Contamination Rates of Three Urine-Sampling Methods to Assess Bacteriuria in Pregnant Women

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OBJECTIVE: To estimate and compare contamination rates of three different urine-sampling methods in pregnant women to assess bacteriuria.

METHODS: In this cross-sectional study, 113 pregnant women collected three different midstream urine samples consecutively: morning (first void); midstream (void without further instructions); and clean-catch sample (void after cleaning). The following end points were considered contaminants: epithelial cells, Gram-positive rods or mixed bacteria in the Gram stain, and mixed growth or skin flora in the urine culture. Intraindividual variability in contaminants was quantified with Fleiss-Cohen's weighted κ statistic. Differences between samples were assessed using generalized estimating equations.

RESULTS: Mainly low numbers of Gram-positive rods were more likely to be present in Gram stains of midstream samples compared with clean-catch samples (77.7% compared with 66.7%, P=.022). Morning samples showed more mixed growth compared with midstream samples (6.2% compared with 0.9%, P=.050). No consistency in quantity of contaminants was found in midstream samples compared with morning and clean-catch samples. No differences were found between the other end points in all three urine samples (P>.05). The study could detect an odds ratios of 2.0 for differences in urine-sampling methods with 80% power and 5% significance for most end points.

CONCLUSION: In pregnant women, the contamination rate of midstream samples is comparable with the contamination rates of morning and clean-catch samples. The quantity of contaminants varied among the three samples collected by one woman. These results show that more complex, unpractical, and time-consuming morning and clean-catch samples are not superior. Therefore, we recommend a midstream sample to assess bacteriuria in pregnant women.

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LEVEL OF EVIDENCE: II

Urine is supposed to be sterile but may become contaminated during micturition with flora and epithelial cells from both the vagina and urethra.[1,2] Contamination of a urine sample can contribute to under- or overdiagnosis of bacteriuria.

It is important to adequately diagnose bacteriuria in pregnant women given the possible complications of asymptomatic bacteriuria and urinary tract infections for both mother and fetus.[3,4] However, collection of uncontaminated voided urine samples is difficult and sometimes not feasible in pregnant women because of practical factors like weight gain and increased vaginal discharge.

Present guidelines underscore the need to collect a midstream urine sample because the first portion of urine may contain epithelial cells and microorganisms originated from the urethra or vagina, which may cause contamination.[5] More elaborate sampling methods such as midstream clean-catch urine sample and collection of the first concentrated urine sample in the

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morning have been introduced to further minimize contamination and optimize diagnosis of bacteriuria. However, a clean-catch sample is time-consuming and frequently performed incorrectly. Collection of a morning sample is not practicable because the patient may visit the physician during the daytime. Contaminated urine samples may lead to unnecessary treatment, need for second sample, and additional costs.

The question remains whether these more laborious urine-sampling methods outweigh the extra effort. In this present study, we compared contamination rates detected with Gram stain and urine culture of two more complex urine-sampling methods (morning and clean-catch samples) with midstream sample without instructions (reference test) in pregnant women.

PATIENTS AND METHODS

We conducted a cross-sectional study from April 2010 to April 2011. A convenience sample of 113 women was selected, because the study could not be adequately powered by lack of information on urine contamination levels and correlation between contamination levels of urine samples taken from the same women during pregnancy. For discordant proportions of more than 0.4, our sample size would be adequate to detect odds ratios (ORs) of 2.5 or more for testing correlated proportions (McNemar) with 80% power and a (two-sided) significance level of .05. A post hoc power analysis would be conducted to determine the minimal detectable OR in our study.

All pregnant women (18 years or older) with an uncomplicated singleton pregnancy attending the obstetrics clinic Vida in Amsterdam, The Netherlands, for routine prenatal visits with a pregnancy duration of at least 22 weeks of gestation were eligible.[6] After women gave informed consent, they were asked to collect three urine samples consecutively within 24 hours using three different sampling methods the day of the next prenatal visit. Oral and written sampling instructions were provided. Women were excluded for the following reasons: urine samples were not analyzed within 48 hours or the sampling method was not adequately coded. Dates of birth and pregnancy duration were collected from the medical records. The study was approved by the medical ethical committee of the Academic Medical Center in Amsterdam.

Three methods of urine sampling were compared in all pregnant women: 1) midstream urine sample (midstream): midstream urine sample without further instructions (reference urine sample). To collect a midstream urine sample, women were instructed to discharge their first and to collect their second urine portion; 2) morning midstream urine sample (morning): midstream urine of the first concentrated urine sample in the morning; and 3) midstream clean-catch urine sample (clean-catch): midstream urine of the urine sample voided after local disinfection of the meatus and adjacent mucosa with cotton balls with water while spreading the labia during urinating.

The urine samples were always collected in the same order: morning, midstream, and clean-catch sample. This order would probably provide the best unbiased estimates of contamination rates for each urine-sampling method.

The coded urine samples were refrigerated and sent to the laboratory of the Academic Medical Center and tested by a combination of leukocyte esterase and nitrite dipstick test, a Gram stain (semiquantitative test), and a urine culture following standard operating procedures. The sampling method was blinded.

Manual interpretation of the complete Gram-stained slide was done with a magnification 12.5×100 and read semiquantitatively per item as none (score 0: 0 cells or organisms per Gram stain), sporadic (score 1: 1 to 10 cells or organisms per Gram stain), few (score 2: one to two cells or organism per high-power field), moderate (score 3: 2 to 10 organisms or 2 to 5 cells per high-power field), many (score 4: 10–50 organisms or 5–10 cells per high-power field), or much (score 5: more than 50 organisms or more than 10 cells per high-power field). The following items were scored: epithelial cells, Gram-positive rods (including lactobacilli), Gram-positive cocci, Gram-negative rods, and leukocytes. The assumption is that Gram-positive rods represent nonuropathogens and Gram-positive cocci and Gram-negative rods may indicate uropathogens. The variable mixed bacteria are defined as the presence of two or more different bacteria species detected with the Gram stain.

The urine culture was examined daily for growth and finally interpreted as follows: negative: defined as no growth or the growth of only skin flora; undefined: in practice some culture results need to be interpreted in combination with the background of the patient, namely mixed growth: growth of at least two organisms or more (mostly nonuropathogens). In most cases, specific identification of these organisms was not made[7,8]; low colony count: growth less than 10e4 colony-forming units (CFU) per milliliter of one uropathogen; positive: defined as the presence of one uropathogen with a growth of at least 10e4 CFU/mL or more. Common uropathogens are *Escherichia coli*,

Proteus mirabilis, *Klebsiella pneumoniae*, *Enterococcus* species, and *Pseudomonas aeruginosa*. Organisms that are normally found on the skin and external genitalia including lactobacilli, corynebacteria, and coagulase-negative staphylococci were considered nonuropathogens and contaminants and therefore the identification of these microorganisms did not define a positive culture[8–10]; the leukocyte esterase dipstick was negative or 1+, 2+, or 3+ (all positive). The nitrite dipstick was read negative or positive.

The following items were considered as contaminants[1,7–12]: Gram stain: epithelial cells, Gram-positive rods (including lactobacilli), mixed bacteria (more than one type); urine culture: growth of skin flora, mixed growth. Because urine is supposed to be sterile, all amounts were gauged as contamination. Attempting to distinguish between clinically relevant and irrelevant contamination, different cutoff points were defined. For the Gram stain, the semiquantitative score many (score 4 out of 5) or much (score 5 out of 5) and for urine cultures growth 10e4 CFU/mL or greater (score 4 or 5 out of 5) was considered relevant.

Another way to evaluate the influence of contamination is to assess the need for a second urine sample. When no interpretation can be made of the original culture as a result of overgrowth of contaminants, the Gram stain can help to distinguish between the possible presence of an infection or simply contamination by differentiating infection parameters (ie, leukocytes) and contaminants (ie, epithelial cells).[2] For this study, we considered that the presence of leukocytes in combination with quantitatively more epithelial cells in the Gram stain warrants a second sample.

Intraindividual consistency and variability in quantities of present contaminants can be assessed because all three different urine samples were collected by one woman within a couple of hours, mostly the same day. The midstream sample was used as the reference test and was compared with either morning or clean-catch samples using Fleiss-Cohen's weighted κ statistic.[13] We used the following interpretation for the κ values: less than 0.00 poor agreement; 0.00–0.20 slight agreement; 0.21–0.40 fair agreement; 0.41–0.60 moderate agreement; 0.61–0.80 substantial agreement; 0.81–0.90 almost perfect agreement.[14]

To estimate and to test an effect of morning and clean-catch samples on urine contamination with respect to the more practical midstream sample, generalized estimating equations were applied on the ordinal test results.[15] This type of analysis takes into account the possible correlation between multiple results from participants. The correlation between urine samples was assumed to be the same for any pair of urine samples (ie, exchangeable correlation matrix). The probability on an ordinal outcome was modeled with the cumulative logit function (similar to logistic regression), which would result in ORs for morning and clean-catch samples with respect to midstream samples. Furthermore, confidence intervals on the ORs were determined using the empirical or sandwich variance estimator to provide an estimate of the standard error on the OR that is valid for possible misspecifications of the correlation matrix. Finally, an overall effect of any difference between the urine samples was determined first with a test statistic called the generalized score test. A P value $<.05$ was considered significant. Models were adjusted for gestational age. Data were analyzed using both PASW Statistics 19 and SAS 9.2.

RESULTS

Table 1 shows the participants' characteristics. During the study period, 118 pregnant women were enrolled. Five women were excluded for either incomplete or inadequate coded urine samples. The ages (n=113) ranged from 19.8 to 42.5 years with a mean of 30.4 years. The gestational age ranged from 23.0 to 39.4 weeks with a mean of 32.0 weeks.

In total 336 urine samples were collected: 112 midstream, 113 morning, and 111 clean-catch urine samples; 110 women collected three and three women only two urine samples.

No consistency in quantity of contaminants was found between midstream samples and morning and clean-catch samples. The weighted κ statistic for epithelial cells was established at 0.19 (−0.00 to 0.38) and 0.10 (−0.10 to 0.29) for the morning and clean-catch samples compared with midstream samples, respectively. This indicates a slight agreement or consistency between samples of the same women during pregnancy. For Gram-positive rods, these weighted κ statistics were given by 0.19 (0.00–0.38) and 0.35 (0.18–0.52), respectively, presenting a slight or possibly fair consistency. For skin flora they were determined at 0.44 (0.28–0.59) and 0.48 (0.31–0.65), which indicates moderate agreement.

Table 1. Demographic Characteristics

Characteristic	Pregnant Women (n=113)
Age at day of collection (y)	30.4 (19.8–42.5)
Gestational age at day of collection (wk)	32.0 (23.0–39.4)

Table 2 shows the Gram stain results. The presence of epithelial cells in the urine samples were determined at 58.9% (midstream), 50.4% (morning), and 56.8% (clean-catch). The generalized score test on the ordinal test outcomes did not demonstrate a difference among the urine samples (P=.201). Only 2.1% of all urine samples contained relevant contaminating numbers of epithelial cells (score 4 or 5 out of 5).

Gram-positive rods were present in 72.0% of all urines samples, but they seem more present in midstream samples (midstream 77.7%; morning 71.7%; clean-catch 66.7%) compared with clean-catch samples. Indeed, the generalized score test applied to the ordinal test outcomes gave a P value equal to .022 (midstream compared with morning: 0.68, 95% confidence interval [CI] 0.45–1.04; midstream compared with clean-catch: OR 0.60, 95% CI 0.42–0.86). After interpretation of the absolute numbers, this difference was mainly the result of variation in low numbers of Gram-positive rods.

No differences were seen in the presence of mixed bacteria (P=.272). These percentages were

Table 2. Gram Stain Results

	Midstream Samples (n=112)	%	Morning Samples (n=113)	%	Clean-Catch Samples (n=111)	%
Epithelial cells*						
None (0)	46	41.1	56	49.6	48	43.2
Sporadic (1)	35	31.3	33	29.2	30	27.0
Few† (2)	—	—	—	—	—	—
Moderate (3)	29	25.9	22	19.5	30	27.0
Many (4)	2	1.8	2	1.8	3	2.7
Much (5)	0	—	0	—	0	—
Gram-positive rods*						
None (0)	25	22.3	32	28.3	37	33.3
Sporadic (1)	9	8.0	14	12.4	12	10.8
Few (2)	19	17.0	22	19.5	16	14.4
Moderate (3)	44	39.3	31	27.4	35	31.5
Many (4)	11	9.8	11	9.7	9	8.1
Much (5)	4	3.6	3	2.7	2	1.8
Gram-negative rods*						
None (0)	91	81.3	87	77.0	86	77.5
Sporadic (1)	3	2.7	8	7.1	6	5.4
Few (2)	7	6.3	8	7.1	8	7.2
Moderate (3)	4	3.6	6	5.3	5	4.5
Many (4)	3	2.7	1	0.9	6	5.4
Much (5)	4	3.6	3	2.7	0	—
Gram-positive cocci*						
None (0)	82	73.2	76	67.3	82	73.9
Sporadic (1)	11	9.8	13	11.5	6	5.4
Few (2)	10	8.9	12	10.6	13	11.7
Moderate (3)	8	7.1	10	8.8	6	5.4
Many (4)	1	0.9	1	0.9	3	2.7
Much (5)	0	—	1	0.9	1	0.9
Presence of bacteria						
No bacteria	11	9.8	17	15.0	20	18.0
One type of bacteria	72	64.3	57	50.4	60	54.1
More than one type of bacteria‡	29	25.9	39	34.5	31	27.9
Leukocytes*						
None (0)	100	89.3	100	88.5	102	91.9
Sporadic (1)	7	6.3	8	7.1	5	4.5
Few (2)	4	3.6	3	2.7	3	2.7
Moderate (3)	1	0.9	2	1.8	1	0.9
Many (4)	0	—	0	—	0	—
Much (5)	0	—	0	—	0	0

* Numbers (0–5) in parentheses indicate the semiquantitative scores.
† "Few" (score 2 out of 5) is not used with epithelial cells.
‡ "More than one type of bacteria" represents the outcome mixed bacteria.

25.9% (midstream); 34.5% (morning); and 27.9% (clean-catch). See Tables 2 and 3 for further details of other bacteria and leukocytes.[1]

In total 14 (4.2%) positive cultures were found in six (5.3%) pregnant women. *E coli* was found in all three urine samples of four women (10e4 CFU/mL or greater). In one woman *P mirabilis* (10e4 CFU/mL) was only found in the morning sample and in another woman *P aeruginosa* (10e4–10e5 CFU/mL) was only found in the midstream sample. In 12 of the 14 positive urine samples, also growth of skin flora was found. In total 18 urine cultures were interpreted as undefined. Low colony count bacteriuria was found in seven urine samples. Mixed growth was found in 11 (3.3%) urine samples (midstream 0.9%; morning 6.2%; clean-catch 2.7%), mainly in morning samples. The generalized score test on the ordinal test results showed a significant difference (0.050). The presence of skin flora (midstream 87.5%; morning 90.3%; clean-catch 86.5%) was high but comparable in all three sampling techniques (P=.565). The presence of an irrelevant quantity of skin flora (83.9% less than score 4 of 5) in all cultures was ubiquitous. For further details, see Tables 3 and 4.

In 285 urine samples, a nitrite and leukocyte dipstick test was performed. A positive leukocyte test was present in 143 of the 285 urine samples (50.2%) and a positive nitrite test in 14 (4.9%) samples. Only in 23 of 143 (16.1%) samples with a positive leukocyte test were leukocytes also seen in the Gram stain. Only three samples, all collected by one woman, of 14 positive nitrite tests were correlated with a positive culture.

Using the ratio of epithelial cells and leukocytes, a second sample was warranted in 11 (3.3%) of 336 samples because more epithelial cells then leukocytes were present. These equivocal results were seen in all sampling methods (midstream four; morning four; clean-catch three).

Based on the standard errors for the estimated effects of morning and clean-catch samples with respect to midstream samples, a minimal detectable OR was determined for each outcome in Table 3. For mixed growth and leukocytes, an OR of minimally 14.0 and 3.3 could be detected, respectively, with 80% power. For all other variables, this OR was less than or equal to 2.0.

Table 3. Generalized Score Test Results*

Variable	Effect*	$P^{\dagger}$
Epithelial cells Gram stain	0.71 (0.46–1.10)	.201
	1.00 (0.63–1.60)	
Gram-positive cocci Gram stain	1.46 (0.94–2.28)	.438
	1.14 (0.69–1.89)	
Gram-negative rods Gram stain	1.06 (0.68–1.66)	.888
	1.10 (0.69–1.75)	
Gram-positive rods Gram stain	0.68 (0.45–1.04)	.022
	0.60 (0.42–0.86)	
Leukocytes Gram stain	1.09 (0.49–2.47)	.588
	0.73 (0.32–1.65)	
Mixed bacteria Gram stain	1.51 (0.91–2.49)	.272
	1.10 (0.70–1.74)	
Skin flora urine culture	1.46 (1.01–2.11)	.565
	0.91 (0.64–1.30)	
Mixed growth urine culture	7.40 (1.17–46.79)	.050
	3.08 (0.30–31.29)	

Data are odds ratio (95% confidence interval) unless otherwise specified.

* An odds ratio (OR) above 1 indicates that the midstream sample has a lower probability of contamination than the other sample. Of all end points, the first OR concerns the comparison between midstream and morning samples and the second OR concerns the comparison between midstream and clean-catch samples. For example, the OR for midstream compared with morning samples for epithelial cells in the Gram stain is 0.71 and the OR for midstream compared with clean-catch is 1.00.

† P value is calculated with the generalized estimated equations-test. P<.05 was considered significant.

DISCUSSION

In this study, we demonstrated comparable contamination rates among midstream, morning, and clean-catch samples. Only midstream samples showed significantly more Gram-positive rods, although mainly in low numbers, compared with clean-catch samples; however, morning samples showed more mixed growth compared with midstream samples. The overall prevalence of clinically irrelevant quantities of contamination was high in all three samples. These results show that midstream samples are equivocal to morning and clean-catch samples to assess bacteriuria in pregnant women.

The strength of this study is that all three different samples were collected consecutively by one pregnant woman within a few hours, which enabled us to investigate intraindividual consistency in quantity of contaminants. More insight is given in the composition of urine samples with or without cleaning by presenting the uncensored data instead of using a composite outcome.

A limitation of this study is that although instructions were given to the pregnant women, we did not verify whether cleansing or midstream collection was accurately performed. However, this reflects clinical practice.[9] Furthermore, the study was not powered for differences between samples as a result of lack of information on the frequencies of the ordinal outcomes and the correlation between these outcomes from samples of the same women. The sample size was too small to detect differences in collection

Table 4. Urine Culture Results

	Midstream Samples (n=112)	%	Morning Samples (n=113)	%	Clean-Catch Samples (n=111)	%
Microorganisms (10e4 CFU/mL or greater)						
Escherichia coli	4	3.6	4	3.5	4	3.6
Proteus mirabilis	0	—	1	0.9	0	—
Pseudomonas species	1	0.9	0	—	0	—
Urine culture						
Negative	104	92.9	100	88.5	100	90.1
Mixed growth*	1	0.9	7	6.2	3	2.7
Low colony count† (less than 10e4 CFU/mL)	2	1.8	1	0.9	4	3.6
Positive (10e4 CFU/mL or greater)‡	5	4.5	5	4.4	4	3.6
Skin flora						
None	14	12.5	9	8.0	14	12.6
Few	51	45.5	49	43.4	54	48.6
Moderate (10e4 CFU/mL)	44	39.3	47	41.6	37	33.3
Many (10e4–10e5 CFU/mL)	3	2.7	5	4.4	5	4.5
Much (10e5 CFU/mL or greater)	0	—	1	0.9	0	—
No result available	0	—	2	1.8	1	0.9

CFU, colony-forming unit.
* "Mixed growth" represents growth of at least two organisms or more.
† "Low colony count" represents the presence of one uropathogen with a growth of less than 10e4 CFU/mL.
‡ "Positive" represents the presence of one uropathogen with a growth of 10e4 CFU/mL or greater.

methods for leukocytes and mixed growth but not for the other variables because ORs below 2.0 could have been detected with 80% power and 5% significance. This suggests that the study was appropriately powered for nearly all end points.

Earlier studies concluded that perineal cleaning has no role in reducing contamination in either pregnant or nonpregnant women.[8,9,12,16] Morning urine samples and Gram stain results were not investigated in studies conducted in pregnant women. In addition in the study of Holliday et al, the samples were not collected consecutively by one woman and Schlager et al only looked at bacterial contamination and not at other end points such as epithelial contamination.[9,12] We also analyzed our data as ordinal outcomes, which makes our results less dependent on differences in threshold values used to determine contamination.

Gram-positive rods were more often seen in midstream samples compared with clean-catch samples. Because it mainly concerned low quantities, we conclude that the influence on the culture results is negligible. In six (5.3%) pregnant women, we found positive cultures. In two women, only one in three samples showed significant bacteriuria. This discrepancy in culture results has been described earlier.[9] In the present study, the results of 18 urine cultures were undefined, meaning mixed growth or a low colony count was found. After earlier research, both mixed growth and low colony count can indicate "true" bacteriuria and therefore may need extra evaluation in combination with symptoms, especially in high-risk patients.[2,7] In healthy pregnant women, it probably suggests contamination. In this study, morning samples showed significantly more mixed growth compared with midstream samples. The increased concentration of morning urine may explain this difference. In accordance with earlier research, both leukocytes and nitrite dipstick test results did not correspond well with Gram stain and culture results.[17,18]

Nearly all our samples could be considered as contaminated. Nonetheless, the definition of contamination is arbitrary and the influence of contamination on culture results varies between patient groups. A more uniform and clinically useful definition of contamination is needed. We propose to distinguish between relevant and irrelevant contamination based on the need for a second sample because the original sample is not interpretable as a result of contamination. This implicates that in our population, in less than 4%, a second sample was warranted independently of the sampling method. We recognize that the need for a second sample is a subjective parameter.

The found contamination rate can be an overestimation because laboratory technicians may be

focused on scoring contamination or an underestimation because women who participated in this study were possibly more eager to collect urine samples properly.

In general, broad intraindividual variability was found in quantities of contaminants among the three urine samples. This further emphasizes the irrelevance which sampling method is used.

In conclusion, the overall contamination was high with all three urine-sampling methods; nonetheless, the need for a second sample was low. On the basis of these results, we recommend the use of the easy and practical midstream sample to assess bacteriuria in pregnant women because the morning and clean-catch samples do not outweigh their associated extra time and costs.

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