

Manual Liquid-Based Cytology: A Clinical Pilot Study of the VitroPrep™ Cytology Processing Kit

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Key Words

Cervical cytology · Liquid-based cytology · Cytology in low-resource settings · Pap smears · ThinPrep™ · VitroPrep™ · PreservPlus · CytoBase

Abstract

Objective: It was the aim of this study to assess the utility of the manual liquid-based cytology (LBC) product VitroPrep™ Cytology Processing Kit (ChemQ Bioscience LLC, Research Triangle Park, N.C., USA). **Study Design:** This is a descriptive pilot study. Women underwent cervical sampling processed by the ThinPrep™ automated LBC system followed by cervical sampling for the VitroPrep manual system. The following criteria were assessed on a scale of 1–5 (1 = unsatisfactory, 2 = borderline, 3 = acceptable, 4 = good, 5 = excellent): monolayer cell adhesion, overall cellularity, background clarity, preservation of cellular morphology, red cell lysis, and elimination of mucus/debris. Cytological diagnosis was compared to results from ThinPrep samples. In addition, VitroPrep samples were taken prior to conization procedures and compared to pathology results. Descriptive statistics were performed. **Results:** Forty-two of 47 women who underwent dual cytologic sampling had satisfactory samples. All scores were 3–5, with >90% graded 4–5. The VitroPrep

diagnosis correlated with the ThinPrep diagnosis in 90% (38/42) of cases. All specimens obtained from 15 women prior to conization were satisfactory and correlated abnormal cytologic findings with cervical intraepithelial neoplasia 1–3 pathology. **Conclusions:** The VitroPrep Cytology Processing Kit was able to provide adequate specimens for evaluation and diagnosis. This low-cost processing kit may provide a useful alternative in settings where automated LBC systems may not be feasible.

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Introduction

Liquid-based cytology (LBC) is a standard method of collecting and processing cervical cytology samples throughout the US. Though conventional cytology methods are also acceptable, LBC is reported to carry the advantage of having better-quality specimens collected in the setting of bleeding or inflammation [1–6]. Though prior studies have reported that LBC specimen adequacy

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was better than conventional cytology overall, the largest systematic review of studies did not report an advantage in decreasing the number of unsatisfactory slides or increasing the number of high-grade lesions detected [4]. LBC does confer the advantage of being able to perform additional testing, such as human papillomavirus (HPV) DNA testing, on samples. However, a disadvantage of the LBC systems is the need for high-cost automated processing equipment. Therefore, the LBC option is limited to settings where such equipment is financially feasible.

Cervical cancer is the most common gynecologic cancer worldwide [7]. The majority of cases are in low-resource areas where screening programs are limited [7]; the costs of LBC processing equipment may be prohibitive. This is a pilot study of a novel LBC method (ViroPrep™ Cytology Processing Kit, ChemQ Bioscience LLC, Research Triangle Park, N.C., USA) using manual processing with centrifuge and vortex equipment only to assess the quality of specimens for interpretation compared to standard cytological and pathological specimens taken for clinical care.

Materials and Methods

This is a descriptive pilot study conducted between June and August 2013. The study was approved by the University of North Carolina Institutional Review Board; all women underwent written informed consent procedures.

Sample Collection

Samples were collected in 2 phases. In phase 1, we recruited women aged 21 years or older undergoing cervical cytology screening. Participants underwent concurrent cervical sampling for processing by both the ThinPrep™ (Hologic, Boston, Mass. USA) automated liquid-based cytology system (routine care Pap) and the ViroPrep manual system (study Pap). Steps for sampling occurred as follows: spatula sample for routine care Pap followed by spatula sample for study Pap; then endobrush sample for routine care Pap followed by endobrush sample for study Pap.

The second phase of sample collection occurred in women planning loop electrosurgical excision procedures (LEEP). These women had cervical cytological specimens that were sampled (spatula and endobrush) for processing by the ViroPrep manual system prior to LEEP. The LEEP procedures were performed due to diagnoses of cervical intraepithelial neoplasia (CIN) 2/3 or persistent CIN 1 on colposcopic examination. This second phase was conducted to further evaluate the ability of the ViroPrep manual system to detect cytologic abnormalities in the setting of known cervical dysplasia.

Pap Smear Processing

ThinPrep samples were processed on the ThinPrep automated imaging system (Hologic) as per standard industry protocols. All processing for study slides was performed by a single investigator

using the following protocol: (1) vortex each PreservPlus solution vial at high speed for 20 s; (2) pour the preservative vial solution into a labeled 15-ml conical tube and centrifuge for 10 min at 1,000–1,200 g; (3) gently decant the supernatant liquid; (4) add 250–300 µl of CytoBase solution into the conical tube and vortex the tube at high speed for 30 s; (5) withdraw 40–45 µl of the mixture (step 4) using a micropipette and spread gently on a standard labeled glass slide by forming a small circle; (6) allow the slides to dry at room temperature for 2–3 h; and (7) follow the Papanicolaou staining protocol.

Pathologic Evaluation

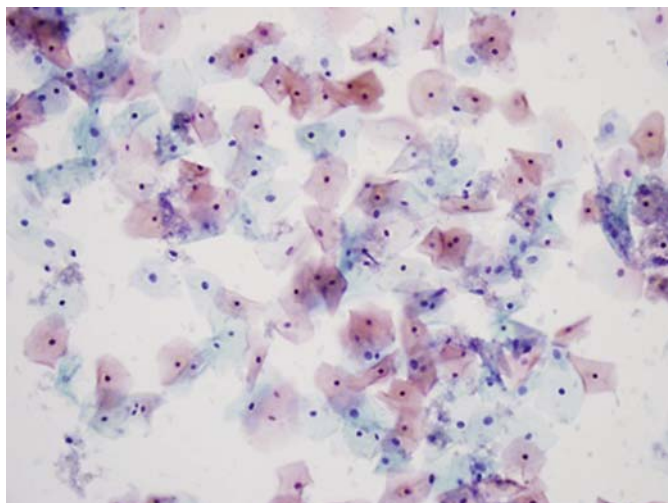
All samples were evaluated by a single blinded gynecologic pathologist/cytopathologist who evaluated both the ThinPrep and ViroPrep samples in sample collection phase 1 and the ViroPrep samples in phase 2. Pathologic evaluation of LEEP specimens were conducted by another board-certified pathology faculty at the University of North Carolina, Chapel Hill, N.C., USA. ThinPrep specimens were evaluated for clinical purposes and data were recorded in the medical record. The following characteristics were assessed in the ViroPrep system specimens: (1) monolayer cell adhesion, (2) overall cellularity, (3) background clarity, (4) preservation of cellular morphology, (5) red cell lysis, and (6) elimination of mucus and debris. The following scoring system was used for assessment: 1 = unsatisfactory, 2 = borderline, 3 = acceptable, 4 = good, 5 = excellent. Specimens were also evaluated for adequacy based on Bethesda criteria and received a Bethesda diagnosis [8]. For the purposes of this comparative study, cytology results were considered to correlate when the ViroPrep and ThinPrep diagnoses for matched pairs were exactly the same or within one degree of change, as it is done in many current standard cytopathology quality assurance practices. One degree of change was defined as follows: ASCUS (atypical squamous cells of undetermined significance)/normal, ASCUS/ASCH [atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion (HSIL)], ASCUS/LSIL (low-grade squamous intraepithelial lesion), or ASCH/HSIL.

High-Risk HPV Testing

High-risk HPV testing was performed on ViroPrep samples collected at the same time as ThinPrep specimens which had tested positive for high-risk HPV using the Digene Hybrid Capture 2™ High-Risk HPV Assay (Qiagen, Inc., Hilden, Germany).

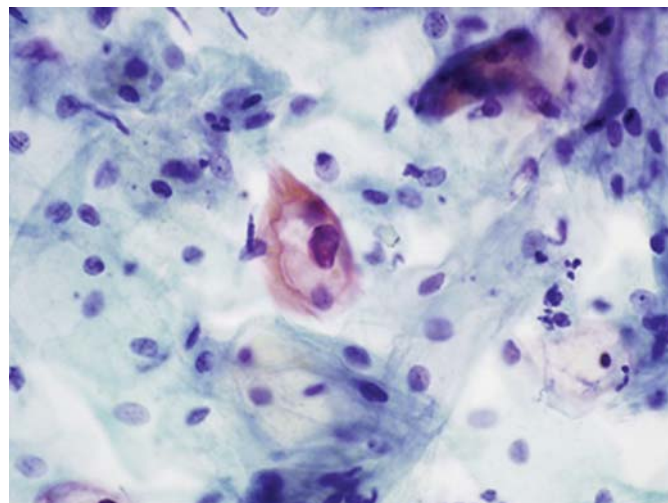
Results

Fifty-seven women who presented for routine gynecological care were recruited for dual cytologic sampling. Fifty-five agreed to participate. Eight of these women were ineligible after enrollment because no Pap smear testing was performed at the visit. Forty-seven women underwent dual cytologic sampling. The processing time for one ViroPrep sample was approximately 13 min. For a group of 6 ViroPrep samples using a 6-tube centrifuge, the processing time was approximately 18–20 min; for a group of 12 samples using a 12-tube centrifuge, the processing time was 25 min.



Color version available online

Fig. 1. Case ID No. 201. Negative for intraepithelial lesion and malignancy: medium-power view demonstrates good to excellent spread of cells within the monolayer, background clarity and elimination of mucus and debris. Normal flora is present in the background. Papanicolaou. $\times 200$.



Color version available online

Fig. 2. Case ID No. 228. LSIL: high-power view shows a central cell of LSIL with koilocytotic changes, including an enlarged hyperchromatic nucleus with irregular nuclear contours and punctate perinuclear clearing, compared to the surrounding normal squamous cells. Papanicolaou. $\times 600$.

Table 1. Discrepancies in cytologic results between VitroPrep and ThinPrep

ID No.	Cytologic result	
	VitroPrep	ThinPrep
119	ASCUS	negative
210 ¹	ASCH	negative
211 ¹	ASCH	negative
221	ASCUS	negative
224	ASCUS	negative
227	ASCUS	ASCH
228 ¹	LSIL	negative
229 ¹	negative	LSIL

Negative = Negative for intraepithelial lesion and malignancy.
¹ Analyzed as a discordant result.

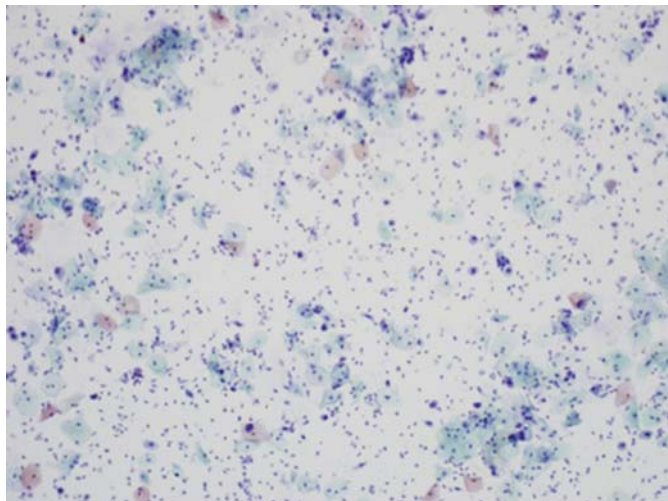
Five manual samples were unsatisfactory due to low cellularity. In 3 unsatisfactory samples, no collection (pellet) was formed after centrifugation. The centrifugation was repeated two more times but no collection of pellet was observed. These samples were then processed according to the procedure outlined above and were evaluated by our pathologist, confirming the low cellularity. The other 2 excluded samples were determined to be hemodiluted with blood on pathologic evaluation. These samples were unable to be scored according to the criteria described in the above Materials and Methods section.

All satisfactory VitroPrep specimens were scored between 3 and 5, with $>90\%$ scored at 4–5. The VitroPrep manual system diagnosis correlated with the ThinPrep automated system diagnosis in 90% (38/42) of cases. Thirty-four matched pairs (81%) had exactly the same results, and the results of 4 additional cases were within one degree of change (table 1). See figures 1–4 for sample images of collected VitroPrep samples. All VitroPrep specimens obtained from 15 additional women prior to LEEP were satisfactory and correlated an abnormal cytologic finding with CIN 1–3 pathology (table 2). Of note, these specimens were collected within 2 months of a colposcopic cervical biopsy that resulted in the referral for LEEP procedure.

There were 6 ThinPrep samples that tested positive for high-risk HPV DNA. Correlating VitroPrep specimens were sent for HPV DNA testing. Four of the 6 (67%) VitroPrep specimens also tested positive for high-risk HPV DNA.

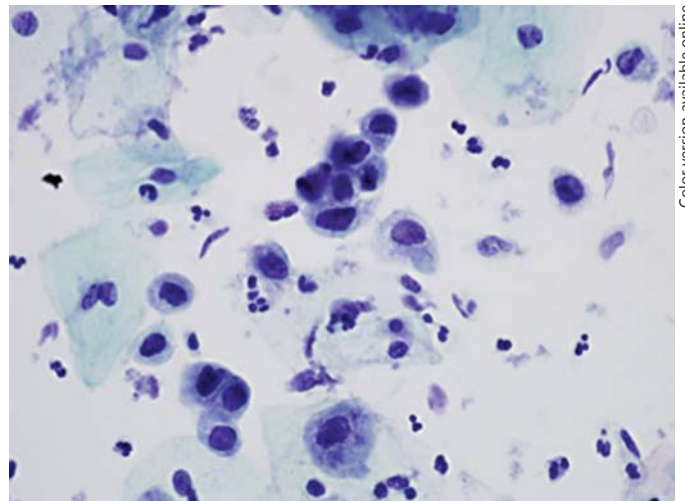
Discussion

Cervical cytology evaluation is the backbone of cervical cancer screening programs. In this pilot study, we found that the VitroPrep manual LBC system was able to provide adequate specimens for evaluation and diagnosis. VitroPrep results correlated with ThinPrep liquid-



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Fig. 3. Case ID No. 129. HSIL: low-power view shows excellent spread of epithelial cells within the monolayer. There is increased acute inflammation in the background which is non-obscuring. Papanicolaou. $\times 100$.



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Fig. 4. Case ID No. 129. HSIL: high-power view of several cells with increased nuclear to cytoplasmic ratios and densely hyperchromatic nuclei with irregular nuclear contours. Papanicolaou. $\times 600$.

Table 2. VitroPrep cytology results at the time of the LEEP conization procedure

ID No.	VitroPrep cytology result	Histopathology result
129	HSIL	CIN 2
130	ASCH	CIN 1
243	HSIL	CIN 2
244	ASCUS	CIN 2
245	ASCUS	CIN 2
246	HSIL	CIN 3
247	HSIL	CIN 3
248	ASCH	CIN 2
249	ASCUS	CIN 2
250	LSIL	CIN 2
251	ASCH	CIN 2
252	HSIL	CIN 3
253	ASCUS	CIN 3
254	ASCUS	CIN 3
255	HSIL	CIN 3

based cytology results in 90% of cases. In the 3 out of 4 cases that did not correlate, the VitroPrep system detected abnormal findings while the ThinPrep did not detect any abnormality. Moreover, no cases of ASCH or HSIL by ThinPrep were interpreted as negative on the corresponding VitroPrep cytology slide.

The goal of our study was to compare VitroPrep to an automated LBC method due to the advantages of LBC

testing: better quality specimens collected in the setting of bleeding or inflammation [1–6], the ability to store samples at room temperature and to batch test, and the ability to perform additional microbiological testing on LBC samples. This study did not compare LBC results to conventional cytology, also a standard method of cervical cytology screening. Manual LBC and conventional cytology are both low-cost procedures at USD 2–3 per slide, especially compared to the infrastructure investment of over USD 55,000 for current automated LBC systems [9]. Other manual LBC methods have been described [9–12] and compared to conventional cytology [13, 14]. The studies that directly compared to conventional cytology reported unsatisfactory slides due to obscuring blood or clumped cells [13] and correlation with conventional cytology was reported as only 68% [14]. The other manual LBC studies describe satisfactory slides for interpretation though there was no comparison with conventional cytology [9–12]. Another low-cost method of cervical cancer screening used in low-income countries is visual inspection with acetic acid which requires no testing or expertise of a pathologist in making clinical diagnoses. However, a comparison with this method of screening was beyond the scope of our study.

This study is limited in being a small pilot study. Additionally, the sampling protocol may have affected the results. Prior LBC studies have had the advantage of using residual fluid from the initial LBC Pap screening for eval-

uation of manual LBC methods [9–12]. However, since we were comparing 2 different LBC methods on women in clinical care, the VitroPrep sample was always taken second to ThinPrep sampling and this may have affected the quality of samples. Obtaining VitroPrep samples after ThinPrep sampling may also have played a role in the discordant HPV testing results in 2 cases. This reasoning is further supported by the fact that there were no unsatisfactory samples in the second phase of sample collection when only a VitroPrep sample was obtained for analysis. We should also comment on the 8 of 15 VitroPrep results that returned as ASCUS or ASCH prior to the LEEP conization procedures. These specimens were collected within 2 months of initial colposcopic biopsy; therefore, subsequent inflammation may have contributed to these equivocal cytologic findings.

Conclusions

This new low-cost cytology processing kit may provide a useful alternative in settings where automated LBC systems may not be feasible. The VitroPrep Cytology Processing Kit provides the advantage of being an LBC method that can be stored and batched for testing. It is also a

medium in which HPV screening is possible if this is required. A centrifuge and a vortex are the only equipment needed to process the samples, and therefore, a laboratory technician in most labs around the world can process these specimens. Despite growing interest in HPV testing becoming primary to screening for cervical cancer, cytology will still be part of the screening algorithm. Therefore, a simple low-cost method is appealing. Larger population-based studies will be required to establish the sensitivity and specificity of cytology and HPV DNA testing abilities of the VitroPrep manual testing system.

Acknowledgements

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Disclosure Statement

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