Evaluation of Urine Specimen Integrity in a Public Health STD Screening Program

Nathan C. Birch, MD,1 Douglas F. Stickle, PhD,1 Anita Young,1 Philip Medina,2 and Steven H. Hinrichs, MD1

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Abstract

Detection of Chlamydia trachomatis and/or Neisseria gonorrhoeae infection in urine using molecular amplification assays has permitted institutions with limited medical facilities to offer testing for these sexually transmitted diseases (STDs). The Nebraska Public Health Laboratory (NPHL) investigated the validity of urine samples submitted for C. trachomatis and/or N. gonorrhoeae amplification after receiving a substantial number of clear specimens. Approximately 75% of all urine specimens submitted for STD testing to the NPHL were from correctional facilities. The falsification of urine specimens submitted for microbiology studies is not evaluated routinely, and this problem was previously undocumented. By using the criteria for specific gravity of 1.001 or less and a creatinine concentration of less than 5 mg/dL (442 µmol/L), approximately 8% of all specimens submitted during the study interval were determined to be inconsistent with urine. The microbiology laboratory should be aware of the possibility for specimen manipulation to identify facilities submitting falsified specimens, to initiate appropriate intervention, and to minimize false-negative reporting.

In the United States, sexually transmitted diseases (STDs) constitute an epidemic, with an estimated 15 million persons acquiring a new STD each year.1 Infections with Chlamydia trachomatis and/or Neisseria gonorrhoeae were the first and second most common STDs in the United States reported to the Centers for Disease Control and Prevention in 2000 and accounted for 80% of all notifiable diseases.2

The highest rates of chlamydial and gonococcal infection occur in the 20- to 24-year age group, which accounts for approximately 75% of all cases. Small minorities of infected individuals maintain a high rate of new partner acquisition and, thus, enhance transmission of infection. In addition, a large number of asymptomatic or minimally symptomatic infected persons continue sexual activity, resulting in further transmission of disease. Infections acquired from asymptomatic persons underscore the importance of tracing sexual contacts and the detection of subclinical infection.3,4

The application of molecular amplification technologies has had a substantial impact on the detection rate of C. trachomatis and N. gonorrhoeae infections.5 Amplification assays such as ligase chain reaction and polymerase chain reaction have greater sensitivity than culture.5-8 First-generation amplification technologies used material collected on a swab from the cervix or the urethra. Such an approach necessitated appropriate examination equipment and trained medical personnel. The advent of high-sensitivity amplification procedures expanded the range of specimen types to include urine.9

Urine amplification testing not only offers a noninvasive means of specimen collection but also eliminates the need for a private examination room and medical personnel. Institutions
with a high prevalence of disease and limited medical facilities, such as state penitentiaries and youth correctional facilities, can provide routine STD testing with the expectation that expanded screening will reduce the number of subclinical infections and ultimately the number of new cases.\textsuperscript{10,11} While this approach has proved successful in small target communities, the ability to successfully implement urine-based screening for STDs on a larger and more sustained scale is unknown.\textsuperscript{10}

The Nebraska Public Health Laboratory (NPHEL), Omaha, provides infectious disease testing for a variety of state-sponsored medical clinics and institutions. Of the urine samples submitted for \textit{C. trachomatis} and/or \textit{N. gonorrhoeae} amplification, approximately 45\% are from a youth correctional facility, with the state penitentiary accounting for an additional 30\%.

The validation of swab specimens submitted for the detection of chlamydial infection, using molecular techniques, has been a major focus of the public health community.\textsuperscript{12} Adulteration of urine used in screening for drugs of abuse is a well-known problem. However, to our knowledge, falsification of urine specimens submitted for \textit{C. trachomatis} or \textit{N. gonorrhoeae} amplification testing has not been described previously. Therefore, no procedures were in place for the detection of potentially manipulated specimens.

The specific characterization of a specimen as urine is difficult, and no single test is able to validate or refute the source of a sample. The characteristic yellow color of urine is imparted by the presence of urobilinogen, a breakdown product of hemoglobin. Between 95\% and 99\% of random urine specimens submitted to the clinical laboratory for urinalysis testing are yellow.\textsuperscript{13} Interest in determining the validity of urine specimens developed when NPHEL technologists noted a substantial number of colorless urine specimens submitted for \textit{C. trachomatis} and/or \textit{N. gonorrhoeae} amplification.

To characterize the sample as compatible with urine or not, criteria derived from the studies of Cook et al\textsuperscript{14} were used. A specimen with a urine creatinine concentration of less than 5 mg/dL (442 \(\mu\)mol/L) and a specific gravity of 1.001 or less was judged to be incompatible with urine, and review of urine specimens using these criteria was initiated.

### Materials and Methods

All testing was performed in 1 laboratory on urine samples submitted from public health clinics. Clients were instructed to urinate into a collection cup and deliver the sample to an attendant. All specimens were transported on ice to the laboratory and tested within 5 days of collection.

The study consisted of 2 parts. The first determined the validity of the adopted visual screening criteria, and the second determined the prevalence of falsified urine samples from various submitting sites.

All specimens were screened initially by appearance according to the criteria of Cook et al.,\textsuperscript{14} which include color (nominally yellow, not colorless), clarity (normally transparent), and foaming properties (subjectively, those characteristic of a dilute protein solution rather than of detergent). Laboratory technologists performed visual screening based on these criteria, and all specimens were classified as visually consistent with or inconsistent with urine. Specific gravity measurements were performed on all flagged specimens. A concurrent urine creatinine concentration was obtained on visually suspect specimens with a specific gravity of 1.001 or less.

To determine the validity of appearance alone as a screening tool, consecutive specimens, irrespective of visual classification, were submitted for paired measurements of specific gravity and creatinine concentration. The opacity of purulent specimens precluded a creatinine concentration measurement, and these samples were excluded from the validation study.

Specific gravity was measured by using a light refractometer following the standard procedures recommended by the manufacturer (Reichert Cambridge, Buffalo, NY).

Creatinine concentration measurements were performed with an enzymatic assay (creatinine amidohydrolase) on the Vitros 250 analyzer (Ortho Clinica Diagnostics, Rochester, NY) using the reagents and procedures developed by the manufacturer. The lower reportable limit of the assay, as determined by the manufacturer, was 0.05 mg/dL (4.4 \(\mu\)mol/L).

Specimens with a specific gravity of 1.001 or less with a concurrent creatinine concentration of less than 5 mg/dL (442 \(\mu\)mol/L) were classified as inconsistent with urine. The reference range for human urine specific gravity is 1.003 to 1.030.\textsuperscript{15} Hyposthenuria, a potentially serious medical condition, is defined as a specific gravity of less than 1.007.\textsuperscript{16,17} Specimens with a specific gravity of less than 1.007 but greater than 1.001 were classified as hyposthenuric. Flagged samples with a subsequent specific gravity of 1.007 or more were classified as consistent with urine. A graphic representation of the classification scheme is provided in Figure 1.

Testing for \textit{C. trachomatis} and \textit{N. gonorrhoeae} was performed on all specimens, including those flagged for further evaluation. Testing using the BDProbeTec ET system (Becton Dickinson, Franklin Lakes, NJ) was performed according to the manufacturer’s recommended procedure. The BDProbeTec ET system has a reported sensitivity and specificity of 90.7\% and 96.6\%, respectively, in the detection of chlamydial infection, and a reported
sensitivity and specificity of 96.0% and 98.8%, respectively, in the detection of gonococcal infection.

Coded specimen accession numbers were assigned during the initial screening, which permitted retrospective pairing of the sample with the submitting institution.

Results

During a period of 27 days, 397 specimens were screened visually. Based on appearance, 66 (16.6%) were visually inconsistent with urine. Thirty-one of the 66 samples had a specific gravity of 1.001 or less, and all had a creatinine concentration of less than 5 mg/dL (442 µmol/L).

Therefore, 31 (47.0%) of the 66 flagged specimens, or 7.8% of the total specimens submitted during the screening interval, were classified as inconsistent with urine. An additional 27 (40.9%) of the flagged specimens, or 6.8% of the total specimens submitted during the screening interval, were classified as hyposthenuric. Only 8 (12.1%) of the 66 flagged specimens, or 2.0% of the total specimens submitted during the screening interval, were classified as consistent with urine. A graphic representation of these results is provided in Figure 2.

Of the 31 specimens inconsistent with urine, 18 (58.1%) were submitted from the state penitentiary. A youth correctional facility submitted 6 (19.4%), a county correctional facility submitted 2 (6.5%), a county health center submitted 3 (9.7%), and an off-campus hospital submitted 2 (6.5%) of the specimens.

The hyposthenuric specimens had a mean ± SD specific gravity of 1.003 ± 0.001. The percentages of sources submitting hyposthenuric specimens were similar to those for specimens inconsistent with urine.

Of the 66 flagged samples, 1 specimen (1.5%) that was classified as hyposthenuric (specific gravity = 1.004), tested positive for *C trachomatis* infection. In contrast, 5.8% of samples not flagged were positive for *C trachomatis*.

It was anticipated that the screening method would not detect falsely colored specimens, and a validation study was conducted. To validate the screening criteria, a subset of 122 consecutive specimens, irrespective of visual screen, were submitted for paired specific gravity and creatinine concentration measurements. The consecutive specimens were received during 10 of the 27 days of screening. Twenty-two purulent specimens were excluded, resulting in 100 (25.2%) of the total 397 specimens undergoing further examination. The mean ± SD specific gravity and creatinine concentration of the 100 nonpurulent samples were 1.011 ± 0.0077 and 66.1 ± 64.5 mg/dL (5,843 ± 5,702 µmol/L, respectively. A graphic representation of these results is shown in Figure 3.
Of the 100 specimens, 39 were flagged by the screening criteria, and the remaining 61 were screened as consistent with urine. Of the 39 flagged specimens, 16 (41.0%) were found to be inconsistent with urine based on creatinine concentrations and specific gravity. Characterization of the specimens screened as visually consistent with urine, using specific gravity and creatinine concentration measurements, failed to detect any additional falsified samples. Therefore, the screening criteria had a sensitivity of 100% in this study. The specificity of the screening criteria, after examination of all flagged specimens, was 73%.

Discussion

The detection of a potential problem with the validity of urine samples prompted the present study. The need for validation of urine specimens for drug testing is well known, while the requirement for such validation on samples submitted for microbiologic studies has not been described. By using the criteria of Cook et al., we found that approximately 8% of urine specimens submitted to NPHL for C. trachomatis and N. gonorrhoeae screening were inconsistent with urine.

Manipulation of urine specimens has been categorized to occur in 1 of 3 general ways: dilution, substitution, or adulteration. Urine dilution may be induced in vivo by ingestion of diuretics, large quantities of water, or both. The effect of dilution on the urine creatinine concentration is neither linear nor immediate. Dilution also may involve the addition of liquid to a previously voided sample and is one possible explanation for a specimen with a specific gravity less than 1.007 (hyposthenuric). Substitution occurs when another individual’s urine or a liquid resembling urine (carbonated beverages, lemon juice, and water) is submitted to the laboratory for testing. Substitution of water is a common method of manipulating urine samples submitted for drug testing. Adulteration occurs when a substance is added to interfere with drug testing.

The screening criteria used in this study, based on color, clarity, and foaming, proved to be highly effective in identifying specimens subsequently shown to be falsified. While the possibility of specimen substitution using a colored liquid may have occurred, no such specimens were detected in the 84 samples screened as consistent with urine, which subsequently were submitted for paired specific gravity and creatinine concentration measurements. Thus, screening based on appearance is an effective method not only for quickly reducing the number of manipulated specimens tested but also for reducing overall costs to the screening program and to the microbiology laboratory.

In our study, the paired means of specific gravity and creatinine concentration, obtained on the screening validation subset, were lower than those reported in the medical literature. However, the distributions of paired measurements were similar to those reported in the drug testing literature. This distribution further served to highlight the large number of hyposthenuric specimens received during the study interval.

A specific gravity measurement of less than 1.007 is considered hyposthenuric and may be a manifestation of any variety of serious medical conditions, including sickle cell disease, analgesic nephropathy, diabetes insipidus, and various nephrotic syndromes. Since hyposthenuria may indicate a clinical condition, the laboratory should provide a statement regarding this finding rather than
The impact of an extremely low specific gravity on the ability of the BDProbeTec ET system to detect infection is unknown. However, false-negative results due to extraordinarily low specific gravity measurements are well known in urine drug testing in the workplace.\textsuperscript{19} Therefore, we recommend such results be brought to the attention of the attending physician.

The motivation for submission of falsified specimens from clients who have been offered optional STD screening at no cost is unknown. Discussions with staff from submitting institutions indicate that the suspicion of random drug testing was the most likely reason for the apparent submission of water in place of urine. The consensus of the institutional staff was that patient education and reassurance would provide the most effective and cost-efficient method of reducing the substitution rate. Reitmeijer et al,\textsuperscript{11} in a field-based screening program, concluded that mistrust of the authorities offering STD testing was common among inner-city youth and potentially could result in the falsification of specimens submitted to the laboratory. However, in their study, no measures were taken to validate the specimens as urine.

Several specimens inconsistent with urine were received from facilities other than incarceration institutions. Three specimens were submitted from a walk-in STD clinic, and 2 were from a hospital. While the motivation again is unknown, it is possible that fear of partner notification may have had a role. In addition, this suggests that the problem may be more common than expected and warrants further study.

Amplification testing procedures or molecular assays using amplification provide a patient-friendly and clinically convenient method of screening for STDS. This form of testing, if properly applied, may increase the detection of disease through increased screening in high-prevalence institutions with limited medical facilities. In addition, given the ease of specimen collection, it is likely that urine will become the specimen of choice for testing for chlamydial and gonococcal urethritis. Thus, the clinical laboratory should be aware of the problem of falsification in order to educate submitting facilities to initiate appropriate screening and to decrease false-negative reporting.

The widespread use of this testing method in the arena of public health with limited funding makes reducing the number of altered specimens a priority. An understanding of the patient population served and coordination with submitting facilities likely will provide an inexpensive and timely solution. Alternatively, other approaches used by drug screening programs such as temperature monitoring, on-site specific gravity measurements, and direct observation could be instituted.

Knowledge of specimen manipulation is necessary to effectively implement a successful urine-based \textit{C trachomatis} and \textit{N gonorrhoeae} screening program and, ultimately, to reduce the incidence of subclinical infections and their associated complications.

\textbf{References}


\textbf{Address reprint requests to} Dr Hinrichs: 986495 Nebraska Medical Center, Omaha, NE 68198-6495.


