Accuracy of ThinPrep liquid-based cytology: a systematic review. Cytopathology 18: 137-144.
32. Ronco G, Cuzick J, Pierotti P, Cariaggi MP, Dalla PP, et al. (2007) Accuracy of liquid-based cytology versus conventional cytology: systematic review. BMJ 335: 28.