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ORIGINAL ARTICLE

Determination of appropriate urine volume cutoff values for voided urine specimens to assess adequacy

Juan Xing, MD[a,*], Yan Qi, PhD[b], Sara E. Monaco, MD[a], Liron Pantanowitz, MD[a]

[a] *Department of Pathology, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania*
[b] *School of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania*

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Introduction Incorporating urine volume into adequacy assessment was recommended by The Paris System for Reporting Urinary Cytology. The concept was relatively new, however, and supportive studies were sparse. We accordingly aimed to determine the role of urine volume in adequacy assessment and cutoff values for urine samples using ThinPrep (Hologic, Inc, MA) processing.

Material and methods Archived consecutive urine cytology cases (n = 2117) were analyzed. Patient age, sex, collection method, urine volume and fixative (CytoLyt, Hologic, Inc) added, adequacy and diagnoses were documented. Adequate samples were defined as samples with >50 well-preserved, well-visualized urothelial cells. Diagnoses of suspicious and positive for high-grade urothelial carcinoma were combined for analysis. Statistical analysis was performed using IBM SPSS Statistics for Windows.

Results There was a correlation between urine volume and the unsatisfactory/less than optimal cellularity versus satisfactory samples ($P \leq 0.001$) in voided urine specimens. A minimum of 10 mL of fresh voided urine was found to be a reasonable cutoff to achieve sufficient cellularity. Cutoff values of 30 mL for voided urine for the high-risk diagnosis were associated with the highest χ^2 statistic, although this was not statistically significant.

Conclusions Urine volume was justified as an adequacy criterion in voided urine. Although 10 mL of fresh voided urine might achieve sufficient cellularity, higher volume (≥30 mL) is recommended in order to maximize the chance of detecting a high-risk diagnosis. Nevertheless, the presence of high-grade urothelial carcinoma can still be detected in low-volume (<20 mL) specimens. Hence, correlation of clinical information with voided urine volume cutoff values for individual cases might also be beneficial.

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*Corresponding author: Juan Xing, MD, University of Pittsburgh Medical Center, 5150 Centre Avenue, POB, Suite 201, Pittsburgh, PA 15232. Tel.: (412) 623-3765; Fax: (412) 623-4779.
E-mail address: xingj@upmc.edu (J. Xing).

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Introduction

An adequacy statement in non-gynecologic cytopathology reports is a challenging and controversial topic, despite the well-adopted quantitative adequacy criteria used for years in gynecologic cytology as recommended in The Bethesda System for Reporting Cervical Cytology.[1] The definition and criteria of adequacy varies by anatomic location as well as the type of specimen in non-gynecologic cytopathology. The adequacy criteria for thyroid fine-needle aspiration specimens have been defined by The Bethesda System for Reporting Thyroid Cytopathology and also widely accepted by the cytopathology community, despite the fact that these criteria were arbitrarily assigned rather than being included because they were evidence-based.[2] To date, the adequacy statement has not been as well defined in other non-gynecologic cytology specimens. Determining adequacy is particularly difficult in urine cytology given the wide variety of collection methods (eg, voided, barbotage, washing and brushing), the sampling nature of exfoliative cytology, and sparse research studies.[3] Hence, some cytopathologists believe that an adequacy statement may not be needed in urine cytology.

In almost all organ systems, cellularity is the most important and a criterion required to determine adequacy for cytology samples. Currently, most cytopathologists likely agree that for urine cytology the presence of well-preserved urothelial cells is essential in order to evaluate the adequacy of a urine specimen; however, the exact adequate number of urothelial cells has not yet been well defined. An arbitrary cutoff value of 15 well-preserved and well-visualized urothelial cells has been proposed by some authors.[4,5] This criterion has not been universally accepted, however. Prather et al performed an evidence-based adequacy study with bladder barbotage cytology specimens and showed that a minimum of 20 well-visualized, well-preserved urothelial cells per 10 high-power fields was needed to increase the positive predictive value.[6] Recently, The Paris System for Reporting Urinary Cytology, the first evidence- and consensus-based reporting system in urine cytology, was published and subsequently adopted by many institutions. In the textbook chapter of The Paris System about adequacy of urine specimens, an adequacy algorithm was recommended and the authors also emphasized the lack of solid data regarding this subject.[7] In addition to urothelial cellularity, urine volume was also recommended as an adequacy criterion in The Paris System based on a study from VandenBussche and colleagues. They suggested that a cutoff value of 30 mL, for SurePath preparations, be applied to fresh unfixed voided urines.[8] Later on, a similar study was undertaken that advocated a cutoff value of 25 mL for ThinPrep (Hologic, Inc, MA) preparations, also performed on fresh unfixed voided urines.[9] As this was the first time that urine volume has been suggested as an adequacy criterion, data and studies were sparse. It remains challenging to assess the role of urine volume adequacy in clinical practice for the following reasons: 1) the volume of urine received in the cytology laboratory may not be always accurately documented; 2) a variety of urine specimens may be received; 3) addition of fixative (eg, CytoLyt, Hologic, Inc, MA) may be uncertain; and 4) the accurate amount of fixative added rarely is provided to the laboratory. Therefore, additional data are necessary to further evaluate the role of urine volume adequacy in clinical practice. We accordingly conducted a study to determine the appropriate cutoff values for urine volume in a variety of urine specimens at our institution.

Materials and methods

A retrospective review was performed using our anatomic pathology laboratory information system (CoPath, Cerner, North Kansas City, MO). A total of 2117 archived consecutive urine cytology cases were analyzed. Patient age, sex, collection method, urine volume, fixative (eg, CytoLyt) added or not, adequacy, and diagnosis (according to The Paris System) were documented. Papanicolaou-stained ThinPrep slides were used for cytology assessment of all cases.

Adequacy and diagnostic categorization

At our institution, adequate samples were defined as having sufficient cellularity (ie, more than 50 well-preserved, well-visualized urothelial cells). An unsatisfactory statement in our cytology reports for urine samples indicated that the minimum criteria needed to assess the case were not met (eg, <15 urothelial cells, the slide was completely obscured by blood, inflammation or another contaminant such as lubricant gel). There were instances when urine specimens were considered to meet the minimum criteria but were less than optimal (LTO) due to limited cellularity (eg, 15 to 49 urothelial cells) or the presence of focal-obscuring factors. In order to determine a threshold for minimum volume needed to render a specimen satisfactory for diagnostic assessment, the cases were grouped into 2 categories, unsatisfactory and satisfactory (which included LTO specimens). Although LTO specimens were considered satisfactory for diagnostic assessment, a second adequacy grouping was created to evaluate the threshold for a minimum volume needed to have a specimen with adequate cellularity. For this second grouping, specimens that were interpreted to be LTO due to having too few cells present were grouped with unsatisfactory cases, and all other causes (non-cellularity) of LTO specimens were grouped with the satisfactory samples.

For the purpose of this study, the cytology diagnostic categories of nondiagnostic, benign, atypical, suspicious for and positive for malignant cells were used to classify each case. Specimens with suspicious or positive diagnoses were grouped as high-risk diagnoses and nondiagnostic, benign, and atypical cases were classified as non-high-risk diagnoses.

Statistical analysis

Bivariate correlations using Kendall's tau were performed on urine volume with each of the 3 binary groupings (unsatisfactory versus satisfactory, unsatisfactory/LTO cellularity versus satisfactory, non-high-risk versus high-risk diagnoses) by collection methods as well as on the overall data set. Bivariate correlations using Kendall's tau were also performed on the total urine volume when CytoLyt was added for each of the 3 binary groupings.

Urine volumes were stratified into 10 bins for each addition of 10 mL through to 90 mL (ie, ≤10 mL, ≤20 mL, ≤30 mL, ≤40 mL, ≤50 mL, ≤60 mL, ≤70 mL, ≤80 mL, ≤90 mL, 91+ mL). For each binary grouping, the fraction of unsatisfactory, unsatisfactory/LTO cellularity, and high-risk was calculated for each method of collection (eg, voided, instrumented, and diversion) and overall (including specimen collection methods that were not otherwise specified). In addition, for specimens in which there was documentation about the volume of CytoLyt fixative added, similar calculations were performed for voided specimens as well as for overall specimens received (ie, all collection methods). The percentage of unsatisfactory, unsatisfactory/LTO cellularity, and high-risk per each bin were plotted for each collection method and for the overall data set.

Using 10 mL increases, specimens above and below each cutoff were compared. A binary logistic regression was used in order to determine minimum threshold for volume of urine most strongly associated with satisfactory cellular specimens and high-risk diagnoses for each urine collection method as well as voided urine samples with the addition of CytoLyt. When cutoff values demonstrated statistically significant results for different intervals, the lowest volume was defined as a minimum threshold. The results include odds ratios, 95% confidence intervals, likelihood ratio χ^2 statistics, and corresponding P-value, with significance assumed at $P \leq 0.05$.

Statistical analysis was performed using SPSS Statistics for Windows, version 22.0 (IBM, Armonk, NY).

Results

Median age of patients was 69 years and 67.0% of them were men. There were 1590 (75.1%) voided samples, 204 (9.6%) diversion samples, 162 (7.7%) instrumented samples, and in 161 (7.6%) cases the sampling method was not specified. Addition of CytoLyt was documented in 255 (12%) samples and 224 (88%) of them were voided urine specimens. For each specimen, 10 mL CytoLyt was added.

Statistical correlation

For the overall data set including all cases, there was a correlation between urine volume and unsatisfactory/LTO cellularity versus satisfactory specimens ($P \leq 0.001$) but not the other 2 groupings (satisfactory versus unsatisfactory, $P = 0.132$; high-risk versus non-high-risk, $P = 0.184$). For voided urine specimens, there was a correlation between urine volume and unsatisfactory/LTO cellularity versus satisfactory ($P \leq 0.001$) but not the other 2 groupings (satisfactory versus unsatisfactory, $P = 0.313$; high-risk versus non-high-risk, $P = 0.331$). Urine volume for specimens collected by diversion and instrumentation were not correlated with any of the 3 binary groupings. There was also no correlation between any of the 3 binary groupings for urine samples with the addition of CytoLyt.

Minimum adequate urine volume

Binary logistic regression analysis for each cutoff volume for voided urine specimens with unsatisfactory or LTO cellularity showed that each cutoff starting from 10 mL up to 70 mL had a statistically significant result. The 10 mL cutoff was associated with the highest odds ratio (3.6) and highest χ^2 statistic (25.148), along with the most significant P-value ($P < 0.001$). Therefore, the data supported that a minimum of 10 mL urine could achieve adequate cellularity in voided urine specimens (Fig. 1 and Table 1). Similar findings were seen in the overall data set. A cutoff value of a minimum of 20 mL was needed for voided urine samples with CytoLyt added (Fig. 2 and Table 2). Similar findings were also seen in the overall dataset with CytoLyt added.

Binary logistic regression analysis for each cutoff volume for all urine specimens with a high-risk diagnosis demonstrated that cutoff at 20 mL was statistically significant ($P = 0.012$). Cutoff values of 30 mL for voided urine and 40 mL for instrumented urine for the high-risk diagnosis were associated with the highest χ^2, statistic although this was not statistically significant at either the 90% or 95% levels. The analysis was not performed in diversion urine cases because there was only 1 high-risk diversion specimen in the data set.

Discussion

Incorporation of urine volume into the adequacy assessment of urine cytology is a relatively new concept. To the best of our knowledge, only 2 studies to date have assessed the role of urine volume in urine cytology adequacy.[7,8] As alluded to in the Introduction, however, it is often difficult to assess the role of urine volume in daily practice because of the variety of specimen types received, the inaccuracy of the documented volume received in the cytology laboratory, and lack of information about any added fixative to these samples. As a result, we retrospectively reviewed 2117 consecutive urine cytology specimens and assessed the significance of urine volume to specimen adequacy in a variety of settings (ie, different collection methods and with/without added fixative) using ThinPrep preparations.

We found that there was a correlation between urine volume and unsatisfactory/LTO cellularity versus satisfactory grouping ($P \leq 0.001$) in voided urine specimens, as

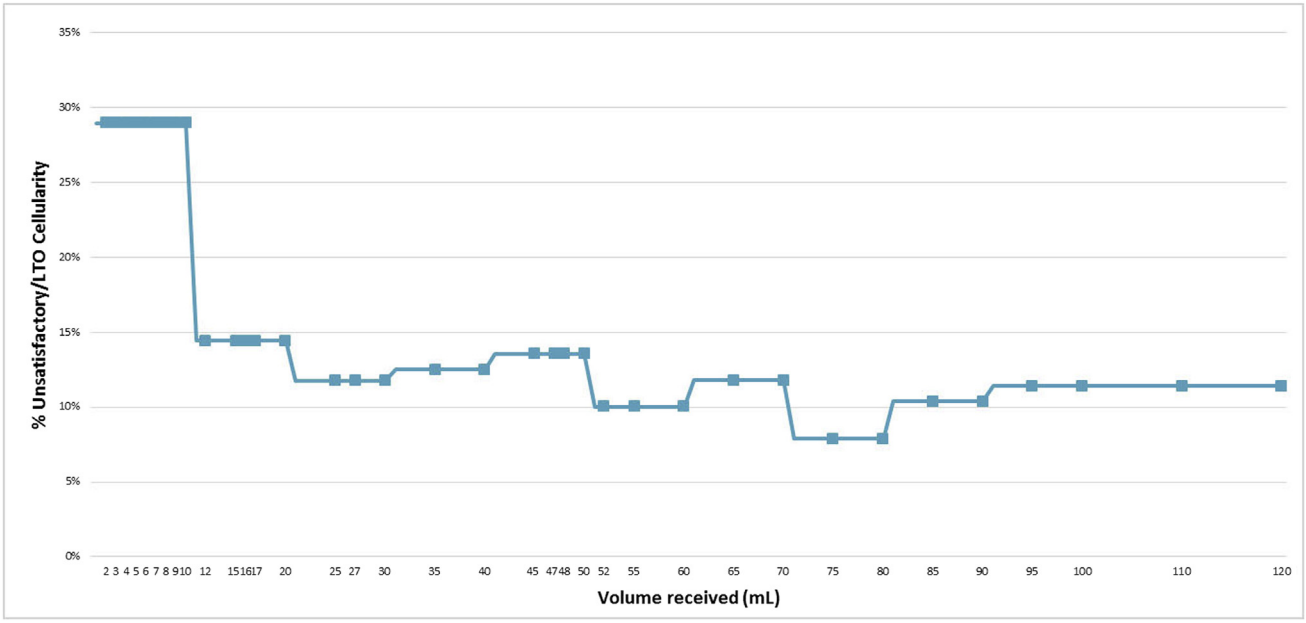

Figure 1 The rate of voided urine specimen assessed as unsatisfactory/LTO cellularity is plotted against urine specimen volume. LTO, less than optimal.

well as in the overall data set. These data suggest that urine volume does correlate with urothelial cellularity and that this parameter can therefore be used as an adequacy criterion, particularly in voided urine samples.

Determination of exact urine volume cutoff values is difficult based on our data. Our data suggest that a minimum of 10 mL urine could achieve adequate cellularity in voided urine specimens; however, these voided samples included samples with and without CytoLyt. Therefore, we performed a binary logistic regression analysis for each cutoff volume for voided urine samples with documented CytoLyt volume (n = 224, CytoLyt volume = 10 mL) and found that a cutoff value of a minimum of 20 mL was needed in order to achieve an adequate diagnosis, indicating that a minimum of 10 mL of fresh voided urine is a reasonable volume in order to achieve adequate cellularity.

Furthermore, our data also demonstrated that a minimum of 20 mL of urine was more likely to be associated with a high-risk diagnosis in all cases ($P = 0.012$). A cutoff value of 30 mL for voided urine was more likely to have a high-risk diagnosis, but no statistical significance was observed. Although there was no statistical significance observed, a cutoff value of 30 mL of voided urine for the high-risk diagnosis was associated with the highest χ^2 statistic, suggesting a minimum of 30 mL of urine could be used as a cutoff value for high-risk diagnosis in voided urine samples.

Overall, exact cutoff values for high-risk diagnosis cannot be determined based on these data. Nevertheless, we

Table 1 Binary logistic regression analysis for each cutoff volume for voided urine specimens with unsatisfactory or less than optimal (LTO) cellularity adequacy.

Cutoff volume, mL	Total samples	Unsatisfactory or LTO due to cellularity (n)	Unsatisfactory or LTO due to cellularity (%)	Odds ratio	95% Confidence interval	χ^2 Statistics	*P*-value
≤10	95	29	30.5	3.641	2.284-5.803	25.158	<0.001
≤20	215	46	21.4	2.327	1.609-3.365	18.191	<0.001
≤30	329	58	17.6	1.831	1.308-2.562	11.7	0.001
≤40	518	81	15.6	1.638	1.202-2.230	9.563	0.002
≤50	713	108	15.1	1.731	1.275-2.350	12.489	<0.001
≤60	913	127	13.9	1.575	1.144-2.169	8.011	0.005
≤70	1087	144	13.2	1.517	1.069-2.153	5.738	0.017
≤80	1396	168	12.0	1.07	0.667-1.715	0.079	0.779
≤90	1498	181	12.1	1.267	0.626-2.566	0.459	0.498
Total	1590	190	11.9				

Cutoff values from 10 mL to 70 mL have statistically significant results.
The data support 10 mL as a minimum threshold.

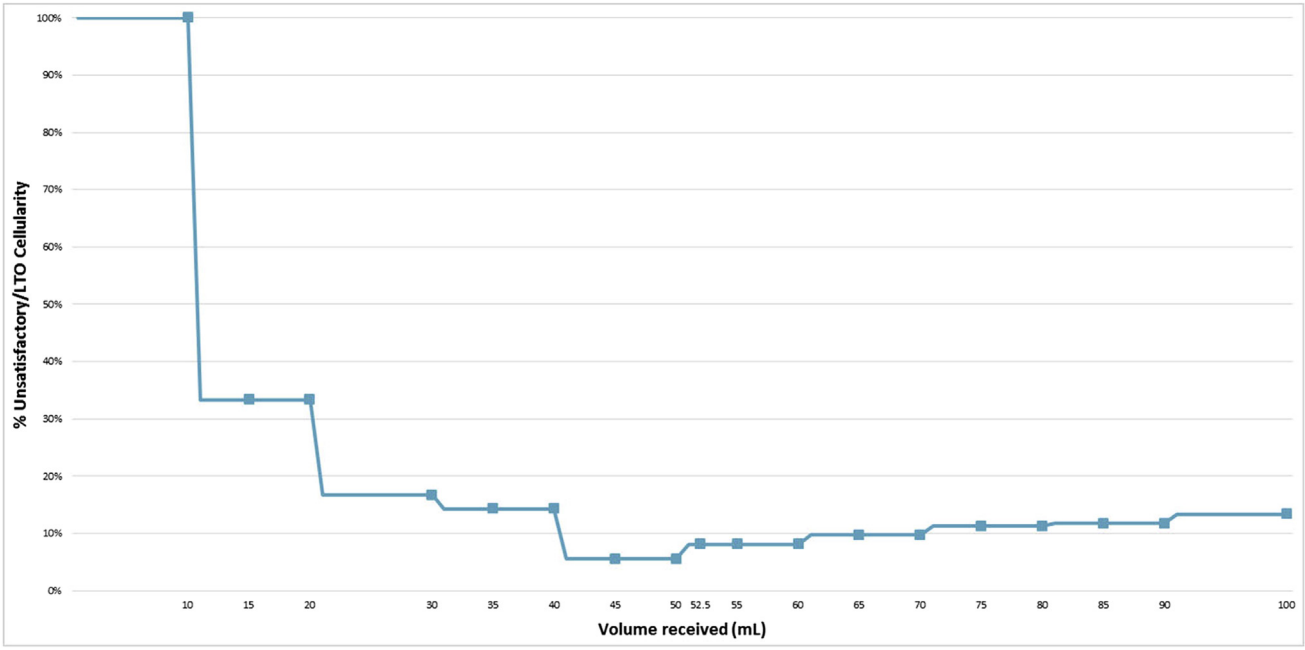

Figure 2 The rate of voided urine specimen with CytoLyt added assessed as unsatisfactory or LTO cellularity is plotted against specimen volume. LTO, less than optimal.

recommend a cutoff value of 30 mL of fresh voided urine to at least increase the chance for detecting high-grade urothelial carcinoma for ThinPrep preparations, despite the fact that high-grade urothelial carcinoma can still be detected in low-volume (<20 mL) urine samples. In patients with small lesions or early stage of disease, there is probably a higher chance of false-negative diagnosis in low-volume specimens. Therefore, more studies regarding the rate of false-negative diagnoses in low-volume urine specimen will be needed to further assess the role of urine volume in voided urine cytology adequacy.

As a result, we postulate that urine volume might best be assessed based on clinical settings rather than a restricted limit. A cutoff value of 10 mL of fresh voided urine might be adequate for patients with low-risk factors (eg, no history of urinary tract high-grade urothelial carcinoma, no risk factors for bladder cancer, and no radiological/cystoscopic worrisome findings). However, at least 30 mL of fresh voided urine might be needed in order to maximize the chance of identifying a high-risk diagnosis in high-risk patients. Compared with a cutoff value of 25 mL of fresh urine for ThinPrep processing, our data suggested 30 mL of fresh voided urine might be a reasonable volume for a high-risk diagnosis.[9]

There are a few limitations associated with our study. First, this is a retrospective study and a few variables may affect the results. For example, the addition of CytoLyt was only documented in a subset of samples (n = 255). Hence,

Table 2 Binary logistic regression analysis for each cutoff volume for voided urine specimens (with CytoLyt added) with unsatisfactory or less than optimal (LTO) cellularity adequacy.

Cutoff volume, mL	Total samples	Unsatisfactory or LTO due to cellularity (n)	Unsatisfactory or LTO due to cellularity (%)	Odds ratio	95% Confidence interval	χ^2 Statistics	*P*-value
≤10	2	2	100.0	*comparison group has 0			
≤20	8	4	50.0	8.391	1.965-35.837	7.28	0.007
≤30	20	6	30.0	3.735	1.297-10.754	5.174	0.023
≤40	34	8	23.5	2.769	1.100-6.972	4.228	0.040
≤50	52	9	17.3	1.791	0.751-4.268	1.641	0.200
≤60	89	12	13.5	1.247	0.554-2.806	0.282	0.596
≤70	130	16	12.3	1.059	0.467-2.400	0.019	0.891
≤80	192	23	12.0	0.953	0.306-2.963	0.007	0.934
≤90	209	25	12.0	0.883	0.188-4.145	0.024	0.876
Total	224	27	12.1				

Cutoff values from 20 mL to 40 mL have statistically significant results.
The data support 20 mL as a minimum threshold for voided urine with CytoLyt added.

additional prospective studies with better control of CytoLyt addition is warranted to further validate the recommended cutoff values. Second, a variety of urine samples (eg, voided, instrumented, and diversion urine) were analyzed in our study. Although the role of urine volume in assessing adequacy is quite different among different specimen types, the correlation observed in overall data set might not be practical. Therefore, we focus on the cutoff values for voided urine only.

Conclusions

Our data suggest urine volume is associated with cellularity in urine cytology and is therefore justified as an adequacy criterion in voided urine. However, determination of exact urine volume cutoff values is difficult because of the variety of variables. Based on our study, a minimum of 10 mL of fresh voided urine without additives is needed for adequate cellularity. At least 30 mL of fresh voided urine without additives is more likely to be associated with a high-risk diagnosis. It might be of further benefit in each case to incorporate clinical information apart from using urine volume cutoff values to assess specimen adequacy.

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Conflict of interest disclosures

None.

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