Orawell® Oral Fluid Drug Screen Device

6-AM/AMP/BAR/BZO/BUP/COC/FEN/MET/K2
MTD/OPI/OXY/PPX/MDMA/THC/TRA/ALC

The Orawell® Oral Fluid Drug Screen Device is a rapid, one-step immunoassay for the qualitative detection of Heroin, Amphetamine, Barbiturates, Benzodiazepines, Buprenorphine, Cocaine, Fentanyl, Methamphetamine, Methadone, Opiates, Oxycodone, propoxyphene, Tetrahydrocannabinol, Tramadol and their metabolites, plus Alcohol at the following cut-off concentration in human oral fluid.

6-AM	6-Acetyl Morphine	10 ng/ml
AMP	d-Amphetamine	25/50 ng/ml
BAR	Secobarbital	25 ng/ml
BZO	Oxazepam	10/20 ng/ml
BUP	Buprenorphine	10 ng/ml
COC	Cocaine	20 ng/ml
FEN	Fentanyl	10 ng/ml
K2	JWH-018	10 ng/ml
MET	d-Methamphetamine	25/50 ng/ml
MTD	Methadone	30 ng/ml
OPI	Morphine	10/40 ng/ml
OXY	Oxycodone	40 ng/ml
PPX	Propoxyphene	25 ng/ml
THC	Delta-9-Tetrahydrocannabinol	20 ng/ml
MDMA	3,4-Methylenedioxymethamphatmine	50 ng/ml
TRA	Tramadol	25 ng/ml
ALC	Alcohol	0.02% B.A.C.

This device provides only preliminary drug test results. To obtain a quantitative result or a confirmation of a presumptive positive result, a more specific alternative method must be used. GC/MS or LC/LC/MS is the preferred confirmatory method. Professional judgment should be applied to any drug of abuse test results, particularly when preliminary positive results are indicated.

Technology and Explanation

6-AM: 6-Acetylmorphine is unique active metabolites of heroin, and further metabolized to morphine or excreted from body. Its present in saliva confirms that heroin was opiates used.

AMP: Amphetamine is a potent sympathomimetic amine related to the human body"s natural catecholamine, epinephrine, and norepinephrine. Depending on the route of administration, amphetamine can be detected in oral fluid as early as 10 minutes and up to 72 hours[1].

BAR: Barbiturates are central nervous system depressants and used therapeutically as sedatives, hypnotics and anticonvulsants.

BZO: Benzodiazepines are frequently prescribed sedative and hypnotic drug for treatment of anxiety, insomnia, sleep and seizure disorders. It can be detected in oral fluid up to 24 hours.

BUP: Buprenorphine is a potent analgesic often used in the treatment of opioid addition.

COC: Cocaine is a potent central nervous system stimulant, a local anesthetic derived from the leaves of the coca plant. Depending on the route of administration, cocaine and its metabolites benzoylecgonine can be detected in oral fluid as early as 10 minutes and up to 24 hours.

FEN: Fentanyl is an extremely fast acting synthetic opioid related to the phenylpiperidines, a potent narcotic analgesic with short duration of action.

K2: K2 and spice is brand name for variety of synthetic cannabinoids, including JWH-018, JWH-073, JWH-250 and HU-210.

MET: Methamphetamine is a potent sympathomimetic agent with therapeutic applications. Methamphetamine use in acute higher doses lead to enhanced stimulation of the central nervous system and induce euphoria, alertness, and a sense of increased energy and power.

MTD: Methadone is an opioid used to treat pain and maintenance therapy for opioid dependence.

MDMA: 3,4-Methylenedioxymethamphatmine (Ecstasy) is a designer drug synthesized for the treatment of obesity. It increases blood pressure and heart rate

OPI: Heroin, morphine, and codeine are opiates that are derived from the resin of the opium poppy. Heroin is quickly metabolized to 6-acetyl morphine and morphine.

OXY: Oxycodone is a semisynthetic opioid provides pain relief by acting on opioid receptors. The plasma half-life is about 14 hours.

PPX: Propoxyphene is a narcotic analgesic with similar structure to methadone.

THC: Tetrahydrocannabinol, the active ingredient in marijuana plant is detectable in saliva shortly after use mainly due to the direct exposure of the drug via smoking. The window of detection for THC in saliva is up to 14 hours after use.

TRA: Tramadol is a narcotic likr pain reliever and can be detected in saliva up to 72 hours.

Test principle

Orawell® Oral Fluid Drug Screen Device is a rapid lateral fluid immunoassay utilizing monoclonal antibodies to selectively detect specific drug at above cutoff levels in human saliva. The sample collection and immunoassay testing was integrated into one step. The assay is based on competitive immunoassay procedure in which the drug conjugates immobilized on nitrocellulose membrane compete with the drugs if present in specimen for the limited amount of antibody on colloidal gold conjugates. If there is no drug present or the drug concentration in the specimen is below cutoff level, the red colloidal gold conjugate will bind to the drug conjugate at the specific test region, to form a visible band which indicated a negative result. If there is drug present in the specimen at above cutoff level, the drug will bind to the limited antibodies on colloidal gold, leaving no antibody available for binding to the drug conjugates on membrane. Thus, the absences of a test line band present at specific test region indicate a presumptive positive result for that particular drug.

Alcohol Test is an enzyme assay which a pad coated with highly specific enzymes, turns to color shades of green and blue on contact with alcohol in the oral fluids. When oral fluid is collected has no alcohol present, the alcohol pad remains colorless. If alcohol is present in the oral fluid, the alcohol reacts with alcohol oxidase to produce Alde Hyde and peroxide. The peroxide reacts with peroxidase in the presence of hydrogen donor to produce a blue color.

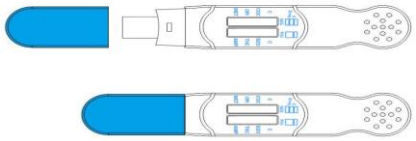


Fig. a

Reagents

The Orawell® Oral Fluid Drug Screen Device contains up to 4 membrane strips and a collection pad. Each strip consists of a membrane immobilized with drug-protein conjugates and corresponding specific drug monoclonal antibody colloidal gold conjugate pad, a sample pad and an absorbent pad. Alcohol test: a cellulose pad coated with highly specific alcohol oxidase, tetramethylbenzidine, and peroxidase.

Precautions

- For *Forensic, Insurance and workplace* Use.
- The test device is for single use and should remain in its original sealed pouch until ready for use.
- Do not use after the expiration date indicated on the kit.
- Handle all oral specimens as potentially infectious. The used device should be discarded according federal, state and local regulation.

Materials Provided

1. 1 Package Insert
2. Test devices packaged individually in a foil pouch with desiccant.

Storage and Stability

1. Store at 4°C-30°C. Do not open pouch until ready to perform the assay.
2. Keep away from direct sunlight moisture and heat.

Test Procedure

Allow the test device to reach room temperature 15 - 30°C, and instruct the donor not to eat, drink, smoke or chew tobacco products for at least 10 minutes prior to collection of fluid specimen.

1. Remove the test device from the sealed pouch and use the device as soon as possible.
2. Pull the blue cap off gently by holding the sides to expose the collection pad.
3. Hold the top portion of the device and place the collection pad into the mouth.
4. Rub the collection pad against the cheek and tongue gently in a circular motion about 10 times. And then place the collection pad underneath the tongue for about one minutes

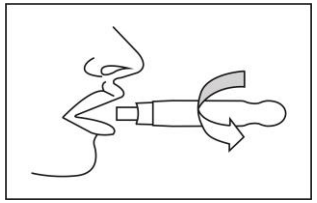


Fig. b Gently rub the collection pad against each cheek several times.

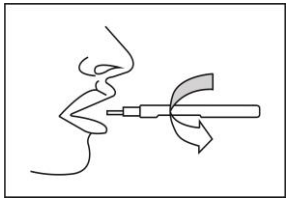


Fig. c Gently rub the collection pad of the tongue.

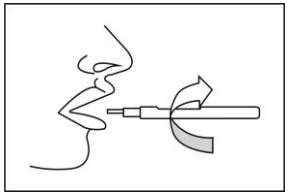


Fig. d Place the collection pad on top underneath.

5. Instruct the donor to hold the device with their hand until the red color liquid show up in window. This should take less than 5 minutes.

6. Remove the device from mouth as soon as the red color liquid moving at both test windows.
7. Place the cap onto the device; lay it on a flat surface.
8. Read results at 5 minutes after removing device from mouth. Do not read results after 15 minutes.

Interpreting Test Results

Negative Results

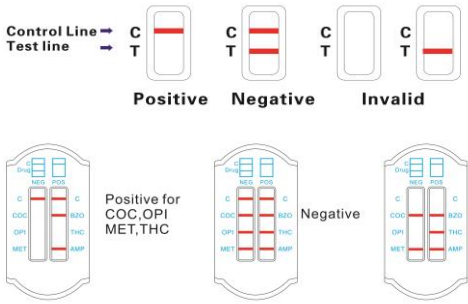
A red colored band should be observed in control region (C), and specific drug test region.
The color and density of the test band may vary for control and drug test region.

Presumptive Positive Results

When the control band is visible in the control region (C) and **no** band appears at the specific test region, the result is a **presumptive positive** for that particular drug.

Invalid

When **no** band appears in the control (C) region, **the test is invalid** regardless of the results in the test region. If the test is invalid, check testing procedures. **Repeat the test using a new device.**



Alcohol Test Result: The presence of green to blue color at the alcohol pads window indicates a presumptive positive result for alcohol.

Important: Do not compare color intensity of one test band to another. Read each test independently.
Any darker or light red band for a specific test is observed in the test region along with the presence of the control line (C), the sample should be considered negative. For confirmation of a presumptive positive result, a more specific quantitative method (GC/MS or LC/MS/MS) must be used.

Quality Control

The device has built-in control band in each window at the control regions (C) to indicate that the test has performed properly. If the control bands do not appear, the test device should be discarded. The use of external controls is strongly recommended as good laboratory testing practice to verify test performance. Negative and positive controls should give the expected results when tested by pipetting 0.5 ml of the controls onto the collection pad.
Laboratories should comply with all federal, state, and local laws, guidelines and regulations.

Limitations of Procedure

- The assay is designed for human oral fluid use only.
- The test only provides a qualitative, preliminary result. Positive results only indicate the presumptive presence of drugs and do not indicate or measure intoxication. A more specific analytical method like LC/MS/MS is preferred to confirm the results.
- Technical or procedural errors as well as substances in certain foods and certain medications may interfere with the test and cause false results.

Performance Characteristics

Analytical sensitivity: For each specific drug test, pooled oral fluid solution was spiked with a drug standard at various concentrations (0%, 50%,and 150% of cutoff level). The results for each drug of the Orawell® Oral Fluid Drug Screen Device Tests are summarized below:

Cut-off level	Drug Test											
	COC		OPI		MET		BZO		THC		AMP	
	-	+	-	+	-	+	-	+	-	+	-	+
0%	60	0	60	0	60	0	60	0	60	0	60	0
-50%	30	0	30	0	30	0	30	0	30	0	30	0
+50%	0	30	0	30	0	30	0	30	0	30	0	30
Cut-off level	BAR		BUP		MDMA		PPX		FEN		MTD	
	-	+	-	+	-	+	-	+	-	+	-	+
0%	60	0	60	0	60	0	60	0	60	0	60	0
-50%	30	0	30	0	30	0	30	0	30	0	30	0
+50%	0	30	0	30	0	30	0	30	0	30	0	30
Cut-off level	6-AM		OXY		TRA		Alcohol					
	-	+	-	+	-	+	-	+				
0%	60	0	60	0	60	0	60	0				
-50%	30	0	30	0	30	0	30	0				
+50%	0	30	0	30	0	30	0	30				

Specificity
The specificity of each drug test was evaluated by adding its structurally related compounds to pooled oral fluid sample. The results are expressed as the lowest concentration of the compound, in ng/ml, that produced a positive result.

Drug Test	Approximate Concentration (ng/ml)	Approximate % Cross Reactivity
6-Acetylmorphine (6-AM)		
6-Acetylmorphine	10	100%
6-Acetylcodein	5,000	2.5%
Codeine	10,000	<0.1%
Heroin	1000	12.5%
Hydrocodone	10,000	<0.1%
Morphine	10,000	<0.1%
Hydromorphine	10,000	<0.1%
Amphetamine (AMP)		
d-Amphetamine	25	100%
l-Amphetamine	1,000	2.5%
d,l-p-Chloramphetamine	200	12.5%
MDA	200	12.5%
Phentermine	50	50%
β-Phenylethylamine	5,000	0.5%
Tyramine	5,000	0.5%
Barbiturates (BAR)		
Secobarbital	25	100%
Allobarbital	20	125%
Alphhenal	20	125%
Aprobarbital	25	100%
Barbital	25	100%
Butalbital	25	100%
Phenobarbital	40	62.5%
Marijuana (THC)		
Δ-9-Tetrahydrocannabinol	40	100%
Cannabinol	100	40%
Δ-8-Tetrahydrocannabinol	100	40%
11-nor-Δ-8-THC-9-COOH	20	200%
11-nor-Δ-9-THC-9-COOH	10	400%
11-Hydroxy-Δ9-THC	400	10%
Cocaine (COC)		
Cocaine	20	100%
Benzoylecgonine	100	20%
Ecgonine HCl	800	2.5%
Ecgonine methylester	200	10%
Benzodiazepine (BZO)		
Oxazepam	10	100%
Alprazolan	20	50%
a-Hydroxyaprazolan	100	10%
Bromazepam	20	50%
Clobazam	10	100%
Clonazepam	400	2.5%
Delorazepam	25	40%
Chlordiazepoxide	500	2%
Opiates (OPI)		
Morphine	40	100%
6-Acetylcodeine	40	100%
6-Acetylmorphine	100	40%
Codeine	75	53%
Dihydrocodeine	250	16%
Ethyl morphine	100	40%
Heroin	50	80%
Hydrocodone	250	16%
Hydromophone	400	10%
Methamphetamine (MET)		
d-Methamphetamine	25	100%
d,l-Ephedrine	5,000	0.5%
1R, 2S l-Ephedrine	3,000	0.8%
p-Hydroxymethamphetamine	1,000	2.5%
MDEA	1,000	2.5%
MDMA	75	33.3%
d,l-Methamphetamine	50	50%
l-Methamphetamine	750	3.4%
Methoxyphenamine	5,000	0.5%
Buprenorphine(BUP)		
Buprnorphine	10	100%
Norbuprenorphine	5	200%
Fentanyl(FEN)		
Fentanyl	10	100%
Norfentanyl	100	10%
Oxycodone(OXY)		
Oxycodone	40	100%
Hydrocodone	10,000	0.04%
Morphine	>100,000	<0.01%
Hydromorphone	>100,000	<0.01%
Methadone(MTD)		
Methadone	30	100%
Hydrocodone	10,000	0.04%
Morphine	>100,000	<0.01%
Hydromorphone	>100,000	<0.01%
Propoxyphene(PPX)		
Propoxyphene	25	100%

Norpropoxyphene	100	25%
ECSTASY(MDMA)		
3,4-Methylenedioxymethamphetamine	50	100%
3,4-Methylenedioxyamphetamine	200	25%
MDE	100	50%
PMMA	150	67%
Tramadol(TRA)		
Tramadol	25	100%
N-Desmethyltramadol	50	50%
Morphine	>100,000	<0.01%
Hydromorphone	>100,000	<0.01%
K2 (K2)		
JWH-018	10	100%
JWH-073	5	200%
JWH-250	100	10%
AM-2201	200	5%

Interference

The Orawell® Oral Fluid Drug Screen Test performance at ±50% cut-off levels is not affected by oral fluid samples with pH range of 2.0 to 8.5. The following compounds were tested no interfering with assay performance when tested at concentration of 10 µg/ml (10,000ng/ml).

Acetaminophen	Hemoglobin
Albumin from human serum	Human IgA
l-Ascorbic Acid	Human IgG
Aspartame	Human IgM
Benzocaine	Ibuprofen
Benzoic acid	Ketamine
Bilirubin	Lidocaine
Caffeine	Naloxone
d-Chlorpheniramine	Naltrexone hydrochloride
Cholesterol	d-Naproxen
Dextromethorphan	Pentazocine
Diphenhydramine	Promazine
Doxylamine	Promethazine
1R, 2S l- Ephedrine *(except MET assay)*	Ranitidine
	Riboflavin
1S, 2R d-Ephedrine	Salicylic acid
l-Epinephrine	Serotonin
Erythromycin	Tetracycline
Ethanol	Thiamine
Glutethimide	Tryptamine

Food/Beverage/Hygiene Products Interference

Foods, drinks and hygiene products were spiked at 1% concentration in ±50% oral fluid controls to evaluate the interference with Orawell® test results. For interference of cigarette, oral fluid samples were collected from 6 subjects within 15 minutes after consuming a cigarette and then spiked with drug standards. The following substances were found not to interfere with Orawell® Oral Fluid Drug Screen Test performance.

Mouth Wash	Orange Juice	Alcohol
MSG	Apple Juice	Tea
Toothpaste	Food color: Red	Carbonated Cola
Gum	Food color: Green	Baking Soda
Coffee	Food color: Blue	Cigarette
Cough Syrup	Sugar	Salt

Bibliography of Suggested Reading

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