

Do Liquid-Based Preparations of Urinary Cytology Perform Differently Than Classically Prepared Cases?

Observations From the College of American Pathologists Interlaboratory Comparison Program in Nongynecologic Cytology

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• **Context.**—The cytomorphology of liquid-based preparations in urine cytology is different than classic slide preparations.

Objectives.—To compare the performance of liquid-based preparation specimens to classically prepared urine specimens with a malignant diagnosis in the College of American Pathologists Interlaboratory Comparison Program in Nongynecologic Cytology.

Design.—Participant responses between 2000 and 2007 for urine specimens with a reference diagnosis of high-grade urothelial carcinoma/carcinoma in situ/dysplasia (HGUCA), squamous cell carcinoma, or adenocarcinoma were evaluated. ThinPrep and SurePath challenges were compared with classic preparations (smears, cytospins) for discordant responses.

Results.—There were 18 288 pathologist, 11 957 cytotechnologist, and 8086 “laboratory” responses available. Classic preparations comprised 90% (n = 34 551) of urine challenges; 9% (n = 3295) were ThinPrep and 1% (n = 485) were SurePath. Concordance to the general category of “positive-malignant” was seen in 92% of classic preparations, 96.5% of ThinPrep, and 94.6% of SurePath chal-

lenges ($P < .001$). These results were statistically different for the exact reference interpretation of HGUCA ($P < .001$) but not for adenocarcinoma ($P = .22$). Cytotechnologists demonstrate statistically better performance for the general category of “positive-malignant” compared with pathologists for all urinary slide types and for the exact reference interpretation of HGUCA (94% versus 91.1%; $P < .001$) but not adenocarcinoma (96.3% versus 95.8%; $P = .77$) or squamous cell carcinoma (93.6% versus 87.7%; $P = .07$).

Conclusions.—Liquid-based preparations performed significantly better in urinary cytology challenges when evaluating malignant categories in the College of American Pathologists interlaboratory comparison program. The liquid-based preparation challenges also performed better for the exact reference interpretation of HGUCA, but no difference was observed for adenocarcinoma challenges. Cytotechnologists perform better than pathologists for all slide types, as well as those demonstrating HGUCA. These results suggest that liquid-based preparations facilitate a more accurate diagnosis than conventional preparations.

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Urine cytology specimens are commonly encountered in daily practice and include voided/catheterized urines and washings from the bladder, ureters, and renal

pelvis. Cytologic examination of urine specimens is often the initial step for bladder cancer screening and diagnosis. However, there is a wide range in the sensitivity and specificity in the diagnosis of low-grade versus high-grade urothelial carcinomas, partially due to the bland nuclear features exhibited by the low-grade lesions.^{1–9} The sensitivity of urine cytology for detecting low-grade lesions varies between 26% and 45%, with a specificity of 98%.^{5,6} In contrast, cytologic samples from high-grade urothelial carcinoma/carcinoma in situ/dysplasia (HGUCA) exhibit a sensitivity of 70% to 80% with a specificity that approaches 100%.^{1–9}

Instrumentation for preparation of liquid-based preparations (LBPs) in the cytology laboratory has been available for use with nongynecologic specimens since 1991. Because of potential to improve slide quality and the ensuing familiarity with LBPs from gynecologic cytology, many laboratories are switching to this technology for a variety of nongynecologic specimens including respiratory specimens, body cavity fluids, and urines. The College of

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American Pathologists Interlaboratory Comparison Program in Nongynecologic Cytopathology provides an educational program with urine cytology cases using glass slide material. As a result of this shift in the processing paradigm of nongynecologic samples, this program contains both classical slide material and LBPs.

We reviewed the responses of pathologists and cytotechnologists for urine cytology cases distributed from 2000 to 2007 in the College of American Pathologists Interlaboratory Comparison Program in Nongynecologic Cytopathology program. Our primary goal was to determine if there was a significant difference in the participant's ability to diagnose a variety of neoplastic urothelial lesions comparing classically prepared specimens to LBPs in this educational interlaboratory comparison program.

MATERIALS AND METHODS

The members of the College of American Pathologists Cytopathology Resource Committee (CRC) submit cases used for the College of American Pathologists Interlaboratory Comparison Program in Nongynecologic Cytopathology program. Cases are selected for the nongynecologic cytology program by consensus at CRC screening sessions. Each case is reviewed by 2 members of the CRC, who agree on general and specific interpretations. The 2 members also agree that the slide is a good representation of the entity and that it is technically adequate for assessment. Each slide distributed in the program has a specific reference diagnosis assigned to a body site as well as a general category of unsatisfactory, negative/benign, positive/malignant, or suspicious.

The College of American Pathologists Interlaboratory Comparison Program in Nongynecologic Cytopathology consists of 4 educational challenges per year, with each challenge composed of 5 slides mailed to participants. The slides are circulated to participants who voluntarily subscribe to the continuing education program. After review of the slides, participants are able to choose a general category (benign/negative, suspicious, positive/positive, unsatisfactory) and a specific reference diagnosis, in which participants hone their responses to a more specific diagnosis. After circulation to many laboratories all slides have a unique performance profile that can be compared against others in the same reference category.

The quality and performance of the slides is continuously monitored. Every 6 months, each slide associated with a slide set is evaluated for stain quality and diagnostic ability, as assessed by a College of American Pathologists cytotechnologist. Any slide with a discordant rate of 70% or higher after circulation is reviewed by a CRC member to determine if it will continue circulation in the program. Any slide that has been identified by a participant on the result form as technically "unacceptable" is reviewed by the CRC twice per year. Slides may be retired most often due to breakage or fading stain. This quality control system ensures that the slide challenges remain equivalent over time, such that newer slides entering into the program perform similarly to older slides in circulation.

We reviewed the cumulative histories of all participant responses for urine cytology challenges with the reference diagnosis of HGUCA, squamous cell carcinoma, or adenocarcinoma obtained through the College of American Pathologists SCORES computer system, which circulated in the program from 2000 to 2007. Urines samples included voided/catheterized specimens and bladder washings. The preparation type was noted for each challenge. Classic preparations in the program included both conventional concentrated smears and cytospin slide preparations. Liquid-based preparation slides consisted of ThinPrep (Hologic, Inc, Bedford, Massachusetts) and SurePath (Becton, Dickinson Company, Franklin Lakes, New Jersey) preparations. Millipore (Millipore Corporation, Billerica, Massachusetts) filter preparations have not been circulated in the program. The total number of urinary cytology slide types circulated in the program pres-

Table 1. Participant Concordance Rate for Urinary Challenges in the College of American Pathologists Interlaboratory Comparison Program in Nongynecologic Cytopathology by Slide Preparation Type and the General Category of "Positive-Malignant" ($P < .001$)

Performance	Classic, % (N = 34 551) ^a	SurePath, % (N = 485)	ThinPrep, % (N = 3295)
Concordance	92.0	94.6	96.5
Discordant	8.0	5.4	3.5

^a Classic includes cytospin and concentrated direct smears.

ently includes 200 cytospin (19.6%), 596 smears (58.5%), 146 ThinPrep (14.3%), and 77 SurePath (7.6%).

Participant results were identified for misclassification for both the general categorization (eg, negative vs positive) and the exact reference diagnosis on a given slide (eg, HGUCA). A participant response was considered discordant (incorrect) if the participant general category response was unsatisfactory or negative and the reference interpretation was positive. A correct response was a response that was concordant to either the general category or the exact reference interpretation. We did not include any "suspicious" response categories in our analysis. General and exact diagnosis discordant rates between slide preparation type as well as participant type (pathologist vs cytotechnologist) were analyzed.

Statistical analysis was performed using Pearson χ^2 test and Fisher exact test to test the null hypothesis that there is no association between the participants' performance and 2 factors: slide preparation and reader type. All tests were run at the $P < .05$ significance level. All statistical analyses were performed using SAS v9.1 (SAS Inc, Cary, North Carolina).

RESULTS

A total of 38 331 participant responses for urine cytology challenges were reviewed which included 18 288 pathologists, 11 957 cytotechnologists, and 8086 "laboratory" responses. Malignant/positive general category diagnoses included the specific reference diagnoses of HGUCA, squamous cell carcinoma, and adenocarcinoma.

Overall, there was concordance to the general category of positive-malignant in 92% of classic slide preparations, 96.5% of ThinPrep, and 94.6% of SurePath ($P < .001$) challenges (Table 1). When the classic preparation results were broken down further into either cytospin or concentrated smear, the concordance rate remained the same at 92% for both. When the results were segregated into the 2 exact reference diagnostic categories of HGUCA and adenocarcinoma, LBPs performed statistically better with the HGUCA challenges ($P < .001$) but not for adenocarcinoma challenges ($P = .22$) (Table 2). There were an insufficient number of squamous cell carcinoma slides to make a statistical comparison between slide types and general category.

When general category responses by participant type were evaluated, cytotechnologists demonstrated a statistically overall better performance (94% vs 91.2%; $P < .001$) compared with pathologists on all slide types combined (Table 3). There was a statistically different rate of performance for cytotechnologists compared with pathologists for the exact reference diagnosis of HGUCA. Ninety-four percent of the cytotechnologists correctly diagnosed the HGUCA challenges compared with 91.1% of the pathologists ($P < .001$). This statistical difference was not identified in the adenocarcinoma (96.3% vs 95.8%; $P = .88$) or squamous cell carcinoma diagnostic categories (93.6% vs 87.7%; $P = .07$) (Table 4).

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Table 2. Participant Concordance Rate for Urinary Challenges in the College of American Pathologists Interlaboratory Comparison Program in Nongynecologic Cytopathology by Slide Preparation Type and an Exact Reference Diagnosis^a

Diagnosis	Classic, % ^b	SurePath, %	ThinPrep, %	P Value
HGUCA	91.9 (N = 33 179)	94.4 (N = 461)	96.6 (N = 3227)	<.001
Adenocarcinoma	96.3 (N = 810)	100.0 (N = 24)	91.8 (N = 49)	.22

Abbreviation: HGUCA, high-grade urothelial carcinoma/carcinoma in situ/dysplasia.

^a N represents total participant responses for specific reference diagnosis.

^b Classic includes cytospin and concentrated direct smears.

Table 3. Concordance Rate by Participant Type for Urinary Challenges in the College of American Pathologists Interlaboratory Comparison Program in Nongynecologic Cytopathology and General Category of "Positive-Malignant" (P < .001)

Performance	Cytotechnologist, % (N = 11 957)	Pathologist, % (N = 18 288)
Concordance	94.0	91.2
Discordant	6.0	8.8

COMMENT

Urine cytology specimens are one of the most common samples in a busy cytopathology laboratory and are of primary importance for screening and diagnosis of bladder cancer. Traditionally, urine samples for cytology have been prepared using filters, concentrated smears, or cytocentrifugation. With the advent of LBP technology in the laboratory, this instrumentation is being used with increased frequency for processing of nongynecologic samples, including urine specimens. Advantages of this processing system over conventional methods include uniformity of the cytologic sample with respect to smear thickness ("monolayered" smear), increased cell concentration and cellularity, lack of obscuring background material, improved morphology, and decreased rate of unsatisfactory and suboptimal specimens. Placing the cellular material directly into the cytofixative in the LBP vial provides more immediate and better preservation, and the proprietary fixatives in LBP have been validated for ancillary testing of residual material in the vial using immunohistochemistry or fluorescence in situ hybridization.¹⁰⁻¹² The concentrated and larger cell deposit available for evaluation in the LBP slides compared with cytospin preparations may reduce the need to prepare multiple slides from the sample for cytologic evaluation.

The method of cytologic slide preparation, in our study, showed a significant increase in the diagnostic accuracy of participants on LBP challenges compared with the classic preparations. Malignancy was correctly diagnosed in 92% of the classic preparations, compared with 95.6% of the ThinPrep slides and 94.6% SurePath samples. This statistically higher rate of concordance for the LBPs was also

noted in the HGUCA group but not for adenocarcinoma. Luthra et al¹³ and Wright and Halford¹⁴ also reported similar results in their evaluation of abnormal urothelial samples using LBPs versus conventional preparations. Wright and Halford noted a higher detection rate of atypical urothelial cells in their ThinPrep material compared with the cytospin preparations (5.3% versus 4.2%, respectively). They also stated that the cellular morphology of the ThinPrep samples showed enhanced nuclear detail with crisper chromatin and more prominent nucleoli. A similar finding was also seen with their Autocyte PREP (eg, SurePath) slides. Voss et al¹⁵ reported slightly higher positive rates for ThinPrep versus filters (3.3% versus 2.8%), but the difference was not statistically significant. On the other hand, others have reported higher detection rates with cytospin material compared with LBPs.^{16,17} In the study by Sng et al,¹⁶ they reported higher rates of sensitivity and positive and negative predictive values for cytospin samples compared with ThinPrep specimens (94.7%, 94.7%, and 83.3% versus 84.2%, 94.1%, and 62.5%, respectively). Part of the reason for this discrepancy may be related to the cytologic appearance of the diagnostic cells. Nassar et al¹⁷ noted that cytospin urine samples were superior to the ThinPrep material in cellular detail. In their study, the diagnostic cells in the ThinPrep slides were dispersed singly with loss of papillary fragments, making them difficult to find. A similar qualitative superiority with centrifuged samples was reported by Zardawi and Duncan¹⁸ and Piaton et al.¹⁹ They also noted greater cellular preservation when comparing cytospin preparations with the ThinPrep and SurePath LBP systems.

The lack of statistical significance between participants' (both pathologists and cytotechnologists) ability to correctly diagnose adenocarcinoma and squamous cell carcinoma in the urinary challenges may be a reflection of the infrequency of adenocarcinoma and squamous carcinoma compared with urothelial carcinomas in routine practice. Roughly 95% of bladder neoplasms are of urothelial (transitional) cell origin. In contrast, squamous cell carcinomas comprise 2% to 15% of bladder tumors, and adenocarcinomas represent less than 2%.²⁰ These cases are proportionately represented in the program, and the smaller number of cases could also influence the lack of statistical significance between slide types.

Table 4. Concordance Rate by Participant Type for Urinary Challenges in the College of American Pathologists Interlaboratory Comparison Program in Nongynecologic Cytopathology and an Exact Reference Diagnosis^a

Diagnosis	Cytotechnologist, %	Pathologist, %	P Value
HGUCA	94.0 (N = 11 482)	91.1 (N = 17 609)	<.001
Adenocarcinoma	96.3 (N = 296)	95.8 (N = 409)	.77
Squamous cell carcinoma	93.6 (N = 140)	87.7 (N = 211)	.07

Abbreviation: HGUCA, high-grade urothelial carcinoma/carcinoma in situ/dysplasia.

^a N represents total population of responses for specific diagnosis.

An interesting finding in our study was the performance of cytotechnologists in comparison to pathologists in the evaluation of the urinary challenges. Tables 3 and 4 demonstrate a statistically better overall general performance for cytotechnologists and specifically for the HGU-CA cases. This difference may in part be due to the cytotechnologists' familiarity with LBP cytology screening of cervicovaginal smears, their superior locator skills, and their expertise as primary screeners of cytologic cases. Studies have shown that pathologists perform worse than cytotechnologists as primary screeners of cervicovaginal smears.²¹ Finally, interobserver variability and lack of standardized nomenclature in urinary cytology also could have contributed to the discordant results between cytotechnologists and pathologists. These 2 variables have been highlighted as interpretive problems in several studies focused on urinary cytology.^{22,23} This study was limited to assessing sensitivity for the detection of malignancy in urine cytology specimens. The participants' ability to accurately eliminate the various benign mimics of urine cytology neoplasia (specificity) was not specifically measured. Cytotechnologists may have superior detection skills because of their primary role in screening; however, pathologists demonstrate better specificity for identifying malignancy (perhaps at the cost of sensitivity), but this was not specifically measured in this study.

In summary, LBP challenges performed significantly better than classic preparations of malignant urinary cytology in an educational interlaboratory comparison program. The LBP challenges also performed better for the exact reference interpretation of HGUCA, but no difference was observed for adenocarcinoma challenges. Cytotechnologists appear to perform better than pathologists in interpretation of high-grade urothelial carcinoma in urologic specimens. Use of LBPs is becoming widespread for urologic specimens and the results confirm that LBPs perform better than classic preparations in an interlaboratory comparison program.

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