Introduction

- For many years, urine was the only biological specimen used for workplace drug testing
- Currently, urine is still the only sample allowed for analysis in the Federal workplace drug testing program
- Other matrices have been suggested:
  - Oral fluid
  - Hair
  - Sweat
  - Blood
- Oral fluid is extensively used for workplace drug testing (outside Federal regulations)
- Regulations to allow oral fluid testing in Federal workplace testing program are in final approval stages

Why Oral Fluid?

- Observed collection
- No special facilities required
- More difficult to adulterate
  - Major problem with urine program is unobserved sample collections
- Easy, rapid collection
- Minimally invasive
- Indication of volume adequacy
- Accurate, quantitative result
- Active / parent drug detection shows recent intake
- In general, the parent drugs (not metabolites) are present in higher concentration
- Ideal specimen for “post-accident” testing
Preferred Matrix

- When given a choice, patients (in an in-patient setting) opted for oral fluid over urine 85% to 15%
- Main reason for preferring urine was “not wanting to try something different”
- Not surprisingly, the medical staff overwhelmingly preferred oral fluid collection to urine

Positivity Rates in Urine versus Oral Fluid

- Most major drug testing laboratories who test for both oral fluid and urine in the workplace population indicate similar positivity rates
- Data from Redwood Toxicology: Urine and oral fluid results based on same statistical population (predominantly criminal justice)
- No significant difference in positivity rate for cannabis in the two specimen types
- Similar positive rates for opiates and benzodiazepines
- Cocaine and amphetamines positive rates were higher in oral fluid than urine
- 6-AM (heroin metabolite):
  - detected in 28.5% of opiate oral fluid positives
  - detected in 13.6% of opiate urine positives

<table>
<thead>
<tr>
<th>Drug Type</th>
<th>THC-T</th>
<th>THC-THCA</th>
<th>AMP</th>
<th>Opiates</th>
<th>Barbiturates</th>
<th>COC-BZE</th>
<th>PCP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral Fluid</td>
<td>6</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>15</td>
<td>2</td>
</tr>
<tr>
<td>Urine</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

Data from Redwood Toxicology
Positivity Rates in a Pain Population

- Oral fluid positivity rates are often higher in programs where urine is collected in a non-observed way

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Federal Workplace Drug Testing

- Substance Abuse and Mental Health Service Administration (SAMHSA) Mandatory Guidelines for Federal Workplace Drug Testing Programs
  - Oral Fluid: released May 15, 2015
- Public comment period
- Final rules still pending

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Drug Testing for State Employees

- Oral fluid for workplace drug testing is prohibited in:
  - Hawaii
  - Vermont
  - Maine
  - Puerto Rico
Considerations
1. Method of sample collection
2. Characteristics of the collection device chosen
3. Split or simultaneously collected specimens ("A" and "B")

1. Method of Sample Collection
a) Neat oral fluid: expectoration, "spitting"
b) Pad with no transportation buffer, e.g. Salivette®
c) Pad with transportation buffer, e.g. Quantisal®, Intercept®, Oral-Eze®

* Oral fluid collection devices are not standardized
* Different volumes collected
* Different dilution factors

Pad versus No Pad
Why use a pad?
* Hygiene and general disgust of observing spitting
* Frothing of saliva – hard to see volume collected
* Speed of specimen collection greatly improved
* Most devices indicate when adequate volume has been collected

Why use a buffer?
* Stabilizes drugs during transportation
* Removes drug from the collection pad
* Prevents bacterial growth in biological specimen
2. Characteristics of the Collection Device

SAMHSA requirements:

a) Volume adequacy indicator
   - Minimum 1 mL ± 10% neat oral fluid
   - Buffer requirements (reproducible volume)

b) Drug recovery from pad systems
   - Minimum 90% recovery at or near the cutoff

c) Drug stability in buffer (THC not stable in expectorated oral fluid)
   - At least 90% stability of drugs/metabolites for one week at room temperature (18-25°C) and under intended shipping and storage conditions

These variables are different for each commercial device

Split Specimens: DATIA Website

Split Sample:

- A split sample is created when an initial urine sample is split into two
- One sample is used for the initial screen and, if positive, the second sample is used for the confirmation test
- If there is a positive result, the individual being tested may request the confirmation test be done at a different laboratory. Department of Transportation (DOT)'s alcohol and drug testing regulations require all tests be performed using a "split sample" collection process

Split Specimens

- Website language is misleading
- Bottle A is screened and if positive, confirmed
- The donor can then request that the split specimen (Bottle B) be retested to confirm the non-negative result
- If Bottle B has already been opened and tested, there would be no split to use for reconfirmation purposes
- Test laboratories do not open sample B
Question 1

Do you currently utilize a split specimen collection program for URINE in a non-regulated testing situation?

3. Split or Simultaneously Collected Specimens ("A" and "B")

- Proposed testing guidelines include acceptable methods of collecting an adequate oral fluid volume to accommodate a split sample
- Analogous to a urine specimen which is poured into two separate containers after collection – A & B
- Stability of drugs in the “B” sample must also be assessed

Question 2

Do you currently utilize a split specimen collection program for ORAL FLUID in a drug testing situation?
Proposed Guidance

Section 8.8: How does the collector prepare the oral fluid specimens?

a) All federal agency collections are to be split specimen collections

An oral fluid split specimen collection may be:

1. Two specimens collected simultaneously with two separate collection devices;
2. Two specimens collected serially with two separate collection devices. Collection of the second specimen must begin within two minutes after the completion of the first collection and recorded on the Federal CCF; OR
3. Two specimens collected simultaneously using a single collection device that directs the oral fluid into two separate collection tubes

Proposed Guidance (cont’d)

1. Two specimens collected simultaneously with two separate collection devices

Currently, there are FDA 510(k) cleared oral fluid collection devices available which adhere to the proposed SAMHSA guidelines by allowing the collection of 1 mL ± 10% of neat oral fluid

- Examples include Quantisal® and OralEze®
- Two devices in the mouth at the same time

Proposed Guidance (cont’d)

Drawbacks: two devices in the mouth at the same time

* Donors may not be comfortable with two devices placed in the mouth at the same time, especially if devices are relatively large
* Difficult to determine if the rate of saliva deposition onto each pad is the same, potentially causing discrepant results between the two samples
* Positioning of the device in the mouth may cause one collection to be complete before the other – again, discrepant results are possible
* No available data or peer-reviewed literature reports supporting this approach for any of the SAMHSA drug classes
Proposed Guidance (cont’d)

2. Two specimens collected serially with two separate collection devices. Collection of the second specimen must begin within two minutes after the completion of the first collection and recorded on the Federal CCF.

Drawbacks: serial collections

- Limited, if any, published data showing that drug concentration in the second sample is similar to the first (within a specific tolerance range)
- Many drugs to be analyzed within the testing program and none have peer-reviewed literature to support this approach

Proposed Guidance (cont’d)

3. Two specimens collected simultaneously using a single collection device that directs the oral fluid into two separate collection tubes

- Some devices can collect expectorated neat oral fluid and divide the sample into two separate graduated containers e.g. Ultra-Sal 2
- Issues with drug stability in neat oral fluid especially for THC
- So, more common to use devices which contain a collection pad and buffer:
  - assists in drug removal from the pad
  - stabilizes drugs in the oral fluid during transportation
  - stabilizes drugs in storage

Question 3

*Would you implement split specimen ORAL FLUID collections for non-regulated testing if a single collection device was commercially available?
Quantisal® II

- Design phase:
  - Based on industry and governmental requirements
- Patented device
- Allows simultaneous collection of oral fluid comprising two pads on a single stem
- Design is based on the existing FDA 510(k) cleared Quantisal® device, manufactured with the same materials in the same facility

Collection Volume

As with Quantisal®, the new collection device is placed into the mouth until the blue dye is activated in the plastic stem indicating that adequate volume has been collected

Volunteers (n=50) provided oral fluid / Results from first 10 donors

<table>
<thead>
<tr>
<th>Donor #</th>
<th>Weight of unused complete system (g)</th>
<th>Weight of completed collected system (g)</th>
<th>Total collected volume (mL)</th>
<th>Weight of dry A</th>
<th>Weight of collected A</th>
<th>Volume of A (mL)</th>
<th>Weight of dry B</th>
<th>Weight of collected B</th>
<th>Volume of B (mL)</th>
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<tbody>
<tr>
<td>1</td>
<td>20.1343</td>
<td>22.2304</td>
<td>2.0961</td>
<td>10.0745</td>
<td>11.1233</td>
<td>1.0488</td>
<td>10.0588</td>
<td>11.0069</td>
<td>0.9481</td>
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<tr>
<td>3</td>
<td>19.9678</td>
<td>21.9684</td>
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<td>0.9934</td>
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<tr>
<td>4</td>
<td>20.1009</td>
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<td>0.9143</td>
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<td>0.9600</td>
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<td>20.1352</td>
<td>22.2842</td>
<td>2.1490</td>
<td>10.1132</td>
<td>11.2095</td>
<td>1.0963</td>
<td>10.0207</td>
<td>11.0745</td>
<td>1.0538</td>
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<td>19.9926</td>
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<td>21.9761</td>
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<tr>
<td>8</td>
<td>20.0486</td>
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<td>0.9733</td>
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<td>0.9159</td>
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<td>10</td>
<td>20.0986</td>
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<td>1.8613</td>
<td>9.9129</td>
<td>10.8608</td>
<td>0.9479</td>
<td>10.1838</td>
<td>11.0997</td>
<td>0.9159</td>
</tr>
</tbody>
</table>

Mean: 20.095 ± 0.099
SD: 0.178
CV (%): 0.806

Time to Adequate Volume

Results from first 10 donors
Time to activation of blue dye: 2.95 minutes

<table>
<thead>
<tr>
<th>Donor #</th>
<th>Time of collection (sec)</th>
<th>Time of collection (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>166</td>
<td>2.77</td>
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<tr>
<td>2</td>
<td>393</td>
<td>6.55</td>
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<tr>
<td>3</td>
<td>159</td>
<td>2.65</td>
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<td>4</td>
<td>163</td>
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<td>5</td>
<td>157</td>
<td>2.62</td>
</tr>
<tr>
<td>6</td>
<td>150</td>
<td>2.50</td>
</tr>
<tr>
<td>7</td>
<td>141</td>
<td>2.35</td>
</tr>
<tr>
<td>8</td>
<td>129</td>
<td>2.15</td>
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<td>9</td>
<td>165</td>
<td>2.75</td>
</tr>
<tr>
<td>10</td>
<td>150</td>
<td>2.50</td>
</tr>
</tbody>
</table>

Average: 177.3 ± 2.95
Quantisal® II (cont'd)

- Pads are then separated
- Placed into two individual transportation tubes containing buffer (3 mL) and capped ("A" and "B")
- Shipped to laboratory for analysis of the "A" sample
- The "B" specimen is handled as a "B" sample would be in a urine test program

Demonstration?
**Design Phase:** Customer and Laboratory Requirements

- As many similarities as possible between the Quantisal® II and its predecessor Quantisal® were maintained
- Minimizes laboratory validation requirements when a new device is introduced
- Same materials (plastics, pad, etc.) to ensure customer safety is not compromised

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Quantisal®</th>
<th>Quantisal® II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biological sample</td>
<td>Neat oral fluid</td>
<td>Neat oral fluid</td>
</tr>
<tr>
<td>Time to complete collection (no collection assistance)</td>
<td>9 min (20 subjects)</td>
<td>9-11 min (20 subjects)</td>
</tr>
<tr>
<td>Collection volume</td>
<td>1 mL ± 10%</td>
<td>1 mL ± 10% (use 1 mL, total volume)</td>
</tr>
<tr>
<td>Transportation buffer volume</td>
<td>3 mL</td>
<td>3 mL</td>
</tr>
<tr>
<td>Drug recovery from collection pad(s)</td>
<td>&gt;80% all drugs tested near proposed SAMHSA cutoffs</td>
<td>&gt;80% all drugs tested near proposed SAMHSA cutoffs</td>
</tr>
<tr>
<td>Drug stability in storage and transportation</td>
<td>Meets proposed guidelines</td>
<td>Meets proposed guidelines</td>
</tr>
</tbody>
</table>

**Design Recovery (25% of Cutoff)**

**Storage at Room Temperature**
Specimens from Drug Users: Authentic Samples

- Oral fluid specimens collected from known drug users
- Three different collection procedures in a randomized protocol
  - Neat, expectorated oral fluid (“spitting”)
  - Quantisal® collection
  - Quantisal® II collection

Drug Concentrations in Simultaneously Collected Oral Fluid Specimens

<table>
<thead>
<tr>
<th>Drug</th>
<th>Specimens tested to date</th>
<th>Concentration range (ng/mL)</th>
<th>Number of splits, 25th of each other</th>
</tr>
</thead>
<tbody>
<tr>
<td>THC</td>
<td>131</td>
<td>4 - 1,677</td>
<td>131</td>
</tr>
<tr>
<td>Amphetamine</td>
<td>118</td>
<td>15 - 4,475</td>
<td>115</td>
</tr>
<tr>
<td>Methamphetamine</td>
<td>94</td>
<td>48 - 23,737</td>
<td>94</td>
</tr>
<tr>
<td>Cocaine</td>
<td>70</td>
<td>8 - 51,082</td>
<td>75</td>
</tr>
<tr>
<td>Benzoylpyrrolgamma</td>
<td>150</td>
<td>8 - 30,517</td>
<td>150</td>
</tr>
<tr>
<td>PCP</td>
<td>44</td>
<td>3 - 2,952</td>
<td>44</td>
</tr>
<tr>
<td>Morphine</td>
<td>86</td>
<td>15 - 2,366</td>
<td>86</td>
</tr>
<tr>
<td>6-acetylmorphine</td>
<td>42</td>
<td>13 - 2,496</td>
<td>42</td>
</tr>
<tr>
<td>Codeine</td>
<td>37</td>
<td>15 - 648</td>
<td>37</td>
</tr>
<tr>
<td>Hydrocodone</td>
<td>17</td>
<td>15 - 652</td>
<td>17</td>
</tr>
<tr>
<td>Oxycodone</td>
<td>46</td>
<td>30 - 9,737</td>
<td>46</td>
</tr>
</tbody>
</table>
\[ \Delta^9\text{-THC in Split Samples} \]

\[ \text{Cocaine Metabolite (BZE) in Split Samples} \]

**Authentic “B” Samples**

* Unopened “B” samples from many donors remain refrigerated and will be analyzed in real time after storage of 1 year
* But in order to meet internal design specifications, used accelerated stability timepoints (Arrhenius equation)
* Increased temperature is usually greatest contributor to instability
* Samples are stored at 37°C (98.6°F)

<table>
<thead>
<tr>
<th>Timepoint</th>
<th>Equivalent long-term storage timepoint (refrigerated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 days</td>
<td>3 months</td>
</tr>
<tr>
<td>7 days</td>
<td>6 months</td>
</tr>
<tr>
<td>10 days</td>
<td>12 months</td>
</tr>
<tr>
<td>15 days</td>
<td>18 months</td>
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</table>
**$^{119}$-THC**

Original value assignment: 6.41 ng/mL
40% of confirmation cutoff (2 ng/mL): 0.8 ng/mL

<table>
<thead>
<tr>
<th>Day</th>
<th>Pad A</th>
<th>Pad B</th>
<th>Pad A</th>
<th>Pad B</th>
<th>Pad A</th>
<th>Pad B</th>
<th>Pad A</th>
<th>Pad B</th>
<th>Pad A</th>
<th>Pad B</th>
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</thead>
<tbody>
<tr>
<td>0</td>
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<td>5.06</td>
<td>3.31</td>
<td>3.08</td>
<td>2.06</td>
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<td>3</td>
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<td>1.66</td>
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<td>2.05</td>
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<td>1.63</td>
<td>1.07</td>
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</tr>
<tr>
<td>Mean</td>
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<td>5.03</td>
<td>3.27</td>
<td>3.09</td>
<td>2.07</td>
<td>2.07</td>
<td>1.63</td>
<td>1.63</td>
<td>1.07</td>
<td>1.07</td>
</tr>
</tbody>
</table>

**THC in Oral Fluid Collected with Quantisal® II**

**Cocaine Metabolite (BZE)**

Original value assignment: 22.5 ng/mL
40% of confirmation cutoff (8 ng/mL): 3.2 ng/mL

<table>
<thead>
<tr>
<th>Day</th>
<th>Pad A</th>
<th>Pad B</th>
<th>Pad A</th>
<th>Pad B</th>
<th>Pad A</th>
<th>Pad B</th>
<th>Pad A</th>
<th>Pad B</th>
<th>Pad A</th>
<th>Pad B</th>
<th>Pad A</th>
<th>Pad B</th>
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</thead>
<tbody>
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<tr>
<td>10</td>
<td>22.38</td>
<td>23.20</td>
<td>22.60</td>
<td>22.12</td>
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<td>22.31</td>
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<td>22.08</td>
<td>23.20</td>
<td>22.60</td>
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<td>22.31</td>
<td>22.31</td>
<td>22.31</td>
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<tr>
<td>Mean</td>
<td>22.73</td>
<td>23.15</td>
<td>22.54</td>
<td>22.61</td>
<td>23.08</td>
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<td>23.31</td>
<td>23.31</td>
<td>23.31</td>
<td>23.31</td>
<td>23.31</td>
</tr>
</tbody>
</table>

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4/30/18
Other Drugs

- Several other drugs (not just those likely to be included in the SAMHSA guidelines) were also tested under these conditions:
  - Tramadol
  - Buprenorphine
  - Benzodiazepines (nordiazepam)
  - Methadone

Other Drugs

- All drugs were recovered from pad >80%
- All drugs stable under the storage and transportation conditions
Summary

- Testing oral fluid is appropriate for workplace situations
- An oral fluid collection device has been developed and patented which allows the simultaneous collection of 2 mL of neat oral fluid
- Oral fluid is equally divided and absorbed into two separate collection pads
- The pads can be separated and placed into individual transportation tubes containing stabilizing buffer for laboratory analysis
- First commercial device to provide truly split samples
  - one sample for analysis (A) and
  - one for storage (B), with the potential for future testing if required

Summary (cont’d)

- Drug recovery for all SAMHSA target compounds >80%
- Drug recovery for other included drugs >80%
- Drug stability for all target compounds achieves proposed guidelines
- Drug detection in second specimen (B sample) is possible for at least 12 months when stored refrigerated – determined by accelerated stability testing
- Authentic unopened “B” specimens will be tested in next few months to compare against concentrations in “A” specimens

Thank you.