

OraLine[®] IV s.a.t

One-Step, Rapid, Qualitative Drugs of Abuse Oral Fluid Immunoassay Test Kit for
Cannabinoids, Opiates, Cocaine, and Methamphetamine

INTENDED USE

The OraLine[®] IV s.a.t. (substance of abuse test) is an immunoassay designed for the qualitative detection of the above listed four (4) drugs of abuse in human oral fluid. The OraLine[®] IV is intended for forensic use only; to be used in such facilities as court systems, police departments, probation/parole officers, juvenile detention centers, prisons, jails, and correction centers. The OraLine IV was developed to achieve optimal test results using fresh oral fluid samples. The manufacturer does not recommend using altered or aged saliva samples, as these samples may have inconsistent flow patterns and yield unsatisfactory results with the test.

The OraLine[®] IV assay provides only a preliminary screening test result. A more specific alternative method must be used to obtain a confirmed analytical result. Although other chemical confirmation methods are available ^(1, 2), Liquid or Gas Chromatography/Mass Spectrometry (LC or GC/MS) are the preferred confirmatory methods ⁽¹⁾. Clinical considerations and professional judgment should be applied to the analysis involving drugs of abuse, particularly when positive results are indicated ⁽³⁾.

SUMMARY

In drug analysis, oral fluid drug screening has certain advantages over the screening of other biological fluids such as urine and blood. Oral fluid is readily accessible, and is less likely to be adulterated. With oral fluid tests, the drugs may be detected immediately after ingestion, even before they are metabolized and would show up in urine. In general, OraLine[®] IV is designed to work at a lower detection level for all test drugs than those detected in urine samples. OraLine[®] IV oral fluid screening for drugs of abuse detects the presence of parent compounds and drug metabolites ^(3,4). The OraLine[®] IV uses an immunochromatographic technique that provides the accuracy of an immunoassay without the need for laboratory equipment ^(6,7,8). The concentrations of the drugs or drug metabolites are at levels greater than or equal to the cutoff value of the assay specified on the attached table. The immunoreaction causes a displacement in the antibody-antigen complex that is visually detected on a chromatographic membrane. The test is designed to use a fresh oral fluid sample directly, and to provide rapid test results for on-site testing purposes ^(9,10,11,12). The OraLine[®] IV detects recent drug usage, and is particularly useful for screening of drug intoxication related to any impairment situations ⁽¹²⁾.

The OraLine[®] IV is designed to detect the presence of the following drugs (at levels equal to or greater than the cutoff level as indicated):

THC – Cannabinoids delta 9-THC 11-nor-delta 9-COOH-THC (Marijuana, Hash, Pot)	4 ng/mL 40 ng/mL
COC – Cocaine Benzoylecgonine (Crack, Coke)	25 ng/mL 8 ng/mL
MET – Methamphetamine (Speed, Crank, Ecstasy)	3.75 ng/mL
OPI – Morphine (Heroin, Morphine, Codeine)	2 ng/mL

PRINCIPLES OF PROCEDURE

The OraLine[®] IV assay is an immunochromatographic test based on the principle of antigen/antibody complexation and is used for the analysis of parent drugs, and their metabolites in human oral fluid samples. The assay is based on the competition for limited antibody sites between the drug or drug metabolite in the sample and a drug conjugate immobilized on a porous membrane support ⁽⁶⁾. All test components are housed in the plastic device. A green colored-dye is added to the test membrane to mark the test line positions where the reactions will take place after the sample is added ⁽⁸⁾. A defined volume of the oral fluid sample is collected with the sample collection spoon, which is an integral part of the test device. The test sample is channeled to the chromatographic membrane to mobilize the microspheres coated with the monoclonal or polyclonal antibody specific to the drug(s). These microspheres then wick along the membrane via capillary action to the probe area on the membrane. After the sample is applied, the green lines will be replaced by pink-colored lines in the case of negative samples. The absence of the colored line is an indication of a positive test result. In the absence of the drug, the colored microspheres attach to the drug conjugate probes, forming visible, pinkish-colored lines as the antibodies complex with the drug conjugate. Therefore, the formation of any visible precipitin at the conjugate probe line area indicates that the oral fluid sample is negative for the drug. However, when the drug is present in the test sample, the drug competes for the limited antibody sites on the microspheres. When a sufficient amount of the drug is present, it occupies all the antibody binding sites and prevents attachment of the colored microspheres to the probe line area on the membrane. A positive oral fluid sample, therefore, will not form the line at the probe area ⁽⁶⁾.

A reference or control line “C” with a different antigen/antibody reaction is also added to the immunochromatographic membrane to indicate that there was proper capillary flow of the sample and that the antigen/antibody reaction is viable. This control line should always be present in a valid test. Normal negative oral fluid sample will produce five (5) colored lines. If an oral fluid sample is positive for a particular drug, there will be no test line formed at the corresponding location on the OraLine[®] IV test membrane for that drug.

REAGENTS

The OraLine® IV test is housed in a plastic device ⁽⁹⁾. Each device contains a membrane with a defined amount of microspheres coated with anti-drug or drug metabolite antibodies. Drug conjugates and purified bovine serum proteins are adsorbed onto the membrane to form the probe line, and a second antibody is used to form the control line. The entire membrane is dried prior to assembly and is used in the dry form. All necessary reagents are included in the test device. No additional equipment or reagents are needed.

MATERIALS PROVIDED

Each OraLine® IV assay kit provides:

1. One package insert
2. 25, 4-drug test devices individually sealed in foil pouches and boxed.
3. 25 Oral fluid collecting cups (for alternative sample collection)

Each foil pouch contains:

1. One OraLine® IV device
2. Desiccant

Materials that may be needed but not supplied:

1. Timer
2. Disposable gloves
3. Paper towel
4. External positive and negative controls
5. Optional oral fluid collection device, such as a straw or syringe

STORAGE

1. The components of this test are stable until the marked expiration date when stored in the sealed pouch at 2⁰ – 30⁰C.
2. Do not allow test device to be subjected to temperatures below freezing (<0⁰C) or above 30⁰C.

PRECAUTIONS

1. Use a new test device for each oral fluid sample.
2. If a foil pouch is torn or perforated, the device should not be used.
3. Do not use the assay beyond the expiration date indicated on the pouch.
4. Open the sealed pouch containing the device immediately prior to use. Prolonged exposure to ambient humidity will cause product deterioration.
5. Always place the OraLine® IV device on a flat, level surface, when running the test.
6. Do not move the test device while the test is still running.
7. Always read the test results under adequate lighting

WARNING: Oral fluid specimens and all materials coming in contact with them should be handled and disposed of as if infectious and capable of transmitting infection. Wear disposable gloves.

SPECIMEN COLLECTION AND HANDLING

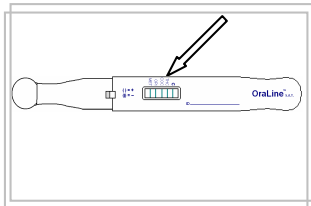
The OraLine® is formulated for use with fresh oral fluid specimens. Oral fluid specimens are collected directly using the OraLine® IV test device. For normal application, do not collect or deliver oral fluid samples using other means. Do not use any altered or processed, or aged saliva samples, as these samples may have inconsistent flow pattern and yield different results as compared with the fresh samples. The amount of oral fluid collected using the OraLine® spoon is optimal for the test. Oral fluid specimens do not require any special pretreatment, but the samples should be free from gross debris from food, juices, or soft drinks.

CAUTION:

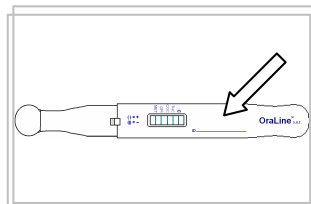
1. If the test device is stored refrigerated, allow the test device to come to room temperature prior to opening the pouch.
2. The person being tested must be able to produce a sufficient oral fluid sample in order for the test to function properly. Frothy samples, and air-bubbles not only reduce the volume of sample delivered to the test, but also may stop the capillary flow on the absorbent material.
3. Make sure that the person being tested has had nothing in his/her mouth for at least five (5) minutes before oral fluid sample is collected; including food, chewing gum, and tobacco products, etc.

NOTE: If the person being tested is experiencing dry mouth prior to sample collection, have the person drink a glass of water or have a piece of hard candy or sugarless gum, remove from the mouth, wait five (5) minutes and then proceed with the test. Alternatively, saliva could be collected into a collection device such as a 5cc syringe or a collection cup. If using a cup, deliver a spoonful of saliva while holding the spoon in a horizontal position with the handle held in an upward position as shown in Procedure II below.

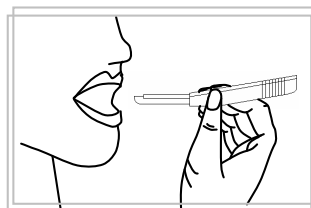
TEST PROCEDURE I



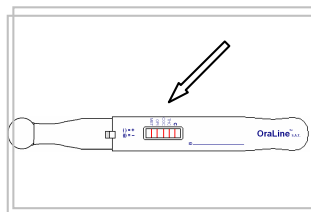
1. Remove the test device from the pouch. Verify that five (5) green lines are present in the test window. If the green lines are not present, a new device with 5 visible green lines should be used.



2. Mark the device with the subject's name or ID number. Remove the cap.



3. Let the test subject accumulate sufficient oral fluid in the mouth first, then place the spoon end of the device into the subject's mouth and collect a spoonful of oral fluid sample. When collecting the sample, make sure the spoon is in a horizontal position with the handle upward as illustrated.



4. Once the spoon is filled, do not replace the cap until the sample shows up in the view window. Place the device on a protected flat surface, and allow running for 10-12 minutes.

5. The test results are read between 10-12 minutes in the viewing window. Make sure the control line (C) is clearly visible before reading and recording the results.

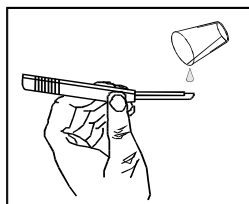
TEST PROCEDURE II

Use this alternate procedure when direct collection and testing are difficult, due to the test environment or other medical conditions.

Step 1 and 2 is same as in Procedure I.



3. Collect oral fluid into a plastic cup.



4. Fill the spoon by transferring the oral fluid into the spoon, by pouring or pipetting from the cup. Make sure the spoon is held horizontally with the handle in an upward position, as shown, when filling the device.

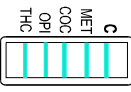
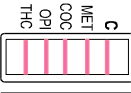

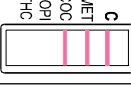
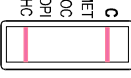
NOTE: If the test is read before ten (10) minutes, the intensity of the colored lines may still change, or a new line may appear after ten (10) minutes. Thus, it is always important to record the results within 10-12 minutes for consistency.

INTERPRETATION OF RESULTS

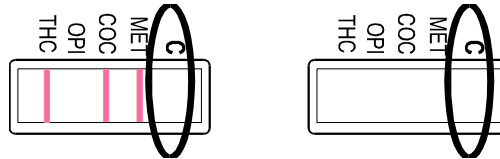
The test results are determined by the presence or absence of red lines. With a POSITIVE sample, the drug or drug metabolite from the sample solution blocks the limited antibody binding sites on the colored microspheres. This reaction prevents the attachment of the colored microspheres to the antigenic probe sites on the membrane. Therefore, a POSITIVE test result for one of the four drugs is indicated by the absence of its corresponding red line. A NEGATIVE test result for all of the four drugs is indicated by the presence of all five (5) red lines. Lines MET, OPI, COC, and THC are the drug probe lines, and line C is the control or reference line (see diagrams). Line C is not related to the presence of any drug, but shows that the microsphere particles have completely traveled along the membrane and that the test is viable. The line intensity may vary for each drug. Do not compare line intensities when interpreting results.

The OraLine® IV is a qualitative test and the intensity of the lines does not correspond to drug concentration. The OraLine® IV only indicates the presumptive presence or absence of a specific drug in the oral fluid sample, and provides preliminary qualitative test results.

Below are examples of a few possible test results (more variations are possible):

	Green lines indicate an intact test device before the test strip exposed to the sample solution (8).
	NEGATIVE test results for all four drugs (THC, COC, OPI and MET) are indicated by the presence of all FIVE red lines.
	POSITIVE for OPI and NEGATIVE for THC, COC and MET.
	POSITIVE for THC & OPI and NEGATIVE for MET, and COC.
	POSITIVE for OPI, COC & MET and NEGATIVE for THC.

NOTE: Interpret the test results after the control line ‘C’ is clearly defined in the test window. No test results should be interpreted until the control line ‘C’ can be clearly identified in the viewing area. In the event that the control line ‘C’ does not form, the test results are inconclusive and the test should be repeated using a new OraLine® IV device.



In both examples, the control line is absent. The test results are INVALID

LIMITATIONS OF PROCEDURE

The OraLine® IV assay is designed for use with human oral fluid samples only. The person being tested must be able to produce and deliver sufficient oral fluid in order for the test to function properly. A positive result with any of the tests may indicate only the presence of the parent drug or drug metabolite and does not indicate or measure level of drug present or intoxication. There is a possibility that other substances and/or factors not listed may interfere with the test and cause false results, such as technical or procedural errors. See INTERFERENCE STUDY for lists of substances that are known to produce positive results and substances that do not interfere with test performance.

QUALITY CONTROL

Each time a new test kit lot number is received or a new operator is performing the test for the first time, an established positive control and a negative control should be tested. In regular operation, quality control of OraLine® IV should be performed periodically, as necessary, depending on the frequency and the volume of tests used.

INTERFERENCE STUDY

The following substances were tested in drug-negative oral fluid and in drug-positive oral fluids. The negative sample testing was done by adding the compounds listed below to an artificial oral fluid base. The positive sample testing was done by adding these compounds to drug positive sample pools of artificial oral fluid. The drug concentrations were made at 25% over previously listed cutoff levels.

Table I: Compounds that will not interfere with negative or positive test results:

SUBSTANCE (100 ug/ml)				
Acetaminophen	Caffeine	Doxylamine	Indomethicin	Phenobarbital
Acetylsalicylic Acid	Chloroquine	Ethanol	Ketamine	Pyroxidine (B6)
Albumin	Dextromethorphan	Fluoxetine	Methadone	Secobarbital
l-Amphetamine	Dimenhydrinate	Haloperidol	Oxazepam	Simethicone
Ascorbic Acid	Diphenhydramine	Ibuprofen	Phencyclidine	L-Tryptophan
Biotin	Doxepin	Imipramine	Phenmetrazine	

Testing was also done after the ingestion or usage of the following substances. The subject ingested or used the substance according to package instructions. The subject then drank some water, waited 5 minutes, and then supplied the oral fluid sample. The results show no significant variance.

Bismuth Subsalicylate (Pepto Bismol)
Chewing Gum (Trident, Eclipse)
Magnesium Hydrochloride (Milk of Magnesia)
Orange Juice
Thymol (Found in anti-bacterial mouth rinse)
Vinegar

ORALINE® IV PRECISION

Three lots of product and two levels of analyte were tested in duplicate, in the AM and PM for five consecutive days. The data indicate that the Day-to-Day, Lot-to-Lot precision exceeded 99% for each drug tested. Results were based on blind studies with spiked samples in synthetic oral fluid.

Sample	Day 1	Day 2	Day 3	Day 4	Day 5
ng/ml	% Positive				
THC 0	0	0	0	0	0
THC 63	>99	>99	>99	>99	>99
COC 0	0	0	0	0	0
COC 63	>99	>99	>99	>99	>99
OPI 0	0	0	0	0	0
OPI 13*	>99	>99	>99	>99	>99
MET 0	0	0	0	0	0
MET 75	>99	>99	>99	>99	>99

*precision studies of February 2004

CANNABINOIDS

SUMMARY AND EXPLANATION OF THE TEST

Cannabinoids (marijuana, THC) is a hallucinogenic agent derived from the flowering portion of the hemp plant. Smoking of marijuana/cannabis is the primary method of consumption. Higher doses used by abusers produce central nervous system effects, altered mood and sensory perceptions, loss of coordination, impaired short-term memory, anxiety, paranoia, depression, confusion, hallucinations, and increased heart rate. A tolerance to the cardiac and psychotropic effects can develop. Symptoms such as restlessness, insomnia, anorexia, and nausea can take place during withdrawal from the drug. When marijuana is ingested, the drug is metabolized by the liver. The OraLine® Cannabinoids test detects the native component, delta-9 THC, and the primary metabolite, 11 nor-delta 9-COOH-THC. Detection of the native compound indicates recent marijuana/cannabis use.

PERFORMANCE CHARACTERISTICS

1. SENSITIVITY/SPECIFICITY

In the configuration of the OraLine® Cannabinoids test, oral fluid concentrations of the analytes at levels equal to or greater than the cut-off value of the assay cause a change in an antigen/antibody complex that is read on a chromatographic membrane. The OraLine® Cannabinoids assay is specifically designed to perform at a threshold of 4ng/mL for native THC. Results can be reported in qualitative terms as "positive" above 4 ng/mL or as "negative" below this cutoff threshold. Positive results should be confirmed by an appropriately sensitive and specific methodology using a different chemical principle. If performed under conditions that provide sufficient sensitivity, HPLC, LC, GC, or GC/MS are generally acceptable alternative methods of confirmation of the OraLine® Cannabinoids assay^(2, 3). While confirmation techniques other than LC or GC/MS may be adequate for some drugs of abuse, LC or GC/MS is generally accepted as a rigorous confirmation technique for all drugs, since it provides the best level of confidence in the results.

Table I: Compounds that will produce a positive result⁽¹³⁾.

Substance	Cutoff ng/ml
11-nor-delta 9-Tetrahydrocannabinol-Carboxylic Acid (metabolite)	40
delta 9-THC (native)	4
Delta 8-THC	10

Table II: Δ9-THC Measured in Spiked Samples and Correlation with the OraLine® IV Result

Spiked Δ9-THC Concentration (ng/mL)*	Average Measured Concentration by LC/MS ⁽¹⁴⁾ (ng/mL)	OraLine® IV THC % Positive (N=20)
0	0	0
12.5	5	80
25	11	85
50	21	95
100	43	100
200	85	100
400	170	100

*Δ9-THC Stock: Cerilliant, 1.0 mg/mL in Methanol, Lot#FCO80603-01A

Table III: OraLine® Time Course Study⁽¹³⁾ Δ 9-THC detection in Saliva

Donor	Descrip.	ID#	Time from Initial Drug Use	OraLine Result	Δ 9 THC LC/MS ng/ml	Donor	Descrip.	ID#	Time from Initial Drug Use	OraLine Result	Δ 9 THC LC/MS ng/ml
A	86 kg Male	08640-01	30 mins.	+	420	C	Male, few puffs	F12540-07	1.25 hr.	+	47
		08640-02	40 mins.	+	90			F12540-08	13 hr.	-	1
		08640-03	1 hr.	+	85			F12540-09	21.5 hr.	-	2
		08640-04	3 hr.	+	21	D	Male, 1 puff only,	F12540-11	5 mins.	+	7
		08640-05	4 hr.	+	13			F12540-12	45 mins.	-	1
B	Male (Beer also consumed)	F12540-01	10 mins.	+	882	E	63 kg Female	F12540-13	30 mins.	+	71
		F12540-02	15 mins.	+	19			F12540-15	50 mins.	+	51
		F12540-04	1 hr.	+	3	F	60kg Female	F12540-16	1 hr. 5 mins.	+	24
		F12540-06	3.5 hr.	+/-	3			F12540-17	1.5 hr.	+	10
								F12540-18	2.3 hr.	+	7

Table IV: OraLine® Screen vs. GC/MS – Human samples, expressed in increasing exposure times.⁽¹⁵⁾

Case#	Time From Last Exposure	OraLine THC	Intercept Collector GC/MS ng/ml	Comments
K3	0 hr.	-	0	Negative Subject
K11	0.083 hr.	+	9	
K8	0.083 hr.	+	147	
K2	0.25 hr.	+	13	
K12	0.5 hr.	-	1	
K7	0.5 hr.	+	3	
K5	1.0 hr.	+	43	
K1	1.0 hr.	+	114	
K10	2.0 hr.	-	11	Coffee before test
K6	2.0 hr.	+	265	
K4	3.0 hr.	-	13	
K9	16.0 hr.	-	4	

2. ACCURACY**Table V: Combined Correlation of OraLine® Δ 9-THC Results with GC/MS or LC/MS**

Sample#	M/F	OraLine® THC	GC/MS or LC/MS Δ 9-THC ng/ml
12540-01	M	+	882
8640-01	M	+	420
12540-20	F	+	293
12540-14	F	+	289
K6	-	+	265
K8	-	+	147
K1	-	+	114
8640-02	M	+	90
8640-03	M	+	85
12540-13	F	+	71
12540-15	F	+	51
12450-07	M	+	47
K5	-	+	43
12540-16	F	+	24
8640-04	M	+	21
12540-02	M	+	19
8640-05	M	+	13
K2	-	+	13
K4	-	-	13
12540-19	F	+	12
K10	-	-	11*
12540-17	F	+	10
K11	-	+	9
12540-10	M	+	8
12540-11	M	+	7
12540-18	F	+	7
12540-03	M	+	4
K9	-	-	4**
K7	-	+	3
12540-04	M	+	3
12540-06	M	+/-	3
12540-09	M	-	2
K12	-	-	1
12540-05	F	-	1
12540-08	M	-	1
12540-12	M	-	1
K1	-	-	0

* coffee before the test, ** 16 hrs after THC use.

MORPHINE/OPIATES

SUMMARY AND EXPLANATION OF TEST

Opiates such as morphine, heroin, and codeine are derived from the resin of the opium poppy. This class of drugs is known as the CNS depressants. At therapeutic doses, opiates have an analgesic action, which reduces the severity of traumatic pain. Acute higher doses, as used by abusers or addicts, produce euphoria and release from anxiety. Signs of physical dependence include depressed coordination, disrupted decision-making, decreased respiration, hypothermia, and coma. Tolerance develops to the analgesic and CNS effects with prolonged use. Narcotic antagonists are used to maintain addicts. Withdrawal symptoms from these opiates are manifested by excitability, anxiety, insomnia, anorexia, diarrhea, and muscle/joint aches. Heroin is quickly metabolized to morphine. Thus, morphine and morphine glucuronide might both be found in the urine of a person who has taken only heroin. The body also changes codeine to morphine. Therefore, a positive result obtained from the morphine part of the test may indicate heroin, morphine (or its metabolite, morphine glucuronide), and/or codeine use^(5, 6).

PERFORMANCE CHARACTERISTICS

1. SENSITIVITY/SPECIFICITY

In the configuration of the OraLine[®] Morphine assay is specifically designed to perform at a threshold of 40 ng/mL. Results can be reported in qualitative terms as presumptive "positive" above 40 ng/mL or as "negative" below this threshold. Positive results should be confirmed by an appropriately sensitive and specific methodology using a different chemical principle. If performed under conditions that provide sufficient sensitivity, HPLC, LC or GC/MS are generally acceptable alternative methods of confirmation of the OraLine[®] Morphine assay^(2, 3). While confirmation techniques other than LC or GC/MS may be adequate for some drugs of abuse, LC or GC/MS is generally accepted as a rigorous confirmation technique for all drugs, since it provides the best level of confidence in the results.

In laboratory testing where a reference cutoff of 40 ng/mL was used, OraLine[®] Morphine tests were calculated as >99% sensitive using the formula TP/(TP+FP); (TP = true positive population and FP = false positive population measured). The test sensitivity is also demonstrated with two test lot preparations tested in replicates of 10 each at different concentration levels of morphine controls. The OraLine[®] Morphine test is >99 % sensitive at 40 ng/mL at 10-12 minutes.

2. ACCURACY

The following compounds will produce positive readings with the OraLine[®] Morphine test. Table I: Cross-reactive substances:

Substance	Cutoff ng/ml
Morphine	2
Diacetylmorphine	1.875
6-Monoacetylmorphine	30
Hydromorphone	1.75
Hydrocodone	1.6875
Codeine	0.78125
Morphine 3, B-D-glucuronide	2
Nalorphine	0.5
Thebain	1,000-2,000
Oxymorphone	500
Oxycodone	250
Levorphanol	250
Buprenorphine	> 100,000
Meperidine	> 7,500
Naltrexone	250
Ranitidine	> 17,500
Propoxyphene	> 100,000
Acetylmorphine	2
Ethylmorphine	15
Naloxone	50

COCAINE

SUMMARY AND EXPLANATION OF TEST

Cocaine is a natural alkaloid product obtained from the leaves of the coca plant. It can also be synthesized in the laboratory from ecgonine. Although cocaine is a local, topical anesthetic, it has limited medical use. Cocaine is the most potent of the naturally occurring central nervous system (CNS) stimulants with sympathomimetic properties similar to the actions of the amphetamines. The CNS stimulation produced by cocaine induces euphoria, hyperactivity, and a false sense of decreased fatigue, enhanced energy, and a feeling of self-confidence. The acute toxicity associated with these psychological effects sometimes leads to anxiety, confusion, psychosis, seizures, cardiac dysrhythmias, and subsequent strokes. Cocaine is usually administered by nasal inhalation (snorting) or smoked as the free base "crack". The psychological effects of cocaine are intense but short-lived and the drug is rapidly converted to metabolites. Benzoylcegonine is one of the major metabolites produced by the body after ingestion of cocaine, and benzoylcegonine is the analyte usually tested for in oral fluid to demonstrate drug abuse^(4, 5). Tolerance has been observed with some chronic, high dose users. Abusers do not appear to be physically dependent on cocaine, but the development of strong psychological dependence is well known. With continued high dose use, a true toxic psychosis can result causing symptoms of paranoia and violent behavior.

PERFORMANCE CHARACTERISTICS

1. SENSITIVITY / SPECIFICITY

In the configuration of the OraLine[®] Cocaine tests, oral fluid concentrations of the analytes at levels equal to or greater than the cut-off value of the assay cause a change in an antigen/antibody complex that is read on a chromatographic membrane. The OraLine[®] Cocaine assay is specifically designed to perform at a threshold of 10 ng/mL of Benzoylcegonine or 25 ng/mL of cocaine. Results can be reported in

qualitative terms as "positive" above 25 ng/mL or as "negative" below this threshold for cocaine. Positive results from the OraLine[®] Cocaine tests should be confirmed by an appropriately sensitive and specific methodology using a different chemical principle. If performed under conditions that provide sufficient sensitivity, HPLC, LC or GC/MS are generally acceptable alternate methods of confirmation of the OraLine[®] Cocaine Test⁽²⁻⁴⁾. LC or GC/MS are generally accepted as a confirmation technique for all drugs, since it provides the best level of confidence in the results.

In laboratory trials where a reference cutoff of 10 ng/mL of Benzoylcegonine was used, the OraLine[®] Cocaine test was calculated as 100% sensitive using the formula TP/(TP + FN) (TP = true positive population and FN = false negative population measured). The test sensitivity was also demonstrated with a series of diluted benzoylcegonine controls in replicates of 10 at each concentration level.

The OraLine[®] Cocaine detects benzoylcegonine and cocaine in the oral fluid. The following compounds will produce positive (Table I) readings with the OraLine[®] Cocaine test.

Table I: Compounds that will produce a positive result:

Substance	Cut off ng/ml
Cocaine	25 ng/ml
Benzoylcegonine	8 ng/mL

2. ACCURACY

Table II: Cocaine Measured in Spiked Samples and Correlation with the OraLine[®] IV Result

Spiked Cocaine Concentration (ng/mL)*	Average Measured (BE/COC) Concentration by LC/MS ⁽¹⁴⁾ (ng/mL)	OraLine [®] IV COC % Positive (N=20)
0	0	0
4	2/3	0
8	3/6	0
15	5/11	80
30	9/23	90
60	18/45	100
120	35/89	100

*Cocaine Stock: Alltech, 1.0 mg/mL in Methanol, Lot#609-4053

METHAMPHETAMINE

SUMMARY AND EXPLANATION OF THE TEST

Methamphetamine is the most popular synthetic derivative of the amphetamines. These drugs are particularly potent central nervous system (CNS) stimulants. The most common amphetamines are d,l-amphetamine, d-amphetamine, and methamphetamine. They are sympathomimetic agents, which at therapeutic doses, have been used as diet pills, to overcome narcolepsy, to treat attention deficit disorders in children, and during surgery to maintain blood pressure of patients under anesthesia. These qualities have spread its use to many population groups including students in universities⁽⁴⁾. Acute higher doses, as when abused, lead to enhanced CNS stimulation, manifested euphoria, decreased fatigue, and anorexia. Responses that are more acute include anxiety, confusion, paranoia, psychosis, seizures, and cardiac dysrhythmias. Consequently, there is a strong tendency to continue to use the amphetamines to maintain the high, but tolerance develops and increasingly larger doses are required to maintain the original levels of stimulation. Oral ingestion or intravenous injection of the amphetamines gives a rapid onset of action due to the rapid absorption after administration. Amphetamine is largely inactivated by the liver yielding metabolites, which hydroxylate and deaminate the compounds, while some unchanged amphetamine is excreted in the urine⁽⁵⁾. Methamphetamine is also excreted to some extent unchanged, but major metabolites of methamphetamine are amphetamine and oxidized deaminated derivative⁽⁵⁾. The relative rate of drug elimination depends on the urinary pH.

PERFORMANCE CHARACTERISTICS

1. SENSITIVITY / SPECIFICITY

In the configuration of the OraLine[®] Methamphetamine tests, oral fluid concentrations of the analytes at levels equal to or greater than the cut-off value of the assay cause a change in an antigen/antibody complex that is read on a chromatographic membrane. The OraLine[®] Methamphetamine assay is specifically designed to perform at a threshold of 50 ng/mL of d-Methamphetamine. Results can be reported in qualitative terms as "positive" above 50 ng/mL, or as "negative" below this threshold. It is important that all positive results from OraLine[®] Methamphetamine be confirmed by an appropriately sensitive and specific methodology using a different chemical principle. If performed under conditions that provide sufficient sensitivity, HPLC, LC or GC/MS are generally acceptable alternate methods of confirmation of the OraLine[®] Methamphetamine Assay⁽²⁻⁴⁾. While confirmation techniques other than LC or GC/MS may be adequate for some drugs of abuse, LC or GC/MS is generally accepted as a rigorous confirmation technique for all drugs, since it provides the best level of confidence in the results.

In contrast to LC or GC/MS, which detects a specific drug or drug metabolite, OraLine[®] Methamphetamine immunoassay procedures can detect the parent methamphetamine molecule and methamphetamine-like metabolites. The OraLine[®] Methamphetamine has been shown to detect an average of 17-30 ng/mL for methamphetamine in clinical oral fluid samples.

The following compounds will produce positive readings with the OraLine® Methamphetamine test.

Table I: Interference substances (compound detected)

Substance	Cutoff ng/ml
d-Methamphetamine	3.75
(+)-3,4-Methylenedioxymethamphetamine (MDMA) ecstasy	5
d-Amphetamine	2,500
l-Methamphetamine	375
Ephedrine	> 2,500
Pseudoephedrine	1,750
(+)-3,4-Methylenedioxyamphetamine	1,000
Benzphetamine	55,000

2. ACCURACY – Table II: Detection of Methamphetamine from Saliva Samples-Time Study⁽¹³⁾

Donor	Descrip.	ID#	Time Elapse from Initial Drug Use	OraLine Result	MET/AMP LC/MS ng/ml*
A	Male 86 kg	2540-01	15 mins.	+	17
		2540-02	1 hr. 10 mins.	+	20
		2540-04	2 hr.	+	63
		2540-06	3 hr. 40 mins.	+/-	17
B	Male	2540-07	1 hr. 15mins.	+	4142 (1)
		2540-08	3 hr.	+	58
		2540-09	21.5 hr.	+	38
		2540-10	23.5 hr.	+	20
		2540-11	33 hr. 45 mins.	+	232 (2)
		2540-12	34 hr. 30 mins.	+	30
C	Male, 75 kg	2540-3	1.5 hr	+	160
D	F, 58 kg	2540-5	3 hr.	+	35

Notes: (1) Intake via smoking “Liquid Metal”

(2) Possible Methamphetamine intake via a mixture in Marijuana.

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