FORENSIC SCIENCE JOURNAL SINCE 2002

Forensic Science Journal
2007;6 (2) : 45-58
Available online at:fsjournal.cpu.edu.tw

# Elucidation of the US urine specimen validity testing (SVT) policies and performance evaluation of five clinical parameters for pre-screening adulterants in Taiwan's opiates urinalysis

**Su-Lien Chou,** [1,2] **M.S. ; Yun-Seng Giang** [2,*] **Ph.D.**

*[1]Department of Criminal Investigation, Central Police University, Taoyuan 33334, Taiwan (ROC)*
*[2]Department of Forensic Science, Central Police University, Taoyuan 33334, Taiwan (ROC)*
*Received: November 15, 2007 / Received in revised form: November 30, 2007 / Accepted: December 7, 2007*

## ABSTRACT

Throughout the history of workplace drug testing, countless testees have attempted to subvert their drug test results to mask their use of illegal drugs. In order to reduce sample adulteration, the US Federal government has demanded all certified laboratories to conduct specimen validity tests (SVTs) on every specimen, namely, to pre-screen and pre-confirm as many adulterants as possible. This study was conducted to better understand the performances of five clinical urine parameters in pre-screening adulterants, taking opiates-containing urine specimens for example. Yet, this paper was also written to elucidate and recommend the US SVT policy to those who were unfamiliar with but might afterwards sprout the interest in the issue, especially those outside the States. Based on our experimental data, when used alone, none of the five parameters was all-sensitive to the seven tested Taiwan-produced adulterants in urine. However, the combinative use of the five parameters was able to pre-detect the abnormities caused by adulteration (but not to identify the adulterants themselves) or show different degrees of promise of the like, depending on the kind and amount of the adulterant added and how strict the criteria of adulteration recognition was. Considering the actual efficiency, we suggest conclusively that, while retaining and complying with those strict Department of Health and Human Services (HHS) provisions for reporting "adulterated," "substituted," "dilute," or "invalid result," each laboratory just cover as many clinical parameters and physical characteristics of urine and place more emphasis on using these parameters and characteristics as indicators with reasonably tolerant criteria to probably trigger additional specific SVTs that then confirm as many adulterants as possible. Meanwhile, from the standpoint of scientific and forensic validity, the importance of SVT data quality cannot be overemphasized. In order to meet the strict and rigid criteria, relevant instruments, especially those for the confirmatory tests, must be appropriately upgraded. Another interesting finding in this study was that nitrite could test for not only nitrite-containing adulterants but also non-nitrite oxidizing adulterants.

**Keywords:** forensic science, urine drug testing, opiates, adulterant, creatinine, nitrite; colorimetry, refractometry, gas chromatography-mass spectrometry (GC-MS)

## Introduction

Urine has been the most commonly used specimen in workplace drug testing programs and, currently, it is the only specimen type allowed for US federal workplace programs [1]. Meanwhile, the problems associated with sample substitution [2], dilution [2-4], or adulteration [5-10] by the sample donor with the intention to avoid detection of previous drug use have also caused major concern as to the validity of test results. Over the past several years, there have been an increasing number of chemical adulterants marketed on the Internet and in counter-culture, pro-drug use magazines [11]. These so-called "masking agents" and/or "cleansing agents"

*Corresponding author, e-mail: ysgiang@mail.cpu.edu.tw

are often toxic or corrosive and are advertised as able to prevent laboratories from detecting drugs or metabolites in physiological specimens (e.g., urine, hair, oral fluid) that are collected as part of a drug-testing program. Examples of adulterants include various nitrites (Klear, Whizzies), pyridinium chlorochromate (Urine Luck, LL481, Sweet Pee's Spoiler), surfactant (Mary Jane SuperClean 13), and acid (Amber-13, THC-Free). As of this time, approximately 400 different products (although many contain the same adulterant) are available for adulterating urine specimens.

In order to reduce the adulteration of specimens, the US Federal government has demanded all certified laboratories to conduct specimen validity tests (SVTs; i.e., to "pre-screen" and "pre-confirm" as many adulterants as possible) on every specimen [11]. While the penalty for sample adulteration may vary from sector to sector, it is usually more severe to the employee than a positive drug test itself; that is, an adulterated specimen is typically treated as a "refusal to drug test," which often results in delay or denial of application for employment/appointment or may result in removal from the federal service or other disciplinary action including employee termination. It is so serious from the standpoints of human right and judicial justice that the SVT, like the drug test itself, requires no false positives occur. A certified laboratory that reports a false positive result for a blind performance testing (PT) sample may be subject to suspension or revocation of its certification.

Compared to blood analysis whose sample is typically collected by trained medical personnel, the less invasive urinalysis is particularly vulnerable to in vivo and in vitro sample substitution, dilution, or adulteration. In as early as 1990, Cody described in a review a variety of means of carrying out and detecting urine specimen adulteration [12]. He also discussed briefly the effects of adulterants on such adulterant detection parameters as pH, specific gravity, temperature, and appearance of the specimen. As the ways to adulterate the specimen are progressive and the adulterant pre-screen test results are crucial to the potential penalty for an individual suspected of adulteration, that small number of conventional clinical urine parameters appear to be inadequate or not stringent enough for urine specimens with various backgrounds. It has been desirable to add some other parameters to the existing adulterant detection battery as first suggested by Lambert et al. [4]. During the past decade, a wide variety of specific or general-purpose on-site adulterant detection devices (e.g., spot tests, dipsticks, test strips, test pads, test cards, etc) have become commercially available. Methodologies employed include detecting pyridinium chlorochromate by chromium (VI) quantification [13-15], detecting peroxidase/peroxide (Stealth) by using a chromogen to produce an immediate color change from clear to dark brown [16], detecting nitrite by rapid color tests based on appropriate redoxidation reactions [14,15], etc. It was also based on these advances that the US Department of Health and Human Services (DHHS or simply HHS) started requiring each Federal drug testing agency to have SVTs conducted on all urine specimens collected under the Mandatory Guidelines and to establish the criteria that must be used by a laboratory to report an altered specimen as adulterated, substituted, dilute, or invalid [11]. Specifically, a certified laboratory shall do the following five Do's (as opposed to Don'ts): 1) determine the creatinine concentration on every specimen; 2) determine the specific gravity on every specimen for which the creatinine concentration is less than 20 mg $dL^{-1}$; 3) determine the pH on every specimen; 4) perform one or more SVTs for oxidizing adulterants on every specimen; and 5) perform additional SVTs when the following conditions are observed: i) abnormal physical characteristics; ii) reactions or responses characteristic of an adulterant obtained during initial or confirmatory drug tests; or iii) possible unidentified interfering substance or adulterant [11].

Opiates have long been among the most prevalent drugs of abuse in Taiwan. There have also been a number of real cases associated with detected urine specimen substitution, where the specimen donors shall have been liable on summary conviction to considerable criminal penalties. In contrast, probably because in today's Taiwan advanced adulterants are not so accessible as in the States and the labor and cost for conducting adulterant pre-screen tests is unprofitable, few institutions responsible for forensic drug urinalysis would include these SVTs in their routine work. This loophole could have produced some actually erroneous results, as some adulterants are rumored and reputed in Taiwanese drug community to interfere with the drug testing process.

For years our laboratory has been engaged in the analysis of opiates in various matrices. We have also been pacing the Taiwan official version of forensic drug testing program, which has mostly been trasplanted from the States under the instruction of Prof. Dr. Ray H. Liu from the University of Alabama at Bermingham

but, unfortunately, has not yet included the SVT policy. To better understand the performances of some USA-recommended adulterant detection parameters in pre-screening Taiwan-produced in vitro adulterants in terms of the USA-adopted SVT criteria, we feel necessary to conduct a native validation study. This study becomes even more interesting now that 1) some adulterants may not be detected at the urine collection site [12] and 2) most adulterants when added to drug-containing urine specimens at 5-15 % (w/w) will render substantial impacts on the fluorescence polarization immunoassay (FPIA) and gas chromatographic-mass spectrometric (GC-MS) test results, particularly the negative-directed influences falling on near-cutoff case specimens, yet whether or not a case specimen has been adulterated is far from perceptible based on the cross-examination of TDx (a trade name of FPIA instrument) and GC-MS data (being a long story, the results on the latter issue will be reported separately). It should also be emphasized that another objective of this report is to elucidate and recommend the HHS SVT policy, with our data being comparatively examined, to those who are unfamiliar with but may afterwards sprout the interest in forensic urine drug testing, especially those outside the States.

## Experimental

### *Materials*

Authentic opiates, i.e., morphine (Mo) and codeine (Co), both as 1 mg $mL^{-1}$ in methanol, were purchased from Cerilliant Co., USA. The Mo/Co binary working solution was prepared as 10 μg mL-1 with respect to each authentic compound in D.I. water.

Seven Taiwan-produced in vitro adulterants were tested in this study: table salt (99.5 % sodium chloride containing 20-35 ppm of potassium iodide), liquid soap (containing surfactant, anti-fungus formula, lamb lotion; pH 5 ± 1), chlorine bleach (containing 6% of hypochlorite; pH 12.0 ± 0.5), Vitali drinking soda water (containing vitamins C, B1, and B2, sucrose, citric acid, sodium citrate, carbonic acid, perfume), tap water, potassium dichromate (99.5 %), alum (98 % aluminum ammonium sulfate). The former four were obtained from a local supermarket and the latter two from the First Chemicals Co., Taiwan. Whether originally in liquid form (liquid soap, chlorine bleach, Vitali soda water, and tap water) or as powder (table salt, alum, and potassium dicromate), all adulterants were added directly to the urine at the below stated w/w percentages.

### *Urine collection, spiking and adulteration*

Caffeine/drugs free, unadulterated urine as a mixture (pH 6.0; referred to as normal urine) was contributed by ten students from the Central Police University. Two unadulterated urine spikes were prepared by adding the Mo/Co binary working solution to the normal urine to make each drug's final concentration equivalent to 125 % and 175 %, respectively, of the Taiwanese 300-ng mL-1 cutoff. To 10 mL each of the two opiates-fortified yet unadulterated spikes were then added an appropriate adulterant at 5, 10, and 15 % (w/w), respectively. Four real-case urine specimens (previously confirmed by GC-MS to be opiates-positive with Mo/Co levels being 6073.5/623.1, 4958.3/239.8, 3635.3/165.8, and 3048.6/210.1 ng $mL^{-1}$, respectively) scheduled for disposal were used for a 10 % (w/w) adulteration test and were provided as gifts by the Taiwan Union Clinical Laboratory. All urinary spikes and case specimens were subjected to the adulterant pre-screen tests described in the following subsection before and after appropriate adulteration, and the differences from the normal urine in the measured parameter values were noted. To simulate the actual situation and comply with the Taiwan Bureau of Controlled Drugs Guidelines for Urine Specimen Collection and Urine Drug Testing, we performed all the adulteration steps while the urine specimens had not yet been refrigerated or, for real-case specimens, after warming up the previously −20℃ frozen urine to ambient temperature, but all specimens to be tested were refrigerated at 5℃ for at least 24 h upon 3 min of adulteration at ambient temperature, and mixed thoroughly prior to tests. [Relevant rules listed in the Taiwanese version of Guidelines include: 1) check the temperature of the specimen within 4 min after the donor hands the officer the specimen; 2) specimens that do not receive an initial test within 1 day of arrival at the laboratory shall be refrigerated at temperatures not exceeding 6℃; 3) positive and split (bottle B) specimens shall be stored frozen at −20℃ or colder.]

### *Adulterant pre-screen test*

Creatinine level was determined by means of a colorimetry-based Beckman Synchron LX20 Analyzer, with the absorption wavelength set at 520 nm (creatinine combined with an alkaline picrate reagent to produce a red color complex) and the sample size being 1mL. A

fully automated Roche Urisys 2400 test strip analyzer capable of testing up to 12 clinical urine parameters in a ca. 1-min total run was used for the following four simultaneous measurements, with the sample size being 2 mL: Color was determined by reflectance colorimetry on the blank pad of a test strip using 470, 555, and 620 nm; pH, by reflectance colorimetry on the same test strip using 555 and 620 nm; nitrite, by reflectance colorimetry on the same test strip using 555 nm; specific gravity, via a built-in refractometer using 650 nm. All the data were acquired instrumentally; even the straightforward-looking colors were determined via instrumental colorimetry (i.e., spectrophotometry) rather than visual color recognition or comparison. The respective factory-set normal ranges of the five tested parameters are listed in Table 3.

## Results and Discussion

***Key points about the HHS SVT policy***

In a broad sense, dilution is a form of adulteration which, in turn, is a form of substitution. To make it forensically specific, however, the HHS Mandatory Guidelines requires each certified laboratory to conform to appropriate SVT criteria to report a specimen as adulterated, substituted, dilute, or invalid [11]. In case the laboratory assumes a urine specimen to be invalid, it must report an "invalid result" to the medical review officer (MRO). This invalid result is then used by the MRO to direct the responsible agency to have the donor immediately submit another urine specimen using a "direct observed collection." Additionally, before a specimen can be reported adulterated or substituted, its SVTs as a valid scientific identification must include both the initial and confirmatory tests using two different analytical methods on two separate aliquots obtained from the original urine specimen [11]. The nature of the analytical methodology to be chosen is based on the chemical composition of the substance to be tested, and the combination of techniques is a function of both the expected prevalence of the substance to be tested and the nature of the analytical technique. [The Guidelines describes in detail the combination of methods that a laboratory must use to report a specimen as adulterated for a specific adulterant [11].] If a laboratory uses the same test for both the initial and confirmatory tests, the laboratory may only report an "invalid result" for a specimen rather than an adulterated result. The only exceptions to this requirement pertain to the tests used to measure the creatinine concentration, specific gravity, and pH. [Specific reasons for that will be elucidated in the respective subsections that follow.]

The HHS views it most important that the performance characteristics of the method (e.g., validity, reliability, accuracy, precision, etc) used for each type of SVT can be documented by the laboratory prior to using the method, as is the case for the drug tests used by the laboratory [11]. Establishing the performance characteristics of a method prior to its use ensures that the method can provide accurate measurements on donor specimens which are verified by simultaneously obtaining results for quality control samples. However, unlike drug tests where both limits of quantitation (LOQ) and detection (LOD) shall be fully documented in the quality control program for each test, the SVT at most uses LOD in the confirmation and retest (i.e., reconfirmation) of the presence of an adulterant; e.g., confirming the presence of chromium (VI) requires the first laboratory to test the second aliquot of a single specimen or the primary (Bottle A) specimen from a split specimen using its confirmatory test to determine the presence of chromium (VI) above the LOD; retesting the presence of chromium (VI) requires the second laboratory to test the third aliquot of a single specimen or an aliquot of the split (Bottle B) specimen using its confirmatory test to determine the presence of chromium (VI) above the LOD. Currently, no LOD documentations are required for the pH and nitrite confirmatory tests (the former is obvious by nature, and the latter is due to a substantial amount of human endogenous nitrite).

The preparation of SVT PT samples currently used for the HHS certification of applicant laboratories has been directed typically toward such test parameters and/or components as creatinine (0-20 mg dL-1), pH ($<2.75$, or $>11.25$), specific gravity ( $\leqq 1.0050$, or 1.0170-1.0230), nitrite (at least 20 % above the cutoff), and at least an oxidant (at a level sufficient to challenge a laboratory' s ability to identify and confirm the oxidant) [11]. That also means any methods/instruments used for the SVTs shall fully satisfy the HHS criteria. In summary, all such strict practice stated above is particularly vital for minimizing the number of problematical cases associated with marginal readings. However, basically, each laboratory still has the flexibility to develop the specific testing requirements, to validate the methods used, and to establish quality control procedures using good laboratory practices. The HHS has essentially just recommended a general scientific approach.

Reportedly, some adulterants show no appreciable influence on clinical urine parameters, but several adulterants do depending on their concentrations [6,23]. Old urine specimens might give measurements exceeding the normal ranges due to bacterial growth, formation of ammonia (hence, pH changes), color changes, or odor changes, but not because of adulterations [12]. The presence of a precipitate or turbidity in a specimen might also result from age or refrigeration [12]. On the other hand, some of the apparent physical characteristics may often seem too transient and/or too subject to borderline to be truly characteristic or practically useful. Even so, after a long conferring with many experts and/or commenters, the HHS still recommends that such physical characteristics as color, odor, or excessive foaming can be used to identify specimens that may require some additional SVTs [11]. However, because of the large number of adulterants being marketed, the HHS does not intend to define or specifically describe all the possible abnormal characteristics to avoid limiting the possible characteristics that may be used to trigger additional SVTs.

As indicated by the five Do's cited in the Introduction section, the HHS has been paying much attention to the SVTs for the increasingly varying oxidizing adulterants [5,11] and the so-called "other adulterants." At present, besides the intrinsic urine parameters (creatinine, specific gravity, and pH), the mainly targeted adulterants/analytes include (in the officially shown order) nitrite, chromium (VI), halogen (e.g., bleach, iodine, fluoride), glutaraldehyde, pyridine (pyridinium chlorochromate), surfactant, and "any other adulterant [11]." The recommended initial and confirmatory tests are tabulated as Table 1. The HHS leaves the protocols for verifying "any other adulterant" open so long as they are scientifically and forensically valid according to the above stated criteria. Since individuals attempting to subvert the drug testing program may use an increasing number of different oxidizing adulterants, the testing requirements are intentionally drafted broadly to permit the flexibility needed to combat such tampering with the testing process. The HHS simply expects the laboratories

**Table 1** HHS-recommended tests for commonly encountered adulterants [1,11]

| Adulterant | Initial tests | Confirmatory tests |
|---|---|---|
| Nitrite | General oxidant colorimetric test with a calibrator (≧200 $\mu$g mL$^{-1}$ nitrite-equivalent), or Initial nitrite colorimetric test | Multi-wavelength spectrophotometry (MWS), Ion chromatography (IC), or Capillary electrophoresis (CE) (No LOD/LOQ specified in the Guidelines.) |
| Chromium (VI) | General oxidant colorimetric test with a calibrator (≧50 $\mu$g mL$^{-1}$ Cr (VI)-equiv.), or Initial Cr (VI) colorimetric test | MWS, IC, Atomic absorption spectrophotometry (AAS), CE, or Inductively coupled plasma-mass spectrometry (ICP-MS) [Cr (VI) ≧LOD] |
| Halogen (e.g., bleach, iodine, fluoride) | Odor, General oxidant colorimetric test with a calibrator (≧200 $\mu$g mL$^{-1}$ nitrite-equiv.), or Initial halogen colorimetric test (Halogen ≧ LOD) | The same halogen colorimetric test as for the initial test, MWS, IC, ICP-MS (Halogen ≧ LOD) |
| Pyridine (pyridinium chlorochromate | General oxidant colorimetric test with a calibrator [≧200 $\mu$g mL$^{-1}$ nitrite-equiv., or ≧50 $\mu$g mL$^{-1}$ Cr (VI)-equiv.] | GC-MS (Pyridine ≧ LOD) |
| Glutaraldehyde | Aldehyde test, or Caracteristic immunoassay(s) | Gas chromatography-mass spectrometry (GC-MS) (glutaraldehyde ≧LOD) |
| Surfactant | Surfactant colorimetric test (≧100 $\mu$g mL$^{-1}$ dodecylbenzene sulfonate-equiv.) | MWS (≧100 μg mL$^{-1}$ dodecylbenzene sulfonate-equiv.) |

to validate each adulterant test before it is used to test donor specimens, and to apply the specified quality control requirements to ensure the proper performance of each test on donor specimens.

The five clinical parameters chosen for this evaluation study are the most basic (all are covered by but do not exceed the foregoing five Do's), simplest, speediest, cheapest ones that are commercially available in Taiwan and have been supposed to be most likely to be applied to Taiwan's SVT. Being one of the 12 options available for the Roche Urisys 2400 instrument, the clarity (or turbidity) test was also automatically performed via a built-in turbidimeter. However, a closer look of the data indicated that the test performed in such a "static" fashion was far less adulteration-indicative than what would have directly, dynamically been observed on the appearances of the specimens. While it did not hurt that the clarity test on refrigerated normal urine after thawing would still show "clear" despite the presence of precipitate at the bottom, it was misleading, for example, for the test on specimens adulterated with too much alum, table salt, or chlorine bleach to still show "clear" even though some adulterants did not completely dissolve or at first produced some short-lived bubbles. Therefore, it is the real-time visual, not static instrumental, observation of specimen appearance that should be included in the routine SVT battery.

***Performance of color as an adulterant pre-screen parameter***

Table 2 displays in one of the columns the instrumental readings of colors obtained for urine fortified with 1.25 × and 1.75× 300-ng $mL^{-1}$ cutoff of Mo/Co before and after addition of appropriate adulterant at 5, 10, and 15 % (w/w). The data reflect the following facts: 1) There existed little or no difference between unadulterated drugs-containing specimens and normal urine in the yellowish color. 2) According to the Roche factory-defined normal urine color, pale yellow-yellow, while the other six adulterants did not cause critical color changes, potassium dichromate even though added at as little as 5 % (w/w) did darken the urine into unnatural reddish brown (instrumentally printed as "brown") indicating the necessity to perform additional SVTs for adulterants. We also tested the seven adulterants each at 10 % (w/w) on the foregoing four case specimens, and, as expected, the only abnormal color, reddish brown, was resulting from potassium dichromate (results unshown).

Generally speaking, measuring the color of a urine specimen is far from a scientifically standard method and discerning an unnatural color change upon adulteration is not easy. Nevertheless, facing the increasingly wide variety of adulterants, the unique function of color as an indicator of adulteration is unsubstitutable, and is therefore highly recommended for inclusion in the routine SVT battery to see if it is required to perform further SVTs for adulterants. In this context, the Roche Urisys 2400 instrument, using its sophisticated spectrophotometry with "pale yellow-yellow" being the criterion for normal urine color, performs more objectively and reliably than variable human visual recognition.

***Performance of creatinine as an adulterant pre-screen parameter***

Creatinine is an amino acid waste product of creatine contained in muscle tissue and found in urine. A person may attempt to foil a drug test by drinking excessive amounts of water or diuretics to dilute or "flush" the system. Therefore, too low a level or absence of creatinine in a urine specimen is indicative of sample dilution or substitution (the inclusion of creatinine measurement in the HHS SVT program was not directed toward the detection of typical adulterants [11]). On the other hand, literally, any endogenous substance that may interfere with the creatinine colorimetric test is going to produce a positive rather than negative error, raising the creatinine level above the upper limit for reporting "substituted" (creatinine $<2$ mg $dL^{-1}$, Table 3) [24], and therefore the specimen will not meet the criteria to report it as substituted. It is benefiting from such specificity that the HHS believes it is scientifically acceptable to use the same Jaffe's or modified Jaffe's colorimetric procedure for both the initial and confirmatory creatinine tests.

Unfortunately, an inherent drawback of creatinine as an adulterant pre-screen parameter is its insufficient sensitivity. Table 2 displays in one of the columns the creatinine readings obtained for the two urine spikes before and after the said adulterations and the respective after-before ratios. The data reflect the following facts: 1) All the adulterated specimens had lower creatinine levels than the unadulterated ones. For a certain adulterant, the more the added adulterant, the lower the creatinine. However, the slopes of variance were generally mild and actually did not appear linear. 2) For a certain adulterant, the creatinine reading of a specimen and its susceptibility to the influences of the adulterant did not have much to do with the drug levels in the specimen. 3) Table salt and alum, which are

**Table 2** Readings [a,b] of color, creatinine, specific gravity, pH, and nitrite obtained for (a) normal urine, (b) morphine/codeine-fortified (375 ng $mL^{-1}$ each c) urine before and after adulteration, and (c) morphine/codeine-fortified (525 ng $mL^{-1}$ each c) urine before and after adulteration

| Adulterant added (%, w/w) | | Color [d,e] | Creatinine [mg $dL^{-1}$] [f,g] | Spec. gravity [g,h] | pH [i,j] | Nitrite [k] |
|---|---|---|---|---|---|---|
| (a) Normal urine | | Pale yellow | 80.5 | 1.014 | 7.0 | − |
| (b) Mo/Co-fortified (375 ng $mL^{-1}$ each) urine | | | | | | |
| Unadulterated | | Pale yellow | 78.5 (1.00) | 1.015 (1.000) | 7.0 (±0 ) | − |
| Table salt [e] | 5 | Yellow | 78.2 (0.99) | *1.037* (1.022) | 7.0 (±0 ) | − |
| | 10 | Yellow | 76.3 (0.97) | *1.052* (1.037) | 6.5 (±0.5) | − |
| | 15 | Yellow | 73.7 (0.94) | *1.086* (1.067) | 6.5 (±0.5) | − |
| Liquid soap | 5 | Pale yellow | 77.7 (0.99) | 1.015 (1.000) | 6.5 (±0.5) | − |
| | 10 | Pale yellow | 73.7 (0.94) | 1.016 (1.001) | 6.5 (±0.5) | − |
| | 15 | Pale yellow | 70.8 (0.90) | 1.016 (1.001) | 6.3 (±0.7) | − |
| Chlorine bleach | 5 | Pale yellow | 69.0 (0.88) | 1.016 (1.002) | 8.0 (+1.0) | ± |
| | 10 | Pale yellow | *56.6* (0.72) | 1.019 (1.004) | *8.5* (+1.5) | ± |
| | 15 | Pale yellow | *55.9* (0.71) | *1.021* (1.006) | *9.0* (±0 ) | ± |
| Vitali soda water | 5 | Pale yellow | 76.6 (0.98) | 1.015 (1.000) | 7.0 (±0 ) | − |
| | 10 | Pale yellow | 72.5 (0.93) | 1.018 (1.003) | 7.0 (±0 ) | − |
| | 15 | Pale yellow | 67.3 (0.86) | 1.019 (1.004) | 7.0 (±0 ) | − |
| Alum | 5 | Pale yellow | 77.0 (0.98) | *1.027* (1.012) | 5.0 (-2.0) | − |
| | 10 | Pale yellow | 75.4 (0.96) | *1.032* (1.017) | 5.0 (±2.0) | − |
| | 15 | Pale yellow | 73.0 (0.93) | *1.034* (1.019) | 5.0 (±2.0) | − |
| Tap water | 5 | Pale yellow | 75.8 (0.97) | 1.013 (0.999) | 7.0 (±0 ) | − |
| | 10 | Pale yellow | 72.4 (0.93) | 1.012 (0.997) | 7.0 (±0 ) | − |
| | 15 | Pale yellow | 66.9 (0.86) | 1.012 (0.997) | 7.0 (±0 ) | − |
| Potassium dichromate | 5 | *Brown* | 75.8 (0.97) | *1.038* (1.023) | 6.5 (±0.5) | ± |
| | 10 | *Brown* | 75.5 (0.96) | *1.048* (1.033) | 6.5 (±0.5) | ± |
| | 15 | *Brown* | 71.3 (0.91) | *1.051* (1.036) | 6.5 (±0.5) | ± |

(To be cont'd)

**Table 2 (Cont'd)**

| Adulterant added (%, w/w) | | Color [d,e] | Creatinine [mg $dL^{-1}$] [f,g] | Spec. gravity [g,h] | pH [i,j] | Nitrite [k] |
|---|---|---|---|---|---|---|
| (c) Mo/Co-fortified (525 ng $mL^{-1}$ each) urine | | | | | | |
| Unadulterated | | Pale yellow | 78.3 (1.00) | 1.015 (1.000) | 7.0 (±0 ) | – |
| Table salt | 5 | Yellow | 77.3 (0.99) | *1.038* (1.023) | 7.0 (±0 ) | – |
| | 10 | Yellow | 75.6 (0.97) | *1.056* (1.040) | 6.5 (±0.5) | – |
| | 15 | Yellow | 74.2 (0.95) | *1.089* (1.073) | 6.5 (±0.5) | – |
| Liquid soap | 5 | Pale yellow | 71.8 (0.92) | 1.015 (1.000) | 6.5 (±0.5) | – |
| | 10 | Pale yellow | 68.8 (0.88) | 1.016 (1.001) | 6.5 (±0.5) | – |
| | 15 | Pale yellow | 66.3 (0.85) | 1.017 (1.002) | 6.3 (±0.7) | – |
| Chlorine bleach | 5 | Pale yellow | 63.7 (0.81) | 1.017 (1.002) | 8.0 (+1.0) | ± |
| | 10 | Pale yellow | *56.4* (0.72) | 1.019 (1.004) | *8.5* (+1.5) | ± |
| | 15 | Pale yellow | *50.8* (0.65) | *1.021* (1.006) | *8.8* (+1.8) | ± |
| Vitali soda water | 5 | Pale yellow | 74.7 (0.95) | 1.016 (1.001) | 7.0 (±0 ) | – |
| | 10 | Pale yellow | 70.4 (0.90) | 1.018 (1.003) | 7.0 (±0 ) | – |
| | 15 | Pale yellow | 66.8 (0.85) | 1.019 (1.004) | 7.0 (±0 ) | – |
| Alum | 5 | Pale yellow | 75.0 (0.96) | *1.028* (1.013) | 5.0 (-2.0) | – |
| | 10 | Pale yellow | 73.5 (0.94) | *1.032* (1.017) | 5.0 (-2.0) | – |
| | 15 | Pale yellow | 72.1 (0.92) | *1.034* (1.019) | 5.0 (-2.0) | – |
| Tap water | 5 | Pale yellow | 74.0 (0.95) | 1.014 (0.999) | 7.0 (±0 ) | – |
| | 10 | Pale yellow | 70.1 (0.90) | 1.013 (0.998) | 7.0 (±0 ) | – |
| | 15 | Pale yellow | 66.9 (0.85) | 1.012 (0.997) | 7.0 (±0 ) | – |
| Potassium dichromate | 5 | *Brown* | 73.8 (0.94) | *1.039* (1.024) | 6.5 (-0.5) | ± |
| | 10 | *Brown* | 72.0 (0.92) | *1.047* (1.032) | 6.5 (-0.5) | ± |
| | 15 | *Brown* | 70.6 (0.90) | *1.051* (1.035) | 6.5 (-0.5) | ± |

[a] All numerical readings are means of duplicate analyses on two separate aliquots; all non-numerals are appropriate first-coming consensuses obtained on two successive aliquots.

[b] Italicized, underlined readings are abnormal indicating specimen alteration.

[c] Equivalent to 125 % and 175 %, respectively, of the 300-ng $mL^{-1}$ cutoff.

[d] The colors actually denoted in this table were defined by the instrument itself rather than variable human visual recognition.

[e] Roche factory-defined normal range: pale yellow-yellow.

[f] Beckman factory-set normal range: 60-250 mg $dL^{-1}$.

[g] Shown in parentheses are adulterated-unadulterated ratios.

[h] Roche factory-set normal range: 1.010-1.020.

[i] Roche factory-set normal range: 5.0-8.0.

**Table 3** Comparison of HHS criteria, instrument manufacturer-set normal ranges, and this study's data ranges with regard to the five evaluated urine parameters for pre-screening adulterants

(a) HHS criteria [1,11]

| Urine parameter | Principle(s) of measurement | Criteria |
|---|---|---|
| Color | Does not define or specifically describe all the possible abnormal characteristics to avoid limiting the possible characteristics that may be used to trigger additional SVTs. | |
| Creatinine [a] | Colorimetry on **initial** and **confirmatory** tests on two separate aliquots. | **Substituted:** Creat. $<2$ mg $dL^{-1}$ and sp. gravity $\leqq 1.0010$ or $\geqq 1.0200$ on both initial and confirmatory creat. tests and on both init. and confirm. Sp. gravity tests on two separate aliquots. |
| Specific gravity [a] | Refractometry on **initial** and **confirmatory** tests on two separate aliquots (one control at 1.0020, one control in 1.0040-1.0180, one control in 1.0200-1.0250). | **Dilute:** 2 mg $dL^{-1} \leqq$ creat. $<20$ mg $dL^{-1}$ and $1.0010 <$ sp. gravity $<1.0030$ on a single aliquot.<br>**Invalid result:** Inconsistent creat. conc. and sp. gravity results obtained. |
| PH | **Initial test:** Colorimetry or pH meter (two calibrators at 3, 1; five controls in 2-2.8, 3.2-4, 4.5-9, 10-10.8, 11.2-12).<br>**Confirmatory test:** pH meter (two calibrators at 4, 7, two controls in 2-2.8, 3.2-4; or two calibrators at 7, 10, two controls in 10-10.8, 11.2-12). | **Adulterated:** $pH<3$ or $\geqq 11$ on init. and confirm. tests on two separate aliquots.<br>**Invalid result:** $3 \leqq pH < 4.5$ or $9 \leqq pH < 11$ on init. and confirm. tests on two separate aliquot. |
| Nitrite | **Initial test:** General oxidant colorimetric test with a calibrator ($\geqq 200$ $\mu$g $mL^{-1}$ nitrite-equiv.), or Initial nitrite colorimetric test.<br>**Confirmatory test:** Multi-wavelength spectrophotometry (MWS), Ion chromatography (IC), or Capillary electrophoresis (CE). | **Initial cutoff:** $\geqq 200$ μg $mL^{-1}$<br>**Confirmatory cutoff:** $\geqq 500$ μg $mL^{-1}$ (No LOD/LOQ specified in the Guidelines.)<br>**Adulterated**：Nitrite $\geqq 500$ μg $mL^{-1}$ on either nitrite colorimetric test or general oxidant colorimetric test for init. test on 1st aliquot, and a different confirm. test on 2nd aliquot.<br>**Invalid result:** Nitrite $\geqq 200$ μg $mL^{-1}$ on nitrite colorimetric test or $\geqq 200$ μg $mL^{-1}$ nitrite-equiv. on general oxidant colorimetric test for both init. and confirm. test; or using either init. test and nitrite $\geqq 200$ μg $mL^{-1}$ but $<500$ μg $mL^{-1}$ for a different confirmatory test on two separate aliquots.[b] |

(b) Instrument manufacturer-set normal ranges, and this study's data ranges [c]

| Urine parameter | Principle of measurement | Factory-set normal range | This study's data range | |
|---|---|---|---|---|
| | | | Unadulterated | Adulterated [d] |
| Color | Colorimetry on blank pad of test strip | Pale yellow-Yellow | Pale yellow | Pale yellow-*Brown* |
| Creatinine | Colorimetry based on formation of red complex | 60-250 mg $dL^{-1}$ | 78.2-78.8 mg $dL^{-1}$ | *49.8*-78.2 mg $dL^{-1}$ |
| Specific gravity | Refractometry | 1.010-1.020 [e] | 1.015 [e] | 1.012-*1.086* [e] |
| PH | Colorimetry on test strip | 5.0-8.0 [f] | 7.0 [f] | 5.0-*9.0* [f] |
| Nitrite | Colorimetry on test strip (Griess test) | — [g] | — [g] | —, ± [g] |

[a] By HHS convention, every specimen's creatinine and specific gravity, which are mutually supporting, must be reported together.

[b] The relatively recent inclusion of the range of 200-500 μg $mL^{-1}$ for reporting "invalid results" is aimed to deter the new trend that more and more adulterants are being produced containing those amounts of nitrite mixed with other oxidant compounds in an effort to avoid detection.

[c] Taiwan official version of forensic drug testing program has not yet included the SVT policy.

[d] Italicized, underlined readings are abnormal indicating specimen alteration.

[e] The instrument allows the values to be reported to only three decimal places.

[f] The instrument allows the pH values to be displayed only at intervals of 0.5.

[g] Threshold: 150 μg $mL^{-1}$.

chemically more inert and are added as powder, showed less negative influences on the creatinine. 4) Except for those adulterated with chlorine bleach (an oxidant) at 10 and 15 % (w/w), according to the Beckman factory-set normal creatinine range, 60-250 mg $dL^{-1}$, all the other adulterated spikes remained "normal," but liquid adulterants like tap water, drinking soda water, and liquid soap when added at more than 15 % (w/w) would dilute the creatinine so substantially as to somewhat approach the 60-mg $dL^{-1}$ cutoff. Although chlorine bleach as a degrading oxidant showed a remarkable negative influence on the creatinine, the also oxidizing potassium dichromate at ca. pH 6.5 did not decrease the creatinine appreciably, indicating even lower pH needed for effective oxidation of creatinine. If a lower cutoff like 50, 20, or 5 mg $dL^{-1}$, as suggested by some other researchers [18], or the HHS criteria (Table 3) had been adopted, the discriminating power would have got even worse. Nevertheless, no matter how hard to "fully prove" the intention of dilution, the pre-screen could always be improved. We also tested the seven adulterants each at 10 % (w/w) on the four case specimens, and the corresponding creainine readings (unshown) featured essentially the same characteristics.

Also employing colorimetry, the Beckman instrument is convenient and qualifies for both the initial and confirmatory creatinine tests. However, by the HHS convention, creatinine is not used for reporting "adulterated." And judging from the fact that all our post-adulteration creatinine numerals are far greater than the HHS 20-mg $dL^{-1}$ cutoff, the probability for creatinine to detect what the HHS call "dilution" or "substitution" is low. Now that creatinine has seen in Table 2 some sensitivity to the addition of such degrading adulterant as chlorine bleach, we would suggest each laboratory, while retaining and complying with the HHS provisions for reporting "substituted," "dilute," and "invalid results" for extreme cases, just put some emphasis on using creatinine as an indicator of sample alteration with a reasonably tolerant cutoff (e.g., $\leqq$60 mg $dL^{-1}$ or $\leqq$65 mg $dL^{-1}$) to see if it is necessary to perform additional SVTs for degrading or diluting adulterants. Regardless, the existing Beckman factory-set normal range of creatinine needs to be further specified.

***Performance of specific gravity as an adulterant pre-screen parameter***

By the HHS convention, urine drug testing specimens with abnormal specific gravity values indicate specimen dilution or substitution. However, the inclusion of specific gravity in the SVT program was not directed toward the detection of typical adulterants.

In a column of Table 2 are displayed the measured specific gravity values for the said spikes before and after the said adulterations, as well as the respective after-before ratios. The data reflect the following facts: 1) For a certain adulterant, the specific gravity of a specimen and its susceptibility to the influences of the adulterant did not have much to do with the drug levels in the specimen. 2) Except for tap water that decreased the specific gravity of urine, all the other six adulterants went the opposite way, with table salt, chlorine bleach, alum, and potassium dichromate (which all contain relatively heavier atoms like chlorine, aluminum, potassium, and/or chromium) increasing the specific gravity more. 3) According to the Roche factory-set normal range of specific gravity, 1.010-1.020, while the specimens remained "normal" at up to 15 % (w/w) of adulteration with tap water, liquid soap, or drinking soda water, they did become heavier than "normal" when adulterated with table salt, alum, or potassium dichromate at as little as 5 % (w/w), or adulterated with chlorine bleach at 15 % (w/w). We also tested the seven adulterants each at 10 % (w/w) on the four case specimens, and the specific gravity values all came out (unshown) without significant differences from the spike counterparts.

The HHS directs that all certified laboratories must use refractometry to perform both the initial and confirmatory specific gravity tests on two separate aliquots [11]. A refractometer is considered a reference instrument (i.e., a method to which other tests are compared) by the scientific community. When properly calibrated (Table 3), it gives extremely accurate results and allows specific gravity values to be reported to four decimal places, i.e., within 10-4 from the cutoff rather than being essentially a "yes" or "no" answer. Further, the creatinine and specific gravity of a specimen, being mutually supporting for reporting SVT results and measured based on different scientific principles, must be reported in combination (see Table 3 for HHS criteria). If a specimen is diluted or substituted, its creatinine and specific gravity values are to be so diminished or so divergent as to be inconsistent with normal human urine.

Although the said Roche instrument employs refractometry for measuring specific gravity, it allows the values to be reported to only three decimal places and qualifies for neither the HHS initial nor confirmatory specific gravity test. On the other hand, the relevant

HHS provisions made up of combined creatinine-specific gravity criteria seem to be inclined all the way toward the forensic validity and rigidity of a confirmatory test making none of the data pairs obtained in this study constitute a real so-called "substituted," "dilute," or "invalid result." (Particularly because the creatinine data are all much greater than the HHS 20-mg $dL^{-1}$ cutoff.) As such, it is suggested again that each laboratory, while retaining and complying with the HHS provisions for conclusively reporting "substituted," "dilute," and "invalid results" for extreme cases, just put some emphasis on using specific gravity as a pre-screening indicator of sample alteration with a properly narrowed criterion for normal urine (e.g., 1.0100-1.0200) to see if it is necessary to perform additional SVTs for weight-increasing or decreasing adulterants. At all events, the existing Roche factory-set criterion of specific gravity needs to be further specified.

***Performance of pH as an adulterant pre-screen parameter***

The measurement of pH tests for the presence of acidic or alkaline adulterants in urine. Because pH values represent a logarithmic scale, any small difference in pH represents a large difference in the specimen condition, and any pH value outside the normal range may indicate the specimen has been adulterated or altered somehow. The HHS Guidelines does not include the use of pH values for reporting "dilute" or "substitution."

In a column of Table 2 are listed the measured pH values of the four spikes before and after the said adulterations and the respective differences of after minus before. The data reflect the following facts: 1) For a certain adulterant, the pH of a specimen and its susceptibility to the influences of the adulterant did not have much to do with the drug levels in the specimen. 2) As expected from the noted pH 12.0 ± 0.5 on the bottle label, chlorine bleach turned out to be the only tested adulterant that when added at more than 10 % (w/w) would lift the urine pH to exceed the upper limit of the Roche factory-set normal pH range, 5.0-8.0. However, it should be recalled that formation of ammonia-like species in old specimens might also raise the pH. The other six adulterants typically made the urine weakly acidic, with alum being the only one capable of acidifying the urine down to marginal pH 5.0 indicating a likelihood of adulteration. Again, if any stricter criteria for abnormal urine (see below) or broader criteria for normal urine (e.g., 4.5-8.0, 4.0-9.0, etc. [18]) had been adopted, then even fewer adulterants would have been detected. We also tested the seven adulterants each at 10 % (w/w) on the four case specimens, and the pH values all came out (unshown) without significant differences from the spike counterparts.

Although the number of specimens actually reported with a pH too low or too high is small, the HHS still believes it is required that a laboratory determine the pH for every specimen tested [11], because elimination of this requirement would allow the use of adulterants that alter the pH interfering with obtaining a valid drug or adulterant test result. In the light of quality test, the HHS would recommend using a pH meter for both the initial and confirmatory pH tests because it is considered a reference method by the scientific community, is a highly reliable instrument, and gives extremely accurate results when properly calibrated. However, having understood the fact that most laboratories want the SVTs to be cost-effective, the HHS then recommends using colorimetry (which is readily automated but has a narrow dynamic range and do not sufficiently support the pH cutoffs) for the initial pH test and using a pH meter (a reference yet manual instrument usually capable of measuring pH to one decimal place) for the confirmatory pH test. The initial colorimetric pH test should be performed with the entire pH range covered with appropriate calibrators and controls (Table 3) and the confirmatory pH meter test may use calibrators and controls that are focused on either the lower or upper decision point, as appropriate (Table 3). The HHS criteria for reporting "adulterated" and "invalid result" are shown in Table 3.

Despite using colorimetry for measuring pH, the said Roche instrument suffered setback due to insufficient sensitivity and poor calibration, which allowed the pH values to be displayed only at intervals of 0.5. Unless the instrumentation can be upgraded, this measuring procedure will not qualify for even the initial pH test by the HHS convention. Also, the said Roche factory-set normal pH range is too rough to be practical.

***Performance of nitrite as an adulterant pre-screen parameter***

Nitrite was specifically targeted by the HHS to test for such oxidizing adulterants as "Klear" and "Whizzies" which are commercially available in the States but not commonly encountered in Taiwan. Relatively large amounts of nitrite are not normally found in random urine. Values of 1.0-50.0 μg $mL^{-1}$ may indicate a urinary tract infection or bacterial growth following

improper storage of the specimen [18,19]. Other natural sources shall produce at most 129 μg $mL^{-1}$ of nitrite [19], based on which the Roche instrument considers it an indication of sample adulteration if urinary nitrite is above 150 μg $mL^{-1}$ (HHS cutoffs shown in Table 3).

Setting 150 μg mL-1 as the threshold, Table 2 shows in one of the columns the nitrite positives/negatives for the four spikes before and after the said adulterations. The data reflect the following facts: 1) For a certain adulterant, the nitrite sign of a specimen and its susceptibility to the influences of the adulterant was irrelevant to the drug levels in the specimen. 2) While the other five adulterants always made the specimens show nitrite-negative, both chlorine bleach and potassium dichromate resulted exclusively in nitrite-positive. The latter situation might at first sight look strange or even conflicting in the usual sense that 1) any trace of endogenous or newly generated nitrite should have been oxidized to nitrate by the excessive oxidizing chlorine bleach giving nitrite-negative; 2) although potassium dichromate at ca. pH 6.5 might not oxidize nitrite to nitrate (the Eo for nitrate to nitrite is 0.934), it's addition should still hardly cause strikingly false nitrite-positive. However, a literature search using in combination such keywords as Griess test, false positive, nitrification, nitrite formation, oxidation, ammonia, amine, etc. did return hundreds of reports talking about various nitrification reactions in urinary or aqueous systems (sample references: [20-23]) suggesting that, with the catalysis of some bacteria or chemical substances (it should be kept in mind that literally countless bacteria and thousands of compounds are present in urine), more nitrite could have also originated from in-vitro oxidation of urinary ammonia, amines, hydroxylamines, etc. by such oxidizing adulterants as chlorine bleach and potassium dichromate. If this is really the case, then, in addition to testing for nitrite-containing adulterants, nitrite can also make a useful indicator for the presence of non-nitrite oxidizing adulterants. We also tested the seven adulterants each at 10 % (w/w) on the four case specimens, and the results (unshown) turned out to be the same as the spike counterparts.

With its existing functional capability, the said Roche instrument, which makes use of a Griess test strip to perform nitrite colorimetric test, can hardly qualify for even the HHS initial nitrite test (Table 3) unless the instrumentation is upgraded to allow displaying numerical results instead of just showing positive/ negative signs.

## Conclusions

The results obtained in this study demonstrated that, when used alone, none of the five evaluated clinical urine parameters was all-sensitive to the seven tested Taiwan-produced adulterants in urine. However, according to the instrument-factory-set criteria adopted in this study for recognizing normal urine (which was relatively rough), the combinative use of the five parameters was able to pre-detect the abnormities of the specimen (but not identify the adulterant itself) caused by the addition of over 5 % (w/w) of table salt (specific gravity increased), alum (specific gravity increased), or potassium dichromate (color turned brown and specific gravity increased), and by over 10 % (w/w) of chlorine bleach (creatinine lowered, specific gravity increased, and pH raised), while only showing different degrees of promise of the like toward the other three liquid adulterants provided larger amounts were added (color, creatinine, and specific gravity all diluted). For a certain adulterant, the five parameters and their susceptibility to the influences of the adulterant did not have much to do with the kinds and levels of the tested drugs in the specimen.

If the much stricter HHS criteria had been adopted, none of the total of 42 (i.e., 3 x 7 x 2) test entries would have constituted a real so-called "adulterated," "substituted," "dilute," or "invalid result." However, to the best of our knowledge, scarcely any other parameter could have ever been more basic yet tested sharper than the evaluated five, and this pre-screening procedure is practically necessary for high-volume laboratories. Considering the actual overall efficiency, we would suggest that, while retaining and complying with those strict HHS provisions, each laboratory just cover as many clinical parameters and physical characteristics of urine and place some emphasis on using them as pre-screening indicators to probably trigger additional specific SVTs including the confirmatory tests of nitrite, chromium (VI), halogen, glutaraldehyde, pyridine, surfactant, and any others if necessary. In so doing, some specimens that are suspected of "substituted," "dilute," or "invalid result" but just do not fall within the corresponding HHS narrow window may finally turn out to be exactly "adulterated." In other words, it will be helpful for each laboratory to make the criteria for pre-screening suspected specimens reasonably tolerant so that probable adulterants won't be missed as false negatives.

From the standpoint of scientific and forensic validity, the importance of SVT data quality cannot be overemphasized. In fact, the HHS criteria for quality SVT are virtually as stringent as those for quality drug testing itself [11]. A basic requirement in common is that a laboratory shall not report any false positive result on any workplace or PT specimen after the confirmatory test (for drug testing, there is actually no opportunity to have a false conclusive positive if good forensic procedures are followed including confirmation of initial positive results using a second methodology based on a different chemical principle such as GC-MS [12]). Another common criterion of quality SVT and quality drug testing is that confirmatory test results of quantifiable analytes (e.g., creatinine and nitrite for the SVT) in PT specimens shall be "within ±20 % or ±2 standard deviations of the calculated reference group mean (whichever range is larger) for at least 80 % of the total challenges." The criteria adopted solely for the quality evaluation of pH and specific gravity are "within ±0.3 pH units and within ±0.0003 specific gravity units, respectively, of the calculated reference group mean." In order to meet these criteria, relevant instruments, especially those for the confirmatory tests, must be appropriately upgraded even though it has above been suggested that some of the studied parameters be merely used as pre-screening indicators of sample alteration with properly narrowed criteria for normal urine.

## Acknowledgments

Financial support by the National Bureau of Controlled Drugs, Department of Health, Taiwan (Republic of China), under Grant No. DOH94-NNB-1009 is gratefully acknowledged. The generous offer of real-case urine specimens from the Taiwan Union Clinical Laboratory and their valuable help in operating the Beckman and Roche instruments is highly appreciated. Su-Lien Chou thanks the Central Police University for supporting her in-service advanced study.

## References

1. Crumpton SD, Sutheimer CA. Specimen adulteration and substitution in workplace drug testing. Forensic Sci Rev 2007; 19(1):1-27.
2. Hoffman A. Deep inside urine testing. In: Steal this urine test: fighting drug hysteria in America. New York: Penguin Book; 1987, p.183.
3. Podkowik BI, Smith ML, Pick RO. Experience with a sulfonamide diuretic in a large drug testing program. J Anal Toxicol 1987; 11:215.
4. Lambert PA, Woodbridge PA, Botie PN. Detection of sample contamination of urine submitted for drugs of abuse testing in a high volume setting using the Olympus 5000 analyzer. Clin Chem 1987; 33:977.
5. Paul PD, Dunkley CS. Specimen validity testing (SVT) --- Effects of oxidizing agents on drugs in urine and procedures for detection. Forensic Sci Rev 2007; 19(1):29-47.
6. Cody JT, Schwarzhoff RH. Impact of Adulterants in RIA analysis of urine for drugs of abuse. J Anal Toxicil 1989; 13:277.
7. Kim HJ, Cerceo E. Interference by NaCl with the EMIT method of analysis for drugs of abuse. Clin Chem 1976; 22:1935.
8. Mikkelsen SL, Ash KO. Adulterants causing false negatives in illicit drug testing. Clin Chem 1988; 34:2333.
9. Vu-Duc. EMIT test for drugs of abuse: interference by liquid soap preparations. Clin Chem 1985; 31:658.
12. Warner M. Interference of common household chemicals in immunoassay methods for dugs of abuse. Clin Chem 1989; 35:648.
11. Department of Health and Human Services (US). Mandatory Guidelines for Federal Workplace Drug Testing Programs, Federal Register 69:71 19644-19673, April 13, 2004.
12. Cody JT. Specimen adulteration in drug urinalysis. Forensic Sci Rev 1990; 2:63.
13. Paul BD, Martin KK, Maguilo J Jr, Smith ML. Effects of pyridinium chlorochromate adulterant (urine luck) on testing for drugs of abuse and a method for quantitative detection of chromium (VI) in urine. J Anal Toxicol 2000; 24(4):233-7.
14. Dasgupta A, Wahed A, Wells A: Rapid spot tests for detecting the presence of adulterants in urine specimens submitted for drug testing; Am J Clin Pathol 2002; 117(2):325-9.
15. Dasgupta A, Chughtai O, Hannah C, Davis B, Wells A. Comparison of spot tests with AdultaCheck 6 and Intect 7 urine test strips for detecting the presence

of adulterants in urine specimens. Clin Chim Acta 2004; 348(1-2):19-25.

16. Cody JT, Valtier S. Effects of StealthTM adulterant on immunoassay testing for drugs of abuse; J Anal Toxicol 2001; 25(6):466-470.
17. Tietz NW. Fundamentals of Clinical Chemistry. Philadelphia (PA): WB Saunders; 1976, p.1220.
18. Website of Sciteck Diagnsotics, Inc. Asheville, North Carolina, USA. Available from: http//www.drugtesting-kits.com, visited May 15, 2007.
19. Urry FM, Komaromy-Hiller G, Staley B, Crockett DK, Kushnir M, Nelson G, Struempler RE. Nitrite adulteration of workplace urine drug-testing specimens. I. Sources and associated concentrations of nitrite in urine and distinction between natural sources and adulteration.J Anal Toxicol 1998; 22(2):89-95.
20. Renmin L, Huaisheng W, Ailing S, Daojie L. Reversed flow injection spectrophotometric determination of trace amount of ammonia in natural water by oxidation of ammonia to nitrite. Talanta 1997; 45(2):405-410.
21. Marietta MA, Yoon PS, Iyengar R, Leaf CD, Wishnok JS. Macrophage oxidation of l-aginine to nitrite and nitrate: nitric oxide is an intermediate. Biochem 1988; 27:8706-11.
22. Anderson JH. Studies on the oxidation of ammonia by nitrosomonas. Biochem J 1965; 95:688.
23. Amarger N, Alexander M. Nitrite formation from hydroxylamine and oximes by pseudomonas aeruginosa. J Bacteriol 1968; 95(5):1651-7.