

Methodologies (Collection Devices, Screening Immunoassays, Confirmatory Tests)

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Oral Fluid Collection

Types of OF collection devices

- Neat OF
- Passive pad with timed collection
- Passive pad with a volume indicator
- Chewable pad with & without stimulation
- Active swabbing with & without a volume indicator
- Oral wash
- Others

Oral Fluid Collection

Neat OF by expectoration (spitting)

- Advantages
 - Large volume can be collected
 - Collection time is short
 - No conversion of drug quantitation to neat value
 - Complete recovery of drug from device
- Disadvantages
 - Unsanitary and biohazardous
 - Subject to bacteria and yeast contamination
 - Difficult to manipulate
 - Split specimen
 - Laboratory aliquot for testing

Oral Fluid Collection

Collection devices with absorbent pad

- Pad in donor's mouth becomes saturated with OF
- Removed at designated time or using volume indicator
- Collection paddle placed in tube containing buffer/preservative
- Buffer solution elutes drugs from pad
- Buffer solution used for laboratory drug tests

Oral Fluid Collection

Collection devices with pad but no buffer solution

- Device uses a chewable pad
- Pad is placed in transport tube
- Tube is centrifuged to remove OF from the pad

Oral Fluid Collection

Collection devices with absorbent pads

- Advantages
 - No direct contact with biohazardous OF if using a paddle pad
 - Easy manipulation of OF if eluted into buffer solution
 - Possible drug stability in the buffer solution
- Disadvantages
 - Speed of collection slow for dry mouth (smokers, drug users, viscous)
 - Lack of uniformity among devices (time vs. indicator; buffer or none)
 - Possible inadequate volume for multiple drugs or repeat analyses
 - Number of variables that affect collection device performance

Oral Fluid Collection

Issues with collection devices with absorbent pads

- Volume of OF collected
 - Need adequate amount for multiple drug analyses
 - Accuracy of the volume collected will impact the final drug level
 - Ex: expect 1 mL but collects 1.5 mL then 50% higher drug level
- Pad absorption
 - Pad capable of absorbing required analytes
- Removal of analytes from pad
 - Studies reveal analyte recovery varies among devices
 - Ex: amphetamine 16-97% but cocaine 61-95%*
 - Lipophilic analyte (THC) lowest recovery <12.5-85.4%**
- Analyte Stability
 - Stable at least 2 weeks in buffer or tube when refrigerated

*Crouch et al. *Ther Drug Monit* 2008;30:188

**Langel et al. *JAT* 2008;32:393

Screening Immunoassays

Two immunoassay types for parent drugs and metabolites

- Heterogeneous Enzyme Immunoassays
 - ELISA
 - Advantages
 - Sensitivity (1 ng/mL or lower)
 - Small sample size (25 uL)
 - Specificity (amp with no cross reaction to methamp)
 - Disadvantages
 - Wash or separation step
 - Labor intensive unless automated

Screening Immunoassays

- Homogeneous assays
 - Advantages
 - Automated assays
 - Lower cost per test
 - Disadvantages
 - Specificity
 - Sensitivity challenges

Screening Immunoassays

- Initial test analytes and cutoff concentrations (ng/mL)

| Oral Fluid | | | Urine | |
|--|---------------------|----------------------|-------------------------------|---------------------|
| Analyte | 2004 SAMHSA Cutoffs | Manufacturer Cutoffs | Analyte | 2010 SAMHSA Cutoffs |
| THC/Metab | 4 | 3-30 | Marijuana Metab | 50 |
| Cocaine/Metab | 20 | 10-30 | Cocaine Metab | 150 |
| Opiates (Morphine) | 40 | 20-40 | Opiates (Morphine) | 2,000 |
| 6-AM (optional) | 4 | Not available | 6-AM | 10 |
| PCP | 10 | 3-10 | PCP | 25 |
| Amphetamines- (Methamphet) (Amphetamine) | 50 | 30-120 30-300 | Amphetamines- (Methamphet) | 500 |
| MDMA | 50 | 50 | MDMA | 500 |

Screening Immunoassays

Specimen validity testing

- Urine
 - Specimens can be adulterated or substituted
 - Creatinine, specific gravity, pH and oxidant testing required
- Oral Fluid
 - Immunoglobulin (IgG) concentration should be ≥ 0.1 ug/mL
 - Some say SVT not required due to observed collection

Confirmation Testing

- Chromatography + mass spectrometry
 - Gas and liquid chromatography provide separation of analytes
 - Detector is a mass spectrometer
- New confirmation technologies
 - 1988-2010 only GC/MS permitted for urine confirmation drug testing
 - New technologies permitted with Revised Guidelines (e.g., LC/MS, GC/MS/MS, LC/MS/MS)
 - 2 NLCP labs currently using LC/MS/MS for 6-AM
 - OF testing will require these new technologies

Confirmation Testing

Confirmation test analytes and cutoff concentrations (ng/mL)

| Oral Fluid | | Urine | |
|------------------|---------------------|------------------|---------------------|
| Analyte | 2004 SAMHSA Cutoffs | Analyte | 2010 SAMHSA Cutoffs |
| THC Parent | 2 | Carboxy-THC | 15 |
| Cocaine & BZE | 8 | Benzoyllecgonine | 100 |
| Morphine | 40 | Morphine | 2,000 |
| Codeine | 40 | Codeine | 2,000 |
| 6-acetylmorphine | 4 | 6-acetylmorphine | 10 |
| PCP | 10 | PCP | 25 |
| Amphetamine | 50 | Amphetamine | 250 |
| Methamphetamine | 50 | Methamphetamine | 250 |
| MDMA | 50 | MDMA | 250 |
| MDA | 50 | MDA | 250 |
| MDEA | 50 | MDEA | 250 |