

were misdiagnosed as “benign.” All these lesions were subsequently extirpated, and the diagnosis of papillary thyroid carcinoma-follicular variant (PTC-FV) was established. This is especially important because a small subgroup of patients with PTC-FV exists, in whom the disease runs an aggressive course.⁴ In fact, Dr. Renshaw has himself commented on the importance of accurate diagnosis in similar cases.^{5,6}

With regard to the “overdiagnosis” of a cellular hyperplastic benign nodule as a “follicular lesion,” this would lead in the majority of cases to an ipsilateral hemithyroidectomy with isthmusectomy, a procedure that admittedly carries some morbidity.⁷ In such cases, when complications occur, litigation usually emanates from poor surgical outcome, and is rarely directed against the diagnostician. Conversely, at least in our opinion, the medical (and legal) complications of “underdiagnosing” follicular thyroid carcinoma (or PTC-FV in selected cases) cannot be overemphasized. In addition, the responsibility for the interpretation of cytologically “suspicious” thyroidal lesions is shared to a variable degree with the treating physician because there are several clinical criteria that are, at least partially, capable of offering guidance with regard to further therapy.⁸

Finally, we wish to clarify that, when submitting our commentary, our intentions did not include either increasing the risk of litigation in controversial (and, by default, “difficult”) diagnostic cases or increasing the existing share of disagreements between academic-based and hospital-/practice-based cytopathologists. In addition to drawing attention to the pertinent, widely accepted definitions and a suggested manner for their optimal use, we believe that our commentary emphasized the important need for the adoption of generally “workable” terminology for thyroid proliferative lesions by the entire cytopathology (and clinical) community.

REFERENCES

1. Sarlis NJ, Gougiortis L, Filie AC. Misclassification of cytologic diagnoses in patients with follicular lesions or follicular neoplasms of the thyroid gland: implications for patient care and clinical research. *Cancer (Cancer Cytopathol)*. 2002; 96:323–324.
2. DeMay RM. Follicular lesions. In: DeMay RM, editor. The art and science of cytopathology. Aspiration cytology, American Society of Clinical Pathologists. Chicago: ASCP Press, 1996: 722–729.
3. Kini SR. Differential diagnosis of follicular lesions of the thyroid. In: Kini SR, editor. Guides to clinical aspiration biopsy. Thyroid. 2nd edition. New York: Igaku-Shoin Medical Publishers, 1996:93–103.
4. Ivanova R, Soares P, Castro P, Sobrinho-Simoes M. Diffuse (or multinodular) follicular variant of papillary thyroid carcinoma: a clinicopathologic and immunohistochemical

analysis of ten cases of an aggressive form of differentiated thyroid carcinoma. *Virchows Arch*. 2002;440:418–424.

5. Renshaw AA. How closely do thyroid fine-needle aspirates need to be screened? *Diagn Cytopathol*. 2002;27:259–260.
6. Renshaw AA. Focal features of papillary carcinoma of the thyroid in fine-needle aspiration material are strongly associated with papillary carcinoma at resection. *Am J Clin Pathol*. 2002;118:208–210.
7. Goldstein RE, Nettekville JL, Burkey B, Johnson JE. Implications of follicular neoplasms, atypia, and lesions suspicious for malignancy diagnosed by fine-needle aspiration of thyroid nodules. *Ann Surg*. 2002;235:656–664.
8. Schlinkert RT, van Heerden JA, Goellner JR, et al. Factors that predict malignant thyroid lesions when fine-needle aspiration is “suspicious for follicular neoplasm.” *Mayo Clin Proc*. 1997;72:913–916.

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Stability of Archived Liquid-Based Cytologic Specimens

In our recent article,¹ we reported that long-term storage of cervical Papanicolaou specimens in PreservCyt (Cytyc Corporation, Boxborough, MA), a methanol-based medium used in liquid cytology, resulted in significant losses in DNA stability and nuclear preservation. Because of our interest in us-

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TABLE 1
Results of Testing of Archived, Paired Cervical Specimens

STM ^d specimen	<i>Chlamydia trachomatis</i> ^a			STM ^d specimen	<i>Neisseria gonorrhoeae</i> ^b		
	PreservCyt ^c specimen				PreservCyt ^c specimen		
	Negative	Positive	Total		Negative	Positive	Total
Negative (%)	44 (91.7)	4 (8.3)	48 (100.0)	Negative (%)	58 (98.3)	1 (1.7)	59 (100.0)
Positive (%)	15 (78.9)	4 (21.1)	19 (100.0)	Positive (%)	9 (100.0)	0 (0.0)	9 (100.0)
Total (%)	59	8	67	Total (%)	67	1	68

STM: specimen transport medium.

^a $P = 0.012$ (symmetry chi-square test).

^b $P = 0.011$ (symmetry chi-square test).

^c Cytoc Corporation, Boxborough, Massachusetts.

^d Digene, Gaithersburg, Maryland.

ing these specimens as an alternative source of DNA for measurements related to our studies of human papillomavirus (HPV) and cervical neoplasia,^{2,3} we further examined whether these archived specimens would support polymerase chain reaction (PCR) amplification assays aimed at detecting other infectious agents that might be etiologic cofactors.

To readdress this issue, we tested enrollment cervical specimens from 70 consenting women in our natural history study (approved by the National Cancer Institute and INCIENSA [Instituto costarricense de Investigacion y ensenanza en salud y Nutricion], Costa Rican institutional research boards), initiated in 1993–1994, of HPV and cervical neoplasia in Guanacaste, Costa Rica.² PCR amplification was used to test specimens for the presence of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* DNA. Paired cervical specimens were collected at the same enrollment visit, one stored in PreservCyt at ambient temperature and the other stored in specimen transport medium (STM; Digene, Gaithersburg, MD) at -70°C , were tested for *C. trachomatis* plasmid DNA and *N. gonorrhoeae* genomic DNA using a commercial PCR assay (AMPLICOR; Roche Diagnostics, Indianapolis, IN) to amplify fragments of 207 and 201 base pairs (bp), respectively.^{4,5} These target fragments were smaller than the smallest β -globin fragment (268 bp) that we measured in our previous report.¹

For increased prevalence of these sexually transmitted infections, we selected a subset of women who had either at least 3 different sexual partners before age 30 years or at least 5 different sexual partners before age 48 years. As controls, PreservCyt specimens that were positive for either *C. trachomatis* or *N. gonorrhoeae* ($n = 10$; it was unknown for which infection each specimen was

positive) and specimens that were negative for *C. trachomatis* and *N. gonorrhoeae* ($n = 10$) were provided by Digene and included in the testing, which was performed in a masked fashion.

PreservCyt aliquots of 4.5 mL were pelleted by centrifugation and suspended in 100 μL TE buffer (10 mM Tris, 1 mM ethylenediamine tetraacetic acid, pH 8.0) for testing; 100 μL of the STM specimen was used without additional manipulation. Specimens were lysed and DNA was extracted using the MagNA Pure LC Robot (Roche Diagnostics), and magnesium chloride concentration was adjusted to 1.5 mM for PCR amplification. DNA was amplified using biotinylated primers specific for either *C. trachomatis* or *N. gonorrhoeae*; amplicons were captured by hybridization to microwells coated with oligonucleotides that were specific for either *C. trachomatis* or *N. gonorrhoeae*; and bound, biotinylated amplicons were detected using avidin–horse-radish peroxidase and a colorimetric substrate.^{4,5} Results from paired specimens were compared using a symmetry chi-square test.

Among the 70 pairs of specimens selected, a PreservCyt specimen was missing from 1 pair and an STM specimen from another pair did not amplify; these pairs therefore were excluded from the analysis, leaving a final set of 68 paired specimens. The results of the testing are shown in Table 1. We found that only 8 of 67 (11.6%) PreservCyt specimens were positive for *C. trachomatis*, significantly less than the 19 of 67 (28.4%) STM specimens found to be positive ($P = 0.012$; symmetry chi-square test). (One pair was excluded from the *C. trachomatis* analysis because the result for the STM specimen was indeterminate and the corresponding PreservCyt specimen was negative.) Similarly, we found that only 1

of 68 (1.5%) PreservCyt specimens was positive for *N. gonorrhoeae*, significantly less than the 9 of 67 (13.2%) STM specimens found to be positive ($P = 0.011$; symmetry chi-square). The PreservCyt positive control specimens, all less than 24 months old, were found to be positive for either *C. trachomatis* ($n = 9$) or *N. gonorrhoeae* ($n = 1$), and all negative control specimens were found to be negative for both infections.

We confirmed that archival PreservCyt specimens stored at ambient temperature for approximately 10 years were not suitable for PCR testing, even when 201–207 bp fragments were being targeted. Previous studies have demonstrated high sensitivity for detection of these infections in PreservCyt specimens,⁶ and our PreservCyt positive control specimens were found to be positive; this finding suggests that the poor test performance of the archival specimens was the result of long-term storage. We previously reported that our ability to detect HPV using a nonamplifying method (Hybrid Capture 2; Digene) was acceptable¹; nonetheless, unless special measures (e.g., frozen storage) are taken to store specimens, they may have limited use in retrospective studies of HPV, infectious cofactors, and cervical neoplasia.

REFERENCES

1. Castle PE, Solomon D, Hildesheim A, et al. Stability of archived liquid-based cytologic specimens. *Cancer Cytopathol.* 2003;99:89–96.
2. Herrero R, Schiffman MH, Bratti C, et al. Design and methods of a population-based natural history study of cervical neoplasia in a rural province of Costa Rica: the Guanacaste Project. *Rev Panam Salud Publica.* 1997;1:362–375.
3. Bratti MC, Rodríguez AC, Schiffman M, et al. Description of a seven-year prospective study of HPV infection and cervical neoplasia among 10 000 women in Guanacaste, Costa Rica. *Rev Panam Salud Publica.* In press.
4. Van Der Pol B, Quinn TC, Gaydos CA, et al. Multicenter evaluation of the AMPLICOR and automated COBAS AMPLICOR CT/NG tests for detection of Chlamydia trachomatis. *J Clin Microbiol.* 2000;38:1105–1112.
5. Martin DH, Cammarata C, Van Der Pol B, et al. Multicenter evaluation of AMPLICOR and automated COBAS AMPLICOR CT/NG tests for Neisseria gonorrhoeae. *J Clin Microbiol.* 2000;38:3544–3549.
6. Koumans EH, Black CM, Markowitz LE, et al. Comparison of methods for detection of Chlamydia trachomatis and Neisseria gonorrhoeae using commercially available nucleic acid amplification tests and a liquid pap smear medium. *J Clin Microbiol.* 2003;41:1507–1511.

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