

Stability of Archived Liquid-Based Cervical Cytologic Specimens

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BACKGROUND. Exfoliated cervical cell specimens collected in PreservCyt®, a methanol-based medium used in ThinPrep® liquid-based cytology, have been archived in epidemiologic studies. However, long-term DNA stability and cytologic stability of these biospecimens have not been evaluated.

METHODS. Cervical specimens were collected into PreservCyt from participants in a natural history study of human papillomavirus (HPV) infection and cervical carcinoma in Guanacaste, Costa Rica (1993–2000), and stored at ambient temperatures. Thirty specimens classified as low-grade squamous intraepithelial lesions by liquid-based cytology were randomly chosen from each collection year (except for 1994) and selectively assessed for molecular and cytologic stability. Specimens were tested in 2001 for 1) HPV DNA by the Hybrid Capture 2 test, 2) β -globin DNA by polymerase chain reaction amplification of multiple length fragments (268, 610, and 1327 bp), and 3) nuclear preservation by visual inspection of newly made liquid-based cytology slides. All testing was done masked to year of collection. Associations of stability and storage time were evaluated using standard contingency tables and chi-square tests for trend.

RESULTS. Human papillomavirus DNA, as detected by the Hybrid Capture 2 test, was unaffected by storage time. Stability of β -globin DNA ($P_{Trend} < 0.0001$) and nuclear preservation ($P_{Trend} < 0.0001$) declined with increasing storage time. Approximately 15% of specimens could not be amplified for any β -globin DNA fragment after 5 years of storage (collected in 1996). In addition, cytology slides made from 41% specimens were rated as marginal (32%) or unsatisfactory (9%) after 8 years of storage (collected in 1993).

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Consent was obtained from all participants in accordance with guidelines of the U.S. Department of Health and Human Services. This study was approved by National Institutes of Health and Costa Rica institutional review boards.

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CONCLUSIONS. Cervical specimens archived in PreservCyt underwent partial DNA and cytologic degradation after several years of storage. Methodologic studies to optimize long-term storage of cervical cells for epidemiologic studies of cervical carcinoma are needed. *Cancer (Cancer Cytopathol)* 2003;99:89–96.

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Liquid-based cytology has emerged as an alternative to the conventional Pap test and is already used for more than 50% of Pap screening in the United States. Because only a portion of the collected cells is typically needed for the preparation of a liquid-based cytologic slide, the remaining specimen can be used for repeat cytology or ancillary testing. For example, human papillomavirus (HPV) DNA testing of ThinPrep cytologic specimens (Cytec, Boxborough, MA) by the Hybrid Capture 2 test (HC2; Digene, Gaithersburg, MD) is as sensitive as testing specimens stored in the initially recommended specimen transport medium (STM; Digene).¹ Recent data from the atypical squamous cells of undetermined significance (ASCUS) low-grade squamous intraepithelial cells (LSIL) Triage Study (ALTS) demonstrated that HPV DNA testing of ThinPrep specimens by HC2 is a sensitive detection method for underlying high-grade cervical neoplasia in women with equivocal cytologic diagnoses.²

The widespread use of liquid-based cytology raises the possibility of cytologic archives analogous to the histologic archives maintained by academic pathology departments. For example, cervical specimens stored in liquid Pap medium have also been archived in a number of large epidemiologic studies of cervical carcinoma. In addition, liquid-based cytologic methods can be used for nongynecologic specimens such as gastrointestinal brushings and fine needle aspirants of the breast and thyroid³ and therefore these specimens might also be archived in liquid media. However, there is little information on whether long-term (> 1 year) archived specimens maintain their cytologic and molecular integrity. To address this issue, we selected archived specimens in PreservCyt® (PreservCyt specimens) collected during a natural history study of HPV and cervical carcinoma in Costa Rica.^{4,5} Specimens from different collection years were tested for preservation of HPV DNA, β -globin DNA, and cell nuclear features.

MATERIALS AND METHODS

Population

A population-based natural history study of HPV and cervical neoplasia was established in 1993–1994 in Guanacaste, Costa Rica.^{4,5} At enrollment, 10,049 of the 11,742 female residents of Guanacaste identified in a

door-to-door survey agreed to visit one of our study clinics and participated in the enrollment interview. This study was sponsored by the National Cancer Institute (NCI) and was approved by the NCI and local institutional review boards (IRB).

Specimen Collection

Pelvic examinations were performed on 9205 sexually active women. As part of the standard pelvic examination at enrollment and during follow-up, exfoliated cervical cells were routinely collected by using a Cervex brush (Unimar, Wilton, CT).^{4,5} The brush was smeared onto a glass slide for the preparation of a conventional Pap smear and then placed in the ThinPrep vial containing PreservCyt for preparation and interpretation of liquid-based cytologic slides. Residual PreservCyt specimens were collected and archived at uncontrolled ambient temperatures until the beginning of 1996, when specimens were stored in a controlled ambient environment (10–26.7 °C). An additional cervical specimen, collected using a Dacron swab (Digene), was placed in STM. These specimens were initially stored frozen at –70 °C in Guanacaste until transported on dry ice to an NCI biorepository where the specimens were again stored at –70 °C.

Specimen Selection and Utilization

PreservCyt specimens with an original cytologic interpretation of LSIL by a single, expert cytopathologist (M.L.H.) were selected from subjects who never had a diagnosis of high-grade cervical neoplasia during the course of the study (Table 1). These specimens were selected because we anticipated high HPV DNA positivity among LSIL cytologic results, and the use of these specimens was unlikely to impact our natural history studies of high-grade cervical neoplasia. Although there is a significant variability in cytopathologic interpretation of an individual review, interpretation of LSIL cytology is generally reproducible among experienced U.S. cytopathologists.⁶ Selecting 30 LSIL specimens from each year (1993, 1996, and 2000), as interpreted by the same cytopathologist, provided internal consistency such that we could expect comparable percentages of HPV positivity across all time periods (ALTS noted consistent levels of HPV

TABLE 1
Specimen Selection and Testing by Year of Study^a

Collection year	No. of LSIL specimens available	No. of specimens selected	HPV DNA tests	β -globin tests	Cytologic tests
1993	54	30	30	30	22
1995	29	29			29
1996	30	30	29	28	28
1997	33	30		30	
1998	31	30		30	
1999	32	30		30	
2000	35	30	30	30	26
Fresh	30	30		30	

LSIL: low-risk squamous intraepithelial lesions; HPV: human papillomavirus.

^a The first phase of testing is shown in bold.

positivity for cytologic LSIL across four geographically diverse sites with numerous cytopathologists⁷).

Specimens were tested in two phases for efficient use of specimens. The first phase was used to determine if there were any storage time-dependent effects on stability and the second phase was used to further describe any degradation identified in the first phase. In the first testing phase, we randomly selected 90 specimens: 30 from 1993 (8 years of storage), 30 from 1996 (5 years of storage; one specimen had insufficient volume for any testing), and 30 from 2000 (1 year of storage). Each specimen was tested for HPV DNA by HC2 and β -globin polymerase chain reaction (PCR) amplification and evaluated microscopically for nuclear preservation on freshly made cytologic slides.

In the second testing phase (performed within 3 months of the first phase), additional specimens were selected from either 1995 (6 years of storage), 1997 (4 years of storage), 1998 (3 years of storage), or 1999 (2 years of storage), depending on which time interval (from 1993 to 1996 or from 1996 to 2000) a stability measurement showed the greatest change with storage time as indicated from the first phase of testing. Specifically, 90 specimens from 1997 to 1999 (30 specimens from each year) were selected for β -globin PCR amplification and all 29 specimens from 1995 were selected for microscopic evaluation of nuclear features. No additional specimens were selected for HPV DNA testing by HC2-B (HC2 with probe set B) because there were no significant changes observed in the first phase of testing.

As positive controls used in the DNA stability assay, 30 freshly collected (less than 4 days old), anonymous, residual PreservCyt specimens were obtained from the University of Oklahoma Health Sciences Center during the fall of 2001, following patient diagnosis and in compliance with a University of Okla-

homa IRB-approved protocol. Specimens were primarily collected using a cytobrush and spatula combination but occasionally a Papette™ broom (Wallach Surgical Services, Orange, CT) was used. These fresh specimens were centrifuged and the cell pellets were frozen at -70°C until they were tested for β -globin DNA by PCR in the second testing phase.

Some archived specimens had a limited amount of residual material remaining as the result of their previous clinical use or their use in the natural history study, which necessitated a judicious and hierarchical use of specimens. An aliquot of 2 mL was removed for HPV DNA testing and then 1 mL was drawn for β -globin DNA testing by PCR. If sufficient specimen remained (greater than 1–2 mL), fresh PreservCyt medium was added to the specimen to bring the specimen volume to 20 mL, which is necessary for the production of cytologic slides using the ThinPrep 2000 processor (Cytec). Table 1 indicates the number of tests performed from each year for each measurement. All specimen testing and evaluations were performed by personnel masked to the specimen collection year.

HPV DNA Testing

Human papillomavirus DNA testing by HC2 was performed utilizing probe set B (HC2-B), which contains RNA probes for 13 cancer-associated HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68).⁶ Signal strengths in relative light units (RLU) were compared with 1 pg/mL HPV Type 16 DNA-positive controls (RLU/PC). Specimens with more than one 1 RLU/PC were HPV DNA positive. The RLU/PC values above this threshold were used as semiquantitative measures of viral load among HPV DNA-positive women.⁸ Specimens were prepared per the manufacturer's instructions except that 2-mL specimen aliquots for a single test using probe B were employed in this study, rather than 4-mL aliquots recommended for clinical testing using both probe set A and probe B. Reagents were scaled accordingly. The entire volume of the denatured specimen (75 μL) was committed to hybridization whereas subsequent steps in the HC2-B test followed the manufacturer's instructions.

As a quality control measure, we compared the results of the HC2-B test on a subset of Year 1993 specimens ($n = 12$) with HC2-B test results on the corresponding paired STM specimens tested in a previous study on a stratified sample of the population.⁹

β -Globin DNA Testing by PCR

Aliquots of specimen (1 mL) were centrifuged for 10 minutes ($14,000 \times g$) and all but 0.2 mL of the supernatant was removed and discarded. The cell pellets

were suspended in the residual 0.2 mL of PreservCyt and the DNA was isolated from each sample using the QIAamp DNA mini kit according to the manufacturer's directions (Qiagen, Valencia, CA). The DNA was eluted in 0.4 mL of 10 mM Tris, pH 9.0, 0.5 mM ethylenediaminetetraacetic acid buffer. The DNA concentration was determined using a PicoGreen ds DNA quantitation assay (Molecular Probes, Eugene, OR).

Approximately 20 ng of total DNA from each sample was amplified in three separate PCR reactions using three different human β -globin primer pairs that were designed to amplify fragments varying in size¹⁰: 1) GH20/PC04, which amplifies a 268-bp fragment, 2) PC03/PC07, which amplifies a 610-bp fragment, and 3) KM29/RS80, which amplifies a 1327-bp fragment. The presence of PCR amplicons was determined by ethidium bromide staining after electrophoresis through 1.2% agarose gels and compared with a 100-bp DNA ladder (Amersham Biosciences, Piscataway, NJ). The results were recorded as positive, weak positive, and negative.

Cytology

Cytologic slides were evaluated by a single expert cytopathologist (D.S.) whose primary measure of cytologic stability was nuclear preservation. Nuclear preservation was scored as satisfactory (most cells had good nuclear detail with visible chromatin structures), satisfactory but limited (some cells did not have good nuclear detail and some slightly degenerated nuclei were present), marginal (most cells showed nuclear degradation and many ghost nuclei were present), and unsatisfactory (the specimen could not be evaluated). Slides were also scored for inflammation and cellularity as potential modifiers of nuclear preservation. Inflammation was scored either as absent, present, or gross inflammation suggestive of cervicitis. Cellularity was scored either as good, sparse, or insufficient. We randomly selected 25% of these slides ($n = 26$). We reevaluated, masked to the original score, a random sample of 25% of the slides ($n = 26$) and the three slides originally sited as unsatisfactory for nuclear preservation to assess the reproducibility of this measurement.

Analysis

Standard contingency table methods and chi-square tests for trend were used to assess the association of biomarkers with collection year. In addition to testing the association of collection year with HPV DNA positivity, the association of HPV viral load among the HPV DNA-positive specimens and year of collection was tested by two methods. First, a nonparametric analysis of variance statistic (Kruskal-Wallis) was used

to compare the RLU/PC values between years of collection. Second, RLU/PC values were categorized (1 to 10, 10 to < 100, ≥ 100 RLU/PC) and the association with year of collection was tested using a chi-square test for trend. Geometric mean viral loads and the corresponding 95% confidence intervals (95% CI) were also calculated. To test the association of β -globin DNA stability with collection year, β -globin PCR data were ranked hierarchically by the presence (positive or weak positive) of the largest amplifiable fragment size (1327 bp > 610 bp > 268 bp > no fragment). The association of quartiles of total DNA recovered (0.0–0.5 μ g, 0.5–0.8 μ g, 0.8–1.2 μ g, ≥ 1.2 μ g) with collection year was evaluated by a chi-square test for trend. Associations between each stability biomarker were also evaluated using Pearson's chi-square test, as were the associations of inflammation and cellularity for nuclear preservation. Finally, we evaluated the association of age at clinic visit (18–27, 28–35, 35–44, ≥ 45 years) with each stability biomarker.

Kappa and linear-weighted kappa statistics¹¹ with 95% CIs were used to assess the reproducibility of cytologic evaluations. Kappa statistics were also used to assess agreement between the HC2-B test results on the PreservCyt specimens from this study and the previous results on paired STM specimens.⁹

RESULTS

As part of the first phase of testing, HC2-B testing for oncogenic HPV DNA was performed on specimens from 1993, 1996, and 2000 (Table 2). We found no significant association of HPV DNA positivity by HC2-B with the year of specimen collection ($P_{\text{Trend}} = 0.6$). We found no significant differences in the HPV viral load between collection years ($P = 0.3$, Kruskal-Wallis; $P_{\text{Trend}} = 1.0$); the geometric mean viral loads for 1993, 1996, and 2000 were 37.0 RLU/PC (95% CI = 10.2–133.9 RLU/PC), 118.7 RLU/PC (95% CI = 45.4–310.0 RLU/PC), and 45.1 RLU/PC (95% CI = 11.4–177.6 RLU/PC), respectively. However, we note that HPV positivity and HPV viral load were lowest for 1993. For 12 of the 30 PreservCyt specimens selected from 1993, we also used HC2-B to test the paired, frozen STM specimens collected at the same clinic visit. Eleven of the 12 specimens had the same test results (i.e., two were negative and nine were positive) for an exact agreement of 91.9% and a kappa value of 0.75 (95% CI = 0.47–1.0). The one discordant result was positive for the PreservCyt specimen and negative for the STM specimen.

Polymerase chain reaction amplification of β -globin fragments of differing size was used as a generic marker of DNA stability, a marker that should be unaffected by HPV infection and LSIL. (The 268-bp am-

TABLE 2
Results of Oncogenic HPV DNA Detection by Hybrid Capture 2 Using Probe B (HC2-B) Versus Collection Year^a

HC2-B test result	Collection year (years-of-storage)			Total
	1993 (8)	1996 (5)	2000 (1)	
Negative	14 <i>46.7%</i>	8 <i>28.0%</i>	12 <i>40.0%</i>	34 <i>38.8%</i>
Positive	16 <i>53.3%</i>	21 <i>72.0%</i>	18 <i>60.0%</i>	55 <i>61.8%</i>
Total	30	29	30	88
1 to < 10 RLU/PC	5 <i>31.3%</i>	3 <i>14.0%</i>	6 <i>33.0%</i>	13 <i>24.0%</i>
10 to < 100 RLU/PC	5 <i>31.3%</i>	6 <i>29.0%</i>	5 <i>28.0%</i>	16 <i>30.0%</i>
≥ 100 RLU/PC	6 <i>37.5%</i>	12 <i>57.0%</i>	7 <i>39.0%</i>	25 <i>46.0%</i>
Total	16	21	18	54

RLU: relative light units; PC: positive controls.

^a The analysis of RLU/PC categories versus collection year is restricted to women with a positive HC2-B test (≥ 1RLU/PC). Column percentages are shown in italics below the number. $P_{Trend} = 0.6$ for HC2-B test results for cancer-associated HPV DNA types; $P_{Trend} = 1.0$ for categories of the RLU/PC value among HPV DNA-positive women.

plicon of the β -globin is a common control for PCR methods, including those employed for HPV DNA detection.) In the first phase of testing, one specimen (3.3%) from 2000 four specimens (14.3%) from 1996, and three specimens (10.0%) from 1993 had no detectable β -globin bands from PCR amplification, suggesting that the largest difference in stability was between 1996 and 2000 (Table 3). As a result, additional specimens from 1997 to 1999 were selected for testing. Overall, specimens from 1993 and from 1996 to 2000, in addition to the freshly collected specimens, were tested for amplification of the three β -globin fragments (1327, 610, and 268 bp). Results were ranked hierarchically based on the premise that the presence of larger sized amplicons indicated greater DNA stability. Increased storage time of the specimen was significantly associated with decreased stability ($P_{Trend} < 0.0001$). Specifically, only the 268-bp fragment or no fragment could be amplified from 43% (12 of 28) and 40% (12 of 30) of specimens stored 5 (from 1996) and 8 years (from 1993), respectively, compared with 7% (2 of 30) of specimens stored 1 year (from 2000). Increased total DNA recovery was also associated with greater stability as measured by β -globin amplification ($P < 0.0001$, Pearson chi-square test; data not shown) but did not explain the negative association between storage time and stability.

In the first phase of testing for nuclear stability, 9 of 22 specimens (40.9%) from 1993 were scored as

marginal or unsatisfactory compared with 4 of 28 specimens (14.3%) from 1996 and 0 of 26 (0.0%) from 2000 (Table 4). The largest difference in stability occurred between 1993 and 1996. Consequently, specimens from 1995 were selected for the second, clarifying phase of testing. Overall, liquid-based cytologic slides were made from specimens collected during 1993, 1995, 1996, and 2000 and were evaluated for nuclear preservation using a qualitative index (Fig. 1). We found a strong decrease in the quality of nuclei with longer storage times ($P_{Trend} = 0.0002$). Specifically, only 9.1% (2 of 22) and 34.5% (10 of 29) of specimens stored 8 years and 6 years, respectively, were rated as satisfactory compared with 57.7% (15 of 26) of specimens stored 1 year. Degradation of nuclear quality was not associated with the age of the women at the clinical visit, cervical inflammation, and specimen cellularity, but was marginally associated with DNA stability as measured by β -globin amplification ($P = 0.07$, Pearson chi-square test). The exact reproducibility of this qualitative assessment was fair, as indicated by a kappa value of 0.34 (95% CI = 0.11–0.57) and exact agreement of 55.1%. Although discordance by a single category was common, discordance by two categories never occurred and there was a large increase in the linear-weighted kappa value ($\kappa = 0.55$; 95% CI = 0.30–0.79) compared with the unweighted value.

DISCUSSION

Our study demonstrates that there is some time-dependent degradation of β -globin DNA and cytologic nuclear features of archival specimens stored in PreservCyt. Our specimens were stored at ambient temperatures (and uncontrolled before 1996; average temperature in Guanacaste is 28 °C) rather than at 4 °C. If degradation kinetics are temperature dependent, higher storage temperatures would be expected to accelerate degradation. The associations between poor nuclear preservation and poor DNA stability and between poor DNA stability and poor recovery of total DNA suggest that nuclei are incompletely preserved in this methanol-based medium, resulting in gradual degradation over time. Therefore, storage of cervical specimens in PreservCyt may not be optimal for long-term prospective studies of cervical carcinoma that rely on archival specimens.

There were two time intervals of significant decreases in genomic DNA stability as measured by β -globin, one occurring between fresh collection and Year 2000 specimens and the other between 1997 and 1996 specimens. For the decline after fresh collection, we suggest two possibilities. First, the populations from which the fresh specimens (Oklahoma) and the

TABLE 3
Results of PCR Amplification of Three β -Globin Fragments Versus Collection Year^a

Largest β -globin PCR fragment (bp)	Collection year (years of storage)						Fresh (0)	Total
	1993 (8)	1996 (5)	1997 (4)	1998 (3)	1999 (2)	2000 (1)		
1327	1 <i>3.3%</i>	0 <i>0.0%</i>	2 <i>6.7%</i>	11 <i>36.7%</i>	4 <i>13.3%</i>	2 <i>6.7%</i>	15 <i>50.0%</i>	35 <i>68%</i>
610	17 <i>56.7%</i>	16 <i>57.1%</i>	25 <i>83.3%</i>	17 <i>56.7%</i>	22 <i>73.3%</i>	26 <i>86.7%</i>	14 <i>46.7%</i>	137 <i>65.9%</i>
268	9 <i>30.0%</i>	8 <i>28.6%</i>	3 <i>10.0%</i>	1 <i>3.3%</i>	4 <i>13.3%</i>	1 <i>3.3%</i>	0 <i>0.0%</i>	26 <i>12.5%</i>
None	3 <i>10.0%</i>	4 <i>14.3%</i>	0 <i>0.0%</i>	1 <i>3.3%</i>	0 <i>0.0%</i>	1 <i>3.3%</i>	1 <i>3.3%</i>	10 <i>4.8%</i>
Total	30	28	30	30	30	30	30	208

PCR: polymerase chain reaction.

^a Results were hierarchically ranked according to the largest fragment detected (1327 bp > 610 bp > 268 bp > none). Column percentages are shown in italics below the number. $P_{Trend} < 0.0001$.**TABLE 4**
Rating of Nuclear Preservation Versus Collection Year^a

Nuclear preservation rating	Collection year (years of storage)				Total
	1993 (8)	1995 (6)	1996 (5)	2000 (1)	
Satisfactory	2 <i>9.1%</i>	10 <i>34.5%</i>	16 <i>57.1%</i>	15 <i>57.7%</i>	43 <i>40.9%</i>
Satisfactory but limited	11 <i>50.0%</i>	14 <i>48.3%</i>	8 <i>28.6%</i>	11 <i>42.3%</i>	44 <i>41.9%</i>
Marginal	7 <i>31.8%</i>	4 <i>13.8%</i>	4 <i>14.3%</i>	0 <i>0.0%</i>	15 <i>14.3%</i>
Unsatisfactory	2 <i>9.1%</i>	1 <i>3.5%</i>	0 <i>0.0%</i>	0 <i>0.0%</i>	3 <i>2.9%</i>
Total	22	29	28	26	104

^a Column percentages are shown in italics below the number. $P_{Trend} = 0.0002$

Year 2000 specimens (Costa Rica) were collected are significantly different. Second, specimen transport within Costa Rica was done at ambient temperatures. Therefore, specimens were exposed to tropical climates during transport. The decline in stability was still significant after exclusion of the fresh specimens from the analysis ($P_{Trend} < 0.001$), suggesting that there was still a storage time-dependent effect that was not related to this first loss in stability. The second decline in genomic DNA stability, comparing specimens from 1996 and 1997, suggests that PreservCyt can only be stored for 4–5 years before the specimens accrue significant losses in DNA integrity as detected by PCR.

Our choice to use archived specimens with an original cytologic interpretation of LSIL resulted in an HPV DNA positivity of 61% by HC2-B. A previous study¹⁰ using HC2 found an HPV DNA positivity rate of 83% for PreservCyt specimens with LSIL cytology.

We cannot explain this difference. As a result, the statistical power to detect more subtle differences was decreased. However, within this study, the test groups and specimen collection are comparable over the duration of the study based on the use of the same clinical team and pathology review by a single expert cytopathologist.

We did not observe decreases in the performance of HC2-B for HPV DNA detection and semiquantitative HPV viral load among HPV-positive specimens. Admittedly, this is a crude measure of HPV DNA stability that may be insensitive to subtle changes due to storage. The HC2-B test utilizes full-length cRNA probes that can anneal to any portion of the HPV genome and may be robust enough in design to withstand substantial fragmentation of target DNA. By comparison, PCR methods rely on hybridization of short oligonucleotides but require continuous stretches of intervening DNA that might be more easily affected by DNA cleavage. However, the lower HPV DNA positivity and lower HPV viral load measured in Year 1993 specimens compared with the other test years may hint that some subtle losses in HPV DNA stability also occurred. The absence of significant changes in stability, as was observed in the other measures of stability, could be due to chance if specimens with greater HPV positivity and viral load were preferentially selected from 1993 and 1996. Although this explanation cannot be ruled out, it seems an unlikely one because we randomly selected 30 cytologic LSIL specimens as interpreted by the same expert pathologist. Despite the obvious degradation measured by morphologic evaluation and β -globin PCR methods, we infer from our data that retrospective HPV DNA testing using HC2-B may be possible using PreservCyt specimens archived up to 8 years,

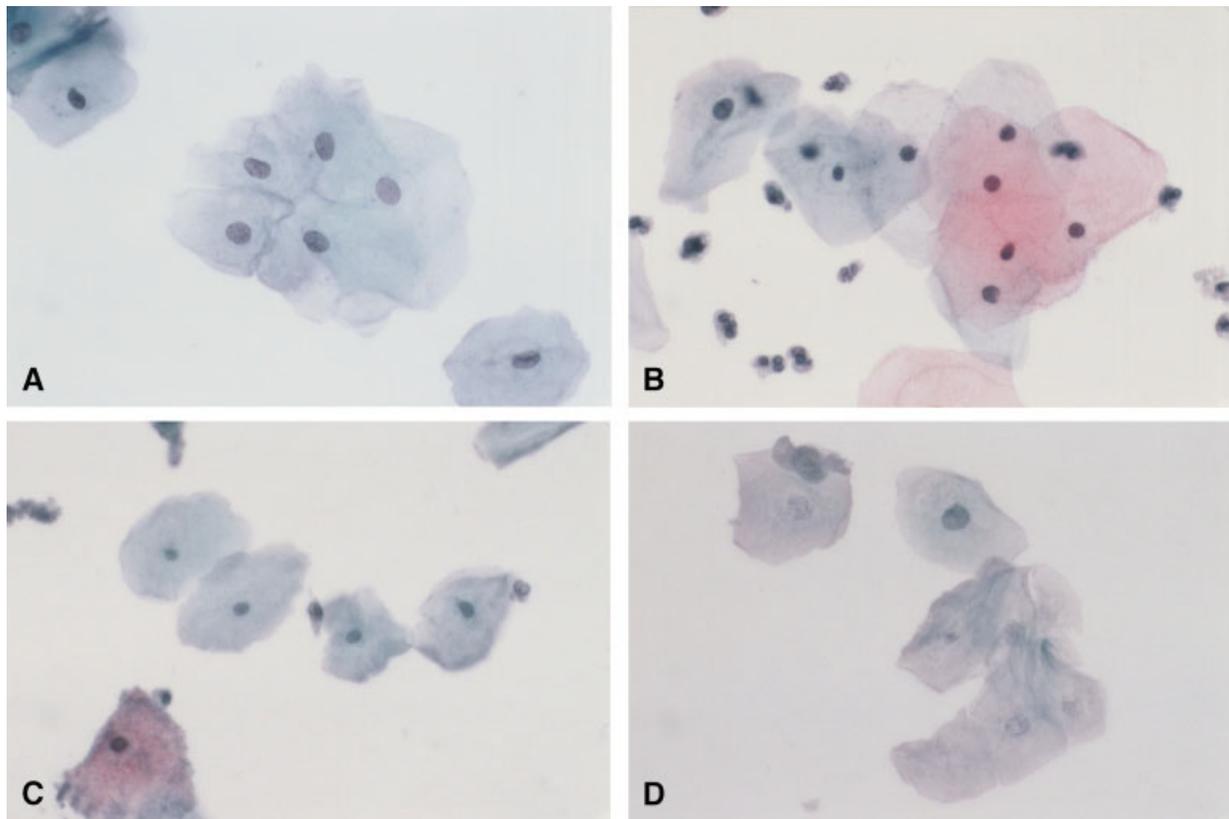


FIGURE 1. Photographs of the different categories of the qualitative cytology index used for scoring slides. Slides were scored as (A) satisfactory (most cells had good nuclear detail with visible chromatin structures); (B) satisfactory but limited (some cells were without good nuclear detail and some slightly degenerated nuclei were present); (C) marginal (most cells showed nuclear degradation and many ghost nuclei were present); and (D) unsatisfactory (the specimen could not be evaluated). Note the variability in the nuclear preservation within a field for specimens scored as either satisfactory but limited (B) or marginal (C).

even under suboptimal storage conditions. This is an important consideration for epidemiologic studies of cervical carcinoma.

We acknowledge that the exact reproducibility of our cytologic indices was only fair. However, there was no gross discordance between repeat measures. In evaluating the slides unmasked, it is evident that there is some variability in the preservation of nuclei from different fields of view within a slide. As the fields of view were randomly selected for each evaluation, we suggest that it is this variability in the sampled fields that contributed to “wobble” in the interpretation of the nuclear details (Fig. 1B,C).

We did not evaluate RNA or protein stability in this study. One study has shown that mRNA from cervical specimens stored in PreservCyt is poorly preserved. Reverse transcriptase (RT)-PCR amplification of the glyceraldehyde-3-phosphate dehydrogenase (GADPH) transcript, a standard RT-PCR intraassay control, was successful for about one-third of cervical specimens stored less than 30 days in PreservCyt.¹² Another study quantitatively measured HPV transcripts

within 3–4 months of collection by using by using real-time RT-PCR and targeting short nucleic acid sequences (< 100 bp).¹³ Finally, PreservCyt storage of mammary tumor cells preserved estrogen and progesterone receptor antigenicity up to 56 days.¹⁴ The longer-term effects of storage of specimens in PreservCyt on RNA and protein need to be addressed in future studies.

In summary, although HPV DNA as measured by HC2-B appeared to be stable over time, cervical specimens archived in PreservCyt underwent measurable degradation of genomic DNA and nuclear features over 8 years of storage at ambient conditions. Some of this degradation is likely the result of uncontrolled temperatures. However, there was degradation of genomic stability ($P < 0.0001$) and morphologic degradation ($P = 0.1$) between 1996 and 2000 after temperature-controlled storage had been instituted. We did not test specimens repeatedly over time but selected specimens with different storage times. As a result, we assumed comparability of the specimens over time. It is conceivable that variability in collec-

tion methods over this time period could explain our findings but a standardized procedure by the same clinical team was used throughout the course of the study. We infer from these data that storage of nongynecologic specimens in PreservCyt could undergo similar degradation. Although storage of PreservCyt specimens at lower temperatures could potentially provide better molecular stability, storage below the freezing point of the medium will damage cell integrity and make specimens unusable for cytology. Storing large numbers of PreservCyt specimens below ambient temperature may be impractical and costly for long prospective studies such as the one we are conducting in Costa Rica in which about 25,000 PreservCyt specimens have been collected. An alternate method of processing specimens by aliquoting and centrifugation so that cell pellets might be stored in cryovials would likely be prohibitively labor intensive and would limit the use of these specimens to molecular testing. We suggest that new liquid media that preserve molecular and nuclear features are needed to optimize archival storage of cervical specimens.

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