"OneScreen" Drug Test Devices: Adulterant Effects and Untrained Subject Use

EMMA ALBRITTAIN, KYLE BOWEN, DIANE DAVIS

ABSTRACT

Objective: To evaluate the CLIA-waived immunochromatographic lateral flow assay "OneScreen" drug test device from American Screening Corporation for its ability to perform accurately in the presence of urine specimen adulterants and to evaluate the ability of untrained subjects to perform the assay with competence.

Design: The device used in this study detected five drugs/drug classes and measured specific gravity and creatinine to detect adulteration. The five drug analytes were tetrahydrocannabinol metabolite (THC), 3,4-methylenedioxy-N-methylamphetamine (MDMA or Ecstasy), methamphetamine, cocaine metabolite (benzoylecgonine) and opiates. A specimen positive for all 5 drugs was created with high enough drug concentrations that dilution alone by an adulterant would not cause the specimen to test negative. This specimen was adulterated with varying concentrations of 11 adulterants: Visine® eye drops, bleach, vinegar, salt, hand soap, ammonia, ethanol, hydrogen peroxide, isopropanol, acetone and baking soda. After the study protocol was cleared by the Salisbury University (SU) Human Subjects Committee, subjects were recruited to test 3 specimens: a specimen positive for one drug, yellow colored water to mimic a diluted specimen and a urine specimen adulterated with enough bleach to give a false negative. After obtaining informed consent and providing safety instruction and protective equipment, subjects were given an expired device kit with all accompanying literature and asked to perform testing on the three specimens without any assistance. Subjects completed a report form and answered questions related to demographics and their reactions to the testing process.

Setting: Salisbury University Medical Laboratory Science Program laboratories, Salisbury, MD

Participants: Sixteen students from Salisbury University volunteered for this study, four students from each of Salisbury University's four schools of learning (Science and Technology, Business, Education and Professional Studies, and Liberal Arts). Each student was a senior in good academic standing. The students were from several majors and their level of self-reported laboratory expertise varied widely.

Main outcome measures: For adulterant testing, the outcomes were the effects on the device testing areas for the control, positive test, creatinine and specific gravity as well as the physical alteration and odor of the specimen. For the subjects performing testing, the outcomes were demographic data, time spent reading instructions, physical performance of the test, interpretation of results, confidence in results and recommendations for further action.

Results: Most adulterants either failed at causing a false negative and/or were detectable as adulterants by odor, physical appearance and/or alterations in specific gravity/creatinine. Vinegar, bleach and ethanol were the only adulterants deemed effective in creating a false negative result; however, vinegar and bleach failed creatinine/specific gravity measurements at fairly low concentrations, and ethanol caused false negatives only at a high concentration. Although the subjects could correctly identify positive, negative and invalid test results, more than a third had difficulty getting proper flow in the devices. Although 87% of subjects reported that they understood the instructions and 81.5% felt confident in their results, no subjects noticed the expired date on the devices or the bleach odor. Subjects either failed to recognize abnormal creatinine and specific gravity or noticed the abnormalities but did not interpret them as signs of adulterated specimens. Seventy-five percent of the subjects recommended additional testing, even though they expressed confidence in their results.

Conclusion: The adulterants that interfered with these devices (bleach, vinegar and ethanol) could easily be detected by alterations in creatinine, specific gravity and/or odor. The data suggests that although the test devices are designed for intuitive and easy use, performance of untrained subjects is imperfect and the potential exists for dangerous misinterpretation of

testing results and failure to identify adulterated specimens.

ABBREVIATIONS: 3,4-methylenedioxy-N-methylamphetamine (MDMA), Clinical Laboratory Improvement Amendments (CLIA), Gas chromatography/mass spectrometry (GC/MS), Food and Drug Administration (FDA), Medical laboratory science (MLS), Point-of-care (POC), Substance Abuse and Mental Health Services Administration (SAMHSA), Tetrahydrocannabinol metabolite (THC)

INDEX TERMS: Point of care technology, Drug abuse screening, United States Substance Abuse and Mental Health Services Administration

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Emma Albrittain, MLS (ASCP)CM, *Johns Hopkins Hospital, Department of Pathology, Chemistry Laboratory Baltimore, MD 21227*

Kyle Bowen, *Salisbury University, Hebron, MD 21830*

Diane Davis, Ph.D., MLS (ASCP)CM, SC CM, SLS CM, *Medical Laboratory Science Program, Department of Health Sciences, Salisbury University, Salisbury, MD*

Address for Correspondence: Dr. Diane Davis, *Professor and Program Director, Medical Laboratory Science Program, Chair, Department of Health Sciences, Salisbury University, 1101 Camden Avenue, Salisbury, MD 21801, 410- 548-4787, dldavis@salisbury.edu*

INTRODUCTION

Point-of-care (POC), Clinical Laboratory Improvement Amendments (CLIA)-waived tests have proliferated in the USA; diabetic self-monitoring, home pregnancy tests, rapid tests in emergency departments and clinics have become accepted and are often the standard for optimal care and/or efficient patient triage and assignment to treatment.[1] Increasingly, testing is being marketed directly to the public, but the literature on how people untrained in medical laboratory science (MLS) perform on these tests is sparse. Results of a Centers for Disease Control survey of CLIA-waived labs "raise quality concerns about practices that could lead to errors in testing and poor patient outcomes",[2] suggesting that the simplicity of these devices is deceptive.

The Food and Drug Administration (FDA) regulates medical devices, including those used for point-of-care testing. It develops guidelines for test complexity and assigns the complexity levels to approved devices. Immunochromatographic lateral flow drug screening devices approved by the FDA for waived testing are being sold over the Internet at low price points (~$1.00 per drug detected). The American Screening Corporation markets these devices as "Immediate results, simply dip & read results. FDA 510K & CLIA Waived & OTC cleared". The devices come in many formats, to include specimen integrity testing within the device, saliva ethanol devices and different panel options, allowing purchasers to select the device which best fit their needs in terms of drugs detected and options for specimen integrity testing.[3]

Drug testing is inherently different from other types of POC testing. Employers, parole officers, family members and others may want to perform drug testing for punitive, not diagnostic, purposes. Virtually all patients have a self-interest in being cooperative with specimen collection and diagnostic testing as it relates to their health and wellness, but with drug testing, the self-interest of the subject may be in concealing drug use. Federal guidelines for specimen collection and handling, including specimen integrity testing, were crafted to address these unique aspects of drug testing.[4] These guidelines include terms and testing methods with which one would not expect the general public to be familiar (creatinine, specific gravity, etc.). Many of the guidelines are geared toward preventing or detecting specimen adulteration. While the physical techniques to perform these POC assays may meet the spirit of waived testing, the knowledge required for appropriate specimen collection, handling and interpretation of testing results may not.

We identified two important potential problems with these testing devices. The first was how the devices perform with various specimen adulterants, and the second was how untrained subjects would use the devices, interpret the results and identify possible specimen adulteration/false negative results. This project was designed to evaluate the extent to which these issues exist.

MATERIALS AND METHODS

The CLIA-waived devices, "OneScreen 5 Panel Dip Card w/ Adulterants", were purchased at a reduced price of $2.27 each because they were close to expiration. All devices were from the same lot and were purchased on-line from American Screening Corporation, Shreveport, LA. No credentials, such as a CLIA certificate, were requested to make the purchase.

The devices detected five drugs/drug classes and measured specific gravity (to detect specimen dilution or excess density from an additive) and creatinine (to validate the specimen as urine). The five drug analytes were tetrahydrocannabinol metabolite (THC), 3,4-methylenedioxy-N-methylamphetamine (MDMA or Ecstasy), methamphetamine, cocaine metabolite (benzoylecgonine) and opiates. All adulterant testing described below was performed prior to device expiration using the manufacturer's instructions.[5] All testing performed by untrained subjects described below was performed with expired devices to assess whether or not the subjects would notice the expiration date.

Certified drug-free urine, negative for human immunodeficiency virus and hepatitis B, was obtained from a commercial source (Utak Labs, Valencia, CA). Drugs and drug metabolites listed above were obtained from commercial sources (Sigma, http://www.sigma aldrich.com; Altech, www.altechchemicals.com; and Analytical Systems, ToxiLab THC control, no longer in business). The purchased drugs were highly concentrated, so very little had to be added to spike the urine, minimizing matrix effects from the solvents in which the drugs were dissolved.

Urine specimen adulterants were chosen based on published literature[6,7] and availability in homes and restrooms. The following were purchased from common retailers: Visine® eye drops, bleach, apple cider vinegar, table salt, hand soap, ammonia floor cleaner, ethanol (190 proof), hydrogen peroxide (30%), isopropanol (70%), acetone, and baking soda.

To test the effect of adulterants, we created a specimen positive for all of the drugs tested. The levels of each drug needed were based on the detection limits indicated in the product literature. Because the addition of an adulterant dilutes drug levels independent of any interference in testing, the drug concentrations in the positive specimen needed to be high enough to compensate. We decided to make the level of each drug in the positive specimen four times the detection limit so that the dilution caused by adulterant would reduce drug concentration to double the detection limit. We did not want any ambiguity in the positive result, but we also didn't want the amount of drug present to overwhelm the adulterant's effect. This multi-positive sample performed satisfactorily for all drugs except opiates. In this system, to consider a specimen positive, no line can appear in the test area (competitive immunoassay). At 4 times cut-off concentration, an extremely faint line was visible in the opiates test area, so the opiate level was increased until the line disappeared, ultimately at eight times the detection limit.

We duplicated the above technique to create the positive specimens for the untrained subjects to test, except that we only put one drug in any positive specimen because this is more realistic. We varied the drug between THC, morphine and methamphetamine and rotated the contents of each "Specimen 1, Specimen 2 and Specimen 3" so that the subjects would not get cues from each other.

To keep the dilution effect of adulterant constant, we devised the dilution scheme shown in Table 1. The negative urine control was used as the diluent to compensate for the varying volumes of adulterant and to eliminate unexpected matrix effects from the use of non-urine diluents. We did not test concentrations of adulterant that exceeded 40% of total specimen volume because we felt that the addition of adulterant beyond 40% was difficult to achieve illicitly. We tested all dilutions of one adulterant at a time to determine the most accurate cut-off point between positive and negative.

Results for each drug/adulterant combination were recorded as positive if no line appeared at the test line, weak negative if a faint line could be seen or negative if a definite line could be seen. The specific gravity and creatinine test areas were matched to the color key provided in the device kit. A physical inspection of each sample with adulterant was performed to check for the smell and appearance because such abnormalities cue the analyst to suspect specimen adulteration.

Table 1. Dilution Scheme for Urine Adulterant Testing

	Negative control	40% adulterant	30% adulterant	20% adulterant	10% adulterant	Positive control
Adulterant	0 uL	800 uL	600 uL	400 uL	200 uL	0 uL
Negative Urine Control Specimen	2000 mL	200 uL	400 uL	600 uL	800 uL	1000 uL
Positive Urine Control Specimen	0 uL	1000 uL	1000 uL	1000 uL	1000 uL	1000 uL

To assess the ability of untrained subjects to use these devices, the study design below was first approved by the Salisbury University Human Subjects Committee. Appropriate instruction and safety equipment were provided to all subjects once the protocol was explained, and informed consent was obtained. The testing devices and sample preparation are described above, and as mentioned, all devices were one year past expiration. Subjects were asked to use the OneScreen drug test kits with all the manufacturer's instructions included. Each was given three specimens to test:

1. Unadulterated control urine specimen that contained one drug, varying between THC, methamphetamine and morphine
2. Water with yellow food coloring, designed to mimic a specimen adulterated with water and fail the test's creatinine and specific gravity measurements
3. Adulterated urine specimen that contained sufficient bleach to cause false negative drug testing (10% of specimen volume), designed to mimic an adulterated specimen with abnormal creatinine, specific gravity, odor and appearance (bubbles).

Sixteen students from Salisbury University volunteered for this study. To increase sample diversity, four students from each of Salisbury University's four schools (Science and Technology, Business, Education and Professional Studies, and Liberal Arts) were recruited as test subjects. Each volunteer was a senior in good academic standing. The students were from several majors and their level of self-reported laboratory expertise varied widely.

Test subjects were asked to read the provided instructions to the extent they deemed appropriate and to perform the drug screening test for each provided specimen. We recorded the amount of time that the subjects spent reading the instructions and observed them the entire time, in case we needed to intervene for safety reasons. We did not intervene for any observed testing problems and did not answer any questions. Subjects were spread out and positioned in the laboratory so that they could not see or speak to each other, preventing influence from the actions of others.

At the completion of testing, the participants were asked to complete a demographics form and record their results on a reporting form, including comments on the information they read, analytical findings, confidence in those findings and difficulties or questions concerning the specimens and/or test devices. The subjects were also asked to suggest any further testing or actions they would perform after analyzing their findings. All subject data was handled to maintain anonymity.

RESULTS

We identified three categories of adulterants. The first is adulterants that had no effect on test results or caused only a weak negative, which could conceivably be interpreted as positive. The second and third categories of adulterants caused clear false negatives at relatively low or relatively high concentrations, respectively. None of the adulterants interfered with appearance of the control line on the device. Table 2 summarizes the adulterant effects.

Table 2. Adulterant Effect on Positive Test Results/Ability to Cause a False Negative

No significant effect on test results	Caused clear false negative at relatively low concentration	Caused clear false negative at relatively high concentration
Visine®	Bleach at 10% of specimen volume (cocaine remained positive)	Ethanol at 40% of specimen volume (MDMA[a] remained positive)
Isopropanol	Vinegar at 20% of specimen volume (cocaine remained positive)	
Peroxide		
Soap		
Ammonia		
Salt		
Baking Soda		
Acetone		

a. MDMA = 3,4-methylenedioxy-N-methylamphetamine

The ability of an adulterant to cause an analytical false negative is mitigated if the adulteration is obvious. These devices were designed to detect adulteration by measuring the specific gravity and creatinine of the urine. Table 3 summarizes physical appearance, odor, creatinine and specific gravity observed for each adulterated urine. Analysis of these results indicated that bleach was chemically the most effective adulterant, so we performed additional testing to see the effect of lower bleach concentrations. We found that below 10% of total volume, bleach begins to lose its ability to interfere with testing, and at a concentration of 2.5% no interference occurs. Notably, even at the lowest bleach concentration tested (2.5%), the bleach smell could still be detected by an alert analyst.

Table 3. Detection of Adulterants in Urine Specimen

Detected by device	Not detected by device or appearance	Detected by smell	Detected by alteration in physical appearance
Bleach at high concentrations (Specific gravity and creatinine)	Visine® eye drops	Bleach at all concentrations	Soap – bubbles, viscosity
Vinegar at all concentrations (Specific gravity)	Acetone	Vinegar at all except low concentrations	Baking Soda – precipitate at high concentrations
Peroxide at all concentrations (Creatinine)	70% Isopropanol	Acetone at all concentrations	Bleach - bubbles form as specimen stands
Soap at all except low concentration (Specific gravity)	Ethanol	Ethanol at all except low concentrations	
Ammonia at all except low concentrations (Specific gravity)		Ammonia at all concentrations	
Baking soda at all but low concentration (Creatinine)			

One of the subjects turned in the drug testing report form, but not the demographic sheet, so demographic data is based on 15 subjects rather than 16. Table 4 presents analysis of selected data. We believe that our results are not skewed by too many subjects from a particular program of study and that we had good variance in prior laboratory experience. The level of confidence most subjects had was striking when compared to the errors that were made. The manufacturer's literature was lengthy and in many places highly technical, and the subjects spent relatively little time reading it. Some subjects with self-reported low levels of experience read for as few as five minutes.

Table 4. Selected Data from 16 Study Subjects Untrained in MLS Who Performed Drug Test

Majors (all subjects Seniors in good academic standing)	Accounting (2), Accounting/Info Studies, Biology (2), Finance, English/Creative Writing, Elementary Ed. (2), English/Ed., History/Philosophy, History/Political Sci/ International Studies, History/Secondary Ed, Math, Music, Nursing
Self-reported level of laboratory experience	High level - 37.5%, Moderate level - 37.5%, Low level - 25%
Time spent reading test instructions	Mode of 10 minutes, with most in 5-10 range. 4 subjects read >20 minutes.
Self-reported comprehension level of test instructions	33% -understood without difficulty, 54% -generally understood, 13%- did not understand, but believed testing correct
Experienced difficulty performing test procedure	37.5% (failure of device capillary action)
Self-reported confidence in testing results	81.5%
Recommended additional testing	75%
Interpreted positive and negative results correctly; recognized when tests were invalid	100%
Recognized expired test devices	0%
Correctly identified cues for adulteration	0%
Recognized test device values suggesting adulteration but did not interpret as such	37.5%

Results were mixed on actual performance of testing and reporting of results. All subjects correctly identified positive and negative tests, drug identities and invalid tests (when present). Abbreviations for each drug were used on the device, and all subjects were able to translate the abbreviation correctly. No misinterpretations occurred due to the disappearance rather than appearance of a line indicating a positive test in this competitive immunoassay.

Despite the preponderance of subjects having self-reported laboratory experience and comprehension of the procedure, the subjects made several errors. No subjects noted that the devices were a year past expiration. Despite the physical simplicity of the testing process, more than a third had difficulties with the test devices themselves, specifically dysfunction in the device where capillary action of the device did not perform in one or more lanes. However, each dysfunctional device was tested after the subject's completion of the testing, and the devices performed correctly, suggesting subject error. Only 37.5% mentioned abnormal specific gravity or creatinine findings, but they did not associate the values with adulterated specimens and none suggested that their results should not be reported. This was somewhat surprising because the instructions related to these tests seemed particularly clear to us, and the bleach specimen had four different cues for adulteration. The mixed results of subject performance, good in some respects and poor in some respects, is reflected by the mixed feelings of the subjects themselves with 81.5% confident in their findings and 75% who felt that additional testing was necessary.

DISCUSSION

Drug testing for non-medical reasons is increasing, but properly performed drug tests can be costly. Among other things, the federal Substance Abuse and Mental Health Services Administration (SAMHSA) mandatory guidelines specify specimen collection procedures, specimen validity testing, confirmation by gas chromatography/mass spectrometry (GC/MS), medical review officers and proficiency testing for certified laboratories.[4] At this writing, the cost of "10 panel" drug testing that meets SAMHSA criteria to students at our institution is $35.00,[8] and the "10 panel" format of this device ranged in price from $3.99-$10.50[3] depending on the amount purchased. Marketing descriptions such as "Immediate results, simply dip & read results. FDA 510K & CLIA Waived & OTC cleared"[3] and the use of SAMSHA mandated cut-off concentrations in the devices suggest that these devices are designed to assure consumers that they can have both valid and rapid results. Why would anyone spend eight times as much to send a subject to an offsite lab which will provide results in days, not minutes?

There are risks when uninformed consumers use these devices. True positive drug tests indicate use, but not necessarily abuse or impairment. Medical review officers can differentiate illicit drug use from interfering substances and prescribed drugs. Confirmatory testing such as GC/MS minimizes false positives, but it is expensive and time consuming. The literature that comes with the product is clear that confirmatory testing should be performed, but it is written in highly technical language and densely printed pages.[9] We commend the company's website product description, "These screening devices will give you a qualitative result, all positives should be confirmed by an alternative method such as GC/MS (gas chromatograph/ mass spectrometry) by a lab"[3] but the extent to which users will notice or follow this instruction is unknown. American Screening Corporation provides extremely clear simplified instructions[5] that do NOT mention confirmatory testing. That said, a preponderance of subjects in our study recommended additional testing, suggesting that those lacking technical knowledge might seek more definitive testing before taking action based on these drug tests.

False negative drug testing due to limitations of the device or specimen adulteration are equally problematic as they promote a false sense of security and continued failure to identify abusers. Unidentified drug abuse leads to continued negative consequences, delay in treatment, crime and harm from impaired individuals. Abuse of substances that are designed to evade drug tests (synthetic cannabinoids, "bath salts", salvia, etc.) is on the rise[10] and specimen adulterants are easily purchased. Typical consumers are unlikely to be well-informed on the technical aspects of these trends. In our study, we did not specifically ask the subjects if they trusted their negative results, but not their positive results; additional work could be performed to ascertain the extent to which untrained individuals believe both negative and positive results should be confirmed. We

note that an inherent flaw of studying subjects in this manner is that it is an artificial situation, and the subjects could behave differently if they were truly responsible for drug testing that had actual consequences.

No formal laboratory credentials or education are required for waived testing, but a certificate for CLIA-waived testing is required in medical settings and the manufacturer's instructions must be followed. Some effort must be expended with respect to proper personnel training, documentation of quality control and the like.[2] With minimal training, analysts can avoid simple mistakes like using expired materials. Products such as these drug tests, directly marketed to the public at low price points, may be inherently dangerous. The product instructions in this study are not well-designed for untrained users, in our opinion. Although our subjects could understand enough to physically perform the test most of the time, they did not glean enough knowledge to reliably identify problems when they existed, and many did not understand what they had read. Given that all subjects in our study had in excess of three years of college education, our study may underestimate the errors that other untrained individuals could make. In addition, we demonstrated that the devices could easily be purchased without presenting a CLIA certificate, so anyone could easily be performing these tests without properly following the manufacturer's instructions.

The devices themselves performed generally well with respect to interference from the adulterants we tested. Interference by ethanol is likely overstated because we elected to use 190 proof ethanol to detect maximum interference. These devices can also be purchased in formats that detect alterations in pH and the presence of more exotic chemicals marketed specifically as adulterants to include oxidants, pyridinium chlorochromate, nitrites and glutaraldehyde. Additional study could be performed with these expanded devices to see how well they perform in the presence of other adulterants, as well as discovering the reaction of subjects to multiple indicators of adulteration. We note that the cost of the devices is approximately doubled when expanded capability for adulterant detection is included.[3] This increased cost could be a deterrent to consumers, who could opt for the lower cost devices with greater risk of false negatives.

These devices meet requirements from the relevant federal agencies such as the FDA and SAMSHA. In our hands, the devices performed as expected, so we have every reason to believe that they could be reliable when used by a trained analyst. In contrast, we have serious reservations about untrained analysts using these devices in situations that could have negative consequences if erroneous results occurred. We question whether or not these devices meet the CLIA definition of a waived test which is "simple tests with a low risk for an incorrect result."

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