



Panel for the Qualitative Detection of Drugs of Abuse in Saliva

INTENDED USE

OralStat-6™* detects drugs of abuse in saliva and provides for the collection, transport, and storage of saliva specimens. OralStat-6 is intended for forensic use in simultaneous, qualitative detection of multiple drugs and metabolites in saliva. The OralStat-6 profile is based, when possible, on the preliminary oral fluid testing requirements of the U.S. Federal Workplace Drug Testing Programs.¹

OralStat-6 Cutoff Concentrations

		ng/mL
COC	Cocaine	20
MOR	Morphine	40
AMP	d-Amphetamine	50
MET	d-Methamphetamine	50
PCP	Phencyclidine	10
THC	Δ^9 -Tetrahydrocannabinol	100

OralStat-6 provides only preliminary analytical test results. An alternate, more specific chemical method must be used to obtain confirmed analytical results. Mass spectrometry (GC/MS or LC/MS/MS) is the preferred confirmation method for saliva specimens.¹ Clinical consideration and professional judgment should be applied to drugs of abuse test results, particularly preliminary positive results.

SUMMARY

Drugs may be ingested, inhaled, or injected. After entering the bloodstream, they are rapidly metabolized by various pathways. Many drugs and drug metabolites are excreted in urine and detected by traditional drug urine screening assays. Alternative laboratory methods have been used to detect drugs in blood or serum.

The utility of saliva as a biological specimen for the detection of recent drug use has been supported by a number of studies.²⁻⁶ A growing body of literature describes the detection and related pharmacokinetics of cocaine, opiates, amphetamines, phencyclidine, and cannabinoids in saliva.^{6,7} Correlation of drug in saliva and blood has been reported.⁸⁻¹¹ Unlike urine drug tests, saliva drug testing detects active drugs present at low concentrations (recent drug use, typically within hours).

Historically, drug screening assays relied on classical chemical methods, such as thin layer chromatography or liquid chromatography, which are accurate but laborious procedures. More recent assays for initial testing are

For Cocaine, Morphine, Amphetamine, Methamphetamine, PCP, and Cannabinoids

based on immunoassay principles. Examples of these methods are radioimmunoassay, enzyme immunoassay, kinetic interaction of microparticles in solution, and fluorescence immunoassay. These methods require sophisticated instrumentation and the handling of laboratory reagents and urine specimens. The classic drug screening technology incorporated in OralStat-6 provides the accuracy of immunoassays but does not require the collector to witness urine collection or handle laboratory reagents and urine specimens.

OralStat-6 is a simple, qualitative, visually read 10-minute test that detects recent drug use using saliva specimens. The design permits convenient collection and lateral flow screening, with a split specimen stored in a tamper-evident container for confirmation testing.

TEST PRINCIPLE

OralStat-6 assays are based on the principle of competitive inhibition. Drug that may be present in the saliva competes with drug conjugate immobilized on the test membrane for binding sites on the antibody-coated microparticles. A visual signal is generated through classical lateral flow technology.

Specimen Collection: Saliva collected by absorption directly into the OralStat-6 saliva collector is delivered into the test cassette by slowly pushing the collector foam into the sample well on the OralStat-6 cassette. A portion of the saliva specimen contacts reagents and initiates the screening test. (Figure 1)

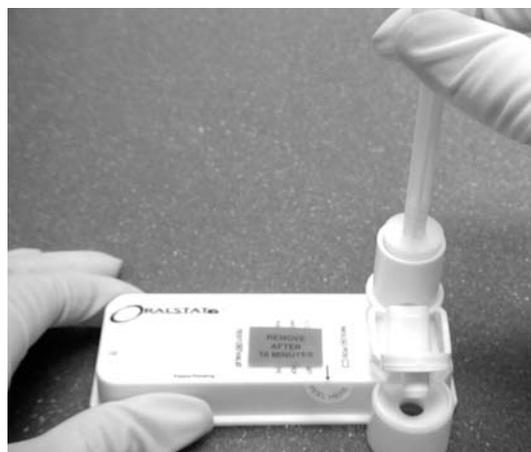


Figure 1

*US Patents 6,372,513; 6,464,939; 6,468,474; and 6,489,172.

The Test: The saliva interacts with antibody-coated microparticles and drug conjugate present on the membrane. In the absence of drug, the antibody is free to react with the drug conjugate, causing the formation of a red/pink band in each drug zone. When drug is present in the specimen, it binds to the antibody-coated microparticles. If sufficient drug is present, the microparticles are inhibited from binding the drug conjugate, and no band is formed. A positive specimen causes the detection area on the membrane to remain blank.

Quality Control: An additional antibody/antigen reaction is designed into the Test Valid area. When the specimen application and procedure are appropriate, antibodies on the reagent membrane bind the antigen on the microparticles and the Test Valid band forms.

Chain of Custody: Excess saliva collects in the confirmation well, which is sealed with the cap and tamper evident tape, if required. The cassette is ready for delivery to a confirmation site for laboratory testing.

PRECAUTIONS & WARNINGS

OralStat-6 test cassettes are for forensic use in testing human saliva for designated drugs. When handling saliva, exercise precautions similar to those used in handling human urine or similar specimens.

STORAGE & STABILITY

Store OralStat-6 cassettes at room temperature, 15–30°C (59–86 °F), in the original foil pouch. If the foil pouch containing the OralStat-6 test cassette is damaged (e.g., a hole or tear), do not use that OralStat-6 cassette. Do not use OralStat-6 cassettes after the expiration date on the foil pouch.

COLLECTION & TESTING

Reagents & Materials Provided

1. An OralStat-6 foil pouch contains:
 - a. One OralStat-6 saliva collector sealed in a bag
 - b. One OralStat-6 cassette, containing:

A sample well which receives saliva from the collector and delivers it to the screening reagents and the confirmation well

Two individual test chambers for detection of multiple drugs or drug metabolites. The reagent strip in each test chamber contains:

 - Microparticles coated with antibodies reactive with the drugs in a buffered solution containing preservative dried on a release pad

- Drug or drug analogue conjugates immobilized on a membrane
- Antibody reactive to mouse immunoglobulin immobilized on a membrane
- Absorbent pads used to filter the specimen and regulate the flow of liquids within the cassette

Confirmation well and cap

c. Desiccant pouch

2. Instruction card and tamper evident labels

3. Biohazard bags for shipment to confirmation site or device disposal, if required

Collect Saliva & Start the Test

Before the test is administered, instruct the donor to drink 4 ounces of water, and to place nothing else in the mouth—such as food, drink, gum, tobacco products, and so forth—for 10 minutes prior to collection.

During the minute or two prior to collection, instruct the donor to draw a pool of saliva into the mouth with a "puckering" action.

Record the donor name and collection date on the cassette.

Collect fresh saliva specimens as follows; no preservative, special handling, or pretreatment is required.

1. Instruct the donor to open the collector bag, place the collector foam inside mouth for 3 minutes, and completely fill the collector foam with saliva. Specifically, instruct that the donor:
 - Bathe the collector foam in the pool of saliva
 - Alternatively, stimulate salivation by continuously moving the collection foam along the sides and lower margin of the tongue
 - Not chew, compress, or suck on the collector foam
2. Observe the collection.
3. Place the OralStat-6 cassette on a flat surface and lift the confirmation well cap to assure that the flow of saliva into the well is not obstructed.
4. Remove the collection foam from the mouth. It should be very wet when removed from the mouth, and must not be compressed when removed.
 - Place the collector foam into the sample well on top of the device, and push the collector **slowly** (approximately 5 seconds) downward until it comes to a firm stop.
 - Saliva flows directly into the test cassette.

Interpret Test Validity and Results

Test validity and results must be interpreted between 10 and 15 minutes after initiating the test.

Seal the confirmation well 10 minutes after initiating the test, by pushing the cap down. The "click" confirms that the well is properly sealed. (Figure 2) Remove the label covering the read windows.



Figure 2

1. Interpret test validity: A valid test is indicated by a band in the Test Valid window. Both of the COC/OPI/AMP and MET/PCP/THC reagent strips pictured below (Figure 3) are valid. Since a valid test may give a faint or incomplete band, any Test Valid band confirms that the test is valid. Due to the high viscosity and variability of some saliva samples, test results may require up to 15 minutes to form.

An invalid test is indicated by the absence of a distinct band in the Test Valid window or by a reddish reagent background in the Results or Test Valid windows which obscures the presence of bands 15 minutes after the test is initiated. If an invalid result is obtained, see Step 3 below.

2. Immediately interpret test results as either negative or positive.

a. A negative result for a given drug (i.e., drug absent or below the cutoff) is the presence of a band in the Results window adjacent to the drug name. The intensity of the bands in the Results windows may vary. Since a negative sample may give a faint or incomplete band, any band in the Result window indicates a negative result. The test results pictured below (Figure 3) are negative for Cocaine, Morphine, Amphetamine, Methamphetamine, PCP, and THC.

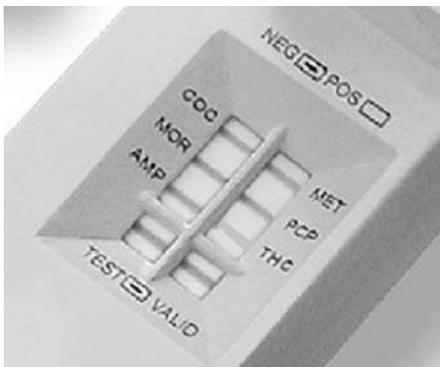


Figure 3

- b. A positive result for a given drug (i.e., drug present above the cutoff, suggesting current drug use) is the absence of a band in the test result window adjacent to the drug name. The adjacent area appears off-white.
- c. If all drug results are negative, or if no further confirmation tests are required, properly discard the test cassette and collector.
- d. When confirmation of a positive screen result is required:
 - Verify that the volume of saliva in the confirmation well is sufficient for laboratory testing by observing the transparent bottom of the confirmation well. A sufficient specimen will fill at least half of the well.
 - Remove and properly discard the OralStat-6 saliva collector.
 - Observe any chain of custody procedures in effect at the collection site. Complete chain of custody paperwork as required.
 - Have the donor initial and date the tamper evident tape. Apply the tape securely over the cap. (Figure 4)
 - Seal the cassette in the bag provided.
 - Send the specimen and paperwork to the confirmation site.



Figure 4

3. If test did not run, if invalid test results are obtained, or if the confirmation well is less than half full, **do not add additional saliva sample**. Use a second OralStat-6 device, after discarding the first.
 - a. Instruct the donor to drink 4 ounces of water. Assure that this water is swallowed and that nothing else is placed in the mouth for 3–5 minutes.
 - b. During the minute or two prior to collection, instruct the donor to draw a pool of saliva into the mouth with a "puckering" action.

- c. Provide a second packaged collector to the donor, and repeat collection instructions with emphasis on:
 - Bathing the collection foam in saliva—and/or
 - Continuous gentle movement of the collector over the tongue throughout the collection
- d. Observe a **4-minute** collection and perform the test according to the instructions in "Collect Saliva & Start Test" section (page 2).
- e. Remove and properly discard the OralStat-6 saliva collector.

Assay Control

Quality control testing at regular intervals is good analytical practice and may be required by federal, state, or local guidelines. Always check with the appropriate licensing or accrediting bodies to ensure your quality control program meets the established standards.

Internal control: Each OralStat-6 cassette has a built-in process control. The Test Valid band should always appear if the test procedure is performed properly and the specimen is adequate. In addition, the red background of reagents in the read windows normally clears to off-white, providing a distinct result 10 minutes after initiating the test. A Test Valid band is an internal process control.

Quality control: The use of positive and negative saliva controls is recommended to test each shipment of product, when a new lot is used, or anytime the product has been stored outside the recommended storage conditions. Contact CLIAwaived, Incorporated for information regarding commercially available controls. These controls should produce the expected results. If these results are not obtained, call Technical Support for assistance.

LIMITATIONS

1. Rare false positive or false negative results may occur as a result of nonspecific interactions (physiological variation, state of health, contamination of saliva with food or other substances, etc.).
2. Antibodies used in these reagents are very specific for targeted drugs of abuse. Nevertheless, structurally similar prescribed and over-the-counter drugs can react with the antibody reagents and cause false positive results. A more specific alternate chemical method such as GC/MS or LC/MS/MS must be used to obtain a confirmed analytical result. A test protocol for simultaneous GC/MS determination of these drugs in saliva is available from Varian, Inc., for validation and

use by commercial laboratories; contact CLIAwaived Incorporated for further information. Clinical consideration and professional judgment should be applied to any drug abuse test result, particularly preliminary positive test results, and should be reviewed by the Medical Review Officer.

3. Saliva collection may be closely observed. As a result, specimen adulteration is unlikely. Nevertheless, if adulteration or substituted sample is suspected, discard that specimen and collect a second specimen for testing.

REFERENCES

1. *Mandatory Guidelines for Federal Workplace Drug Testing Programs*, Draft #3, 12/5/00 Meeting of the Drug Testing Advisory Board. Department of Health and Human Services, Substance Abuse and Mental Health Services Administration.
2. Cone, E.J. Saliva Testing for Drugs of Abuse. *Ann. N.Y. Acad. Sci.*, 694, 1993.
3. Jenkins, A.J. Detecting drugs of Abuse in Saliva. *Ther. Drug Monit. and Toxicol.* 19(3), 1998.
4. Kidwell, D.A.; Holland, J., et al. Testing for drugs of abuse in saliva and sweat. *J. Chromatogr. B* 713, 1998, 111–135.
5. Peel, H.W.; Perrigo, B.J., et al. Detection of Drugs in Saliva of Impaired Drivers. *J. Forensic Sci.* 29(1), 1984, 185–189.
6. Schramm, W.; Smith R.H., et al. Drugs of Abuse in Saliva: A Review. *J. Anal. Toxicol.* 16, 1992, 1–9.
7. Cone, E.J.; Oyler, J., et al. Cocaine Disposition in Saliva Following Intravenous, Intranasal, and Smoked Administration. *J. Anal. Toxicol.*, Vol. 21, 1997, 465–475.
8. Huestis, M.A.; Dickerson, S., et al. Can saliva THC levels be correlated to behavior? A.A.F.S. Publication 92-2, 1992: 190.
9. Menkies, D.B.; Howard, R.C., et al. Salivary THC following cannabis smoking correlates with subjective intoxication and heart rate. *Psychopharmacol.* 103, 1991, 277–279.
10. Jenkins, A.J.; Oyler, J.M., et al. Comparison of Heroin and Cocaine Concentrations in Saliva with Concentrations in Blood and Plasma. *J. Anal. Toxicol.* 19, 1995, 359–374.
11. O'Neal, C.L.; Crouch, D.J., et al. Correlation of Saliva Codeine Concentrations with Plasma Concentrations after Oral Codeine Administration. *J. Anal. Toxicol.* 23, 1999, 452–459.