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Evaluation of On-Site Oral Fluid Drug Screening Technology

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16 Abstract

Oral fluid has emerged as a popular matrix for drug detection in criminal justice, workplace, and impaired-driving populations. The advantages of oral fluid compared to blood and urine specimens include that the sample collection is a noninvasive procedure with minimal potential for adulteration. Additionally, oral fluid samples can be collected proximate to the time of driving, allowing for better correlation between signs and symptoms of impairment observed at the time of the arrest as compared to drugs detected in a biological sample collected later. With the increase in popularity of this matrix, several point-of-contact oral fluid devices have been developed and marketed for use in the field without any controlled assessment to evaluate their applicability and quality. The purpose of this evaluation was to explore the practical aspects of designing and performing tests on the latest generation of oral fluid devices to assess their accuracy, reliability and performance to specification. Five devices, the Dräger DrugTest 5000 (DDT5000), Dräger DrugCheck 3000 (DDC3000), Securetec DrugWipe S 5-Panel (DrugWipe), the Alere DDS2 Mobile System (DDS2) and the AquilaScan Oral Fluids Testing Detection System were included in the evaluation. An appropriate scope of testing and cutoff concentrations was based on two important previous studies: the Roadside Testing Assessment (ROSITA), which recommended greater 90% sensitivity and specificity and greater than 95 percent accuracy, and Driving Under the Influence of Drugs, Alcohol and Medicines (DRUID), which recommended greater than 80 percent sensitivity, specificity and accuracy. Based on the summation of all testing performed for each device, the DDT5000, the DDC3000, and each of their individual assays demonstrated performance consistent with the requirements of the ROSITA group. The DDS2 data, in aggregate, also met the performance requirements for ROSITA; however, the THC assay did not. None of the individual assays on the DrugWipe or the AquilaScan met the performance requirement of ROSITA, nor did the performance of either device in aggregate. The DDT5000, DDC3000 and DDS2 in aggregate also met the performance requirements for DRUID.

In its Moving Ahead for Progress in the 21st Century (MAP-21) Act, Congress directed NHTSA to establish a cooperative program — the National Cooperative Research and Evaluation Program (NCREP) — to conduct research and evaluations of State highway safety countermeasures. NCREP was continued in the Fixing America's Surface Transportation (FAST) Act. This program is administered by NHTSA and managed jointly by NHTSA and the Governors Highway Safety Association (GHSA). Each year, the States (through GHSA) identify potential highway safety research or evaluation topics they believe are important for informing State policy, planning, and programmatic activities. One such topic identified by GHSA, an evaluation of on-site oral fluid drug screening devices, formed the basis for this project.

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Glossary of Terms

Accuracy - The degree to which the result of a measurement, calculation, or specification conforms to the correct value or a standard. The overall proportion of subjects whose drug status as determined by a subsequent confirmatory test was correctly predicted by the immunoassay test.

Aliquot - A small portion of sample that is used for testing.

Ampoule - A hermetically sealed small glass vessel used to hold a solution, in this case, the buffer solution.

Analyte - A substance (drug or drug metabolite) being identified and measured.

AquilaScan - An abbreviation for AquilaScan Oral Fluids Testing Detection System, one of the roadside immunoassay testing systems evaluated.

Cross-reactivity - A measure of the extent in which a non-target analyte can react with an immunoassay test and produce a positive result.

Cutoff - The drug concentration threshold at which the immunoassay test should produce a positive test result.

DDC3000 - An abbreviation for Dräger DrugCheck 3000, one of the roadside immunoassay testing systems evaluated.

DDS2 - An abbreviation for AlereDDS2, one of the roadside immunoassay testing systems evaluated.

DDT5000 - An abbreviation for Dräger DrugTest 5000, one of the roadside immunoassay testing systems evaluated.

Drug standard - standard reference material that certifies the purity and strength of a drug.

DrugWipe - An abbreviation for the Securetec DrugWipe S 5-panel, one of the roadside immunoassay testing systems evaluated.

DRUID - An abbreviation for a European Union research project on Driving Under the Influence of Drugs, Alcohols and Medicines, called the DRUID project for short.

Expectorated - Saliva collected by means of spitting.

Exogenous substance - A foreign material that originated outside of the body or a matter that is not a naturally occurring component of the matrix.

Fortifying - The addition of drugs or other material to the matrix.

Free drug - Drugs not bound by plasma proteins and that are able to penetrate into the tissues and cause effects.

Immunoassay test - A biochemical test that uses antibodies to detect the presence of specific molecules or drugs.

Lateral flow immunoassay - A simple type of immunoassay test where sample is applied to one side of a paper or an absorbent strip. The sample then flows from the one side to the other through capillary flow crossing sections coated with testing antibodies.

Matrix/biological matrix - The biological material used in drug-testing analysis. Matrices include blood, urine, oral fluid (spit/saliva), hair, nails, sweat, and breath.

Metabolites - A by-product produced by the body as it breakdowns a drug to help facilitate its elimination. Metabolites are often used to identify prior drug presence in biological matrix.

Negative predictive value (NPV) - Proportion of subjects whose field test correctly predicted they would test negative in the confirmatory test.

Oral fluid - Also known as saliva, a watery fluid produced by the salivary glands of the mouth to aid the body in chewing, tasting, swallowing, and protection from bacterial infection.

Point-of-contact drug testing - Testing performed immediately at the time the sample is collected.

Positive predictive value (PPV) - Proportion of subjects whose field test correctly predicted they would test positive in the confirmatory test.

Protein-bound drug - The degree to which a drug attaches to plasma proteins, which ultimately may enhance or inhibit a drugs performance. Generally, drugs that are highly protein-bound are not able to penetrate into the tissues.

ROSITA - An abbreviation for the European Union project for the Roadside Testing Assessment, ROSITA for short, which evaluated roadside testing equipment.

Sensitivity - The degree of an immunoassay tests ability to correctly detect a positive result and avoid false negative test result. Proportion of subjects who subsequently test positive in a confirmatory test whose positive status was correctly predicted by the immunoassay test.

Specificity - The degree of an immunoassay tests ability to avoid false positive results. The proportion of subjects who subsequently test negative in a confirmatory test whose negative status was correctly predicted by the immunoassay test.

Test cassettes - Plastic encasement that contains lateral flow immunoassay test strips and facilitates the application of sample to the testing strip.

Executive Summary

Background and Objectives

Oral fluid has emerged as a popular alternative matrix for drug detection in criminal justice, workplace, and impaired-driving populations. Drugs are incorporated into the oral fluid primarily via passive diffusion of the free drug that is not bound by proteins in the blood. Drugs that are protein-bound are not active and thus incapable of causing pharmacological effects. The detection windows for many drugs in oral fluid are similar to those in blood. The advantages of using oral fluid specimens over blood and urine is oral fluid can be collected using a non-invasive sample collection technique, which eliminates the need for a collection facility or same-sex observation, and it has minimal potential for adulteration and contamination, all of which help to save time and resources. Furthermore, oral fluid samples can be collected proximate to the time of driving, allowing for better correlation between signs and symptoms of impairment observed at the time of arrest as compared to any drugs detected in a biological sample collected later.

The increased popularity of oral fluid as a biological matrix in drug screening has led to the development of an increasing number of portable oral fluid drug-testing devices designed for use in the field, which vary in applicability and quality. In the oral fluid drug-testing market, there is no program to evaluate the suitability of point-of-contact oral fluid devices for field use.

The purpose of this study was to evaluate field oral fluid drug-testing devices to assess their accuracy, reliability, performance to specification, susceptibility to interference, and resistance of the consumables to extremes of temperature and humidity.

NHTSA's mission is to save lives, prevent injuries, and reduce economic costs due to traffic crashes, through education, research, safety standards, and enforcement activity. In the Moving Ahead for Progress in the 21st Century (MAP-21) Act, Congress directed NHTSA to establish a cooperative program — the National Cooperative Research and Evaluation Program (NCREP) — to conduct research and evaluations of State highway safety countermeasures. NCREP was continued in the Fixing America's Surface Transportation (FAST) Act. Each year, the States (through the Governors Highway Safety Association, GHSA) identify potential highway safety research or evaluation topics they believe are important for informing State policy, planning, and programmatic activities. One such topic identified by GHSA, an evaluation of on-site oral fluid drug screening devices, forms the basis for this project, reflecting the high level of interest by the States.

Methods

The devices selected for evaluation were based on them having an appropriate test for several drug categories including, at a minimum, cannabinoids, opiates, cocaine/metabolite, methamphetamine/ amphetamine, and in some cases methadone or benzodiazepines. All testing was performed using devices that were currently available on the market and purchased in January 2017. The five devices tested were as follows:

- Dräger DrugTest 5000 (DDT5000);
- Dräger DrugCheck 3000 (DDC3000);
- Securetec DrugWipe S 5-Panel (DrugWipe);
- Alere DDS2 Mobile System (DDS2); and
- AquilaScan Oral Fluids Testing Detection System.

The scope of each device and cutoff concentration for each target analyte is shown in ES-Table 1.

ES-Table 1. Drug category assay and cutoff concentration (ng/mL) for each device.

Drug Category/Assay	Oral Fluid Drug-Testing Device				
	DDT5000	**DDC3000**	**DrugWipe**	**DDS2**	**AquilaScan**
THC	5	15‡	5	25	40
Cocaine	20	20	10	30*	20
Amphetamine	50	35	80†	50	50
Methamphetamine	35	35	80†	50	50
Benzodiazepines	15	-	-	20	15
Opiates	20	20	10	40	20
Methadone	20	-	-	-	15

‡DDC3000 offers a THC cutoff of 15 ng/mL or 25 ng/mL depending on the testing procedure used; the procedure providing the more sensitive cutoff was followed throughout the evaluation.
*DDS2 device targets benzoylecgonine instead of cocaine.
†DrugWipe 5S has a combined amphetamine/methamphetamine panel.

Testing was conducted in five phases. *Phase I* evaluated the feasibility of using a synthetic oral fluid matrix that can be made in-house using a published method or one that is commercially available as a possible alternative to using human saliva to conduct device testing. The subsequent phases of testing involved evaluation of devices. *Phase II* evaluated device performance relative to the manufacturers' claimed cutoff concentrations. *Phase III* assessed cross-reactivity of drug metabolites and other therapeutic or abused drugs. *Phase IV* evaluated the possibility for commonly encountered substances such as beverages (orange juice, coffee milk, soda), oral care products, and tobacco to cause interferences. *Phase V* assessed the impact of temperature and humidity on the shelf life of the device consumables, specifically the cassettes that contain the physical panel of testing strips used in the devices to identify the presence of specific drugs.

An appropriate scope of testing and cutoff concentrations was based on two important previous studies using oral fluid drug-testing devices; the Roadside Testing Assessment (ROSITA), which recommended greater than 90 percent sensitivity and specificity, and greater than 95 percent accuracy; and the Driving Under the Influence of Drugs, Alcohol and Medicines (DRUID) project, which recommended greater than 80 percent sensitivity, specificity and accuracy (Schulze et al., 2012; Viviane et al., 1999). The ROSTIA and DRUID studies were the first large scale evaluations of using oral fluid drug testing in the field and recommended performance criteria for oral fluid drug-testing devices designed for use in the field (point-of-contact testing).

Performance in each phase of the study was evaluated for individual drug classes and in aggregate for each device using Receiver Operator Characteristic (ROC) analysis.

Results and Discussion

Overall Performance

Recommendations related to device performance specifications have been previously described in the ROSITA and DRUID projects. ES-Table 2 provides the performance of each of the five devices when aggregating all the scoreable tests from the cutoff, cross-reactivity and environmental testing experiments (*Phases I-V*).

- The DDT5000 and the DDC3000 performances, in aggregate, demonstrated performance consistent with the requirements of the ROSITA group.
- The DDS2 data, in aggregate, met the performance requirements for ROSITA; however, its THC assay did not.
- None of the individual assays on the DrugWipe or the AquilaScan met the performance requirement of ROSITA, nor did the performance of either device in aggregate.
- The DDT5000, DDC3000 and DDS2 met the performance requirements for DRUID.

ES-Table 2. Aggregate performance data for the five devices evaluated using the described protocol.

Overall Device Test Results									
Device	**TP**	**FN**	**FP**	**TN**	**Sensitivity**	**Specificity**	**Accuracy**	**PPV**	**NPV**
DDT5000	886	8	15	1766	99.1%	99.2%	99.1%	98.3%	99.5%
DDC3000	589	17	0	929	97.2%	100.0%	98.9%	100.0%	98.2%
DrugWipe with DrugRead	289	213	3	489	57.6%	99.4%	78.3%	99.0%	69.7%
DrugWipe with Manual Evaluation	451	73	2	466	86.1%	99.6%	92.4%	99.6%	86.5%
DDS2	635	62	4	1306	91.1%	99.7%	96.7%	99.4%	95.5%
Aquilascan	161	581	5	988	21.7%	99.5%	66.2%	97.0%	63.0%

Phase I: *Suitability of human and synthetic oral fluid mixtures*

This study required oral fluid for use in testing the devices. In *Phase I*, we evaluated daily, pooled, expectorated oral fluid from volunteers in the laboratory, which was verified drug-free for the drugs of interest in this study, and was used without freezing or thawing and discarded at the end of the day. Two synthetic oral fluid matrices (OraSure negative calibrator solution and one solution made in-house) were also evaluated. These experiments revealed acceptable performance of the devices at 30 percent above the cutoff concentration for each target drug across all three oral fluid matrices. None of the matrices evaluated produced any issues in terms of compatibility, as there were no invalid test results, and all testing control lines formed as expected. None of the intermittent failures could be conclusively linked to any combination of device, drug analyte, or oral fluid matrix. Overall, there were the fewest inconsistent results with the human saliva.

Based on these considerations, it was decided that subsequent testing would be carried out using drug-free, pooled human saliva collected daily from volunteers as the testing matrix, to avoid any confounding of device performance in different synthetic recipes.

Phase II: Cutoff performance evaluation
The cutoff evaluation consisted of individually testing each drug at the cutoff concentration claimed by the manufacturer, 30 percent below the claimed cutoff concentration, and 30 percent above the claimed cutoff concentration. To perform the evaluations, certified reference materials of the target drugs were purchased from a company that provides drug standards for analytical evaluations. These drug standards were prepared in freshly collected and pooled, drug-free human saliva, by spiking with verified drug standards at 30 percent above, at, and 30 percent below the manufacturers' claimed cutoffs.

The **DDT5000** produced results that yielded greater than 95 percent sensitivity, specificity, accuracy, positive predictive value (PPV) and negative predictive value (NPV) for all analytes on all tests performed during the cutoff evaluations. For all of the cutoff evaluations (30% above, at, and 30% below), the DDT5000 detected 208 out of a possible 210 drug positive samples. The two times positive results were not obtained were at concentrations below the cutoff level. With respect to tests performed specifically at the cutoff concentration or 30 percent above the cutoff concentration, the DDT5000 detected every drug positive sample (n=140).

The **DDC3000**, the only wholly manually-read device tested, demonstrated sensitivity, specificity and accuracy of greater than 95 percent for all assays except for opiates, for which it had sensitivity of 65 percent and accuracy of 95.3 percent during the cutoff evaluations. This reflects false negatives for seven out of the twenty positive challenge samples for opiates. The seven false negatives were at the cutoff concentration of the device. Overall, the device had sensitivity, specificity and accuracy of 93.1 percent, 100 percent, and 98.8 percent.

The **DrugWipe** with the DrugRead demonstrated more variable results, with the THC, cocaine, and opiate assays all having sensitivity of less than 60 percent. Sensitivity for THC at 5 ng/mL was 40 percent. There were many false negatives; 34 false negatives out of 112 samples spiked at the cutoff or 30 percent above the cutoff. This resulted in overall performance of sensitivity of 69.6 percent, specificity of 100 percent, and accuracy of 91.3 percent. The device produced no false positives during the cutoff evaluations.

The **DDS2** demonstrated overall sensitivity, specificity and accuracy of 91.7 percent, 100 percent, and 98.9 percent, respectively in the cutoff evaluations. The device failed to detect THC in 8 out of 20 positive samples 30 percent above (n=4) and at the manufacturer's cutoff of 25 ng/mL (n=4). The device had no false positives during this phase of testing.

The **AquilaScan** demonstrated overall cutoff concentration sensitivity, specificity and accuracy of 37.9 percent, 100 percent, and 91.4 percent, respectively. These results were due in part to the device's failure to detect THC in any of the positive challenges at 30 percent over the claimed cutoff of 40 ng/mL, the highest cutoff of any of the devices evaluated. It similarly failed to detect cocaine, methadone, or benzodiazepines in any of the positive controls. The device did not produce any false positives.

Phase III: Cross-reactivity evaluation

Immunoassay tests are chemical tests that use an immunological reaction. As such, they are not necessarily specific to one analyte. Drugs within the same drug class and that have similar chemical structures could cross-react triggering a positive test result. When a drug in the same class that is not the specific target drug, reacts with the antibody, it may do so to a different (greater or lesser) degree. The degree to which it reacts is called its cross-reactivity. Cross-reactivity to other drugs within the same class is beneficial since it expands the scope of the test. Cross-reactivity to other drugs, chemicals or artifacts that are not of interest is unfavorable, resulting in false positives. The experiments in *Phase III* and *Phase IV* were designed to assess both favorable and unfavorable cross-reactivity of the devices for a range of drugs and other substances. A series of six mixtures of commonly encountered drugs, some of which are desirable cross-reactants in immunoassay tests as they belong to the same drug class as the target analyte and some of which may cause undesirable cross-reactivity (non-targeted drugs) were evaluated.

None of the non-targeted drugs, which included caffeine, nicotine, non-steroidal anti-inflammatory drugs (NSAIDs), over-the-counter analgesics, selective serotonin/noradrenaline reuptake inhibitors (SSRIs/SNRIs), zolpidem, dextromethorphan, lidocaine or PCP, produced false positives on any of the test platforms at concentrations of 1000 ng/mL. Other members of the drug classes to which the devices are targeted showed variable cross-reactivity. Generally, hydrocodone, codeine and hydromorphone were well detected albeit at higher concentrations across all five devices. Oxycodone was not detected at 100 ng/mL and oxymorphone was not detected at 1000 ng/mL. The DDT5000 showed the best sensitivity for opioids at a cutoff of 100 ng/mL. The DDT5000 also showed good responsiveness to the benzodiazepine category consistently giving positive results at 10 ng/mL for nordiazepam and temazepam, and detecting oxazepam one time. The DDT5000, DDC3000, and DDS2 detected MDMA and MDA at concentrations of 100 ng/mL. Generally, these listed drugs and/or metabolites that have some degree of cross-reactivity may be of interest in an impaired-driving context and are included in the drugs of interest in the National Safety Council guidelines.

Phase IV: Interferent evaluations

A series of experiments were run to evaluate the potential for other substances that may be present in the subject's mouth to cause interferences with the various assay platforms. These consisted of running a series of experiments of solutions of beverages (milk, beer, orange juice, soda), oral hygiene products, tobacco and mint-flavored gum. Saliva was mixed with commonly encountered food, drinks or orally ingested products (tobacco, gum, etc.) at a concentration of 5 percent of the total volume (v/v).

In general, chewing tobacco produced frequent false positives and false negative results across all five devices. Coffee, milk, cola and wintergreen mints produced intermittent and inconsistent false positive or false negatives on one device or another, but there was no consistent pattern of interference. Incorporation of a 10-minute waiting/deprivation period as recommended by the manufacturers prior to testing eliminated all of the effects of the potential interferents.

Phase V: Environmental stressing of the test cassettes
The cassettes containing the physical testing strips for each device were subjected to extremes of heat and humidity in environmental test chambers, then returned to the laboratory for evaluation of performance in testing oral fluid samples spiked at 30 percent over the manufacturers' cutoff concentrations, as described in Phase II.

The DDT5000, DDC3000, and DDS2 demonstrated intermittent low frequency false positives and false negatives, as demonstrated in Phase II, but with little impact on the overall performance of the device, which remained unchanged.

The DrugWipe, demonstrated similarly higher rates of false positives and false negatives to its performance in Phase II as it did in Phase IV, and it was not possible to assign the cause of these to the heat and humidity challenges to the consumables.

The performance of the THC assay in the DDS2 demonstrated the largest difference in performance compared to the consumables that had not been subject to the environmental stressors. The sensitivity of the THC assay dropped from 61.9 percent in Phase II to 27.1 percent after the humidity and temperature exposure, with the device failing to detect 43 of 59 positive samples at 30 percent above the cutoff.

Due to the performance of the AquilaScan during Phase II, it was difficult to evaluate the impact of the environmental stressing on this device.

With the increased popularity of oral fluid drug-testing devices designed for use in the field and the lack of any standardized evaluation protocol to evaluate their performance, this study sought to characterize five commercially available devices using several testing parameters. Recommendations related to device performance specifications have been previously described in the ROSITA and DRUID projects (Schulze et al., 2012; Viviane et al., 1999). The DDT5000, the DDC3000, and each of the individual assays demonstrated performance consistent with the requirements of the ROSITA group. The DDS2 data in aggregate also met the performance requirements for ROSITA; however, the THC assay did not. None of the individual assays on the DrugWipe with DrugRead or the AquilaScan met the performance requirement of ROSITA or DRUID, nor did the performance of either device in aggregate. The DDT5000, DDC3000 and DDS2 met the performance requirements for DRUID. It should also be noted that all of the devices we tested are screening devices. Results in field use would still require confirmatory testing.

Background

Oral fluid has recently emerged as a popular alternative matrix for drug detection in criminal justice, workplace and impaired-driving populations. Oral fluid is a combination of gingival crevicular fluid and fluid produced by three primary salivary glands: the parotid, submaxillary and sublingual (Aps & Martens, 2005; Cone & Huestis, 2007), and may also contain other cellular debris and bacteria. Drugs are incorporated into the oral fluid primarily via passive diffusion of free drug (drug not protein-bound) in the blood through the highly perfuse salivary glands. Drugs that are protein-bound are not active and thus incapable of causing pharmacological effects. Several factors affect both the ease of oral fluid collection and the concentrations of drugs in oral fluid. Dry mouth (xerostomia) and decreased salivary flow rate, which can be attributed to either the effects of the drug itself or a lack of proper hydration, can affect sample volumes and require much longer collection times (Aps & Martens, 2005; Cone, 1993; Drummer, 2006). Factors affecting drug concentrations in oral fluid include salivary pH, lipophilicity of drug, protein binding, concentration of unionized drug, pKa, physical size of the molecule, and membrane characteristics (Aps & Martens, 2005; Bosker & Huestis, 2009; E. J. Cone, 1993; Crouch, 2005; Verstraete, 2004). The factor pKa is among the most important factors with basic drugs being preferentially excreted into oral fluid, while acidic and neutral drugs are retained preferentially in the blood.

The detection windows for many drugs in oral fluid are similar to those in blood (Cone, 1993; Samyn et al., 1999; Verstraete, 2004, 2005). Even at lower concentrations, drugs of abuse can generally be detected in oral fluid between 5-48 hours, but can vary widely based on dose, preparation, route of administration, and acute versus chronic use.

The main advantages of oral fluid compared to blood or urine specimens is that oral fluid can be collected using a noninvasive sample collection technique that can be observed, eliminating the need for a collection facility or same-sex observation, and it has minimal potential for adulteration and contamination, all of which help to save time and resources. With respect to impaired-driving drug testing specifically, the major benefit is that the sample can be collected proximate to the time of the driving event, allowing for better correlation between signs and symptoms of impairment observed at the time of arrest as compared to drugs detected in a biological sample collected later. Limitations of oral fluid as a sample matrix include the fact that drug concentrations cannot be related to a specific degree of impairment in the driver, nor can they be used to predict blood drug concentrations, but neither can any other type of test.

The increased popularity of oral fluid as a biological matrix for use in drug screening has led to the development of several portable oral fluid drug-testing devices designed for use in the field. These devices provide the ability to generate presumptive results in the field, which adds another component to the battery of tests administered to help determine impairment. This, in turn, can help validate and correlate findings of standard field sobriety tests (SFSTs) and the Drug Recognition Expert (DRE) program and aid the officer in an arrest decision.

Employing point-of-contact drug testing in conjunction with observations of a trained officer can help strengthen the case and enhance the strength of the evidence for the prosecution of a drug impaired-driving case. Further, the point-of-contact test results can provide a laboratory with presumptive results and direct the subsequent confirmatory testing.

The increasing interest in the use of oral fluid as an alternative biological matrix has led to numerous on-site drug-testing products continually being released and marketed as forensically suitable, but without any published structured assessment of their effectiveness or performance characteristics. Most of the current generation of oral fluid field testing devices are based on lateral-flow immune-chromatographic technology (Ropero-Miller et al., 2009). As such, the results generated indicate the presence of drug classes, as opposed to individual analytes, and are considered presumptive as they may be susceptible to interferences from other substances. As such, an additional specimen should be collected for laboratory-based confirmatory testing using chromatographic and mass spectrometric methods to meet standards for forensic admissibility in criminal casework.

With respect to oral fluid analysis, recommendations related to the scope of analysis and appropriate screening and confirmatory thresholds for oral fluid analysis were recently published by the National Safety Council's Alcohol, Drugs and Impairment Division (NSC-ADID), which were based on prevalence of driver drug use from various surveys and laboratory databases, and readily available laboratory testing technology. The recommendations do not, however, address criteria for field-based testing. Recommendations related to device performance specifications have been previously described in the Roadside Testing Assessment (ROSITA) and Driving Under the Influence of Drugs, Alcohol and Medicines (DRUID) projects. Under the ROSITA project, the recommended performance criteria was greater than 90 percent sensitivity and specificity and greater than 95 percent accuracy (Viviane et al., 1999). The DRUID project required greater than 80 percent sensitivity, specificity and accuracy (DRUID, 2010; Schulze et al., 2012). The ROSTIA and DRUID studies were the first large scale evaluations of using oral fluid drug testing in the field and recommended performance criteria for oral fluid drug-testing devices designed for use in the field (point-of-contact testing). Sensitivity (true positive rate) refers to a positive confirmatory result that was correctly predicted by the field test. Specificity (true negative rate) refers to a negative confirmatory result that was correctly predicted by the field test, and accuracy refers to the overall proportion of confirmatory tests that were correctly predicted by a field test. At the time of the studies, none of the devices available on the market met the specified criteria for all target analytes, and none of the tested devices in either study were recommended for law enforcement use. Several of the devices, some of which are included in this study, have been evaluated as part of on road studies (Desrosiers et al., 2014; DRUID, 2010; Edwards et al., 2017; Ellefsen et al., 2016a; Gentili et al., 2016; Krotulski et al., n.d.; Logan et al., 2014; Moore et al., 2013; Musshoff et al., 2014; Rohrig et al., 2017; Scherer et al., 2017; Schulze, Schumacher, Urmeew, & Auerbach, 2012; Veitenheimer & Wagner, 2017; Viviane et al., 1999), but there currently is no published literature related to a systematic laboratory-based evaluation.

The United Kingdom has developed A Guide to Type Approval Procedures for Preliminary Drug Testing Devices (Centre for Applied Science and Technology, 2013). The document includes specifications related to the required scope, visualization of the results, minimum requirements related to cutoff performance, and device features, among others. Further, the document details that all test solutions are required to be made up in an artificial oral fluid matrix. In the United States, there is no governing document specifying the battery of testing that should be carried out, number of replicates, or required sensitivity, specificity or accuracy for an oral fluid screening device to be considered reliable for use by law enforcement.

The testing described in this document was designed to assess some of the latest field oral fluid drug-testing devices available and evaluate their sensitivity and specificity, and detection thresholds. A series of laboratory testing steps including performance relative to manufacturers' specifications, detectability of common drugs of abuse, therapeutic compounds and drug metabolites, evaluation of potential interferences and the impact of environmental factors were all included as part of the assessment. In addition, characteristics such as time spent administering the test, ease of use, portability, and robustness were also evaluated. A review of the protocol deployed, suggestions for improvement, and the utility for the described method to be formalized into an assessment all oral fluid screening devices would be evaluated against is also provided at the conclusion of this document.

Objective

As there is no Federally approved process for the evaluation of oral fluid roadside tests to evaluate their precision, accuracy, robustness and overall usability in a law enforcement setting, the current study had to design a series of experiments to objectively evaluate oral fluid devices per those factors. Based on these goals, the following objectives were adopted:

- Evaluate the feasibility of using synthetic oral fluid that is either commercially available or can be made in-house using a published method, compared to expectorated human saliva

- Assess device performance relative to the cutoff levels published by the manufacturer and the ability of the devices to produce accurate results when more than one drug is present in the saliva

- Investigate the cross-reactivity of drugs known to interfere with the performance of immunoassay-based tests

- Evaluate the possibility for commonly encountered substances such as foodstuffs, oral care products, and tobacco that might cause interferences

- Assess the impact of environmental factors, such as temperature and humidity, on the shelf life of the device consumables, the cassettes containing the panel of strips used in the devices to identify the presence of specific drugs, for the test.

- Document issues related to usability, robustness, manufacturing quality or defects, ease of use and readability, and other qualitative factors.

In summary, the goal was to empirically evaluate commercially available oral fluid roadside testing devices using a protocol that would allow for the device performance to be assessed based on the manufacturer's instructions and published sensitivity statements. The project also provided an objective set of testing criteria that could be used to assess available roadside testing devices for their suitability for use in the investigation of drug impaired-driving cases.

Device Selection

Priority features identified for oral fluid drug-testing devices designed for police use include the need for it to be portable, easy to use in field conditions, minimize the amount of time spent administering the test, provide results that can be easily interpreted and not subject to interpretation, and be confirmable by a subsequent toxicological test (Arntson et al., 2013; DRUID, 2009; Logan et al., n.d.).

Additional considerations included selecting devices that are currently being marketed to or used by law enforcement, as well as the scope of the test being consistent with drugs routinely encountered in a driving under the influence of drugs (DUID) environment. With increased interest in the use of oral fluid as a matrix for drug testing, several of the commercially available devices encountered online and sold to law enforcement and the public are simply relabeled urine drug-testing devices, targeting drug metabolites instead of the parent drug, typically with unrealistically high cutoffs, but being represented as oral fluid drug-testing devices. For this study, we selected a mix of manually/visually read tests and instrumented devices.

All testing was performed using devices that were currently available on the market and purchased in January 2017. The following devices were selected for analysis during this assessment: Dräger DrugTest 5000 (DDT5000), Dräger DrugCheck 3000 (DDC3000), Securtec DrugWipe S 5-Panel (DrugWipe), the Alere DDS2 Mobile System (DDS2) and the AquilaScan Oral Fluids Testing Detection System. In addition to these devices meeting the practical needs described for field use, the selected devices had stated performance capabilities for detecting the levels of drugs typically found in oral fluid (Logan et al., n.d.). The testing panel published by the manufacturer for each device and cutoff concentration are shown in Table 1. Details on each of these devices, including photographs and descriptions of how they operate, are provided in **Appendix A**.

Table 1. Drug category/assay and cutoff concentration (ng/mL) for each device.

Drug Category/Assay	Oral Fluid Drug-Testing Device				
	DDT5000	**DDC3000**	**DrugWipe**	**DDS2**	**AquilaScan**
THC	5	15‡	5	25	40
Cocaine	20	20	10	30*	20
Amphetamine	50	35	80†	50	50
Methamphetamine	35	35	80†	50	50
Benzodiazepines	15	-	-	20	15
Opiates	20	20	10	40	20
Methadone	20	-	-	-	15

‡DDC3000 offers a THC cutoff of 15 ng/mL or 25 ng/mL depending on the testing procedure used; the procedure providing the more sensitive cutoff was followed throughout the evaluation.
*DDS2 device targets benzoylecgonine instead of cocaine.
†DrugWipe has a combined amphetamine/methamphetamine panel.

DDT5000
The Dräger DDT5000 is a seven-panel test, testing for amphetamine, methamphetamine, cocaine, opiates, benzodiazepines, delta-9-tetrahydrocannabinol (THC) and methadone. The DDT5000 consists of four main parts, the analyzer, printer, keyboard and test cassette that contains the sample collector. Device dimensions and features can be found in Table 2. The instructions state the user must remove the safety cap from the collector and sample buffer, place the sample collector into the mouth and move it between the gums and cheek. When sufficient volume is collected, an adequacy indicator turns blue to signal that enough sample (approximately 150-200 µL) has been collected. Upon powering on the analyzer, the instrument goes through a series of self-checks, and once completed, the test cassette and buffer cartridge can be inserted into the analyzer. Prior to the start of the analysis, the expiration date of the cassette is verified by the system. In the event the expiration date has passed, the system will warn the user and advise the operator against using the expired cassette; however, continued use is at the discretion of the operator.

Analysis for the device is completed automatically. The system controls and monitors the addition of the buffer, the time of analysis, and the internal temperature. While the testing is in progress, the DDT5000 allows the operator to enter information regarding the test subject. The data collection includes the following fields: last name, first name, date of birth, and address, as well as operator fields with ID code, cause for test, location, and a comment field. The testing is complete in eight minutes. Results are initially displayed on the instrument as positive or negative and can be printed and stored (the device can store up to 500 test results). Printed results include any information that was entered into the system as well as the date of analysis and the lot number and expiration date of the test cassette used in the analysis. Additional information related to the devices operation and pictures can be found in Appendix A.

DDC3000
The Dräger DDC3000 is a device that is manually read (i.e. the operator must visually examine and interpret the test strip results). This device tests for amphetamine, methamphetamine, cocaine, opiates and THC. The DDC3000 consists of a test cassette that contains the sample collector. The DDC300 test cassette contains an internal ampoule of buffer and two test strips. Samples are collected by inserting the sample collector into the mouth and gently moving it between the cheek and gums. A pink adequacy indicator, initially present prior to collecting the sample, fades away after approximately 15-30 seconds to indicate a sufficient volume has been collected. The DDC3000 collects approximately 300 µL of sample. Additional details related to the device can be found in Appendix A.

Once the sample is obtained, the collector is inserted into the test cassette. Through the insertion process, the ampoule containing the buffer is broken, releasing the buffer. Following this, the cassette must be vigorously shaken for 30 seconds to mix the buffer and samples. After mixing, it is recommended that the cassette sit upright for 10 to 60 seconds (10 seconds is recommended for rapid THC results with a cutoff at 25 ng/mL, 60 seconds is recommended for more sensitive THC results with a cutoff at 15 ng/mL). As per the instructions, a sample is negative with the appearance of a testing line and control lines. If after 5 minutes a testing line has not appeared, but the control lines are present, the sample is considered positive. All results must be manually evaluated within 10 minutes of starting the test.

DrugWipe
The Securetec DrugWipe is a lateral flow immunoassay-based test for drugs present in a sample typically collected by wiping the tongue with an absorbent pad, and eluting the sample off with a buffer contained in the device onto the test strip. The DrugWipe is then visually read by checking for the presence of colored lines in the read window. The DrugWipe test cassette can also be read using the DrugRead analyzer with an attached printer. Positive and negative controls are provided with the DrugRead analyzer, and it is recommended they are run once per day when the device is being used, but it is not required. Additional features of the DrugWipe with DrugRead are described in Appendix A. Dimensions of the device are reported in Table 2. The DrugWipe tests for amphetamines (according to the manufacturer this includes methamphetamine), cocaine, opiates and cannabinoids. While amphetamine and methamphetamine are both considered target analytes, the device does not differentiate between them, as it provides only one shared test line for the two drugs. The test cassette contains a plastic cover, sample collector (which is removable from the cassette), test strips and buffer. To collect sample, the person simply wipes the pads across the tongue of the test subject. When enough sample has been collected, the pads will change color from pink to yellow collecting approximately 15 microliters (μL) of sample.

Once the sample has been collected, the sample collector is clicked back into place on the test cassette, and the ampoule containing the buffer must be broken by pressing where the test cassette is labeled, "PRESS." The instructions provided with the DrugRead lists two different approaches at this point. One is to lay the test cassette flat, insert it into the DrugRead analyzer, and press where the cassette is labeled "PRESS" to break the ampoule. The second procedure is to hold the test cassette vertically with the ampoule at the bottom, press where the cassette is labeled "PRESS" to break the ampoule, and to continue to hold the test cassette in the vertical position for 10 seconds, which is what is illustrated on the test cassette foil packaging. Once the cassette is inserted into the analyzer, the operator must select the correct method and manually start the run by pressing start. During development, the test cassette should be kept level and horizontal. The DrugRead analyzer is equipped with a sensor and will alert the operator if the system is not level. After 5 minutes, the results are displayed on the DrugRead screen and followed by a series of questions related to the subject including: name, ID, license number, birthdate, and address, as well as fields for the input of the operator ID, location, and a comment field. The DrugRead can store up to 1,000 results, and they can be downloaded to a personal computer.

DDS2
The Alere DDS2 is a handheld system consisting of the analyzer, printer, test cassette and sample collector. The system details are provided in Table 2. The DDS2 tests for amphetamine, methamphetamine, cocaine, opiates, benzodiazepines, cocaine and THC. Samples are collected by swabbing the inside of the subject's cheeks, gums, and tongue. Once enough sample has been collected the adequacy indicator turns blue, collecting approximately 450 μL of sample. Prior to the analysis of samples, a positive and negative quality control cassette can be analyzed allowing the operator to verify the device is capable of correctly interpreting positive and negative results. These controls are provided with the kit, but are not required to be run as part of the analysis process. It is recommended that the quality controls are run once each day the analyzer is used. Additional device details are provided in Appendix A.

Prior to analysis, the test cassette is inserted into the analyzer followed by the insertion of the collector into the test cassette, which releases the buffer and begins the analysis. The DDS2 analysis is completely automated. Testing is completed in 5 minutes. The DDS2 also contains sensors that alert the operator if the analyzer is not held or placed on a level surface or if the environmental conditions exceed the analyzers operational range of 41°F-95°F. Results are digitally displayed on the analyzer, after which users are prompted to enter information related to the subject (age and gender), reason for the test (pre-employment, random, post-accident/incident, and for cause/intercept) and vehicle type (car, goods vehicle/truck, motorcycle, and other). The questions included in the questionnaire can be edited through optional software. Results then can be printed or stored. The DDS2 can store up to 10,000 test results, which can be downloaded onto a personal computer.

AquilaScan

The AquilaScan is a handheld device consisting of the analyzer, cassette and sample collector, and integrated printer. Additional features are discussed in Appendix A. Device dimensions can be found in Table 2. The device tests for amphetamine, methamphetamine, cocaine, opiates, benzodiazepines, cannabinoids and methadone. Samples are collected by swabbing the inside of the subject's cheeks, gums and tongue. An adequacy indicator turns red when a sufficient volume of sample has been collected (approximately 1.5 mL). Prior to the analysis of the sample, the device prompts the operator for data related to the test including information about the operator and subject, such as name, license plate number, date of birth, test types and remarks. Additionally, the barcode on the test cassette must be scanned prior to the analysis to prevent the use of an expired test kit.

The sample collector is inserted into the test cassette, which releases the sample into the cassette. A paper disk is provided with the kit to help prevent splashing. After insertion of the collector into the test cassette, the sample migrates up the test strip. As per the manufacturer's instruction, the control lines must be developed before the cassette is inserted into the analyzer, which takes approximately 3 to 5 minutes. The remaining portions of the test are completed within 5 minutes, resulting in an 8- to 10-minute total test time. Results are displayed on the screen alongside a picture of the test strip. The instructions state that if the visual results do not match the results on screen, the operator should use the visual results as observed by the human eye, and any presence of a test line, no matter how faint, is a negative result. The analyzer can store up to 100,000 results. The stored results include the text display along with the picture of the test lines. The results can be down loaded to a personal computer through a USB port with separately sold software.

Table 2. Analyzer dimensions, weight, and features.

Device	Length	Width	Height	Weight	Printer	Keyboard	Combined Weight (with printer and keyboard)
DDT5000	20 cm	22 cm	25 cm	4500 g	Separate	Separate	5568 g/12 lbs
DDS2	22.2 cm	8.9 cm	6.4 cm	680 g	Separate	Integrated	1133 g/2.5 lbs
DrugRead	21 cm	10 cm	12 cm	800 g	Separate	Touch Screen	993 g/2.1 lbs
AquilaScan	20.5 cm	9 cm	5.5 cm	476 g	Built-in	Touch Screen	476 g/1 lb

The DDC3000 was not included in Table 2 as it is only the test cassette itself. It has no analyzer.

Methods

In order to evaluate each of the stated objectives outlined above, several sets of experiments were carried out. For all testing, the following designations were made: Positive results were recorded as true positives (TP) if the analyte was present in the aliquot (representative of the sample) and detected by the device, irrespective of its concentration, that is to say the devices were not penalized for having better than advertised performance. The justification for this approach was our interest in assessing the devices' ability to identify confirmable positives (even if they were detected by the device at 30 percent below the claimed cutoff). With all negative results, the concentration of each drug in the aliquot was compared to the manufacturer's advertised cutoff concentration and designated as a true negative (TN) or false negative (FN) relative to that cutoff. In the case of a positive result, when the analyte was not present in the aliquot, the result was designated as a false positive (FP).

The analytical performance of each device was evaluated by drug class and by how the device performed around its stated cutoff concentration calculated using Receiver Operator Characteristic (ROC) analysis. The following were calculated for each experiment.

- Sensitivity (TP/(TP+FN))
- Specificity (TN/(TN+FP))
- Accuracy ((TP+TN)/(TP+TN+FP+FN))
- (PPV) positive predictive value (TP/(TP+FP))
- (NPV) negative predictive value (TN/(TN+FN))

The experiments are detailed below.

Phase I: Oral Fluid Matrix Evaluations

The first phase evaluated the ability to use synthetic oral fluid for the device evaluations to follow. Two synthetic oral fluid solutions were tested through a series of experiments. A synthetic negative oral fluid calibrator solution was purchased from OraSure Technologies, Inc. (Bethlehem, PA). The second synthetic matrix was made in-house using the United Kingdom's guidelines for type-approval testing (Centre for Applied Science and Technology, 2013). The mixture was prepared using the ingredients listed in Table 3. All supplies were purchased from Sigma Aldrich (St. Louis, MO). The components were weighed on an analytical balance and prepared in one liter of deionized water (DI).

Table 3. Ingredients and concentrations used to make an in-house synthetic oral fluid.

Component	Concentration (mg/L)
Potassium Chloride	1360
Bovine Mucin (from sub-maxillary glands)	1300
Potassium Hydrogen Phosphate	950
Sodium Chloride	860
Sodium Azide	500
Sodium Hydrogen Carbonate	440
Potassium Thiocyanate	250
Calcium Chloride	210
Urea	180
Magnesium Chloride	60

Authentic drug-free oral fluid was collected from volunteers in our laboratory using Sarstedt salivettes saliva collection tubes (Sarstedt AG & Co., Nümbrecht, Germany). Volunteers were instructed to insert the plain cotton swab into their mouth and gently chew on the swab for 5 minutes or until the pad was saturated. The cotton swab was then placed back into the collection container and centrifuged at 3,800 rpm for 8 minutes. The residual oral fluid collected was pooled and used only in testing on the day the samples were collected. The pooled oral fluid was verified to be negative for target drug classes via liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF) and liquid chromatography tandem mass spectrometry (LC-MSMS).

The technician applied the oral fluid sample slowly to the collector of each device using a Pasteur pipette to drop the saliva onto the sorbent pad of the collector. The entire surface was evenly wetted until the adequacy indicator changed color indicating a sufficient volume had been collected for each of the devices. Any excessive drips were gently shaken off. The amount added to each collector was not controlled, which was intentional to best mimic how samples would be collected in a real-world setting.

To evaluate the matrices, an aliquot of each matrix was spiked with a mixture of target analytes at 30 percent above the device cutoff. Amphetamine was spiked into a separate aliquot to avoid any potential cross-reactive reaction with the methamphetamine test. Each aliquot was run in triplicate for a total of six tests per type of device. All testing was also split evenly across a pair of devices to counter any instrumental bias or failure. Certified drug standard reference materials were purchased from Cerilliant Corp. (Round Rock, TX), and subject to LC-QTOF analysis to verify identity prior to being used in analysis.

Phase II: Cutoff Evaluations

The cutoff evaluation consisted of two parts. The first part was to test individually each drug at the cutoff concentration claimed by the manufacturer, 30 percent below the claimed cutoff concentration, and 30 percent above the claimed cutoff concentration.

Oral fluid was collected using the procedures described above. Samples were prepared by fortifying the oral fluid to contain a single target analyte at the desired concentration. Each target analyte was tested for 10 replicates at each level, which was split across two units of each device included in the evaluation to counter instrumental bias or failure, initially starting at 30 percent above the stated cutoff concentration (Table 4). Testing proceeded to the next lower concentration only if the device detected the target analyte.

Table 4. Concentrations (ng/mL) used for the cutoff concentration evaluation.

Drug Category/Assay	DDT500			DDC3000			DrugWipe			DDS2			AquilaScan		
	Cutoff	***+30%***	***-30%***	***Cutoff***	***+30%***	***-30%***	***Cutoff***	***+30%***	***-30%***	***Cutoff***	***+30%***	***-30%***	***Cutoff***	***+30%***	***-30%***
THC	5	6.5	3.5	15	19.5	10.5	5	6.5	3.5	25	32.5	17.5	40	52	28
Cocaine/ Metabolite*	20	26	14	20	26	14	10	13	7	30*	39*	21*	20	26	14
Amphetamine	50	65	35	35	45.5	24.5	80†	105†	56†	50	65	35	50	65	35
Methamphetamine	35	45.5	24.5	35	45.5	24.5	80†	105†	56†	50	65	35	50	65	35
Benzodiazepines	15	19.5	10.5	-	-	-	-	-	-	20	26	14	15	19.5	10.5
Opiates	20	26	14	20	26	14	10	13	7	40	52	28	20	26	14
Methadone	20	26	14	-	-	-	-	-	-	-	-	-	15	19.5	10.5

*DDS2 device targets benzoylecgonine instead of cocaine.
†DrugWipe has a combined amphetamine/methamphetamine panel.

The second phase of the evaluation consisted of running a series of mixed drug controls at varying concentrations consistent with concentrations reported in the literature (Bosker & Huestis, 2009; Ellefsen et al., 2016a, 2016b; Krotulski et al., n.d.; Langel et al., 2014; Nordal et al., 2015). This phase of testing was performed blind, meaning that the technician had no knowledge of what drugs were in each mix at the time of testing. A series of nine controls were spiked with mixtures of three to six target analytes at various concentrations (Table 5). An additional positive control containing all target analytes at a concentration of 100 ng/mL and a negative control (blank oral fluid) were run during the analysis. Because the DDS2 targets benzoylecgonine and temazepam, rather than cocaine and diazepam targeted by all of the other devices, separate controls were prepared using these target analytes for the DDS2.

Table 5. Concentrations (ng/mL) used for the mixed drug blind control evaluation.

	Mixed Drug Controls										
Drug	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**Positive**	**Negative**
THC	80	150		20	2	5	-	40	1000	100	0
Cocaine*	-	1000	30		80	-	400	50	10	100	0
d-Amphetamine	1000	60		10	160	50	-	300	40	100	0
d-Methamphetamine	-	-	1000	300	-	20	90	50	-	100	0
Diazepam**	40	-		60	5	30	10	7	20	100	0
Morphine	-	40	90	500	-	80	10	-	30	100	0
Methadone	-	80			200	1000	10	400	15	100	0

*Benzoylecgonine used for analysis on the DDS2.
**Temazepam used for analysis on the DDS2.

Phase III: Cross-Reactivity Evaluations

A series of cross-reactivity experiments were run for each device to see if substances other than the targeted analyte may also be reported as positive results. Common drug metabolites, other therapeutic drugs, and drugs known to cross-react on immunoassay tests were prepared into a series of five controls (Table 6). An additional sixth control was prepared using common drugs unlikely to cross-react (Table 6). Each control initially contained all analytes at a concentration of 1,000 ng/mL and was evaluated in triplicate across the pairs of devices. If any of the analytes produced a positive result at the initial 1,000 ng/mL concentration for any of the assays, the concentration of the drug was decreased by a factor of 10 until a negative result was obtained.

Because DDS2 targets temazepam and benzoylecgonine while the other devices targeted diazepam and cocaine, separate mixtures of Mix 5 were prepared. Mix 5A was used during evaluations with the DDT5000, DDC3000, DrugWipe, and AquilaScan devices and contained L-methamphetamine, hydromorphone, cannabidiol, temazepam, and benzoylecgonine. Mix 5B was spiked with L-methamphetamine, hydromorphone, cannabidiol, diazepam, and cocaine, and was only run on the DDS2.

Table 6. Mixed drug controls used in the cross-reactivity evaluations.[1]

MIX 1
MDMA (3,4-methylenedioxymethamphetamine)
Alpha-hydroxyalprazolam
THC-COOH (11-nor-delta9-THC-COOH)
Hydrocodone
EDDP (2-Ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine)
MIX 2
MDA (3,4-methylenedioxyamphetamine)
Codeine
7-aminoclonazepam

[1] Heroin was not evaluated as part of the testing protocol due to its rapid metabolism in the body. Fentanyl was not evaluated as part of the testing protocol as fentanyl is known not to cross-react on immunoassays targeted to morphine.

MIX 3	
Pseudoephedrine	
Nordiazepam	
Oxymorphone	
MIX 4	
l-Amphetamine	
Oxazepam	
Oxycodone	
MIX 5 (A)	**MIX 5 (B, DDS2 only)**
L-methamphetamine	L-methamphetamine
Hydromorphone	Hydromorphone
Cannabidiol	Cannabidiol
Temazepam	Diazepam
Benzoylecgonine	Cocaine
MIX 6	
Dextromethorphan	
Caffeine	
Nicotine	
Tramadol	
Acetaminophen	
Diphenhydramine	
Salicylic acid	
Naproxen	
Ibuprofen	
Pentobarbital	
Zolpidem	
Fluoxetine	
Sertraline	
Lidocaine	
PCP (Phencyclidine)	

Phase IV: Interferent Evaluations

The interferent evaluation studies consisted of running a limited series of experiments. Saliva was mixed with commonly encountered food, drinks, or orally ingested products (e.g., tobacco, gum, etc.) at a concentration of 5 percent of the total volume (v/v) (Table 7). Two series of experiments were performed in duplicate. One experiment contained the interferent with the target analytes at 50 percent above the published cutoff concentration to evaluate suppression of a positive signal, and the second experiment evaluated oral fluid samples with only the potential interferent to evaluate the risk of false positives.

Table 7. Substances used for the interference studies.

INTERFERENCE PART 1	INTERFERENCE PART 2
Beer (Heineken)	Toothpaste (Colgate: sensitive enamel protect)
Methanol	Chewing tobacco (Skoal: classic straight long cut)
Orange juice	Spearmint gum (Wrigley's)
Milk (2% fat)	Peppermint mints (Altoids)
Mouthwash (Crest Pro-Health)	Wintergreen mints (Altoids)

INTERFERENCE PART 1	INTERFERENCE PART 2
Soda (Pepsi)	
Coffee (Dunkin Donuts, straight black)	

Phase V: Environmental Testing Evaluations

Since oral fluid drug-testing devices are subject to wide ranges of temperature and humidity in roadside traffic enforcement conditions, it was important to evaluate how this effected the devices' performance. A total of 93 test cassettes for each device were shipped to Cincinnati Sub-Zero (known as CSZ, a subsidiary of Weiss Technik North America, Inc., Cincinnati, OH) where simulations of various environmental storage conditions were performed. A full test report from CSZ can be found in Appendix B. The conditions simulated were various static temperatures ranging from 0°F to 100°F in 10-degree increments and various relative humidity levels of 25, 50, 75, 85, and 95 percent were also simulated. Each humidity level was replicated at temperatures of 25, 50, 75, and 100°F. For each environmental condition, three packaged test cassettes from each device were exposed to the conditions over a 2-hour ramp to stabilization, an 8-hour hold, and a 2-hour ramp to ambient conditions. The packaged test cassettes were then shipped back to the Center for Forensic Science Research and Education (CFSRE, Willow Grove, PA) for testing. For the three cassettes stored at each condition, two were tested with a positive sample mix of target analytes at +30 percent above the target cutoff concentration. The third test cassette was run with a negative sample to evaluate if any of the environmental conditions yielded any false positive results.

The total number of possible tests are reflected in Table 8. Any test that produced an invalid result would decrease the totals as they were not included in the ROC analysis. By design, devices that had a larger scope had a greater number of possible test results. Devices that were challenged below the cutoff level would have an increased number of total tests possible.

Table 8. Summary of the number of tests per phase of the evaluation.

Device	Matrix Evaluation	Cutoff Evaluation	Interference Evaluation	Environmental Testing	Total Cassettes	Test per Cassette	Total Tests
DDT5000	6	221	64	93	384	7	2688
DDC3000	6	161	48	93	308	5	1540
DrugWipe	6	101	48	93	248	4	992
DDS2	6	191	53	93	343	6	2058
Aquilascan	6	101	48	93	248	7	1736

Results and Discussion

Phase I: Oral Fluid Matrix Evaluations
To evaluate the matrices, each matrix was spiked with a mixture of target analytes as indicated in Table 4. THC was tested at 26 ng/mL and amphetamine/methamphetamine were tested at 78 ng/mL on the DrugWipe. The DDC3000 was evaluated using 75 ng/mL for methamphetamine and amphetamine. During the analysis, racemic mixtures were used for the amphetamine and methamphetamine standards. Results were evaluated accordingly.

The importance of this evaluation was to ascertain if it was possible to use synthetic fluid. Using a synthetic fluid would eliminate the need to verify that saliva collected from human subjects was drug free. Daily collection of human saliva also adds time due to the need to collect a sufficient volume for testing and increases the overall costs related to the overall project. Generally, results from the oral fluid evaluation were comparable across all three oral fluid matrices (OraSure negative calibration fluid, the United Kingdom recommended synthetic recipe, and verified drug-free human saliva) on all devices. None of the matrices evaluated produced any issues in terms of compatibility, as there were no invalid test results, and all testing control lines formed as expected. None of the intermittent failures could be conclusively linked to any combination of device, drug analyte, or oral fluid matrix. Summary data is provided in Appendix C.

The ***DDT5000*** had 100 percent sensitivity and 100 percent specificity using either the OraSure negative calibration fluid or the United Kingdom recommended synthetic recipe. One false positive result was obtained for THC in the verified drug-free human saliva, resulting in 100 percent sensitivity, 95.2 percent specificity and 97.6 percent accuracy in this matrix across all test panels.

The ***DDC3000*** yielded all true positives and all true negatives for an overall 100 percent for sensitivity, specificity and accuracy in all three matrices.

The ***DrugWipe*** with the DrugRead produced sensitivity, specificity and accuracy of 100 percent when tested using human saliva. When tested using the synthetic recipes, there was one false negative result for opiates with the U.K. synthetic recipe lowering the overall sensitivity to 88.9 percent, accuracy to 96.7 percent and the negative predictive value (NPV) to 95.5 percent. The DrugWipe with the DrugRead results were similar when evaluated using the OraSure fluid but had an additional false negative for THC and opiates resulting in 83.3 percent sensitivity, 92.6 percent accuracy, and 88.2 percent NPV.

The ***DDS2*** results were the same across the three matrices. The DDS2 calculated sensitivity and specificity was 100 percent for cocaine, opiates, benzodiazepines, and methamphetamine in all three matrices. However, with respect to THC, there were false negative or false positive results in each of the three matrices. When tested with human saliva, the device produced three false negative results. When testing using the U.K. synthetic recipe, there were two false negative results. However, with the OraSure negative calibration fluid there were three false positive THC results across the two DDS2 devices. The spiking mix was verified to be negative for THC by

LC-MSMS and LC-QTOF analysis. Additional THC samples were run with the OraSure negative calibration fluid and upon reanalysis the results were correctly identified as negative.

The ***AquilaScan*** produced inaccurate results when tested using both human saliva and the U.K. synthetic recipe. With both matrices, the AquilaScan failed to detect a single positive result with 15 false negatives resulting in a 0 percent overall sensitivity and accuracies of 64.3 percent. For the OraSure negative calibrator fluid, the AquilaScan detected amphetamine and opiates, which improved overall sensitivity of 36.3 percent, but failed to detect the remaining drugs resulting in 12 false negatives and an overall accuracy of 71.4 percent.

With the limited number of tests performed for each matrix type, the data did not lend itself to statistical analysis to confirm if differences in performance were related to the matrix itself, versus random failures of individual consumables. Based on these considerations in addition to the feasibility of acquisition, cost and being able to attribute performance solely to the device and not the matrix, it was decided that subsequent testing would be carried out using drug free, pooled human saliva collected daily from volunteers as the testing matrix, to avoid any confounding of device performance in different synthetic recipes.

Phase II: Cutoff Evaluations

DDT5000

The DDT5000 produced results that yielded greater than 90 percent sensitivity, specificity, accuracy, PPV and NPV for all test performed during the cutoff evaluations. For all of the cutoff evaluations (30% above, at, and 30% below), the DDT5000 detected 208 out of a possible 210 drug positive samples. With respect to tests performed specifically at the cutoff concentration or 30 percent above the cutoff concentration, the DDT5000 detected every drug positive sample (n=140).

It was observed that at 30 percent below the cutoff concentrations, the DDT5000 still produced positive results for the corresponding drug classes spiked in the samples. The data demonstrated that the DDT5000 performed above its advertised performance, and that the device has greater sensitivity for the target analytes than its specified cutoff concentrations across all seven tests.

Both negative results recorded were for THC at 30 percent below the advertised cutoff; however, they were not scored as false negative results as they were both 30 percent below the cutoff concentration. The DDT5000 was the only device to produce false positive results during this stage of testing, which included three false positive THC results and one false positive benzodiazepine result. Out of the 1,470 tests performed on the DDT5000, the four false positives resulting in a false positivity rate of less than 0.5 percent. The combined results for the DDT5000 cutoff evaluation are shown in Table 9. The calculated specificity for the DDT5000 was 98.4 percent or greater across all test analytes and 99.7 percent overall.

Due to the fact that the device is a seven-panel test, more analyses were performed on the DDT5000 than any other device. For the cutoff comparison, 1,470 test results were recorded for the DDT5000. Of note, the DDT5000 had two invalid test cassettes out of the 212 test cassettes used during this phase of the evaluations, both of which were due to control line failures on one

or two of the seven analyte panels. In these cases, the whole cassette was considered invalid and the results discarded. The tests were then resampled and repeated with another test cassette.

Table 9. DDT5000 cutoff evaluation results combined for all testing performed.

DDT5000 Cutoff Evaluation Results									
Drug	**TP**	**FN**	**FP**	**TN**	**Sensitivity**	**Specificity**	**Accuracy**	**PPV**	**NPV**
THC	28	0	3	179	100.0%	98.4%	98.6%	90.3%	100.0%
Cocaine	30	0	0	180	100.0%	100.0%	100.0%	100.0%	100.0%
Amphetamine	30	0	0	180	100.0%	100.0%	100.0%	100.0%	100.0%
Methamphetamine	30	0	0	180	100.0%	100.0%	100.0%	100.0%	100.0%
Benzodiazepines	30	0	1	179	100.0%	99.4%	99.5%	96.8%	100.0%
Opiates	30	0	0	180	100.0%	100.0%	100.0%	100.0%	100.0%
Methadone	30	0	0	180	100.0%	100.0%	100.0%	100.0%	100.0%
Overall	208	0	4	1258	100.0%	99.7%	99.7%	98.1%	100.0%

DDC3000

As this was the only device tested that was only manually read, the interpretation of the results was based on the operator performing the testing. With respect to the test lines formed, the THC line was the hardest test line to interpret as the line was often lighter than the other test bands, slower to form and continued to form or darken if evaluated outside of the instructed evaluation time. Additionally, the opiate line was also difficult to identify (see Figure 1), and there were seven false negatives at the cutoff concentration and the lowest sensitivity of all test analytes at 65 percent. According to the manufacturer's instructions, any perceived test line on the DDC3000 is to be evaluated as a negative. All of the false negative results were very faint lines that could be subject to different interpretation by different operators.

Figure 1. The faint negative THC line and not completely absent opiate line in an opiate positive sample. The presence of a line no matter how faint is considered a negative result with the DDC3000.

During the cutoff evaluation, 153 total test cassettes were used and, of those, three cassettes produced invalid results. In all three instances, the buffer leaked out while shaking the cassettes; therefore, there was no liquid to flow up the test strips. The results of the three invalid tests were discarded, and the testing was repeated with a new test cassette. Summary results are displayed in Table 10.

Table 10. DDT3000 cutoff evaluation results combined for all testing performed.

DDC3000 Cutoff Evaluation Results									
Drug	**TP**	**FN**	**FP**	**TN**	**Sensitivity**	**Specificity**	**Accuracy**	**PPV**	**NPV**
THC	28	1	0	121	96.6%	100.0%	99.3%	100.0%	99.2%
Cocaine	22	0	0	128	100.0%	100.0%	100.0%	100.0%	100.0%
Amphetamine	30	0	0	120	100.0%	100.0%	100.0%	100.0%	100.0%
Methamphetamine	29	1	0	120	96.7%	100.0%	99.3%	100.0%	99.2%
Opiates	13	7	0	130	65.0%	100.0%	95.3%	100.0%	94.9%
Overall	122	9	0	619	93.1%	100.0%	98.8%	100.0%	98.6%

DrugWipe

During the evaluation of the DrugWipe, all three concentration levels of 30 percent above, at, and 30 percent below the cutoff concentration were run for all five of the target analytes. Cocaine, opiate, and methamphetamine were run as a mix, which reduced the number of possible true negatives. The amphetamine and THC were run individually.

At 30 percent above the cutoff concentration, THC was detected 6 out of 10 times. At the cutoff concentration, THC was detected 2 out of 10 times, but was not detected below the cutoff concentration. The sensitivity for both amphetamine and methamphetamine improved with all replicates yielding positive results at 30 percent above the cutoff concentration. At the cutoff, amphetamine was detected 8 out of 10 times, while methamphetamine was detected 9 of 10 times. When tested at 30 percent below the cutoff concentration, amphetamine was detected 3 out of 10 times, and methamphetamine was detected 5 out of 10 times. Opiates were detected by the DrugWipe 7 out of 10 times at 30 percent above the cutoff concentration, 4 out of 10 times at the cutoff concentration, and 1 out of 10 times at 30 percent below the cutoff concentration.

There were two invalid tests. The first invalid test resulted when a control line failed to appear. The DrugRead analyzer flagged the failure and the sample was retested with another cassette. The second failure was a defective or faulty DrugWipe test cassette. The DrugWipe was removed from its sealed foil wrap and it was observed that the normally pink collection pads were already yellow indicating possible moisture exposure despite there being a desiccant pouch (drying agent) in the package. The test cassette was not used and was discarded.

Table 11. DrugWipe cutoff evaluation results combined for all testing performed with the DrugRead analyzer.

DrugWipe Cutoff Evaluation Results with DrugRead Analyzer									
Drug	**TP**	**FN**	**FP**	**TN**	**Sensitivity**	**Specificity**	**Accuracy**	**PPV**	**NPV**
THC	8	12	0	70	40.0%	100.0%	86.7%	100.0%	85.4%
Cocaine	13	10	0	67	56.5%	100.0%	88.9%	100.0%	87.0%
Amphetamine	21	2	0	37	91.3%	100.0%	96.7%	100.0%	94.9%
Methamphetamine	24	1	0	35	96.0%	100.0%	98.3%	100.0%	97.2%
Opiates	12	9	0	69	57.1%	100.0%	90.0%	100.0%	88.5%
Overall	78	34	0	278	69.6%	100.0%	91.3%	100.0%	89.1%

Sensitivity issues and variance observed with the DrugWipe was attributed to faint or incomplete test lines. The user guide provided with the DrugRead states that, “in rare cases a test maybe misinterpreted due to incomplete test lines.” Incomplete test lines occurred throughout our use of the DrugWipe and the DrugRead analyzer. Examples of faint and incomplete test lines on the DrugWipe are shown in Figure 2. In the figure shown below, both cassettes showed faint lines for the opiate assay, with one being detected by the analyzer and one that was not. The user guide also states that the DrugRead analyzer is authoritative as the DrugRead software and sensors evaluate each DrugWipe with the same objectivity under consistent conditions compared to if the DrugWipe being manually evaluated, which imparts subjectivity related to variables such as different ambient lighting conditions, variances in operator’s perception of color, and

their level of training. As such, the results in Table 11 are the results as recorded by the DrugRead analyzer.

Figure 2. Faint or incomplete lines for the opiate test (indicated by the red arrow) on two test cassettes that were interpreted differently by the DrugRead analyzer.

The DrugWipe cassettes were also read manually (unblinded, i.e., the technician was aware of which sample was positive for which drug(s)), and the manually scored results were compared with the DrugRead results, which the manufacturer defines as authoritative result. The results of manually scoring of the incomplete test lines missed by the DrugRead produce the data shown in Table 12. Only THC failed to improve significantly as partial test lines were not observed for THC. Manually reading the DrugWipe cassettes produced improvements in the performance of the cocaine, amphetamine, methamphetamine, and opiates assay, and improved the overall sensitivity from 69.6 percent to 85 percent, and accuracy from 91.3 percent to 94.9 percent.

Table 12. DrugWipe cutoff evaluation results combined for all testing performed with manual interpretation.

DrugWipe Cutoff Evaluation with Manual Interpretation									
Drug	**TP**	**FN**	**FP**	**TN**	**Sensitivity**	**Specificity**	**Accuracy**	**PPV**	**NPV**
THC	8	12	0	70	40.0%	100.0%	86.7%	100.0%	85.4%
Cocaine	26	3	0	61	89.7%	100.0%	96.7%	100.0%	95.3%
Amphetamine	28	1	0	31	96.6%	100.0%	98.3%	100.0%	96.9%
Methamphetamine	30	0	0	30	100.0%	100.0%	100.0%	100.0%	100.0%
Opiates	21	4	0	65	84.0%	100.0%	95.6%	100.0%	94.2%
Overall	113	20	0	257	85.0%	100.0%	94.9%	100.0%	92.8%

DDS2

The DDS2 had false negative THC results both at the cutoff concentration and 30 percent above the cutoff (n=4). These THC false negatives resulted in a THC sensitivity of 61.9 percent. False negative results were also obtained for other analytes, which included one false negative for methamphetamine at 30 percent above the cutoff concentration and false negative results at 30 percent above the cutoff concentration for benzodiazepines (n=1) and amphetamines (n=2). The DDS2 was able to detect some positive results for each target analyte at 30 percent below the

cutoff concentration, including one THC positive. The DDS2 had an overall sensitivity greater than 91 percent and there were no false positives nor invalid test results during this stage of testing. The overall results of the cutoff evaluation are shown in Table 13.

Table 13. DDS2 cutoff evaluation results combined for all testing performed.

DDS2 Cutoff Evaluation Results									
Drug	**TP**	**FN**	**FP**	**TN**	**Sensitivity**	**Specificity**	**Accuracy**	**PPV**	**NPV**
THC	13	8	0	159	61.9%	100.0%	95.6%	100.0%	95.2%
Cocaine Metabolite	28	0	0	152	100.0%	100.0%	100.0%	100.0%	100.0%
Amphetamine	20	2	0	158	90.9%	100.0%	98.9%	100.0%	98.8%
Methamphetamine	24	1	0	155	96.0%	100.0%	99.4%	100.0%	99.4%
Benzodiazepines	24	1	0	155	96.0%	100.0%	99.4%	100.0%	99.4%
Opiates	23	0	0	157	100.0%	100.0%	100.0%	100.0%	100.0%
Overall	132	12	0	936	91.7%	100.0%	98.9%	100.0%	98.7%

AquilaScan

The AquilaScan failed to detect any THC, cocaine, benzodiazepine, or methadone at 30 percent above the cutoff concentration (Table 14). There was one positive methamphetamine detected at 30 percent above the cutoff concentration and seven positives for amphetamine out of the 10 replicates tested. Consequently, additional testing at the cutoff or 30 percent below the cutoff was not performed for these analytes.

The AquilaScan consistently detected the presence of opiates. Morphine was detected 9 out of 10 times at 30 percent above the cutoff concentration and 9 out of 10 times at the cutoff concentration, yielding two false negatives and a sensitivity of 92.6 percent for opiates. At 30 percent below the cutoff concentration, morphine was detected seven out of 10 times.

Table 14. AquilaScan cutoff evaluation results combined for all testing performed.

AquilaScan Cutoff Evaluation Results									
Drug	**TP**	**FN**	**FP**	**TN**	**Sensitivity**	**Specificity**	**Accuracy**	**PPV**	**NPV**
THC	0	10	0	80	0.0%	100.0%	88.9%	N/A	88.9%
Cocaine	0	10	0	80	0.0%	100.0%	88.9%	N/A	88.9%
Amphetamine	7	3	0	80	70.0%	100.0%	96.7%	100.0%	96.4%
Methamphetamine	1	9	0	80	10.0%	100.0%	90.0%	100.0%	89.9%
Benzodiazepines	0	10	0	80	0.0%	100.0%	88.9%	N/A	88.9%
Opiates	25	2	0	63	92.6%	100.0%	97.8%	100.0%	96.9%
Methadone	0	10	0	80	0.0%	100.0%	88.9%	N/A	88.9%
Overall	33	54	0	543	37.9%	100.0%	91.4%	100.0%	91.0%

Phase III: Cross-Reactivity Evaluations

DDT5000

For the eleven control mixtures of target drugs, the DDT5000 recorded a perfect 100 percent score across all drug classes and target analytes. As seen with the cutoff evaluation, the DDT5000 detected 48 out of a possible 51 positives. The 3 positives not detected by the DDT5000 were considered true negatives as they were spiked at concentrations well below the devices advertised cutoff. One sample contained amphetamine 10 ng/mL (cutoff of 50 ng/mL), and the other 2 were the morphine and methadone, each spiked at 10 ng/mL (cutoff of 20 ng/mL for both morphine and methadone). There were no false negatives or false positive results on the DDT5000 during this phase of testing. Results are shown in Table 15.

Table 15. DDT5000 mixed drug blind control testing results.

DDT5000 Mixed Drug Blind Control Results									
Drug	**TP**	**FN**	**FP**	**TN**	**Sensitivity**	**Specificity**	**Accuracy**	**PPV**	**NPV**
THC	8	0	0	3	100.0%	100.0%	100.0%	100.0%	100.0%
Cocaine	7	0	0	4	100.0%	100.0%	100.0%	100.0%	100.0%
Amphetamine	7	0	0	4	100.0%	100.0%	100.0%	100.0%	100.0%
Methamphetamine	6	0	0	5	100.0%	100.0%	100.0%	100.0%	100.0%
Benzodiazepines	8	0	0	3	100.0%	100.0%	100.0%	100.0%	100.0%
Opiates	6	0	0	5	100.0%	100.0%	100.0%	100.0%	100.0%
Methadone	6	0	0	5	100.0%	100.0%	100.0%	100.0%	100.0%
Overall	48	0	0	29	100.0%	100.0%	100.0%	100.0%	100.0%

DDC3000

The DDC3000 had only one false negative result during the mixed drug control analysis, which occurred with a sample containing morphine at 30 ng/mL, 10 ng/mL above the opiate cutoff concentration (Table 16).[2]

As mentioned previously, this was the only device in the evaluation without an analyzer, thus requiring the operator to manually interpret the results. During this phase of the evaluation, there were 55 test results. Out of the 55 results, 5 test lines were noted to be ambiguous with faint color. Any appearance of a test line, no matter how faint, is to be interpreted as a negative result. One of these was the false opiate negative discussed above where the test cassette had leaked. The other 4 tests where results were not definitive were in instances where the target analytes (amphetamine, methamphetamine and THC) were at concentrations below the cutoffs. Samples that had the target analytes above the cutoff concentrations (true positives) or when the target analytes were not in the control mixture (true negatives) were clear and easy to interpret.

[2] The false negative may be accounted for because the test cassette had a leak during the test strip development, which likely decreased the sensitivity causing the false negative.

Table 16. DDC3000 mixed drug blind control testing results.

DDC3000 Mixed Drug Blind Control Results									
Drug	**TP**	**FN**	**FP**	**TN**	**Sensitivity**	**Specificity**	**Accuracy**	**PPV**	**NPV**
THC	6	0	0	5	100.0%	100.0%	100.0%	100.0%	100.0%
Cocaine	6	0	0	5	100.0%	100.0%	100.0%	100.0%	100.0%
Amphetamine	7	0	0	4	100.0%	100.0%	100.0%	100.0%	100.0%
Methamphetamine	5	0	0	6	100.0%	100.0%	100.0%	100.0%	100.0%
Opiates	5	1	0	5	83.3%	100.0%	90.9%	100.0%	83.3%
Overall	29	1	0	25	96.7%	100.0%	98.2%	100.0%	96.2%

DrugWipe

For the DrugWipe, results from the testing as detected by the DrugRead analyzer were compared to results evaluated manually by the operator counting partial, incomplete and faint lines as positive, and that comparison is shown in Tables 17 and 18. In the automated evaluation by the analyzer, there were 10 false negatives across all drug categories. However, with a manual visual evaluation, qualifying lines were observed, and the false negatives were reduced to one, increasing the overall sensitivity, accuracy, and NPV from 64.3 percent, 77.3 percent, and 61.5 percent to 96.4 percent, 97.7 percent, and 94.1 percent, respectively, which is similar in performance to the DDC3000 and DDS2. The remaining single false negative result occurred with THC at the cutoff concentration of 5 ng/mL.

Table 17. DrugWipe mixed drug blind control testing results using the DrugRead analyzer.

DrugWipe Mixed Drug Blind Control Results (DrugRead Analyzer)									
Drug	**TP**	**FN**	**FP**	**TN**	**Sensitivity**	**Specificity**	**Accuracy**	**PPV**	**NPV**
THC	4	3	0	4	57.1%	100.0%	72.7%	100.0%	57.1%
Cocaine	6	1	0	4	85.7%	100.0%	90.9%	100.0%	80.0%
Amp/Methamp	4	3	0	4	57.1%	100.0%	72.7%	100.0%	57.1%
Opiates	4	3	0	4	57.1%	100.0%	72.7%	100.0%	57.1%
Overall	18	10	0	16	64.3%	100.0%	77.3%	100.0%	61.5%

Table 18. DrugWipe mixed drug blind control testing results using operator visualization for interpretation.

DrugWipe Mixed Drug Blind Control Results (Manual Interpretation)									
Drug	**TP**	**FN**	**FP**	**TN**	**Sensitivity**	**Specificity**	**Accuracy**	**PPV**	**NPV**
THC	6	1	0	4	85.7%	100.0%	90.9%	100.0%	80.0%
Cocaine	7	0	0	4	100.0%	100.0%	100.0%	100.0%	100.0%
Amp/Methamp	7	0	0	4	100.0%	100.0%	100.0%	100.0%	100.0%
Opiates	7	0	0	4	100.0%	100.0%	100.0%	100.0%	100.0%
Overall	27	1	0	16	96.4%	100.0%	97.7%	100.0%	94.1%

DDS2

The DDS2 had one false negative result during the mixed drug blind control analysis, which occurred with methamphetamine at the cutoff concentration of 50 ng/mL. A summary of the results in shown in Table 19.

Table 19. DDS2 mixed drug blind control testing results.

DDS2 Mixed Drug Blind Control Results									
Drug	**TP**	**FN**	**FP**	**TN**	**Sensitivity**	**Specificity**	**Accuracy**	**PPV**	**NPV**
THC	6	0	0	5	100.0%	100.0%	100.0%	100.0%	100.0%
Cocaine Metabolite	6	0	0	5	100.0%	100.0%	100.0%	100.0%	100.0%
Amphetamine	7	0	0	4	100.0%	100.0%	100.0%	100.0%	100.0%
Methamphetamine	4	1	0	6	80.0%	100.0%	90.9%	100.0%	85.7%
Benzodiazepines	6	0	0	5	100.0%	100.0%	100.0%	100.0%	100.0%
Opiates	5	0	0	6	100.0%	100.0%	100.0%	100.0%	100.0%
Overall	34	1	0	31	97.1%	100.0%	98.5%	100.0%	96.9%

AquilaScan

As was the case during the cutoff evaluation, the AquilaScan failed to detect positive results in samples where the target analyte was either 30 percent above, or at the cutoff concentration, resulting in numerous false negatives (n=25) (Table 20). On the 11 controls run, the AquilaScan had an overall sensitivity of 35.9 percent, accuracy of 67.5 percent, and NPV of 60.3 percent. With respect to specific analytes, the AquilaScan successfully detected opiates in all samples and amphetamines, except for one false amphetamine negative, which was at the cutoff concentration of 50 ng/mL. This resulted in an amphetamine sensitivity and NPV of 83.3 percent and accuracy of 90.9 percent. The AquilaScan failed to detect a single diazepam or THC positive, at the cutoff or 30 percent above it. The AquilaScan with cutoffs for diazepam at 15 ng/mL and THC at 40 ng/mL failed to detect a diazepam control at 100 ng/mL and failed to detect a THC control at 1,000 ng/mL. (Figure 3). Images captured by the device camera (Figure 3A) clearly show instances where, despite the controls containing concentrations well above the cutoff concentration of the target analytes, a faint line appeared indicating negative results, which were clearly defined and unmistakable. In Figure 3B, based on the concentrations in the control and cutoff concentrations, positive results should have been obtained for opiates, benzodiazepines, methadone, and THC; however, the only positive result obtained was for opiates at 30 ng/mL. In this control, the THC concentration was at 1,000 ng/mL, 25 times higher than the THC cutoff concentration published for this device, and it was not detected.

Table 20. AquilaScan mixed drug blind control testing results**.**

AquilaScan Mixed Drug Bling Control Results									
Drug	**TP**	**FN**	**FP**	**TN**	**Sensitivity**	**Specificity**	**Accuracy**	**PPV**	**NPV**
THC	0	5	0	6	0.0%	100.0%	54.5%	#DIV/0!	54.5%
Cocaine	1	5	0	5	16.7%	100.0%	54.5%	100.0%	50.0%
Amphetamine	5	1	0	5	83.3%	100.0%	90.9%	100.0%	83.3%
Methamphetamine	1	4	0	6	20.0%	100.0%	63.6%	100.0%	60.0%
Benzodiazepines	0	5	0	6	0.0%	100.0%	54.5%	#DIV/0!	54.5%
Opiates	6	0	0	5	100.0%	100.0%	100.0%	100.0%	100.0%
Methadone	1	5	0	5	16.7%	100.0%	54.5%	100.0%	50.0%
Overall	14	25	0	38	35.9%	100.0%	67.5%	100.0%	60.3%

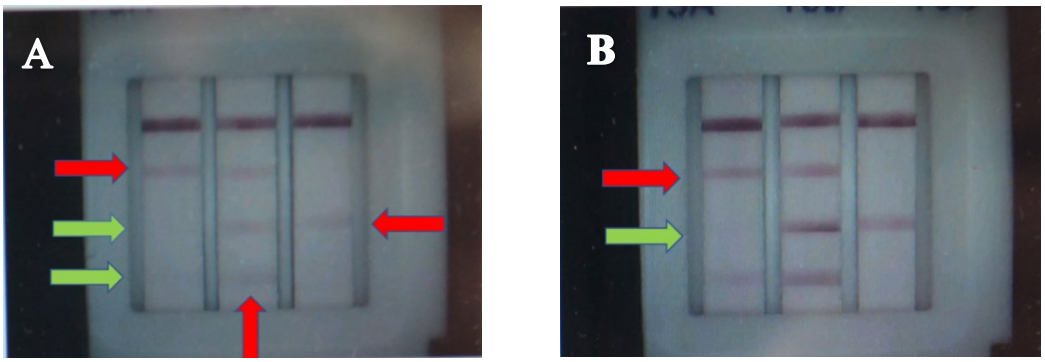

Figure 3. A) Control 10 with all seven target analytes at 100 ng/mL each. The only lines that should be visible are the three dark control lines at the top of each strip. The green arrows indicate positive results (amphetamine and opiates). The red arrows point to false negative test lines for THC, cocaine, methamphetamine, benzodiazepine, and methadone. B) Control 9, the red arrow points to a clearly visible line for THC indicating a negative result. THC was present in the sample at 1000 ng/mL. The green arrow points to the lack of an opiate test line indicating a true opiate positive.

Phase III: Cross-Reactivity of Metabolites and Drugs in the Same Class as the Target Compound.

The final phase of the cross-reactivity experiments included the evaluation of the effects of various common drug metabolites, and other either commonly encountered drugs or drugs known to cause false positive results on immunoassays. Results from the analysis of the mixtures are shown in Table 21.

Table 21. Lowest concentration testing positive (2/3) for indicated analyte.

	DDT5000	DDC3000	DDS2	DrugRead	AquilaScan
Amphetamines/Phenethylamines					
MDMA	100ng/mL	100ng/mL	100ng/mL	1000ng/mL	NCR
MDA	100ng/mL	100ng/mL	100ng/mL	100ng/mL	1000ng/mL
Pseudoephedrine	NCR	NCR	NCR	NCR	NCR
l-Methamphetamine	1000ng/mL	1000ng/mL	1000ng/mL	NCR	NCR
l-Amphetamine	NCR	NCR	NCR	NCR	NCR
Cocaine					
Cocaine	-*	-*	100ng/mL	-*	-*
Benzoylecgonine	1000ng/mL	1000ng/mL	-*	1000ng/mL	100ng/mL
Opiates					
Hydrocodone	10ng/mL	100ng/mL	100ng/mL	100ng/mL	1000ng/mL
Codeine	10ng/mL	100ng/mL	100ng/mL	100ng/mL	100ng/mL
Hydromorphone	10ng/mL	100ng/mL	100ng/mL	1000ng/mL	100ng/mL
Oxycodone	1000ng/mL	1000ng/mL	NCR	NCR	NCR
Oxymorphone	NCR	NCR	NCR	NCR	NCR
Benzodiazepines					
Alpha-hydroxyalprazolam	100ng/mL	N/A	100ng/mL	N/A	NCR
7-aminoclonazepam	100ng/mL	N/A	NCR	N/A	NCR
Nordiazepam	10ng/mL	N/A	100ng/mL	N/A	NCR
Oxazepam	100ng/mL	N/A	100ng/mL	N/A	100ng/mL
Temazepam	10ng/mL	N/A	-*	N/A	100ng/mL
Diazepam	-*	N/A	100ng/mL	N/A	-*
Cannabinoids					
11-nor-delta9-THC-COOH	10ng/mL	100ng/mL	10ng/mL	10ng/mL	1000ng/mL
Cannabidiol	NCR	NCR	NCR	NCR	NCR
Other					
EDDP (methadone metabolite)	NCR	NCR	NCR	NCR	NCR
Dextromethorphan	NCR	NCR	NCR	NCR	NCR
Caffeine	NCR	NCR	NCR	NCR	NCR
Nicotine	NCR	NCR	NCR	NCR	NCR
Tramadol	NCR	NCR	NCR	NCR	NCR
Acetaminophen	NCR	NCR	NCR	NCR	NCR
Diphenhydramine	NCR	NCR	NCR	NCR	NCR
Salicyclic acid	NCR	NCR	NCR	NCR	NCR
Naproxen	NCR	NCR	NCR	NCR	NCR
Ibuprofen	NCR	NCR	NCR	NCR	NCR
Pentobarbital	NCR	NCR	NCR	NCR	NCR
Zolpidem	NCR	NCR	NCR	NCR	NCR
Fluoxetine	NCR	NCR	NCR	NCR	NCR
Sertraline	NCR	NCR	NCR	NCR	NCR
Lidocaine	NCR	NCR	NCR	NCR	NCR
PCP	NCR	NCR	NCR	NCR	NCR

N/A = Assay not available on this device; NCR = No cross-reactivity at 1000 ng/mL; -*= This was the target analyte for the assay and was not scored as a potential cross-reactant

The drugs in mixture six did not cross-react with any of the devices at a concentration of 1,000 ng/mL. Oxycodone, a desirable cross-reactant did not give a positive on the AquilaScan at 1,000 ng/mL. However, the DDT5000 and DDC3000 devices detected oxycodone for all three tests at 1000 ng/mL, while the DDS2 produced one positive result for oxycodone at that concentration.

When tested at the lower concentration of 100 ng/mL, no positive results were obtained for any of the previously positive devices.

Likewise, both Dräger devices and the DDS2 all recorded three positives for the l-methamphetamine at 1000 ng/mL but did not produce any positives when tested at the lower concentration of 100 ng/mL.

Dräger was the only manufacturer who provided cross-reactivity data with the test cassettes. Included in Table 22 are the analytes they identify as having cross-reactivity, and the associated concentrations that should yield a positive result. The results from Table 21 are generally in agreement with those published by the manufacturer. However, during the testing, it was noted for the benzodiazepines tested that the DDT5000 was more sensitive than advertised. Positive results for nordiazepam, oxazepam, and temazepam were obtained at concentrations as low as 10 ng/mL despite cross-reactivity concentrations being published at 45, 40, and 20 ng/mL, respectively.

Table 22. Cross-reactivity results provide with the Dräger devices.

Manufacturer Published Specificity (ng/mL)		
Related Compounds	**DDT5000**	**DDC3000**
Nordiazepam	45	N/A
Oxazepam	40	N/A
Temazepam	20	N/A
Benzoylecgonine	70	100
MDMA	75	200
MDA	100	50
Pseudoephedrine	100,000	100,000
Codeine	25	20
Hydrocodone	20	10
Hydromorphone	30	10
Oxycodone	1,000	1,000
EDDP	7,000	N/A
11-nor-delta9-THC-COOH	2	50
Cannabidiol	90,000	90,000

Phase IV: Interferent Evaluations

Interferences refer to exogenous substances that effect the results of the test by either causing a false positive or false negative result. Summary results for the interference studies are provided in Table 23. Due to the sensitivity limitations observed in earlier phases of testing, the assessment of interferences on the DrugWipe and AquilaScan performance is also limited.

Table 23. Summary results of the interference and its effects on the different devices.

INTERFERENT EFFECT					
Interferent	**DDT5000**	**DDC3000**	**DDS2**	**DrugWipe**	**AquilaScan**
Coffee	FP Benzo	None	None	None	None
Pepsi cola	None	None	None	None	FN Opi
Beer	None	None	None	None	None
Methanol	None	None	None	None	None
Mouthwash	None	None	None	None	None
Orange juice	None	None	None	None	None
Milk (2% fat)	FN THC	None	invalids, FP COC, FP THC	None	None
Wintergreen mint	None	None	FN THC	None	None
Peppermint mint	None	None	None	None	None
Spearmint gum	None	None	None	None	None
Toothpaste	None	None	None	None	None
Chewing tobacco	FN Opi, FP Meth	FN OP	FP Meth	FP Opi	FP Opi

Opi = opiate, COC = cocaine, Meth=methamphetamine, Benzo = benzodiazepine, none = the interferent produced no effect, FP = false positive, FN = false negative

DDT5000

During the interference testing phase, the DDT5000 had seven false positives. Three of these were for THC and occurred one time each when the negative saliva was mixed with beer, toothpaste, and coffee. Though it is possible that the interferents led to the false THC positives, they could also have been random as the result was not replicated upon duplicate testing. Conversely, the DDT5000 had six false negative results with three of them being for THC, which were all repeatable and attributed to the addition of milk to saliva at 5 percent of the total volume (v/v). The false opiate negatives were attributed to interference from the chewing tobacco. Based on the DDT5000's previous performance where there had been no false negative results, the milk and chewing tobacco samples were re-spiked with THC and morphine, and the analysis was repeated. The same results were obtained with false negative results on both the THC and morphine assays. Additionally, the sample containing chewing tobacco produced a false methamphetamine positive. Saliva containing coffee at 5 percent v/v/ produced two false positives out of two tests performed on the benzodiazepine assay.

DDC3000

The DDC3000 was affected by the interferences. False negative results were produced when the saliva was mixed with chewing tobacco. The false negative results were on the opiate assay.

DrugWipe

The impact of interferences was difficult to assess for the DrugWipe. During this phase of testing, the DrugWipe performance was subject to faint or partial, incomplete test lines not being detectable by the DrugRead analyzer (i.e., false negatives), which resulted in 45 false negative results across all testing panels, in spite of the partial lines being observable by the naked eye. These observations were similar to those made during the cutoff evaluation where there were several false negative results when the interpretation was performed by the DrugRead analyzer. Notably, the sensitivity for THC on the DrugWipe was 29.2 percent, but it is difficult to assess if

this was the result of the interferences. With respect to false positives on the DrugWipe, there was one false positive for opiates when evaluated by the DrugRead analyzer. When visually interpreted, there were two false positives for opiates, which occurred when saliva was mixed with chewing tobacco. During previous testing, the DrugRead analyzer had recorded false positive results where there was no visible test line on the DrugWipe test cassette.

DDS2

The overall results for the DDS2 for all interference testing included three THC false negative results and four false positive results across the various test panels. Initially, two false negative results for THC resulted when the saliva was mixed with a wintergreen mint. A third sample was prepared and again a false negative result for THC was obtained, suggesting that wintergreen mints interfere with the THC test panel on the DDS2. When saliva was mixed with chewing tobacco, two false positives for methamphetamine were obtained. The DDS2 was also affected when milk was mixed with the saliva, which resulted in multiple invalids (9 out of 12 test lines were invalid), false positives for cocaine and THC and a red smear up the test strips, which caused the background to turn red instead of white leading to difficulty distinguishing the tests lines from the background.

AquilaScan

Since the AquilaScan failed to detect THC, cocaine, methamphetamine, benzodiazepines, and methadone during the cutoff or blind control evaluations, these drugs were still not detected when potential interferents were added to the spiked samples. However, based on the previous opiate performance during the cutoff and mixed drug blind control analysis where the sensitivity was between 92.6 percent and 100 percent, the sensitivity dropped to 66.7 percent during this phase of testing. When the cola was added to the sample containing morphine at 50 percent above the cutoff concentration, the duplicate testing resulted in two false negative results. At least one false negative of the duplicate samples tested resulted on the opiate assay when the following interferents were added to the sample: coffee, methanol, peppermint, and spearmint gum. During the other phases of testing, the AquilaScan had not yet recorded a false positive; however, when the interferents were added to the samples there were two false positives, one false positive for opiates when drug-free saliva was mixed with chewing tobacco, and one for THC when the saliva was mixed with a wintergreen mint.

Discussion

Beer, methanol, orange juice, mouthwash, peppermint mints, spearmint gum, and toothpaste had no observed interference effects on any of the devices. Chewing tobacco was the one interferent tested that resulted in issues with device performance across all five devices. With coffee and milk causing consistent issues on the DDT5000 and DDS2 at 5 percent v/v interference, they were re-evaluated at 1 percent v/v to represent a more realistic concentration and retested both with the interferent mixed with negative saliva, and with the interferent mixed with saliva containing the drug at 50 percent above the cutoff concentration. Upon reanalysis at 1 percent v/v, milk showed no interfering effects on either the DDS2 or DDT5000. The 1 percent v/v coffee was only run on the DDT5000. The sample containing negative saliva and 1 percent v/v coffee was run in triplicate resulting in one false benzodiazepine positive.

Since adding coffee directly to saliva continued to cause false benzodiazepine positives on the DDT5000 a more realistic test was carried out following the manufacturer instructions. Two volunteers each drank a cup of coffee, waited 10 minutes, and then collected saliva with Sarstedt salivettes. Even after a 10-minute waiting period, the saliva collected by the salivettes was still slightly discolored by the coffee. The blank saliva was then pooled and run four times on the DDT5000 with no issue. The operation manuals of all five devices instruct the operator to wait 10 minutes after any food, beverage, or tobacco product has been consumed or used by a subject before collecting any sample. The results of this phase of testing strongly suggest that the operators of these devices should take heed of this instruction.

Phase V: Environmental Testing Evaluations
Data for false positive, false negative and invalid results relative to the environmental condition associated with the results for all devices can be found in **Appendix D**.

DDT5000
The DDT5000 did not show changes in performance after stressing the cassettes under various environmental conditions including temperature and humidity, with overall performance indicators exceeding 99 percent and no clear impact from the various storage conditions (Table 24). There were two false positive and two false negative results, but they appear to be random and may not be correlated to the environmental conditions as they occurred randomly in isolation on cassettes that had been stored at 10°F, 25°F, and 50°F. Additionally, there were two invalid tests, one that occurred at 10°F, but seemed to be caused by a cassette defect as the sample collector volume indicator did not turn blue, and seemed as if the sample collector was partially blocked. The second invalid test occurred with an invalid amphetamine result on a positive sample run on a cassette stored at 50°F with 75 percent relative humidity. There was no issue with the duplicate positive.

Table 24. Summary data for the environmental testing on the DDT5000.

DDT5000 Environmental Evaluation									
Drug	**TP**	**FN**	**FP**	**TN**	**Sensitivity**	**Specificity**	**Accuracy**	**PPV**	**NPV**
THC	59	1	1	30	98.3%	96.8%	97.8%	98.3%	96.8%
Cocaine	59	1	0	31	98.3%	100.0%	98.9%	100.0%	96.9%
Amphetamine	60	0	0	31	100.0%	100.0%	100.0%	100.0%	100.0%
Methamphetamine	60	0	0	31	100.0%	100.0%	100.0%	100.0%	100.0%
Benzodiazepines	60	0	1	30	100.0%	96.8%	98.9%	98.4%	100.0%
Opiates	60	0	0	31	100.0%	100.0%	100.0%	100.0%	100.0%
Methadone	60	0	0	31	100.0%	100.0%	100.0%	100.0%	100.0%
Overall	418	2	2	215	99.5%	99.1%	99.4%	99.5%	99.1%

DDC3000

The DDC3000 did not show changes in performance across all the storage conditions with overall performance indicators of 96 percent or above (Table 25). During testing, there were five false negative results, four of which came from one isolated test cassette. The corresponding duplicate positive had no issue. All false negative results were very subjective faint lines. The DDC3000 also had one test cassette, which leaked during the test procedure causing an invalid test due to a lack of flow up the testing strip.

Table 25. Summary data for the environmental testing on the DDC3000.

DDC3000 Environmental Evaluation									
Drug	**TP**	**FN**	**FP**	**TN**	**Sensitivity**	**Specificity**	**Accuracy**	**PPV**	**NPV**
THC	61	1	0	30	98.4%	100.0%	98.9%	100.0%	96.8%
Cocaine	61	1	0	30	98.4%	100.0%	98.9%	100.0%	96.8%
Amphetamine	62	0	0	30	100.0%	100.0%	100.0%	100.0%	100.0%
Methamphetamine	61	1	0	30	98.4%	100.0%	98.9%	100.0%	96.8%
Opiates	60	2	0	30	96.8%	100.0%	97.8%	100.0%	93.8%
Overall	305	5	0	150	98.4%	100.0%	98.9%	100.0%	96.8%

DrugWipe

The DrugWipe showed random and varied results with respect to what the DrugRead would detect. Positive samples were run in duplicate across each environmental test condition, and they consistently varied as to what the DrugRead would detect due to faint and incomplete/partial test lines. In some cases, the faint, incomplete or partial lines would be detected, yielding a positive result, while the duplicate test would be deemed negative despite having the same faint, partial or incomplete line that was detectable to the human eye, sometimes being called positive and other times being negative. Summary data for cassettes analyzed by the DrugRead can be found in Table 26. For comparison, summary data for cassettes evaluated by the operator where all faint, partial or incomplete lines were consistently called positive is shown in Table 27. The manual evaluation of results significantly improved device performance, especially for THC sensitivity. When manually read, the resulting sensitivity for THC was 87 percent compared to 32 percent when using the results provided by the DrugRead. The environmental storage conditions seemed to have no impact on the DrugWipe results.

Table 26. Summary data for the environmental testing on the DrugWipe with the DrugRead analyzer**.**

DrugWipe Environmental Evaluation (DrugRead Analyzer)									
Drug	**TP**	**FN**	**FP**	**TN**	**Sensitivity**	**Specificity**	**Accuracy**	**PPV**	**NPV**
THC	20	43	0	30	31.7%	100.0%	53.8%	100.0%	41.7%
Cocaine	37	26	0	30	58.1%	100.0%	72.0%	100.0%	53.6%
Amphet/methamph	52	11	0	30	82.5%	100.0%	88.2%	100.0%	73.2%
Opiates	19	44	0	30	30.2%	100.0%	52.7%	100.0%	40.5%
Overall	128	124	0	120	50.8%	100.0%	66.7%	100.0%	49.2%

Table 27. Summary data for the environmental testing on the DrugWipe with operator evaluation.

DrugWipe Environmental with Manual Evaluation									
Drug	**TP**	**FN**	**FP**	**TN**	**Sensitivity**	**Specificity**	**Accuracy**	**PPV**	**NPV**
THC	55	8	0	30	87.3%	100.0%	91.4%	100.0%	78.9%
Cocaine	57	6	0	30	90.5%	100.0%	93.5%	100.0%	83.3%
Amphet/methamph	62	1	0	30	98.4%	100.0%	98.9%	100.0%	96.8%
Opiates	54	9	0	30	85.7%	100.0%	90.3%	100.0%	76.9%
Overall	228	24	0	120	90.5%	100.0%	93.5%	100.0%	83.3%

DDS2

Based on the information provided by the manufacturer, the DDS2 has the smallest recommended temperature range for cassette storage ranging between 59-77°F. During the cutoff evaluation, the DDS2 had a 61.9 percent sensitivity for THC. THC sensitivity dropped to 27.1 percent during the environmental testing phase, while the other analytes had no decreases in sensitivity, suggesting this test panel is most susceptible to deviations in performance when exposed to various temperatures and humidity (Table 28). However, failure to detect THC also occurred when testing using cartridges stored within the recommended storage range.

Table 28. Summary data for the environmental testing on the DDS2.

DDS2 Environmental Evaluation									
Drug	**TP**	**FN**	**FP**	**TN**	**Sensitivity**	**Specificity**	**Accuracy**	**PPV**	**NPV**
THC	16	43	0	27	27.1%	100.0%	50.0%	100.0%	38.6%
Cocaine	59	0	0	27	100.0%	100.0%	100.0%	100.0%	100.0%
Amphetamine	59	0	0	27	100.0%	100.0%	100.0%	100.0%	100.0%
Methamphetamine	59	0	0	27	100.0%	100.0%	100.0%	100.0%	100.0%
Benzodiazepines	59	0	0	27	100.0%	100.0%	100.0%	100.0%	100.0%
Opiates	59	0	0	27	100.0%	100.0%	100.0%	100.0%	100.0%
Overall	311	43	0	162	87.9%	100.0%	91.7%	100.0%	79.0%

AquilaScan

The AquilaScan performance was consistent with the performance during the cutoff assessment. During the cutoff evaluation, the AquilaScan had sensitivity for amphetamine and methamphetamine at 70 percent and 10 percent respectively, which were nearly the same as the sensitivities calculated during the environmental testing, which were 67.7 percent and the 9.7 percent, respectively (Table 29). The opiate assay has performed well during the cutoff evaluation with a calculated sensitivity of 92.6 percent, which dropped to 35.5 percent during the environmental testing. Positive results were obtained across the temperature range, so the decrease in performance could not be attributed to single storage condition or isolated range. It was noted, however, that different test cassette lot numbers were used during this evaluation than those that were used during the cutoff evaluation and may account for the disparity. The AquilaScan recorded one invalid result on the methadone test strip due to the control line failing to appear. However, none of these results were attributable to the storage conditions as the errors occurred in isolated samples.

Table 29. Summary data for the environmental testing on the AquilaScan.

AquilaScan Environmental Evaluation									
Drug	**TP**	**FN**	**FP**	**TN**	**Sensitivity**	**Specificity**	**Accuracy**	**PPV**	**NPV**
THC	2	60	1	30	3.2%	96.8%	34.4%	66.7%	33.3%
Cocaine	2	60	0	31	3.2%	100.0%	35.5%	100.0%	34.1%
Amphetamine	42	20	1	30	67.7%	96.8%	77.4%	97.7%	60.0%
Methamphetamine	6	56	0	31	9.7%	100.0%	39.8%	100.0%	35.6%
Benzodiazepines	1	61	0	31	1.6%	100.0%	34.4%	100.0%	33.7%
Opiates	22	40	0	31	35.5%	100.0%	57.0%	100.0%	43.7%
Methadone	2	59	1	30	3.3%	96.8%	34.8%	66.7%	33.7%
Overall	77	357	3	214	17.7%	98.6%	44.7%	96.3%	37.5%

Summary Calculations for All Testing Completed

Summary data compiled based on all phases of the testing assessment with respect to true positives, true negatives, false positives and false negatives as well as calculations related to analyte specific performance and overall performance are provided for each device in the tables below (Tables 30 to 35). It should be noted that results from the cross-reactivity experiments were only included if false positive results were generated. Comparing the devices to published recommendations for device performance, the DDT5000 met the recommendations of greater than 90 percent sensitivity and specificity and greater than 95 percent accuracy under the ROSITA project criteria for all target analytes. The DDC3000 and DDS2 met the ROSITA recommendations in aggregate with the exception of the opiate assay on the DDC3000 and THC assay on the DDS2. These three devices also met the criteria proposed by the United Kingdom of 90 percent accuracy and a less than 5 percent false positive rate. If the DRUID criteria of greater than 80 percent performance across all categories was used, the DrugWipe with the DrugRead analyzer would be included, but only for the amphetamine/methamphetamine test panel. The total number of invalid cassettes that resulted from all testing performed was as follows: DDT5000 n=5, DDC3000 n=5, DrugWipe n=2, DDS n=7, AquliaScan n=2.

Table 30. Summary results for DDT5000 for all testing performed.

DDT5000 vs. All Testing									
Drug	**TP**	**FN**	**FP**	**TN**	**Sensitivity**	**Specificity**	**Accuracy**	**PPV**	**NPV**
THC	124	4	10	246	96.9%	96.1%	96.4%	92.5%	98.4%
Cocaine	127	1	0	254	99.2%	100.0%	99.7%	100.0%	99.6%
Amphetamine	128	0	0	254	100.0%	100.0%	100.0%	100.0%	100.0%
Methamphetamine	126	0	1	255	100.0%	100.0%	99.7%	99.2%	100.0%
Benzodiazepines	129	0	4	248	100.0%	98.4%	99.0%	97.0%	100.0%
Opiates	125	3	0	254	97.7%	100.0%	99.2%	100.0%	98.8%
Methadone	127	0	0	255	100.0%	100.0%	100.0%	100.0%	100.0%
Overall	886	8	15	1766	99.1%	99.2%	99.1%	98.3%	99.5%

Table 31. Summary results for DDC3000 for all testing performed.

DDC3000 vs. All Testing									
Drug	**TP**	**FN**	**FP**	**TN**	**Sensitivity**	**Specificity**	**Accuracy**	**PPV**	**NPV**
THC	122	2	0	183	98.4%	100.0%	99.3%	100.0%	98.9%
Cocaine	116	1	0	190	99.1%	100.0%	99.7%	100.0%	99.5%
Amphetamine	126	0	0	181	100.0%	100.0%	100.0%	100.0%	100.0%
Methamphetamine	122	2	0	183	98.4%	100.0%	99.3%	100.0%	98.9%
Opiates	103	12	0	192	89.6%	100.0%	96.1%	100.0%	94.1%
Overall	589	17	0	929	97.2%	100.0%	98.9%	100.0%	98.2%

Table 32. Summary results for DrugWipe for all testing performed.

DrugWipe with DrugRead vs. All Testing									
Drug	**TP**	**FN**	**FP**	**TN**	**Sensitivity**	**Specificity**	**Accuracy**	**PPV**	**NPV**
THC	38	79	2	131	32.5%	98.5%	67.6%	95.0%	62.4%
Cocaine	79	41	0	128	65.8%	100.0%	83.5%	100.0%	75.7%
Amphet/methamph	126	21	0	101	85.7%	100.0%	91.5%	100.0%	82.8%
Opiates	46	72	1	129	39.0%	99.2%	70.6%	97.9%	64.2%
Overall	289	213	3	489	57.6%	99.4%	78.3%	99.0%	69.7%

Table 33. Summary results for DrugWipe for all testing performed with manual interpretation.

DrugWipe with Manual Evaluation vs. All Testing									
Drug	**TP**	**FN**	**FP**	**TN**	**Sensitivity**	**Specificity**	**Accuracy**	**PPV**	**NPV**
THC	80	37	0	131	68.4%	100.0%	85.1%	100.0%	78.0%
Cocaine	110	16	0	122	87.3%	100.0%	93.5%	100.0%	88.4%
Amphet/methamph	157	2	0	89	98.7%	100.0%	99.2%	100.0%	97.8%
Opiates	104	18	2	124	85.2%	98.4%	91.9%	98.1%	87.3%
Overall	451	73	2	466	86.1%	99.6%	92.4%	99.6%	86.5%

Table 34. Summary results for DDS2 for all testing performed.

DDS2 vs. All Testing									
Drug	**TP**	**FN**	**FP**	**TN**	**Sensitivity**	**Specificity**	**Accuracy**	**PPV**	**NPV**
THC	59	57	1	219	50.9%	99.5%	82.7%	98.3%	79.3%
Cocaine	120	0	1	213	100.0%	99.5%	99.7%	99.2%	100.0%
Amphetamine	112	2	0	220	98.2%	100.0%	99.4%	100.0%	99.1%
Methamphetamine	114	2	2	217	98.3%	100.0%	98.8%	98.3%	99.1%
Benzodiazepines	116	1	0	217	99.1%	100.0%	99.7%	100.0%	99.5%
Opiates	114	0	0	220	100.0%	100.0%	100.0%	100.0%	100.0%
Overall	635	62	4	1306	91.1%	99.7%	96.7%	99.4%	95.5%

Table 35. Summary results for AquilaScan for all testing performed.

AquilaScan vs. All Testing									
Drug	**TP**	**FN**	**FP**	**TN**	**Sensitivity**	**Specificity**	**Accuracy**	**PPV**	**NPV**
THC	2	102	2	142	1.9%	98.6%	58.1%	50.0%	58.2%
Cocaine	3	102	0	143	2.9%	100.0%	58.9%	100.0%	58.4%
Amphetamine	73	29	1	145	71.6%	99.3%	87.9%	98.6%	83.3%
Methamphetamine	9	92	0	147	8.9%	100.0%	62.9%	100.0%	61.5%
Benzodiazepines	1	103	0	144	1.0%	100.0%	58.5%	100.0%	58.3%
Opiates	69	53	1	125	56.6%	99.2%	78.2%	98.6%	70.2%
Methadone	4	100	1	142	3.8%	99.3%	59.1%	80.0%	58.7%
Overall	161	581	5	988	21.7%	99.5%	66.2%	97.0%	63.0%

Table 36 reflects the performance of each of the five devices when aggregating all the scoreable tests from the cutoff, cross-reactivity, and environmental testing experiments. The DDT5000, the DDC3000, and each of the individual assays demonstrated performance consistent with the requirements of the ROSITA group (Viviane et al., 1999). The DDS2 data in aggregate also met the performance requirements for ROSITA; however, the THC assay did not. None of the individual assays on the DrugWipe with the DrugRead or the AquilaScan met the performance requirement of ROSITA, nor did the performance of either device in aggregate. However, when the DrugRead was evaluated manually, the amphetamine/methamphetamine assay would meet the ROSITA recommendations. The DDT5000, DDC3000 and DDS2 in aggregate also met the performance requirements for DRUID (2010; Schulze et al., 2012).

Table 36. Aggregate data for all testing completed by device.

Overall Device Test Results									
Device	**TP**	**FN**	**FP**	**TN**	**Sensitivity**	**Specificity**	**Accuracy**	**PPV**	**NPV**
DDT5000	886	8	15	1766	99.1%	99.2%	99.1%	98.3%	99.5%
DDC3000	589	17	0	929	97.2%	100.0%	98.9%	100.0%	98.2%
DrugWipe with DrugRead	289	213	3	489	57.6%	99.4%	78.3%	99.0%	69.7%
DrugWipe with Manual Evaluation	451	73	2	466	86.1%	99.6%	92.4%	99.6%	86.5%
DDS2	635	62	4	1306	91.1%	99.7%	96.7%	99.4%	95.5%
AquilaScan	161	581	5	988	21.7%	99.5%	66.2%	97.0%	63.0%

Conclusions

Discussion of Findings

The appearance of a new, unregulated, point-of-contact oral fluid drug testing frequently leads to a lot of products of variable applicability and quality, and the potential consumers of the technology are often ill-equipped to assess the suitability or reliability of these devices. The purpose of this evaluation was to explore the practical aspects of designing and performing tests on these devices to assess their accuracy, reliability, performance to specification, susceptibility to interference, and resistance of the consumables to extremes of temperature and humidity. We were also interested in more qualitative aspects of the devices performance to include, manufacturing quality, robustness, and ease of use.

This study selected five devices available for purchase in the United States and targeted to law enforcement for oral fluid drug testing for drug impaired-driving enforcement. The devices selected were the Dräger DrugTest 5000 (DDT5000), Dräger DrugCheck 3000 (DDC3000), Securetec DrugWipe S 5-Panel (DrugWipe), the Alere DDS2 Mobile System (DDS2) and the AquilaScan Oral Fluids Testing Detection System. Devices were selected based on having the appropriate scope of drugs, being consistent with the major drug classes of concern in impaired-driving casework, as determined from the National Safety Council's recommendations (Logan et al., n.d.), and contained at a minimum cannabinoids, opiates, cocaine/metabolite, methamphetamine/amphetamine, and in some cases also methadone or benzodiazepines.

The devices had different claimed analytical cutoffs or detection limits. The greatest variability was in the cutoffs for THC, for which ranged from 5ng/mL (DDT5000 and DrugWipe), to 15ng/mL (DDC3000), to 25ng/mL (DDS2), to 40ng/mL (AquilaScan).

The project was conducted in four phases.

Phase I: Assessment of suitability of human and various synthetic oral fluid mixtures for the study

This assessment evaluated daily, pooled, expectorated oral fluid from volunteers in the laboratory, which was verified as being drug-free with respect to the drugs of interest in this study, and was used without freezing or thawing and discarded at the end of the day. Authentic oral fluid was compared to two synthetic matrices (U.K. synthetic matrix and OraSure negative calibration solution). The various matrices were spiked with known amounts of the target analytes selected for the project at 30 percent above the manufacturers cutoff for the device in each of the matrices. Sample was then applied to the device as described by the manufacturer, and the results recorded. These experiments revealed generally good performance of the devices at this threshold across all matrices. None of the intermittent failures could be conclusively linked to any combination of device, analyte, or matrix.

Based on these considerations, in addition to the feasibility of acquisition, cost, and being able to attribute performance solely to the device and not the matrix and given the small scale of the oral fluid assessment and ready availability of volunteers, subsequent testing was conducted using drug free, pooled human saliva collected daily from volunteers as the testing matrix, to avoid any confounding of device performance in different synthetic recipes. The volunteers provided saliva

that was collected daily on the morning of each set of experiments and any excess was discarded at the close of business that day, and a fresh stock collected the following morning.

Phase II: Cutoff performance evaluation
Standards were prepared in freshly collected and pooled, drug-free human saliva, by spiking with verified drug standards at 30 percent above, at, and 30 percent below the manufacturers claimed cutoffs (Tables 9 to 14). Devices were scored as true positives if they detected the drug regardless of the concentration if the drug was present, and true negatives if the device was negative when the drug was below the manufactures' claimed cutoff or the drug had not been added to the control at any concentration. Drugs at the cutoff concentrations not detected by the devices were scored as false negatives for the purposes of this evaluation.

The DDT5000 produced results that yielded greater than 95 percent sensitivity, specificity, accuracy, PPV and NPV for all analytes on all tests performed during the cutoff evaluations. The DDT5000 detected 208 out of a possible 210 drug positive samples. With respect to testing at the cutoff concentration or 30 percent above the cutoff concentration, the DDT5000 detected every drug positive sample (n=140). The DDT5000 had the greatest number of false positive results, three for THC out of 182 drug negative challenge samples, and one for benzodiazepines during the cutoff evaluation. It performed in many cases better than the manufacturers claimed performance, consistently detecting drug when present at 30 percent below the claimed cutoff.

The DDC3000, the one wholly-manually-read device, demonstrated sensitivity, specificity and accuracy of greater than 95 percent for all assays except for opiates, for which it had sensitivity of 65 percent and accuracy of 95.3 percent. This reflects false negatives for 7 out of the 20 positive challenge samples. The 7 false negatives were at the cutoff concentration of the device. Overall the device had sensitivity, specificity and accuracy of 93.1 percent, 100 percent, and 98.8 percent. The DDC3000 readily detected THC, amphetamine, and methamphetamine at 30 percent below the cutoff concentration in all 10 replicates tested with the exception of one THC test. The device had no false positives.

The DrugWipe read with the DrugRead demonstrated variable results, with the THC, cocaine, and opiate assays all having sensitivity of less than 60 percent. Sensitivity for THC at 5ng/mL was 40 percent. This resulted in overall performance of sensitivity of 69.6 percent, specificity of 100 percent, and accuracy of 91.3 percent. This was due to the large number of false negatives, occurring in 34 of 112 positive challenges. The device produced no false positives. As noted above, an experienced operator manually reading the test lines in the DrugWipe cassettes, revealed additional positives, that the DrugRead did not flag.

The DDS2 demonstrated overall sensitivity, specificity and accuracy of 91.7 percent, 100 percent, and 98.9 percent, respectively. The device failed to detect THC in 8 out of 20 positive challenges 30 percent above (n=4) and at the manufacturer's cutoff of 25 ng/mL (n=4). This resulted in sensitivity, specificity and accuracy of 61.9 percent, 100 percent, and 95.6 percent respectively for THC. The device also had two false negatives for amphetamine, one for methamphetamine, and one for benzodiazepines. The device had no false positives.

The AquilaScan demonstrated overall sensitivity, specificity and accuracy of 37.9 percent, 100 percent, and 91.4 percent, respectively. These results were due in part to the devices failure to detect THC in any of the positive challenges at 30 percent over the claimed cutoff of 40ng/mL, the highest cutoff of any of the devices evaluated. It also failed to detect THC at 100 ng/mL and 1000 ng/mL, well above the concentration that would be expected in the oral fluid of a recent cannabis user. It similarly failed to detect cocaine, methadone, or benzodiazepines in any of the positive controls. The device performed best for opiates, with a sensitivity of 92.6 percent. The device did not produce any false positives during this phase of testing.

Phase III: Cross-reactivity evaluation
A series of six mixtures of commonly encountered drugs, some of which are desirable cross reactants in immunoassay tests as they belong to the same drug class as the target analyte, and can be included in confirmatory testing, and some of which may cause undesirable cross-reactivity, either resulting in positives in other drug class assays, or as unrelated compounds, causing false positives that are outside the scope of the assay target (Table 21).

None of the non-targeted drugs, which included caffeine, nicotine, non-steroidal anti-inflammatory drugs (NSAIDs), over-the-counter analgesics, selective serotonin/noradrenaline reuptake inhibitors (SSRIs/SNRIs), zolpidem, dextromethorphan, lidocaine, or PCP produced false positives on any of the test platforms at concentrations of 1000 ng/mL.

Other members of the drug classes to which the devices are targeted showed variable cross-reactivity. Generally, hydrocodone, codeine, and hydromorphone were well detected albeit at higher concentrations across all five devices. Oxycodone was not detected at 100 ng/mL and oxymorphone was not detected at 1000 ng/mL. The DDT5000 showed optimum sensitivity for opioids at a cutoff of 100 ng/mL and showed good responsiveness to the benzodiazepine category consistently giving positive results at 10 ng/mL for nordiazepam and temazepam, and detecting oxazepam one time. The DDT5000, DDC3000 and DDS2 detected MDMA and MDA at concentrations of 100 ng/mL.

Generally, the devices showed appropriate cross-reactivity to drugs that may be of interest in an impaired-driving context, and which are included in the drugs of interest in the NSC guidelines.

Phase IV: Interferent evaluations
A series of experiments evaluated the potential for other substances that may be present in the subject's mouth to cause interferences with the various assay platforms. These consisted running a series of solutions of foods and beverages (milk, beer, orange juice, soda), oral hygiene products, tobacco, and mint flavored gum. Saliva was mixed with commonly encountered drinks or orally ingested products (tobacco, gum, etc.) at a concentration of 5 percent of the total volume. The experiments were conducted spiking the pooled expectorated human saliva with the target analytes at 150 percent of their published cutoff concentration to evaluate suppression of positives, and into drug-free pooled saliva to evaluate the potential for false positives.

Given that the AquilaScan and DrugWipe, both showed some insensitivity to the target compounds around their published cutoffs it was difficult to evaluate the impact of potential interferents at those cutoffs. In general, chewing tobacco produced frequent false positives and

false negatives across all five devices. Coffee, milk, soda, and wintergreen mints produced intermittent and inconsistent false positive or false negatives on one device or another, but there was no consistent pattern of interference.

It is impossible to test every kind of food or beverage that may potentially be in a subject's mouth during the drug test evaluation. The manufacturers recommended best practice is likely to be to have an observation/deprivation period of 10 minutes, analogous to the practice to eliminate mouth alcohol interference in breath alcohol testing protocols.

Phase V: Environmental stressing of the test cassettes

Since oral fluid drug-testing devices are subject to wide ranges of temperature and humidity in roadside traffic enforcement conditions, it was important to evaluate how this effected the devices' performance. Test cassettes for each device were subjected to extremes of heat and humidity in environmental test chambers, then returned to the laboratory for evaluation of performance in testing oral fluid samples spiked at 30 percent over the manufacturers cutoff concentrations, as described in *Phase II*. The conditions simulated were various static temperatures ranging from 0°F to 100°F in 10-degree increments and various relative humidity levels of 25, 50, 75, 85, and 95 percent were also simulated. Each humidity level was replicated at temperatures of 25, 50, 75, and 100 degrees.

For the DDT5000, and the DDC3000, there were intermittent low frequency false positives and false negatives as demonstrated in *Phase II*, but with little impact on the overall performance of the device, which remained unchanged.

For the DrugWipe, the presence of partial lines in spiked positive samples, which the DrugRead did not identify as positives that was described in *Phase II* was also noted in this phase of the evaluation, but the rates of false positives and false negatives remained similar, and it was not possible to assign the cause of these to the heat and humidity challenges to the consumables. Manual reading of the DrugWipe consistently gave better performance (overall sensitivity and accuracy of 90.5 percent and 93.5 percent, compared to 50.8 percent and 66.7 percent, respectively).

The performance of the THC assay in the DDS2 demonstrated the largest difference in performance compared to the consumables that had not been subject to the environmental stressors. The sensitivity of the THC assay dropped from 61.9 percent in *Phase II* to 27.1 percent after the humidity and temperature exposure, with the device failing to detect 43 of 59 positive challenges at 30 percent above the cutoff. The other assay performed comparably to their Phase II performance.

Due to the performance of the AquilaScan during *Phase II*, it was difficult to evaluate the impact of the environmental stressing on this device. The overall sensitivity and accuracy of the device after the environmental stressing were 17.7 percent and 44.7 percent respectively, down from 37.9 percent and 91.4 percent respectively in *Phase II*, mostly due to a decline in the performance of the opiate assay which demonstrated a large increase in the number of false negatives.

Generally, the temperature and humidity exposure of the devices had no impact on the frequency of false positives.

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Appendix A

The Dräger DrugTest 5000

Figure 1. The Dräger DrugTest 5000.

The Dräger DrugTest (DDT5000) tests for a panel of seven drug classes: amphetamine (D-amphetamine at 50 ng/mL), methamphetamine (35 ng/mL), cocaine (D-methamphetamine at 20 ng/mL), opiates (morphine at 20 ng/mL), benzodiazepines (diazepam at 15 ng/mL), cannabinoids (delta9-Tetrahydrocannabinol at 5 ng/mL), and methadone (methadone at 20 ng/mL). The DDT5000 system (DrugTest 5000 analyzer, printer and keyboard) are in Figure 1. The DDT5000 analyzer weights almost 12 pounds. The DDT5000 comes sealed in a foil pouch (Figure 2) in packaged units of 20. Listed on the pouch are the test cassette lot numbers, the test cutoffs, expiration date, and the temperature range for the cassette storage. The DDT5000 sample collector and test cassette are one piece. The sample collector is a hard, white, porous material. To collect a sample, the safety cap and buffer cartridge are removed (Figure 3) and the collector is placed into the mouth of the subject and moved between the cheeks and gums. There is an adequacy indicator at the bottom of the sample collection tube, which will turn blue when enough sample has been collected (Figure 4). For this testing, sample was applied dropwise with a Pasteur pipette to the top of the collection tube until the indicator at the bottom turned blue. The DDT5000 collects approximately 150-200 µL of sample. The test strips are completely obscured from sight and located inside the test cassette, so there is no test line interpretation that is visible to the operator. The results are clearly displayed as positive or negative on the analyzer screen (Figure 8), which can also be printed.

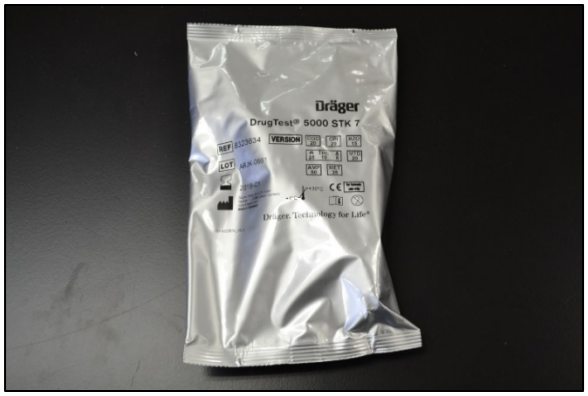

Figure 2. DDT5000 test cassette/sample collector comes in a sealed foil package labeled with the lot number, expiration date, cutoff information, and temperature range for storage.

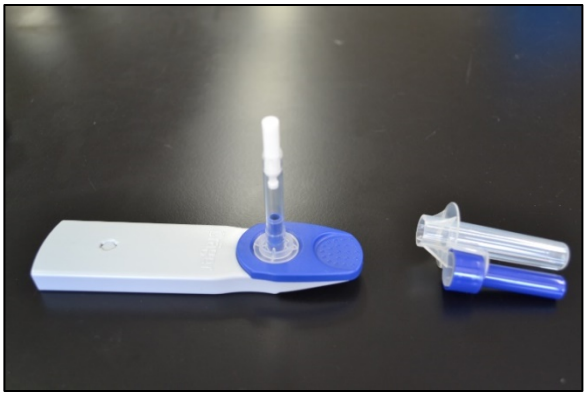

Figure 3. The safety cap and buffer cartridge are removed from the sample collector/test cassette.

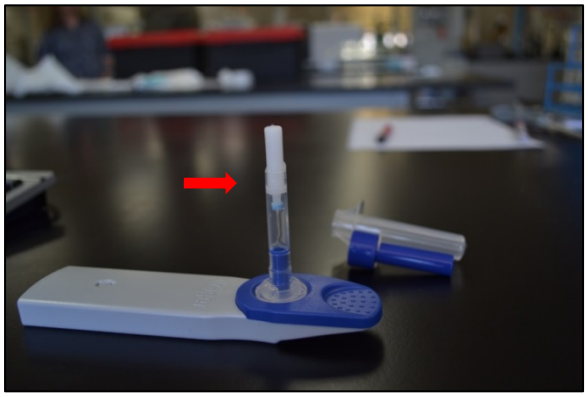

Figure 4. When enough saliva has been collected the indicator turns blue (see red arrow).

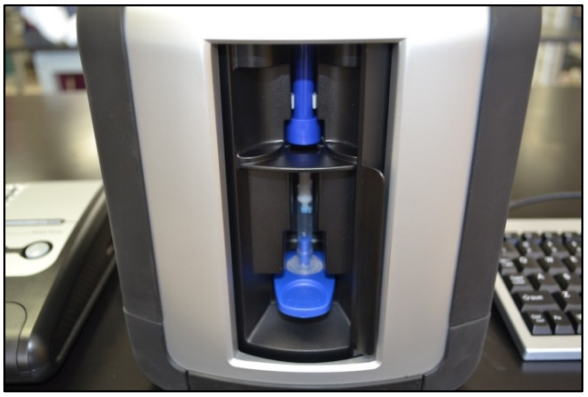

Figure 5. The test cassette and buffer cartridge are inserted into the analyzer.

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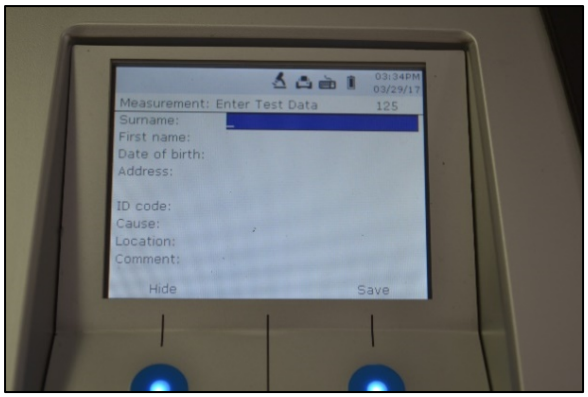


Figure 6. Sample data questionnaire on the DDT5000.

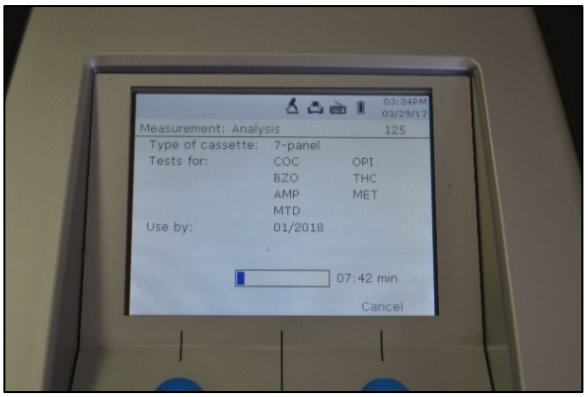


Figure 7. Testing in progress.

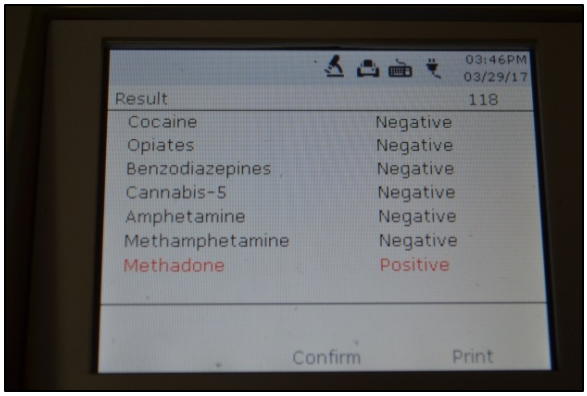


Figure 8. DDT5000 display of test results with the option to print. The "Confirm" is a recommendation/instruction not a separate functionality of the analyzer.

The Dräger DrugCheck 3000:

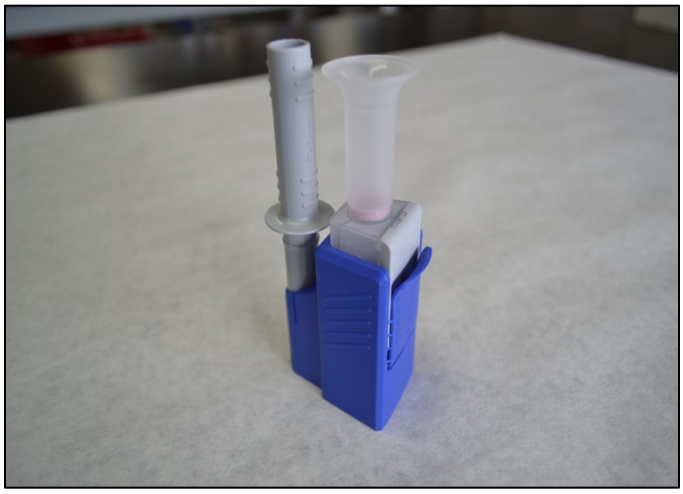


Figure 9. The Dräger DrugCheck 3000.

The Dräger DrugCheck 3000 (DDC3000) (Figure 9) consists of a sample collector and a test cassette. The device tests for a panel of five drug classes: amphetamine, methamphetamine, cocaine, opiates, and THC. The target analytes and cutoffs are listed as: D-amphetamine at 35 ng/mL, D-methamphetamine at 35 ng/mL, cocaine at 20 ng/mL, morphine at 20 ng/mL, and THC at 15 ng/mL. The DDC3000 does not have a test reader. Thus, the operation, result interpretation, and result documentation are completely manual and reliant on the operator. For operation and result interpretation a timer is strongly recommended but not included. The DDC3000 comes sealed in a foil pouch (Figure 10) in packaged units of 20. Listed on the pouch are the test cassette lot numbers, the test cutoffs, expiration date, and the temperature range for the cassette storage. The test cassette contains an internal ampule of buffer and two test strips. The sample collector consists of a gray plastic handle and a hard, porous, white collection material which has a pink color indicator at the tip (Figure 11). The sample collector is placed in the mouth of the subject and the collector is moved between the cheeks and gums. Once enough sample is collected, the pink indicator on the sample collector tip fades away (Figures 12 and 13). Sample collection typically takes 15 to 30 seconds. For our study, sample was applied

slowly to the collector with a Pasteur pipette to the top while rotating and allowed to run down toward the color indicator. The entire surface was evenly wetted until the color indicator changed. Any excessive drips were gently shaken off. The DDC3000 required approximately 300 µL of sample.

Once enough sample is collected, the sample collector is inserted into the funnel tube of the test cassette (Figure 14). It must be pressed completely down until the hilt of the sample collector is completely flush with the funnel top of the test cassette, breaking open the ampule of buffer fluid contained inside the test cassette. Then the test cassette must be shaken vigorously to mix the sample and buffer for approximately 30 seconds until the color indicator fades (Figures 14 and 15). After successfully mixing the sample and buffer, it is recommended to let the cassette sit upright for 10 to 60 seconds. A 10-second wait time is recommended for a fast THC test, which list the THC cutoff at 25 ng/mL. For a lower THC cutoff of 15 ng/mL, operators are instructed to wait a full 60 seconds at this point. Once the wait time has elapsed, the blue front perforated security cover is removed exposing the test strips (Figure 16). The gray inner portion containing the sample collector and funnel tube is then pressed completely down into the blue outer base of the test cassette (Figure 17) initiating the test.

For evaluation of the results, a sample is negative for a drug with the appearance of a test line along with the successful appearance of the control lines. If after five minutes a red line has not appeared, and the control lines have, the sample is considered a positive screen result. In the example shown in Figure 18, the sample is positive for amphetamines while methamphetamine, THC, opiates, and cocaine were not detected. The results must be evaluated within 10 minutes of starting the test. The THC test line takes the longest to appear and is often faint in color intensity. This can be seen in Figure 18 where the THC line appears less defined. The THC test line also continues to develop with time, which can turn a true positive THC sample into a false THC negative if the result is evaluated beyond the 5- to 10-minute window. For temperatures between 50°F and 41°F, more time is required for the test with the results not being evaluated until after 10 minutes from the start of the test.

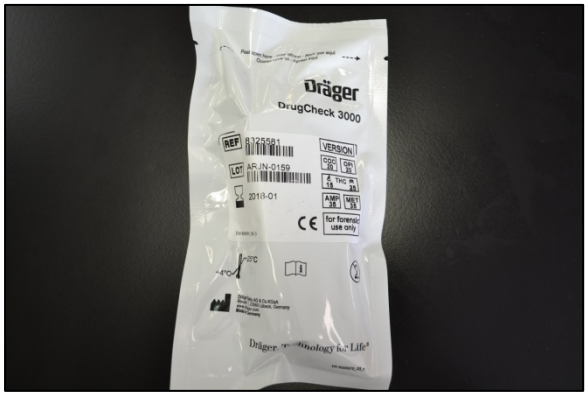


Figure 10. Sealed foil pack containing individual DDC3000.

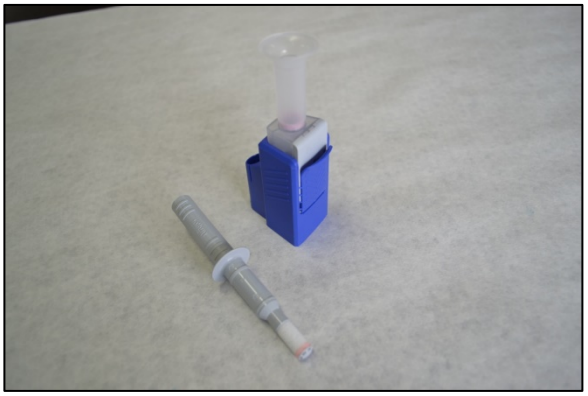


Figure 11. DDC3000 test cassette and sample collector.

Figure 12. A pink line is visible on the sample collector before sampling.

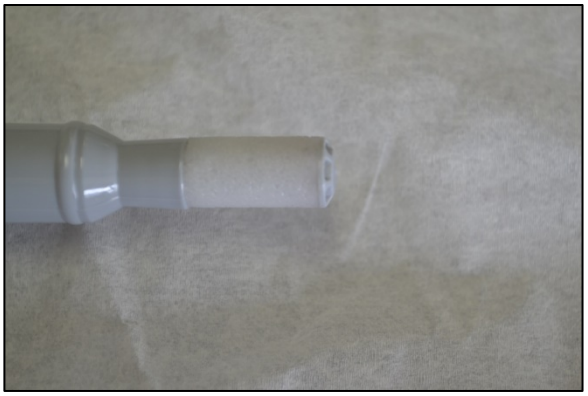


Figure 13. The pink indicator disappears after collecting sufficient sample.

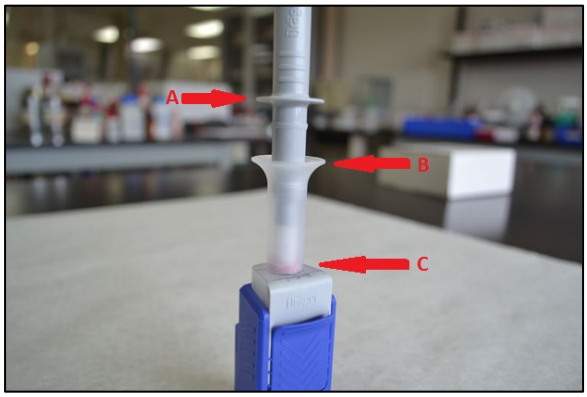


Figure 14. Arrow A: The sample collector hilt. Arrow B: Funnel top. Arrow C: Pink color indicator line for the buffer.

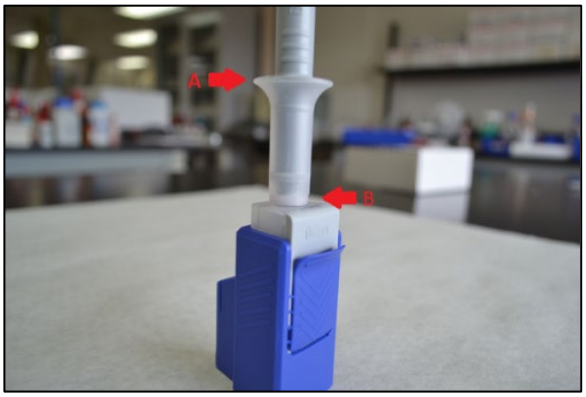


Figure 15. Arrow A: Sample collector hilt now flush with funnel top. Arrow B: Pink color has faded away after shaking indicating mixing of the sample and buffer.

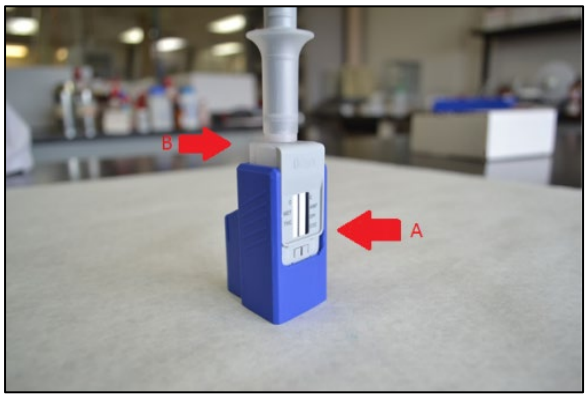


Figure 16. Arrow A: Blue security cover removed, exposing the test strips. Arrow B: note the raised gray portion.

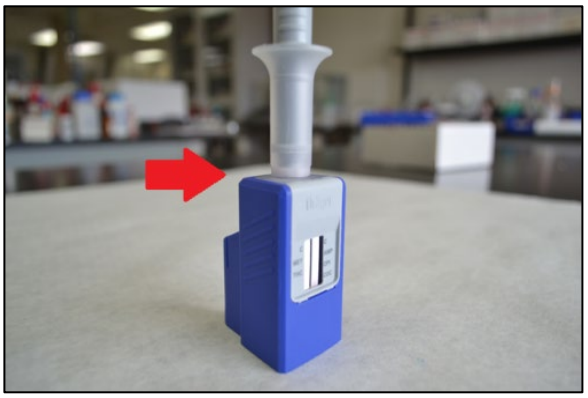


Figure 17. The sample collector and test cassette funnel have been forcefully pressed down completely into the test cassette initiating the test.

Figure 18. Completed test. The appearance of a line no matter how faint indicates a negative test. The absence of a visible line is indicative of a positive. This example is positive for amphetamine.

The DrugRead:

Figure 19. The DrugRead.

The DrugRead system consists of an analyzer with printer (Figure 19) and utilizes the DrugWipe 5S test cassette (Figure 24). The DrugWipe 5S test for four drug classes: amphetamines (including methamphetamine), cocaine, opiates, and cannabinoids. The target analytes and cutoffs are listed as: D-amphetamine at 80 ng/mL, D-methamphetamine at 80 ng/mL, cocaine at 10 ng/mL, morphine at 10 ng/mL, and THC at 5 ng/mL. While D-amphetamine and D-methamphetamine are both considered target analytes, the DrugWipe 5S does not differentiate between the two as it provides for only the one shared, “am/met” test line (Figure 24). The DrugRead measures 21 cm X 10 cm X 12 cm. There is a separate printer that is connected to the DrugRead via a Bluetooth link. The printer measures 9.5 cm X 10 cm X 3.7 cm. Together the printer and DrugRead analyzer weigh approximately 2.1 pounds. The DrugRead is equipped with a touch screen as an interface. It is also equipped with GPS capability allowing for exact documentation of the location of testing. Included with the DrugRead analyzer are two quality control test cassettes (Figure 20) (positive cassette and a negative cassette) to verify that the DrugRead analyzer is capable of correctly interpreting positive and negative results. To run the

quality control cassettes a separate quality control test must be selected from the test menu (Figure 21). A 5-minute test timer appears which may be skipped as there is no test development time. Once the analyzer is done reading the cassette, a quality control test result is displayed on screen (Figure 22). It is recommended that the quality control tests are run once each day the analyzer is used.

The DrugWipe 5S comes individually sealed in a foil package (Figure 23). Each foil package is labeled with the lot number, expiration date, and the temperature range for storing the cassette. On the back of the foil package there is a series of pictures depicting the use of the DrugWipe 5S. The DrugWipe 5S test cassette consist of three parts. There is a gray plastic protective cover which is slid off. The white bottom portion that contains the test strips and the buffer ampoule. The third part is the removable top blue portion which is the sample collector (Figures 24 and 25). With the sample collector unclipped from the test cassette, there are three thin, pink, absorbent pads underneath. To collect the sample, the pads are simply wiped across the tongue of the test subject. When enough sample has been collected, the pads will change color from pink to yellow (Figures 27 and 28). Sample collection with the DrugWipe 5S is the easiest and fastest process in the comparison. For the study, a small drop of sample was applied with a Pasteur pipette. The small drop was then spread around the pad with the pipette needle until the entire pad had contact with sample and turned yellow. The excess sample was gently shaken off. The pads are very thin, and they do not absorb a large volume of sample. Any part or section of the pad which makes any contact with sample instantly changes color from pink to yellow. The DrugWipe 5S used the least amount of sample with approximately 5 µL of sample being applied per pad.

Once the sample has been collected, the sample collector is clicked back into place on the test cassette. The ampoule at the bottom of the test cassette is then broken by pressing where the test cassette is labeled, “PRESS” (Figure 28). The instructions provided with the DrugRead lists two different approaches at this point. One is to lay the test cassette flat, insert it into the DrugRead analyzer, and press where the cassette is labeled “PRESS” to break the ampoule. The second procedure is illustrated on the test cassette foil package. That is to hold the test cassette vertically with the ampoule at the bottom, press where the cassette is labeled “PRESS” breaking the ampoule, and to continue to hold the test cassette in the vertical position for 10 seconds. The test cassette is then inserted into the DrugRead analyzer (Figures 29 and 30). The operator must select the correct method and manually start the run by pressing start. During development, the test cassette should be kept level and horizontal. The DrugRead analyzer is equipped with sensor and will alert the operator if the system is not kept level. The test cassette is not labeled, or bar coded with the expiration date. With the expiration date only on the test cassette foil package it is up to the test operator to manually verify that the test cassette is valid. After 5 minutes, the results are displayed on the DrugRead screen (Figures 31 and 32). They are followed by a three-screen sample questionnaire (one represented in Figure 33). The first two screens concern the test subject data and include: first name, last name, ID, license number, birthday, and address. The third screen has fields for the input of the operator ID, location, and a comment field. The DrugRead can store up to 1,000 results and they can be downloaded to a personal computer.

The results of the DrugWipe 5S are visible and labeled as the DrugWipe 5S is designed to be read visually (Figure 34). This can lead to some ambiguity between the visual results and the

results as interpreted by the DrugRead analyzer. The DrugRead always reads the results under the same conditions, sensors, and algorithms, which may differ from results that are evaluated visually. Due to that, the included manual states that, “the DrugRead result is authoritative.” The manual also states that in rare cases, the DrugRead may misinterpret a result due to partial or incomplete test lines. This issue occurred during our testing (Figures 35 and 36). The DrugRead gave a few false positives during testing when no line was visible. In those instances, a rescan of the DrugWipe cassette produced the correct result.

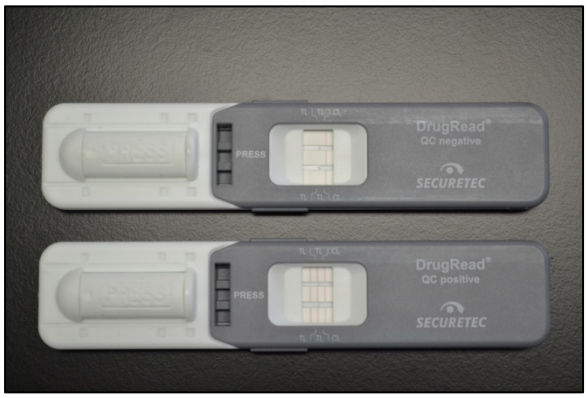


Figure 20. The DrugRead quality control cassettes. One negative control and one positive control.

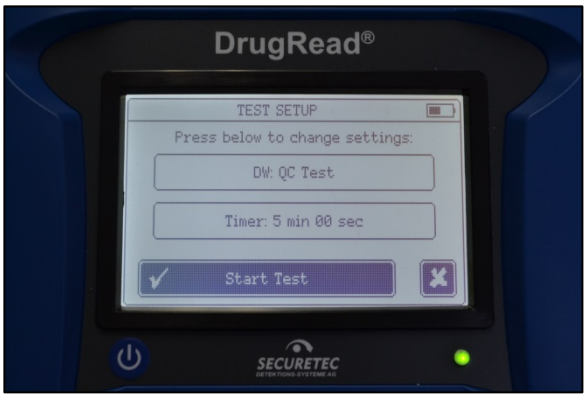


Figure 21. The DrugRead test setup to perform the quality control test.

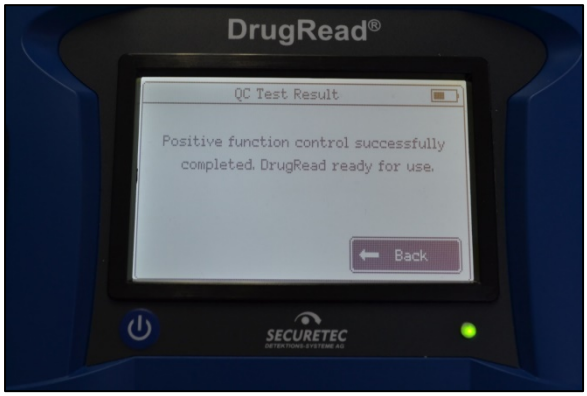


Figure 22. Message displayed after successful qc evaluation.

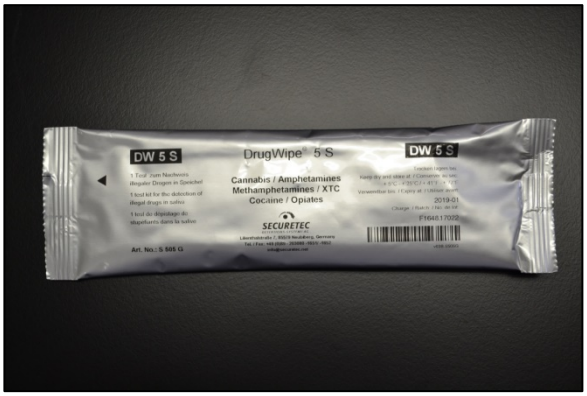


Figure 23. The DrugRead uses the DrugWipe 5S cassette which comes in a sealed foil package labeled with the lot number, expiration date, and temperature range for storage.

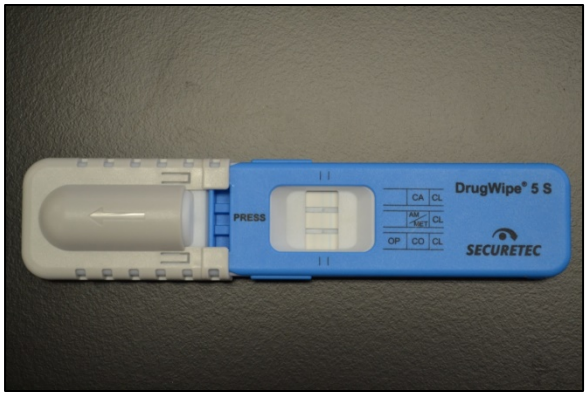


Figure 24. The DrugWipe 5S test cassette.

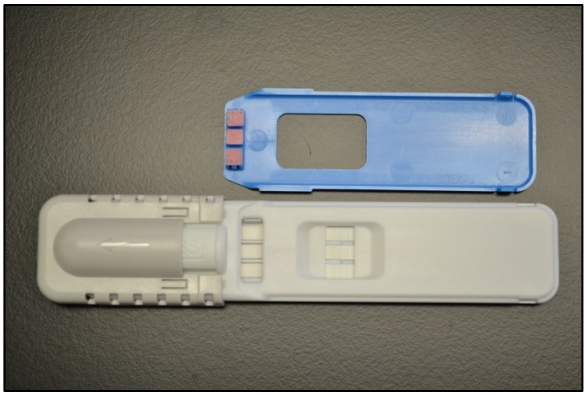


Figure 25. The DrugWipe 5 S test cassette with sample collector removed.

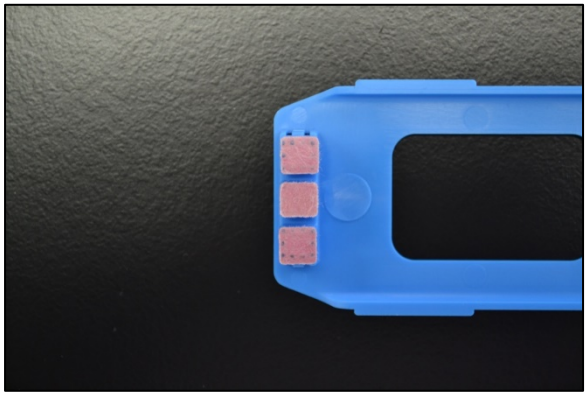


Figure 26. The DrugWipe 5S sample collector uses three thin pink colored pads to collect sample.

Figure 27. The DrugWipe 5S sample collection pads change color from pink to yellow when in contact with sample.

Figure 28. Once sampling is complete the collector is snapped back into the test cassette and an ampoule of buffer is manually broken.

Figure 29. Resting horizontal and level the DrugWipe 5S test cassette is inserted into the DrugRead analyzer.

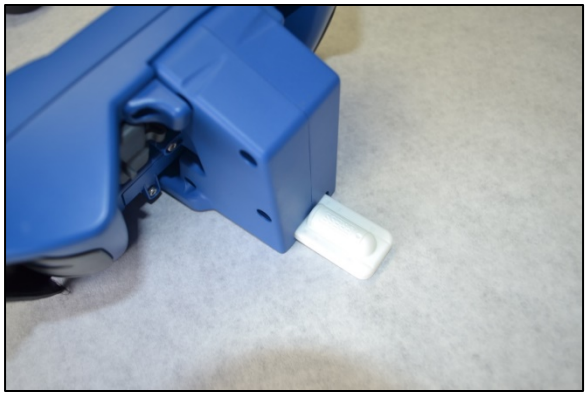


Figure 30. The DrugWipe 5S completely inserted into the DrugRead analyzer.

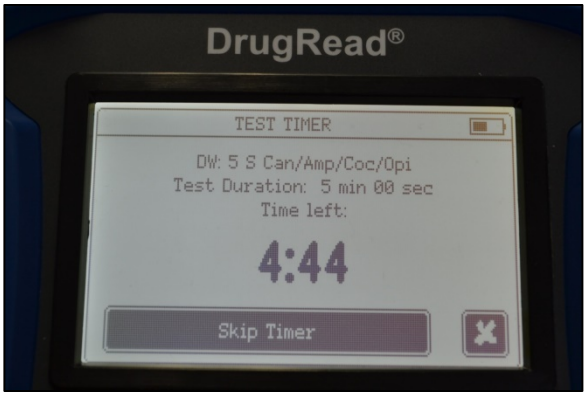


Figure 31. The DrugRead analyzer times the development of the test cassettes before reading the results.

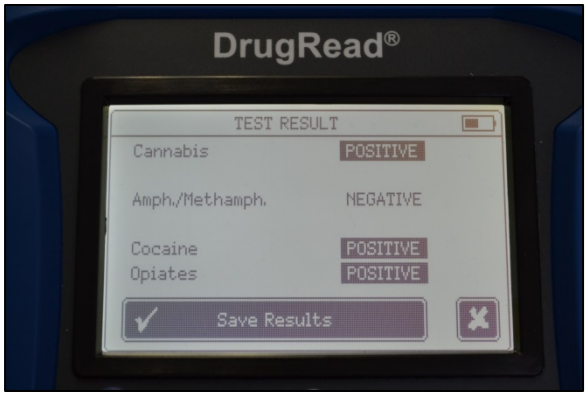


Figure 32. The displayed results.

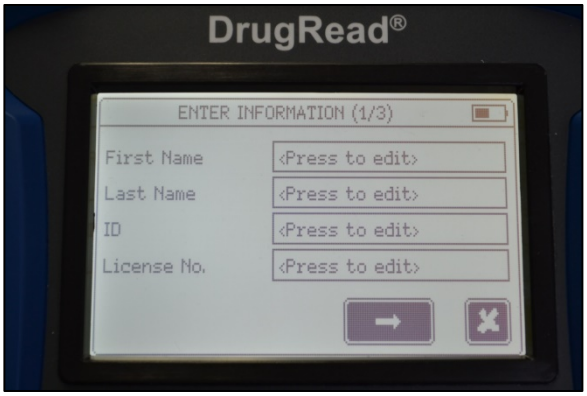


Figure 33. The first screen sample questionnaire.

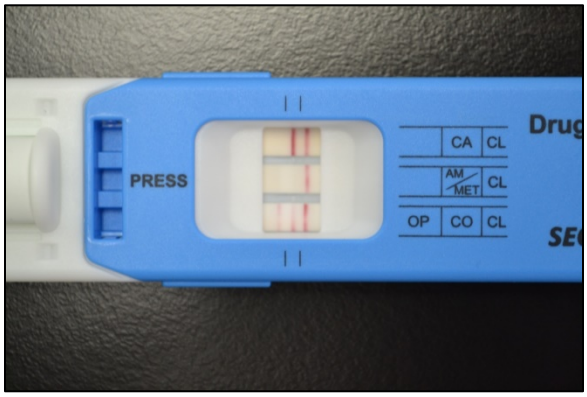


Figure 34. Positives for cannabinoids, cocaine, and opiates on the DrugWipe 5S. A visible line indicates a positive result on the DrugWipe 5S.

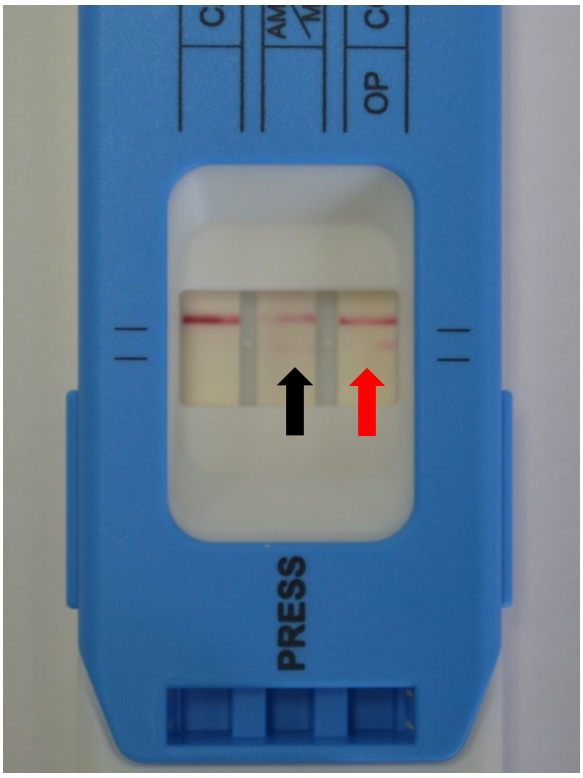


Figure 35. A faint line indicating a positive amphetamine/methamphetamine (see black pointer) and an incomplete/partial developed cocaine test line (see red pointer).

Figure 36. Incomplete or partial opiate test lines. One was detected by the DrugRead analyzer, the other was not.

The Alere DDS2:

Figure 37. The Alere DDS 2.

The Alere DDS2 (DDS2) tests for a panel of six drug classes: amphetamines, methamphetamine, cocaine, opiates, benzodiazepines, and cannabinoids. The target analytes and cutoffs are listed

as: d-amphetamine at 50 ng/mL, d-methamphetamine at 50 ng/mL, benzoylecgonine at 30 ng/mL, morphine at 40 ng/mL, temazepam at 20 ng/mL and THC at 25 ng/mL. The DDS2 system (analyzer, printer, test cassette and sample collector) can be seen in Figures 37 and 38. The DDS2 analyzer is small enough to be hand held with dimensions of 22.2 cm X 8.9 cm X 6.4 cm. The analyzer has a weight of approximately 1.5 pounds. The printer is approximately 1 pound. The analyzer and printer can be used without removing them from the carrying case (Figure 39). The DDS2 includes color changing desiccant as an additional quality control check (Figure 40). If the silica gel is yellow, the test cassette is ok to use; however, if the silica gel is green, the test cassette should be discarded.

The sample collector is a pleated sponge on the end of a clear plastic handle (Figure 41). A sample is collected by swabbing the inside of the subject's cheeks, gums, and tongue. Once enough sample has been collected, a color indicator will turn blue (Figure 42). For the study, sample was applied to the collector slowly up and down the pleats while rotating with a Pasteur pipette, stopping when the indicator turned blue and the sponge was uniformly wetted. This required approximately 450 µL of sample.

Also included with the DDS2 are two quality control test cassettes (one positive control cassette and one negative control cassette) that allow the operator to verify the DDS2 analyzer is capable of correctly interpreting positive and negative results (Figure 43). There is no method or menu option required to run them. The quality control cassettes are inserted into the bottom of the DDS2 (Figure 44) like a normal test cassette. After the analyzer reads the first quality control cassette, it will prompt the operator to remove it and insert the second one. Once complete, the DDS2 will display a green check indicating the analyzer is ok and ready to use (Figure 45). It is recommended that the quality controls tests are run once each day the analyzer is used.

For the analysis, the test cassette is inserted into the bottom of the analyzer as shown in Figure 44. The analyzer reads the bar code on the cassette and determines if the cassette has expired or is ok to use. If the cassette is past its expiration date, the DDS2 will not allow the operator to use it. The expired cassette must be removed from the analyzer and a different cassette that has not expired can be inserted. With the test cassette inside the analyzer, the sample collector is then inserted into the bottom of the test cassette (Figure 46). Inserting the sample collector completely punctures the buffer membrane and automatically triggers the start of analysis. As with the DDT5000, the DDS 2 is completely automated. The DDS2 times the testing (Figure 47). If the analyzer is not held or placed on a level surface, there is a tilt sensor. There is an on-board heater to control the cartridge temperature. The DDS2 will also throw a temperature error code if the environmental conditions are outside of the analyzers operational range of 41°F-95°F. Testing is completed in 5 minutes.

The results on the DDS2 are clearly displayed as positive or negative (Figure 48). The result interpretation is automatically and completely handled by the analyzer. The DDS2 analyzer interprets not only the presence of control and test lines, but also the intensity of the test lines. While test lines maybe visible on the test cassette to the human eye (Figure 49), they cannot be interpreted by the human eye and are not labeled avoiding any subjectivity related to manual interpretation. After the result screen, the DDS2 takes the operator through a four-screen questionnaire for data entry regarding the test subject including: age, gender, reason for test (pre-

employment, random, post-accident/incident, and for cause/intercept) and vehicle type (car, goods vehicle/truck, motorcycle, and other) (Figure 50). The questions included in the questionnaire can be edited through optional software. Once the questionnaire is completed or skipped, the results can be stored or printed. The DDS2 can store up to 10,000 test results. The results can be downloaded to a personal computer with the use of optional software. The DDS2 analyzer also features a micro SD card, which can be used for firmware updates and the addition of new test cassette types to the analyzer.

Figure 38. The DDS2 test cassette and sample collector are further sealed in foil packaging labeled with the lot number, expiration date, and temperature range for storage.

Figure 39. The DDS2 in use from inside the carrying case.

Figure 40. The DDS2 test cassette comes with a color changing desiccant. If it is yellow, testing may proceed; if it is green, the test cassette should be discarded.

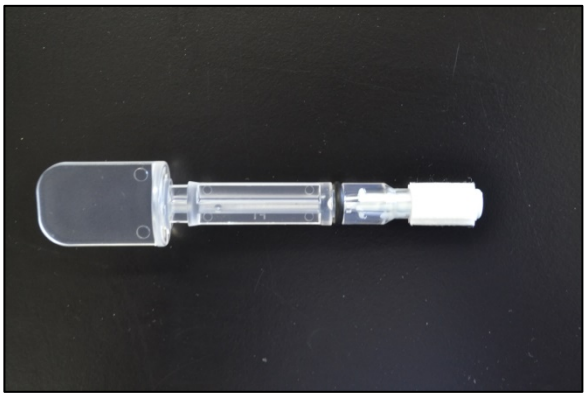


Figure 41. The DDS2 sample collector.

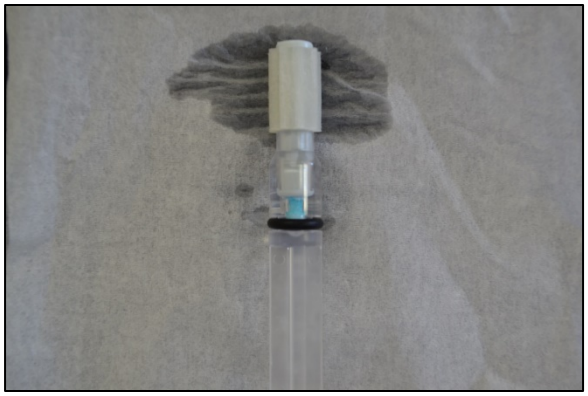


Figure 42. When enough saliva has been collected the indicator turns blue.

Figure 43. The DDS2 comes with a set of external quality control test cassettes.

Figure 44. The test cassette is inserted into the bottom of the DDS2.

Figure 45. The DDS2 after running the quality control test cassettes

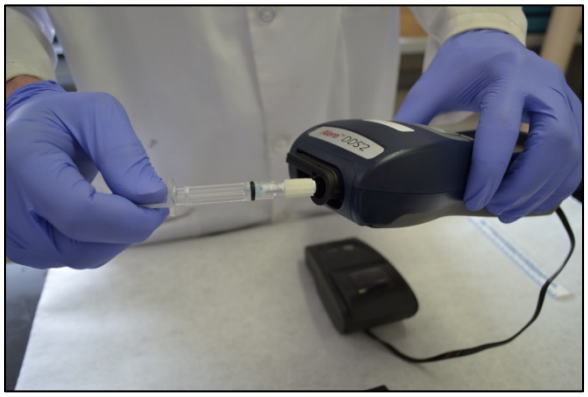


Figure 46. The sample collector is then inserted into the test cassette already inside the DDS2 analyzer**.**

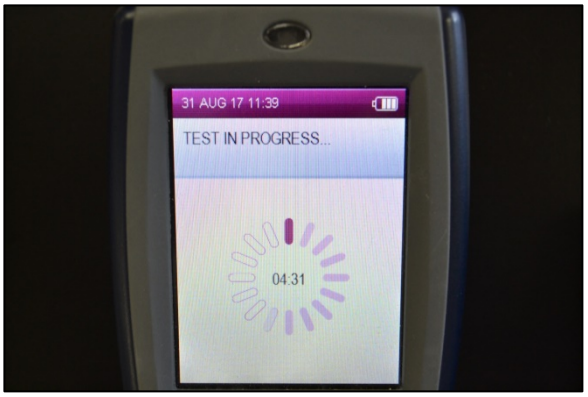


Figure 47. The DDS2 is completely automated. Testing begins automatically when the sample collector is fully inserted into the test cassette already in the analyzer.

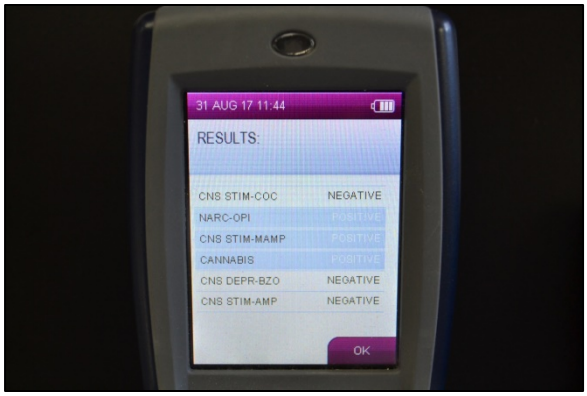


Figure 48. The DDS2 display of the test results.

Figure 49. The test result interpretation is completely reliant upon the DDS2 analyzer. Though some test/control lines maybe visible, they are not labeled and not readable without the analyzer. One of these test cassettes is completely negative, the other is positive for methamphetamines, opiates, and cannabis.

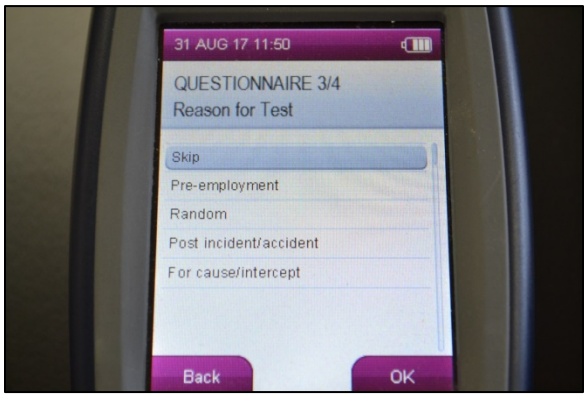


Figure 50. Post analysis questionnaire for entering test subject information.

The AquilaScan:

Figure 51. The AquilaScan.

The AquilaScan consists of the analyzer (Figure 51), the test cassette, and sample collector (Figure 52), and it tests for a panel of seven drug classes: amphetamine, methamphetamine, cocaine, opiates, benzodiazepines, cannabinoids, and methadone. The target analytes and cutoffs are listed as: d-amphetamine at 50 ng/mL, d-methamphetamine at 50 ng/mL, cocaine at 20 ng/mL, morphine at 20 ng/mL, diazepam at 15 ng/mL, THC at 40 ng/mL and methadone at 15 ng/mL. The AquilaScan analyzer measures 20.5 cm X 9 cm X 5.5 cm and weighs approximately 1 pound. It includes an integrated printer. The AquilaScan is equipped with a touch screen as an interface as well as with GPS and 3G/ WiFi capability allowing for exact documentation of the location of testing. The AquilaScan test kits come in zip-lock bags of 10 sealed foil packs, each pack is labeled with the lot number, expiration date, test included, and cutoff values (Figure 53). Each foil pack includes the test cassette, the sample collector, and a paper splash guard. The AquilaScan system does not include a buffer solution.

The test cassette contains the test strips and a bar code on the back (Figure 54). The sample collector is a sponge on the end of a clear plastic handle (Figure 55). Sample is collected by swabbing the inside of the subject's cheeks, gums, and tongue. Once enough sample has been collected a color indicator disk, located in the center between the handle and the sponge, will turn red (Figure 56). For the study, sample was applied to the collector sponge with a Pasteur pipette and then worked into the sponge by mopping inside a small plastic weighing boat until the sponge was completely soft, with no dry spots, and the color indicator was red. Approximately 1.5 mL of sample was required.

At the start of the test process the AquilaScan prompts the operator for the test information. The analyzer takes the operator through three screens of questions. The first screen concerns the particulars of the operator (Figure 57) and screens two and three are for the details of the test subject (Figures 58 and 59). The test cassette bar code is then scanned, and the test kit information is displayed on the screen (Figures 60 and 61). Once enough sample has been collected and the cassette bar code has been scanned, the sample collector is inserted into the test cassette. This requires the sample collector to be snapped down into place and then rotated to

help with wicking (Figures 62 and 63). The insertion of the sample collector into the test cassette compresses the sponge and releases the sample into the cassette. It can also cause the sample to splash outside of the test cassette. To help guard against splashing, a paper disk is included to be slipped over the sample collector handle, which acts as an absorbent cover of the opening.

With the sample collector correctly seated in the test cassette, the sample flows up the test strips. It is written in the instructions to wait until the control lines, at the top of the test strips, are developed before inserting the test cassette into the analyzer. This takes approximately 3-5 minutes. With the controls developed the test cassette is inserted into the analyzer and a 5-minute timer is started (Figures 64 and 65). The total time of analysis is 8-10 minutes.

At the end of the countdown, the results are clearly displayed on the analyzer along with a picture of the test lines (Figure 66). The instructions note that if the visual results do not match the results on screen, the operator should use the visual results as observed by the human eye. Any presence of a test line, no matter how faint, is a negative result. The analyzer can store up to 100,000 results. The stored results include the text display along with the picture of the test lines. The results can be down loaded to a personal computer through a USB port with separately sold software.

During the study, there were instances where it was difficult to interpret the color change of the sample presence indicator in the sample collector. The disk would only turn red on a small edge of the disk or not at all. Other times the disk would clearly turn red. This difficulty may have led to an over saturation of the collection sponge with attempts to collect adequate sample volume, which in turn may have led to the AquilaScan test cassettes to leak out of the cassette edges after the test lines were done developing (Figure 67). The cassettes would leak into the AquilaScan analyzer to the point that saliva could be observed on and outside of the device (Figures 68, 69 and 70).

Figure 52. The AquilaScan test cassette, sample collector, and paper splash guard.

Figure 53. The AquilaScan test cassette and sample collector are sealed in foil packaging labeled with the lot number, expiration date, and testing cutoffs.

Figure 54. The back of the AquilaScan test cassette includes a bar code to be scanned by the analyzer to determine testing and whether the test kit is expired.

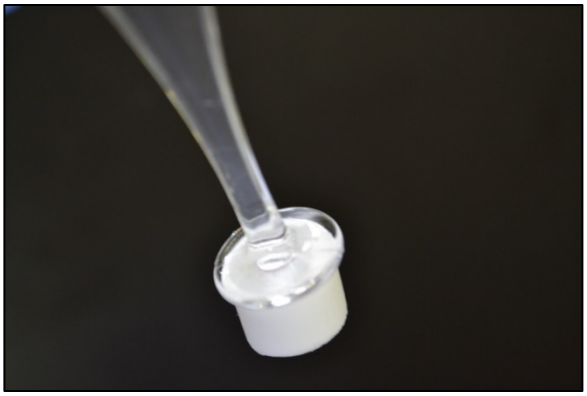


Figure 55. The AquilaScan sample collector consist of a clear plastic handle and collection sponge.

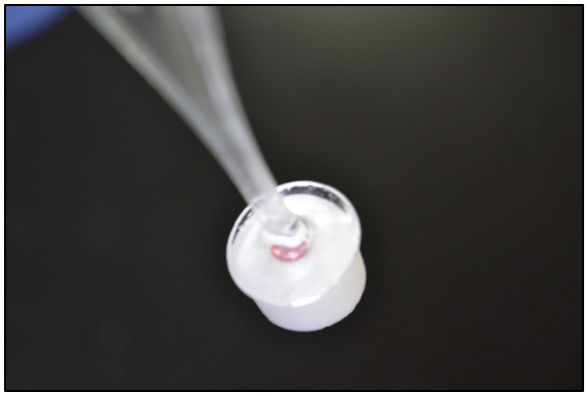


Figure 56. Once enough sample has been collected, a color indicator disk will turn red between the handle and the collection sponge.

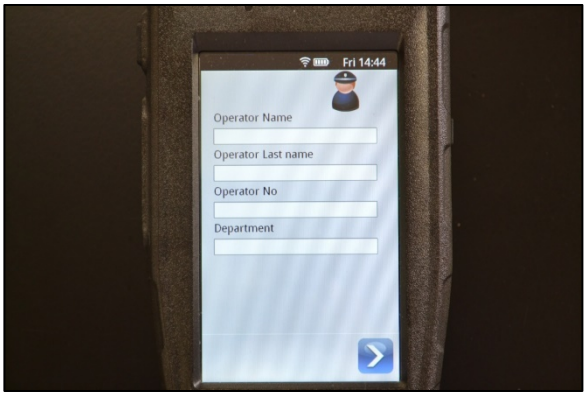


Figure 57. Pretesting questionnaire screen one.

Figure 58. Pretesting questionnaire screen two.

Figure 59. Pretesting questionnaire screen three.

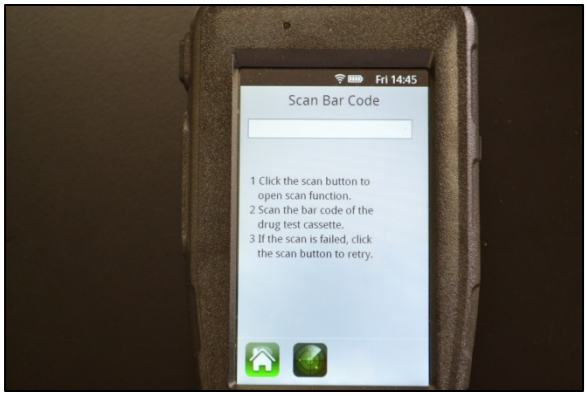


Figure 60. The analyzer prompt for the test cassette bar code prevents the use of an expired test kit.

Figure 61. After scanning the barcode, the analyzer displays the testing included on the cassette as well as the cutoffs.

Figure 62. Once enough sample has been collected and the bar code scanned a paper splash guard is slid onto the base of the sample collector.

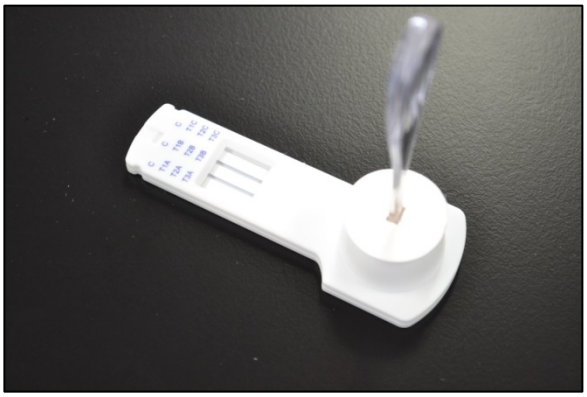


Figure 63. The sample collector is then snapped into the test cassette and twisted compressing the sponge and delivering sample.

Figure 64. Once the control lines have formed the test cassette is inserted into the side of the analyzer.

Figure 65. Once the test cassette is inserted into the analyzer a five-minute timer is automatically started.

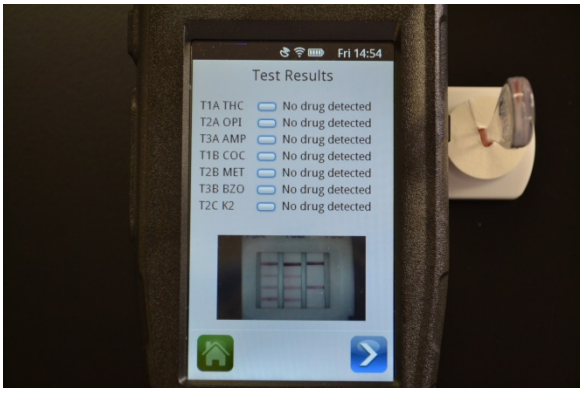


Figure 66. After five minutes the test results are displayed on screen along with a picture of the test lines.

Figure 67. Saliva leaked from the AquilaScan test cassette.

Figure 68. Saliva observed from the AquilaScan analyzer after leaking from the test cassette**.**

Figure 69. Saliva observed from the AquilaScan analyzer after leaking from the test cassette.

Figure 70. Saliva observed from the AquilaScan analyzer after leaking from the test cassette.

Temperature Notes:

Table 1. Recommend storage conditions, operational temperature ranges and humidity for all devices.

Device	Cassette storage temperature		Operating temperature		Humidity	
	Low (°F)	High (°F)	Low (°F)	High (°F)	Low (%)	High (%)
DDT5000	39.2	86	41	104	5	95
DDC3000	39.2	77	41	104	5	95
DDS2	59	77	41	95	20	80
DrugWipe 5S	41	77	41	104	0	90
AquilaScan	35.6	86	-	-	-	-

Table 2. Notes related to operation from each device's instructions.

Device	Notes
DDT5000	Make sure cassettes are at ambient temperature before starting.
DDC3000	In temperatures below 50°F, positive results must not be evaluated until 10 minutes after starting the test (normally 5 minutes). Recommended to warm device in hands before use in temperatures below 50°F.
DrugRead	Storage/transportation 14°F-140°F.
DDS2	Do not freeze. Test cartridges must not be at ≤ 23°F for more than 10 minutes. The Alere DDS 2 has as an onboard heater and thermometer which will throw an error code if outside of operational range.
AquilaScan	No other operating conditions specified.

Appendix B

Testing Certifications # **503.01**

MTO2017-145

NATIONAL FORENSIC SCIENCE TECHNOLOGY CENTER

8285 BRYAN DAIRY RD., STE 125

LARGO, FL 33777

Prepared for: Kevin Lothridge

E-Mail Address: Kevin.Lothridge@NFSTC.org

Office Phone: [Redacted]

Report Date: 10/12/2017

ENVIRONMENTAL TEST REPORT MTO2017-145

for

Temperature Testing &

Temperature/Humidity

applied to

Test Kits

in accordance with Customer Specifications

& Quote Q2017-520

Submitted to

National Forensic Science Technology Center

8285 Bryan Daily Rd., Ste. 125

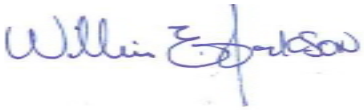
Largo, Fl 33777

Customer PO. [Redacted]

Report Date: 10/12/2017

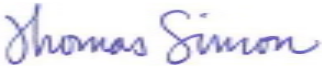
Approved by: William E. Jackson

EMAIL: William.jackson@genthermcsz.com



Reviewed by: Thomas Simon

EMAIL: Tom.simon@genthermcsz.com



Job # MTO2017-145

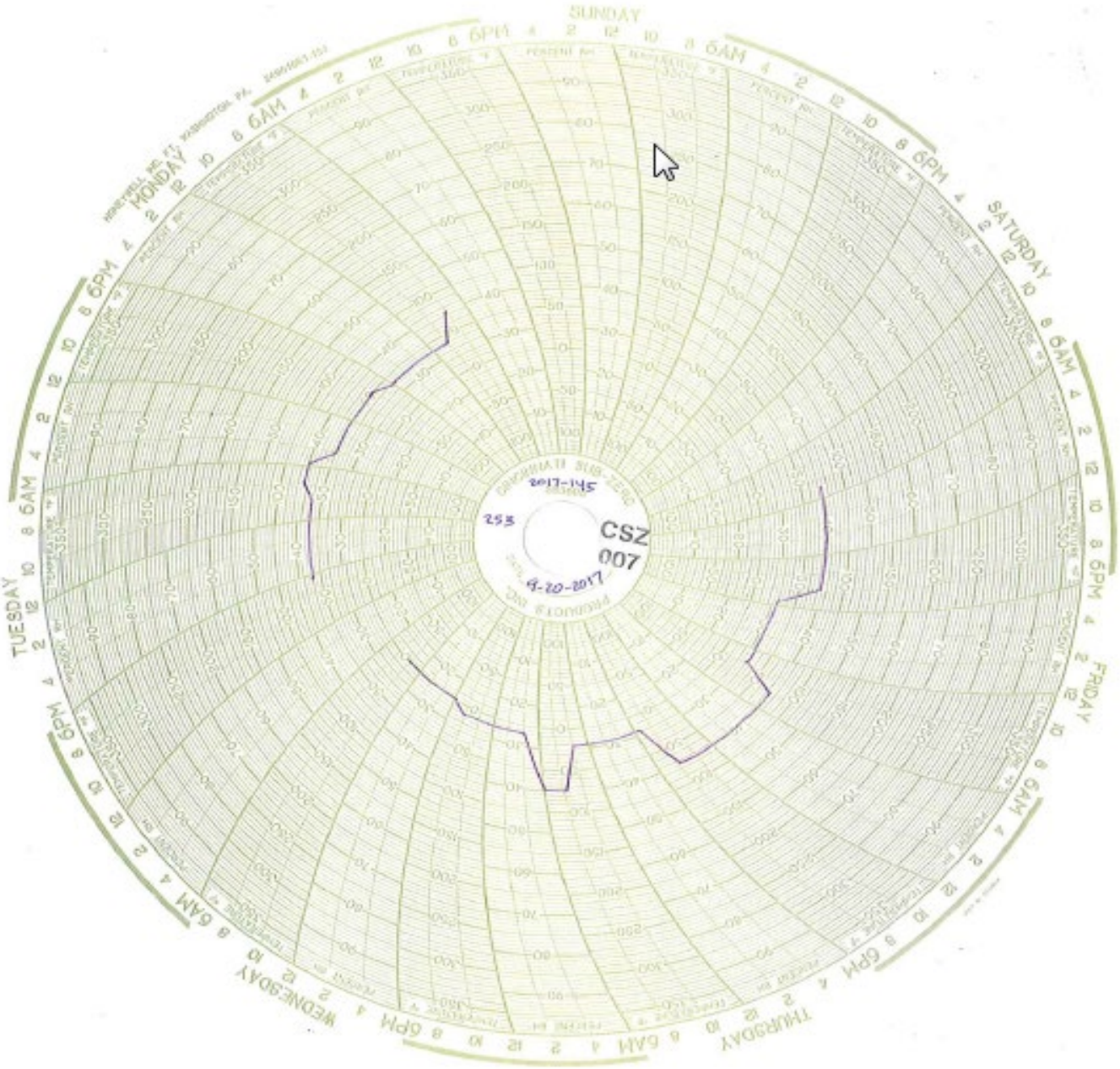
Environmental Test Report
Temperature Test
Test Kits
Part No: N/A
Serial No: CSZ001 – CSZ165
Test Start Date: 9/20/2017
Test Completion Date: 10/4/2017
Test Performed by: Tom Simon

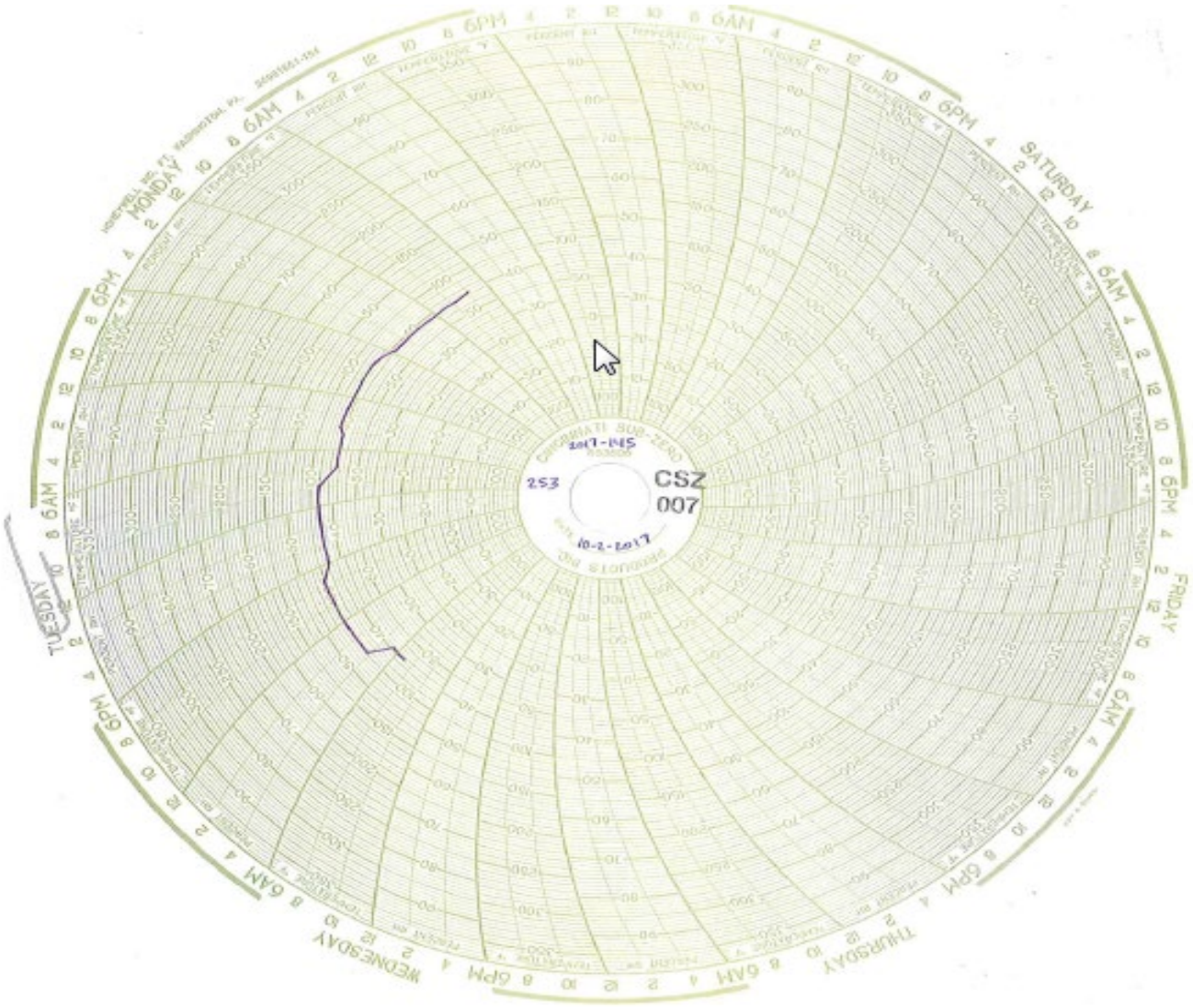
Equipment	Description	Serial No.	Calibration Date	Calibration Due Date
ESSC 253	ZP Chamber	ZP1625126	5/23/2017	5/23/2018

Summary: The test kits were placed in the chamber and exposed to temperature from 0F to 100F at 10F intervals.

Observations & Anomalies:

No anomalies observed


SUNDAY
MONDAY
TUESDAY
WEDNESDAY
THURSDAY
FRIDAY
SATURDAY
6AM
6PM
2017-145
CSZ
007
9-20-2017


MONDAY
TUESDAY
WEDNESDAY
THURSDAY
FRIDAY
SATURDAY
6AM
6PM
CSZ
007

Environmental Test Report
Temperature Humidity Test

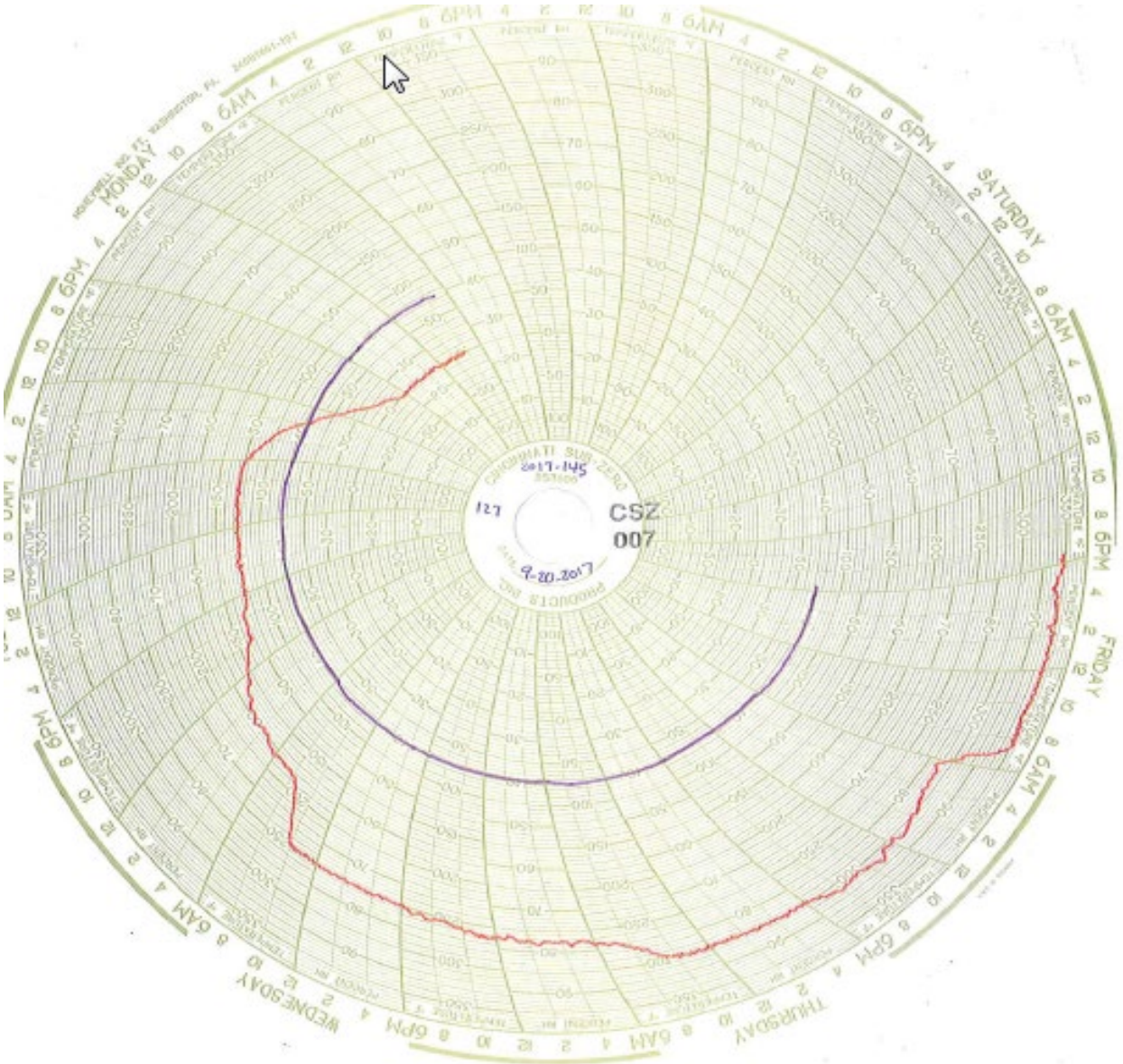
Test Kits
Part No: N/A
Serial No: CSZ166 – CSZ465
Test Start Date: 9/20/2017
Test Completion Date: 10/4/2017
Test Performed by: Tom Simon

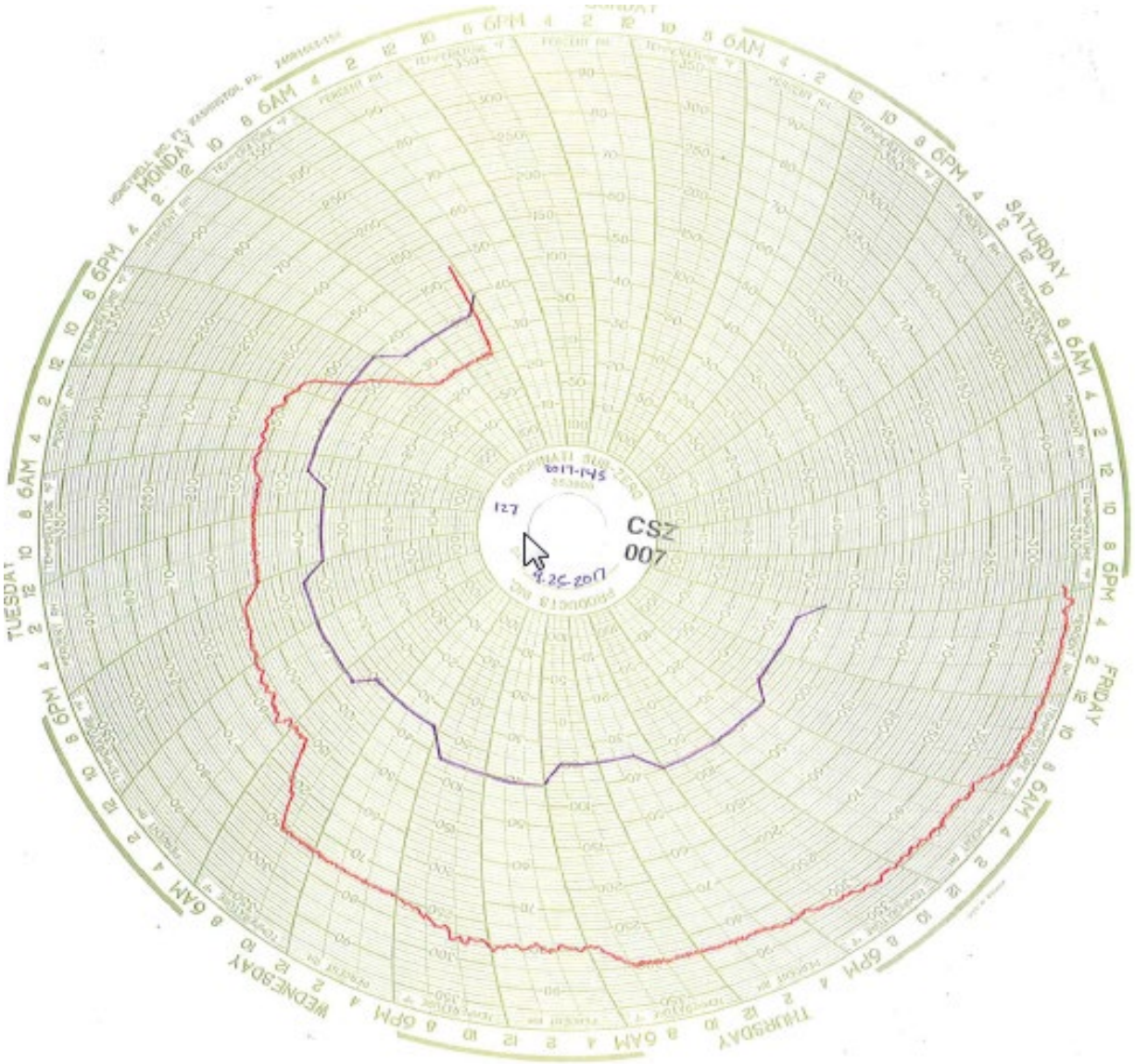
Equipment	Description	Serial No.	Calibration Date	Calibration Due Date
ESSC 127	Walk-In Chamber	05-WM-14394	5/23/2017	5/23/2018

Summary: The test kits were placed in the chamber and exposed to 25%, 50%, 75% & 95% RH at +25F, +50F, 75F & +100F

Observations & Anomalies:

No anomalies observed


MONDAY
SATURDAY
FRIDAY
THURSDAY
WEDNESDAY
CSZ
007
9-20-2017


MONDAY
TUESDAY
WEDNESDAY
THURSDAY
FRIDAY
SATURDAY
CSZ
007

Date Started	ate Complete	Setpoint	Test Profile	esult (outer packaging only)
9/20/2017	9/20/2017	0°F	2hr ramp to stabilization, 8 hr hold, 2hr ramp to ambient	no visual anomalies
9/20/2017	9/21/2017	10°F	2hr ramp to stabilization, 8 hr hold, 2hr ramp to ambient	no visual anomalies
9/21/2017	9/21/2017	20°F	2hr ramp to stabilization, 8 hr hold, 2hr ramp to ambient	no visual anomalies
9/22/2017	9/22/2017	30°F	2hr ramp to stabilization, 8 hr hold, 2hr ramp to ambient	no visual anomalies
9/25/2017	9/25/2017	40°F	2hr ramp to stabilization, 8 hr hold, 2hr ramp to ambient	no visual anomalies
9/25/2017	9/26/2017	50°F	2hr ramp to stabilization, 8 hr hold, 2hr ramp to ambient	no visual anomalies
9/26/2017	9/26/2017	60°F	2hr ramp to stabilization, 8 hr hold, 2hr ramp to ambient	no visual anomalies
10/2/2017	10/2/2017	70°F	2hr ramp to stabilization, 8 hr hold, 2hr ramp to ambient	no visual anomalies
10/2/2017	10/3/2017	80°F	2hr ramp to stabilization, 8 hr hold, 2hr ramp to ambient	no visual anomalies
10/3/2017	10/3/2017	90°F	2hr ramp to stabilization, 8 hr hold, 2hr ramp to ambient	no visual anomalies
10/3/2017	10/4/2017	100°F	2hr ramp to stabilization, 8 hr hold, 2hr ramp to ambient	no visual anomalies

Date Started	ate Complete	Setpoint	Test Profile	esult (outer packaging only)
9/20/2017	9/20/2017	25°F / 25% RH	2hr ramp to stabilization, 8 hr hold, 2hr ramp to ambient	no visual anomalies
9/21/2017	9/21/2017	25°F / 50% RH	2hr ramp to stabilization, 8 hr hold, 2hr ramp to ambient	no visual anomalies
9/22/2017	9/22/2017	25°F / 75% RH	2hr ramp to stabilization, 8 hr hold, 2hr ramp to ambient	no visual anomalies
9/23/2017	9/23/2017	25°F / 85% RH	2hr ramp to stabilization, 8 hr hold, 2hr ramp to ambient	no visual anomalies
9/24/2017	9/24/2017	25°F / 95% RH	2hr ramp to stabilization, 8 hr hold, 2hr ramp to ambient	no visual anomalies
9/25/2017	9/25/2017	50°F / 25% RH	2hr ramp to stabilization, 8 hr hold, 2hr ramp to ambient	no visual anomalies
9/26/2017	9/26/2017	50°F / 50% RH	2hr ramp to stabilization, 8 hr hold, 2hr ramp to ambient	no visual anomalies
9/27/2017	9/27/2017	50°F / 75% RH	2hr ramp to stabilization, 8 hr hold, 2hr ramp to ambient	no visual anomalies
9/28/2017	9/28/2017	50°F / 85% RH	2hr ramp to stabilization, 8 hr hold, 2hr ramp to ambient	no visual anomalies
9/29/2017	9/29/2017	50°F / 95% RH	2hr ramp to stabilization, 8 hr hold, 2hr ramp to ambient	no visual anomalies
10/2/2017	10/2/2017	75°F / 25% RH	2hr ramp to stabilization, 8 hr hold, 2hr ramp to ambient	no visual anomalies
10/3/2017	10/3/2017	75°F / 50% RH	2hr ramp to stabilization, 8 hr hold, 2hr ramp to ambient	no visual anomalies
10/4/2017	10/4/2017	75°F / 75% RH	2hr ramp to stabilization, 8 hr hold, 2hr ramp to ambient	no visual anomalies
10/5/2017	10/5/2017	75°F / 85% RH	2hr ramp to stabilization, 8 hr hold, 2hr ramp to ambient	no visual anomalies
10/6/2017	10/6/2017	75°F / 95% RH	2hr ramp to stabilization, 8 hr hold, 2hr ramp to ambient	no visual anomalies
10/7/2017	10/7/2017	100°F / 25% RH	2hr ramp to stabilization, 8 hr hold, 2hr ramp to ambient	no visual anomalies
10/8/2017	10/8/2017	100°F / 50% RH	2hr ramp to stabilization, 8 hr hold, 2hr ramp to ambient	no visual anomalies
10/9/2017	10/9/2017	100°F / 75% RH	2hr ramp to stabilization, 8 hr hold, 2hr ramp to ambient	no visual anomalies
10/10/2017	10/10/2017	100°F / 85% RH	2hr ramp to stabilization, 8 hr hold, 2hr ramp to ambient	no visual anomalies
10/11/2017	10/11/2017	100°F / 95% RH	2hr ramp to stabilization, 8 hr hold, 2hr ramp to ambient	no visual anomalies

Unless Otherwise noted on the Environmental Exposure Certificate, all testing has been performed at:

CSZ Testing Services
11901 Mosteller Road
Cincinnati, Ohio 45241

Job Instructions:

Perform testing I accordance with Quote # Q2017-520 and specifications outlined on Environmental Exposure Certificate(s)

Appendix C

Detailed below are the tables and device performance for the three matrices evaluated that included drug-free pool human saliva and two synthetic matrices (Tables 1-15).

DDT5000

Table 1. Summary results for the DDT5000 using drug-free pooled authentic oral fluid.

DDT5000 vs. Drug-Free Pooled Oral Fluid									
Drug	**TP**	**FN**	**FP**	**TN**	**Sensitivity**	**Specificity**	**Accuracy**	**PPV**	**NPV**
THC	3	0	1	2	100.0%	66.7%	83.3%	75.0%	100.0%
Cocaine	3	0	0	3	100.0%	100.0%	100.0%	100.0%	100.0%
Amphetamine	3	0	0	3	100.0%	100.0%	100.0%	100.0%	100.0%
Methamphetamine	2	0	0	4	100.0%	100.0%	100.0%	100.0%	100.0%
Benzodiazepines	3	0	0	3	100.0%	100.0%	100.0%	100.0%	100.0%
Opiates	3	0	0	3	100.0%	100.0%	100.0%	100.0%	100.0%
Methadone	3	0	0	3	100.0%	100.0%	100.0%	100.0%	100.0%
Overall	20	0	1	21	100.0%	95.2%	97.6%	95.2%	100.0%

Table 2. Summary results for the DDT5000 using the U.K. synthetic oral fluid.

DDT5000 vs. U.K. Synthetic Fluid									
Drug	**TP**	**FN**	**FP**	**TN**	**Sensitivity**	**Specificity**	**Accuracy**	**PPV**	**NPV**
THC	3	0	0	3	100.0%	100.0%	100.0%	100.0%	100.0%
Cocaine	3	0	0	3	100.0%	100.0%	100.0%	100.0%	100.0%
Amphetamine	3	0	0	3	100.0%	100.0%	100.0%	100.0%	100.0%
Methamphetamine	1	0	0	5	100.0%	100.0%	100.0%	100.0%	100.0%
Benzodiazepines	3	0	0	3	100.0%	100.0%	100.0%	100.0%	100.0%
Opiates	3	0	0	3	100.0%	100.0%	100.0%	100.0%	100.0%
Methadone	3	0	0	3	100.0%	100.0%	100.0%	100.0%	100.0%
Overall	19	0	0	23	100.0%	100.0%	100.0%	100.0%	100.0%

Table 3. Summary results for the DDT5000 using the OraSure fluid.

DDT5000 vs. OraSure Fluid									
Drug	**TP**	**FN**	**FP**	**TN**	**Sensitivity**	**Specificity**	**Accuracy**	**PPV**	**NPV**
THC	3	0	0	3	100.0%	100.0%	100.0%	100.0%	100.0%
Cocaine	3	0	0	3	100.0%	100.0%	100.0%	100.0%	100.0%
Amphetamine	3	0	0	3	100.0%	100.0%	100.0%	100.0%	100.0%
Methamphetamine	3	0	0	3	100.0%	100.0%	100.0%	100.0%	100.0%
Benzodiazepines	3	0	0	3	100.0%	100.0%	100.0%	100.0%	100.0%
Opiates	3	0	0	3	100.0%	100.0%	100.0%	100.0%	100.0%
Methadone	3	0	0	3	100.0%	100.0%	100.0%	100.0%	100.0%
Overall	21	0	0	21	100.0%	100.0%	100.0%	100.0%	100.0%

DDC3000

Table 4. Summary results for the DDC3000 using drug-free pooled authentic oral fluid.

DDDC3000 vs. Drug-Free Pooled Oral Fluid									
Drug	**TP**	**FN**	**FP**	**TN**	**Sensitivity**	**Specificity**	**Accuracy**	**PPV**	**NPV**
THC	3	0	0	3	100.0%	100.0%	100.0%	100.0%	100.0%
Cocaine	3	0	0	3	100.0%	100.0%	100.0%	100.0%	100.0%
Amphetamine	3	0	0	3	100.0%	100.0%	100.0%	100.0%	100.0%
Methamphetamine	3	0	0	3	100.0%	100.0%	100.0%	100.0%	100.0%
Opiates	3	0	0	3	100.0%	100.0%	100.0%	100.0%	100.0%
Overall	15	0	0	15	100.0%	100.0%	100.0%	100.0%	100.0%

Table 5. Summary results for the DDC3000 using the U.K. synthetic oral fluid.

DDC3000 vs. U.K. Synthetic Fluid									
Drug	**TP**	**FN**	**FP**	**TN**	**Sensitivity**	**Specificity**	**Accuracy**	**PPV**	**NPV**
THC	3	0	0	3	100.0%	100.0%	100.0%	100.0%	100.0%
Cocaine	3	0	0	3	100.0%	100.0%	100.0%	100.0%	100.0%
Amphetamine	3	0	0	3	100.0%	100.0%	100.0%	100.0%	100.0%
Methamphetamine	1	0	0	5	100.0%	100.0%	100.0%	100.0%	100.0%
Opiates	3	0	0	3	100.0%	100.0%	100.0%	100.0%	100.0%
Overall	13	0	0	17	100.0%	100.0%	100.0%	100.0%	100.0%

Table 6. Summary results for the DDC3000 using the OraSure fluid.

DDC3000 vs. OraSure Fluid									
Drug	**TP**	**FN**	**FP**	**TN**	**Sensitivity**	**Specificity**	**Accuracy**	**PPV**	**NPV**
THC	3	0	0	3	100.0%	100.0%	100.0%	100.0%	100.0%
Cocaine	3	0	0	3	100.0%	100.0%	100.0%	100.0%	100.0%
Amphetamine	3	0	0	3	100.0%	100.0%	100.0%	100.0%	100.0%
Methamphetamine	3	0	0	3	100.0%	100.0%	100.0%	100.0%	100.0%
Opiates	3	0	0	3	100.0%	100.0%	100.0%	100.0%	100.0%
Overall	15	0	0	15	100.0%	100.0%	100.0%	100.0%	100.0%

DrugWipe

Table 7. Summary results for the DrugWipe with DrugRead using drug-free pooled authentic oral fluid.

DrugWipe with DrugRead vs. Drug-Free Pooled Oral Fluid									
Drug	**TP**	**FN**	**FP**	**TN**	**Sensitivity**	**Specificity**	**Accuracy**	**PPV**	**NPV**
THC	3	0	0	3	100.0%	100.0%	100.0%	100.0%	100.0%
Cocaine	3	0	0	3	100.0%	100.0%	100.0%	100.0%	100.0%
Amphetamine	3	0	0	1	100.0%	100.0%	100.0%	100.0%	100.0%
Methamphetamine	2	0	0	1	100.0%	100.0%	100.0%	100.0%	100.0%
Opiates	3	0	0	3	100.0%	100.0%	100.0%	100.0%	100.0%
Overall	14	0	0	11	100.0%	100.0%	100.0%	100.0%	100.0%

Table 8. Summary results for the DrugWipe with DrugRead using the U.K. synthetic oral fluid.

DrugWipe with DrugRead vs. U.K. Synthetic Fluid									
Drug	**TP**	**FN**	**FP**	**TN**	**Sensitivity**	**Specificity**	**Accuracy**	**PPV**	**NPV**
THC	3	0	0	3	100.0%	100.0%	100.0%	100.0%	100.0%
Cocaine	3	0	0	3	100.0%	100.0%	100.0%	100.0%	100.0%
Amphetamine	0	0	0	6	N/A	100.0%	100.0%	N/A	100.0%
Methamphetamine	0	0	0	6	N/A	100.0%	100.0%	N/A	100.0%
Opiates	2	1	0	3	66.7%	100.0%	83.3%	100.0%	75.0%
Overall	8	1	0	21	88.9%	100.0%	96.7%	100.0%	95.5%

Table 9. Summary results for the DrugWipe with DrugRead using the OraSure fluid.

DrugWipe with DrugRead vs. OraSure Fluid									
Drug	**TP**	**FN**	**FP**	**TN**	**Sensitivity**	**Specificity**	**Accuracy**	**PPV**	**NPV**
THC	2	1	0	3	66.7%	100.0%	83.3%	100.0%	75.0%
Cocaine	3	0	0	3	100.0%	100.0%	100.0%	100.0%	100.0%
Amphetamine	0	0	0	3	N/A	100.0%	100.0%	N/A	100.0%
Methamphetamine	3	0	0	3	100.0%	100.0%	100.0%	100.0%	100.0%
Opiates	2	1	0	3	66.7%	100.0%	83.3%	100.0%	75.0%
Overall	10	2	0	15	83.3%	100.0%	92.6%	100.0%	88.2%

DDS2

Table 10. Summary results for the DDS2 using drug-free pooled authentic oral fluid.

DDS2 vs. Drug-Free Pooled Oral Fluid									
Drug	**TP**	**FN**	**FP**	**TN**	**Sensitivity**	**Specificity**	**Accuracy**	**PPV**	**NPV**
THC	0	3	0	3	0.0%	100.0%	50.0%	N/A	50.0%
Cocaine Metabolite	3	0	0	3	100.0%	100.0%	100.0%	100.0%	100.0%
Amphetamine	2	0	0	4	100.0%	100.0%	100.0%	100.0%	100.0%
Methamphetamine	2	0	0	4	100.0%	100.0%	100.0%	100.0%	100.0%
Benzodiazepines	3	0	0	3	100.0%	100.0%	100.0%	100.0%	100.0%
Opiates	3	0	0	3	100.0%	100.0%	100.0%	100.0%	100.0%
Overall	13	3	0	20	81.3%	100.0%	91.7%	100.0%	87.0%

Table 11. Summary results for the DDS2 using the U.K. synthetic oral fluid.

DDS2 vs. U.K. Synthetic Fluid									
Drug	**TP**	**FN**	**FP**	**TN**	**Sensitivity**	**Specificity**	**Accuracy**	**PPV**	**NPV**
THC	1	2	0	3	33.3%	100.0%	66.7%	100.0%	60.0%
Cocaine Metabolite	3	0	0	3	100.0%	100.0%	100.0%	100.0%	100.0%
Amphetamine	0	0	0	6	N/A	100.0%	100.0%	N/A	100.0%
Methamphetamine	1	0	0	5	100.0%	100.0%	100.0%	100.0%	100.0%
Benzodiazepines	3	0	0	3	100.0%	100.0%	100.0%	100.0%	100.0%
Opiates	3	0	0	3	100.0%	100.0%	100.0%	100.0%	100.0%
Overall	11	2	0	23	84.6%	100.0%	94.4%	100.0%	92.0%

Table 12. Summary results for the DDS2 using the OraSure oral fluid.

DDS2 vs. OraSure Fluid									
Drug	**TP**	**FN**	**FP**	**TN**	**Sensitivity**	**Specificity**	**Accuracy**	**PPV**	**NPV**
THC	3	0	3	0	100.0%	0.0%	50.0%	50.0%	N/A
Cocaine Metabolite	3	0	0	3	100.0%	100.0%	100.0%	100.0%	100.0%
Amphetamine	0	0	0	6	N/A	100.0%	100.0%	N/A	100.0%
Methamphetamine	2	0	0	4	100.0%	100.0%	100.0%	100.0%	100.0%
Benzodiazepines	3	0	0	3	100.0%	100.0%	100.0%	100.0%	100.0%
Opiates	3	0	0	3	100.0%	100.0%	100.0%	100.0%	100.0%
Overall	14	0	3	19	100.0%	86.4%	91.7%	82.4%	100.0%

AquilaScan

Table 13. Summary results for the AquilaScan using drug-free pooled authentic oral fluid.

AquilaScan vs. Drug-Free Pooled Oral Fluid									
Drug	**TP**	**FN**	**FP**	**TN**	**Sensitivity**	**Specificity**	**Accuracy**	**PPV**	**NPV**
THC	0	3	0	3	0.0%	100.0%	50.0%	N/A	50.0%
Cocaine	0	3	0	3	0.0%	100.0%	50.0%	N/A	50.0%
Amphetamine	0	0	0	6	N/A	100.0%	100.0%	N/A	100.0%
Methamphetamine	0	0	0	6	N/A	100.0%	100.0%	N/A	100.0%
Benzodiazepines	0	3	0	3	0.0%	100.0%	50.0%	N/A	50.0%
Opiates	0	3	0	3	0.0%	100.0%	50.0%	N/A	50.0%
Methadone	0	3	0	3	0.0%	100.0%	50.0%	N/A	50.0%
Overall	0	15	0	27	0.0%	100.0%	64.3%	N/A	64.3%

Table 14. Summary results for the AquilaScan using the U.K. synthetic oral fluid.

AquilaScan vs. U.K. Synthetic Fluid									
Drug	**TP**	**FN**	**FP**	**TN**	**Sensitivity**	**Specificity**	**Accuracy**	**PPV**	**NPV**
THC	0	3	0	3	0.0%	100.0%	50.0%	N/A	50.0%
Cocaine	0	3	0	3	0.0%	100.0%	50.0%	N/A	50.0%
Amphetamine	0	0	0	6	N/A	100.0%	100.0%	N/A	100.0%
Methamphetamine	0	0	0	6	N/A	100.0%	100.0%	N/A	100.0%
Benzodiazepines	0	3	0	3	0.0%	100.0%	50.0%	N/A	50.0%
Opiates	0	3	0	3	0.0%	100.0%	50.0%	N/A	50.0%
Methadone	0	3	0	3	0.0%	100.0%	50.0%	N/A	50.0%
Overall	0	15	0	27	0.0%	100.0%	64.3%	N/A	64.3%

Table 15. Summary results for the AquilaScan using the OraSure oral fluid.

AquilaScan vs. OraSure Fluid									
Drug	**TP**	**FN**	**FP**	**TN**	**Sensitivity**	**Specificity**	**Accuracy**	**PPV**	**NPV**
THC	0	3	0	3	0.0%	100.0%	50.0%	#DIV/0!	50.0%
Cocaine	0	3	0	3	0.0%	100.0%	50.0%	#DIV/0!	50.0%
Amphetamine	3	0	0	3	100.0%	100.0%	100.0%	100.0%	100.0%
Methamphetamine	1	0	0	5	100.0%	100.0%	100.0%	100.0%	100.0%
Benzodiazepines	0	3	0	3	0.0%	100.0%	50.0%	#DIV/0!	50.0%
Opiates	3	0	0	3	100.0%	100.0%	100.0%	100.0%	100.0%
Methadone	0	3	0	3	0.0%	100.0%	50.0%	#DIV/0!	50.0%
Overall	7	12	0	23	36.3%	100.0%	71.4%	100.0%	65.7%

Appendix D

Additional details related to device performance during the environmental assessment can be found in the tables below (Tables 1-5):

DDT5000

Table 1. Summary results for false positive (FP), false negative (FN), and invalid tests and associated conditions on the DDT5000 during environmental testing.

DDT5000 False Negative, False Positive and Invalid Results											
Condition	**FN**	**FP**	**Invalid**	**Condition**	**FN**	**FP**	**Invalid**	**Condition**	**FN**	**FP**	**Invalid**
0°F	-	-	-	25°F/25%rh	-	-	-	75°F/25%rh*	-	-	-
10°F	1	-	1	25°F/50%rh	-	1	-	75°F/50%rh*	-	-	-
20°F	-	-	-	25°F/75%rh	-	-	-	75°F/75%rh*	-	-	-
30°F	-	-	-	25°F/85%rh	1	-	-	75°F/85%rh*	-	-	-
40°F*	-	-	-	25°F/95%rh	-	-	-	75°F 95%rh*	-	-	-
50°F*	-	-	-	50°F/25%rh*	-	1	-	100°F/25%rh	-	-	-
60°F*	-	-	-	50°F/50%rh*	-	-	-	100°F/50%rh	-	-	-
70°F*	-	-	-	50°F/75%rh*	-	-	1	100°F/75%rh	-	-	-
80°F*	-	-	-	50°F/85%rh*	-	-	-	100°F/85%rh	-	-	-
90°F	-	-	-	50°F/95%rh*	-	-	-	100°F/95%rh	-	-	-
100°F	-	-	-	-	-	-	-	-	-	-	-

*= Within manufacture recommended cassette storage range; rh= relative humidity

DDC3000

Table 2. Summary results for false positive (FP), false negative (FN), and invalid tests and associated conditions on the DDC3000 during environmental testing.

DDC3000 False Negative, False Positive and Invalid Results											
Condition	**FN**	**FP**	**Invalid**	**Condition**	**FN**	**FP**	**Invalid**	**Condition**	**FN**	**FP**	**Invalid**
0°F	-	-	-	25°F/25%rh	-	-	-	75°F/25%rh*	-	-	-
10°F	-	-	-	25°F/50%rh	4	-	-	75°F/50%rh*	-	-	-
20°F	-	-	-	25°F/75%rh	-	-	-	75°F/75%rh*	-	-	-
30°F	-	-	-	25°F/85%rh	-	-	-	75°F/85%rh*	-	-	-
40°F*	-	-	-	25°F/95%rh	-	-	-	75°F 95%rh*	-	-	-
50°F*	-	-	-	50°F/25%rh*	-	-	-	100°F/25%rh	-	-	-
60°F*	-	-	-	50°F/50%rh*	-	-	-	100°F/50%rh	-	-	1
70°F*	-	-	-	50°F/75%rh*	-	-	-	100°F/75%rh	-	-	-
80°F	-	-	-	50°F/85%rh*	-	-	-	100°F/85%rh	-	-	-
90°F	-	-	-	50°F/95%rh*	1	-	-	100°F/95%rh	-	-	-
100°F	-	-	-	-	-	-	-	-	-	-	-

*= Within manufacture recommended cassette storage range; rh= relative humidity

DrugWipe

Table 3. Summary results for false positive (FP), false negative (FN), and invalid tests and associated conditions on the DrugWipe during environmental testing**.**

DrugWipe False Negative, False Positive and Invalid Results											
Condition	**FN**	**FP**	**Invalid**	**Condition**	**FN**	**FP**	**Invalid**	**Condition**	**FN**	**FP**	**Invalid**
0°F	1	-	-	25°F/25%rh	6	-	-	75°F/25%rh*	3	-	-
10°F	2	-	-	25°F/50%rh	4	-	-	75°F/50%rh*	3	-	-
20°F	8	-	-	25°F/75%rh	4	-	-	75°F/75%rh*	3	-	-
30°F	4	-	-	25°F/85%rh	2	-	-	75°F/85%rh*	4	-	-
40°F	3	-	-	25°F/95%rh	5	-	-	75°F 95%rh	6	-	-
50°F*	5	-	-	50°F/25%rh*	3	-	-	100°F/25%rh	4	-	-
60°F*	4	-	-	50°F/50%rh*	5	-	-	100°F/50%rh	3	-	-
70°F*	5	-	-	50°F/75%rh*	4	-	-	100°F/75%rh	1	-	-
80°F	6	-	-	50°F/85%rh*	7	-	-	100°F/85%rh	2	-	-
90°F	4	-	-	50°F/95%rh	6	-	-	100°F/95%rh	5	-	-
100°F	2	-	-	-	-	-	-	-	-	-	-

*= Within manufacture recommended cassette storage range; rh= relative humidity

DDS2

Table 4. Summary results for false positive (FP), false negative (FN), and invalid tests and associated conditions on the DDS2 during environmental testing.

DDS2 False Negative, False Positive and Invalid Results											
Condition	**FN**	**FP**	**Invalid**	**Condition**	**FN**	**FP**	**Invalid**	**Condition**	**FN**	**FP**	**Invalid**
0°F	2	-	-	25°F/25%rh	2	-	-	75°F/25%rh*	2	-	-
10°F	-	-	-	25°F/50%rh	2	-	-	75°F/50%rh*	2	-	-
20°F	1	-	-	25°F/75%rh	1	-	-	75°F/75%rh*	2	-	1
30°F	1	-	-	25°F/85%rh	2	-	-	75°F/85%rh	2	-	-
40°F	-	-	-	25°F/95%rh	2	-	-	75°F 95%rh	2	-	-
50°F	1	-	-	50°F/25%rh	1	-	1	100°F/25%rh	2	-	-
60°F*	-	-	-	50°F/50%rh	2	-	-	100°F/50%rh	-	-	1
70°F*	1	-	-	50°F/75%rh	2	-	-	100°F/75%rh	2	-	-
80°F	2	-	-	50°F/85%rh	2	-	-	100°F/85%rh	1	-	-
90°F	1	-	-	50°F/95%rh	1	-	1	100°F/95%rh	1	-	-
100°F	1	-	1	-	-	-	-	-	-	-	-

*= Within manufacture recommended cassette storage range; rh= relative humidity

AquilaScan

Table 5. Summary results for false positive (FP), false negative (FN), and invalid tests and associated conditions on the AquilaScan during environmental testing.

AquilaScan False Negative, False Positive and Invalid Results											
Condition	**FN**	**FP**	**Invalid**	**Condition**	**FN**	**FP**	**Invalid**	**Condition**	**FN**	**FP**	**Invalid**
0°F	10	-	-	25°F/25%rh	9	-	-	75°F/25%rh	10	-	-
10°F	13	-	-	25°F/50%rh	9	-	-	75°F/50%rh	13	-	-
20°F	12	-	-	25°F/75%rh	10	-	-	75°F/75%rh	12	-	-
30°F	12	-	-	25°F/85%rh	13	-	-	75°F/85%rh	11	-	-
40°F*	11	-	-	25°F/95%rh	10	-	-	75°F 95%rh	12	-	-
50°F*	12	-	-	50°F/25%rh	12	-	-	100°F/25%rh	11	-	-
60°F*	11	-	-	50°F/50%rh	13	-	-	100°F/50%rh	12	-	-
70°F*	9	-	-	50°F/75%rh	12	-	-	100°F/75%rh	12	-	-
80°F*	14	2	1	50°F/85%rh	10	1	-	100°F/85%rh	12	-	-
90°F	13	-	-	50°F/95%rh	12	-	-	100°F/95%rh	11	-	-
100°F	13	-	-	-	-	-	-	-	-	-	-

*= Within manufacture recommended cassette storage range; rh= relative humidity

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