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HEADQUARTERS AIR FORCE DRUG TESTING LABORATORY
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**AIR FORCE DRUG TESTING LABORATORY STUDY OF SAMPLE LEAKAGE
DURING SHIPMENT**

1. INTRODUCTION

The Air Force Drug Testing Laboratory (AFDTL) applies the fatal BY and PY discrepancy codes to ensure the integrity of the drug testing process and to not put members at risk. During the May monthly DDRP Program Manager's meeting, an attendee expressed concern with AFDTL's assignment of fatal discrepancies to samples and/or boxes that demonstrate signs of leakage, as other service laboratories do not engage in this practice. As such, the Air Force Program Manager asked the AFDTL to examine the issue further. AFDTL conducted the following experiments examining leakage during shipment.

2. EXPERIMENT 1

AFDTL prepared 2 standard sample boxes with 24 total samples (12 samples per box) with each sample containing 30 mL of negative urine. Each box had 6 bottles with the cap either tightly screwed on or with the cap screwed down to finger tight where lids being tightened with 2 fingers until the cap met resistance. Prior to placing the samples in each box all bottles were turned on their side and then upside down to ensure they were not actively leaking. The bottles were then placed in the box alternating between a right side up and upside down orientation to ensure both possible bottle orientations were evaluated as the laboratory has no control over how the box will be oriented during shipping. Figure 1 shows the configuration of both boxes.

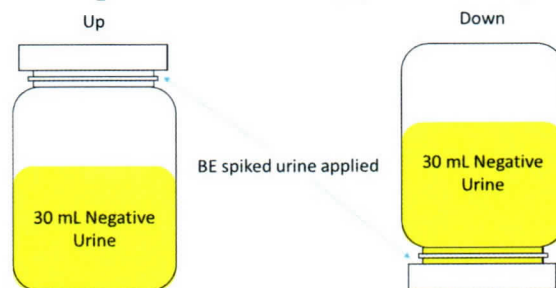
In box 1, the samples and bottle divider were placed directly inside the standard box. In box 2, a plastic bag was first placed inside the box. The samples and bottle divider were then placed inside the bag inside the box. Prior to sealing both boxes, 30 mL of negative urine spiked with the cocaine metabolite benzoylecgonine (BE) at 300,000 ng/mL was added to the box. The urine was added by pipetting it around the neck of the sample bottles as shown in Figure 2 below. Additionally, 1 drop of the BE-spiked urine was added to a 30 mL sample of negative urine as a control. This bottle was not added to the box but was retained at AFDTL. The plastic bag in box 2 was tied at the top before the standard box was taped shut.

The standard boxes were then placed inside a plastic sleeve in accordance with Air Force Instruction 90-507 prior to being placed inside another box. The outer box was also taped shut. On 8 May 2018 the boxes were shipped via FedEx overnight delivery service to Navy Drug Screening Laboratory (NDSL) Jacksonville. Representatives at NDSL Jacksonville then placed a return label on the boxes and shipped them back to the AFDTL via FedEx overnight delivery service. The boxes arrived at the AFDTL on 10 May 2018.

Figure 1. Configuration of bottles in sample boxes for experiment 1. “Tight Cap” means the bottles were tightened completely and “Loose Cap” means caps were screwed down to finger tightness. “Up” indicates the bottle was placed right side up in the box and “Down” indicates the bottle was placed upside down in the box.

Box 1, Shipment 1 No Bag inside the box			Box 2, Shipment 1 – Inside Bag inside the box		
Bottle 1 Up / Tight Cap 30 mL Neg Urine	Bottle 2 Down / Tight Cap 30 mL Neg Urine	Bottle 3 Up / Loose Cap 30 mL Neg Urine	Bottle 13 Up / Tight Cap 30 mL Neg Urine	Bottle 14 Down / Tight Cap 30 mL Neg Urine	Bottle 15 Up / Loose Cap 30 mL Neg Urine
Bottle 4 Down / Loose Cap 30 mL Neg Urine	Bottle 5 Up / Tight Cap 30 mL Neg Urine	Bottle 6 Down / Tight Cap 30 mL Neg Urine	Bottle 16 Down / Loose Cap 30 mL Neg Urine	Bottle 17 Up / Tight Cap 30 mL Neg Urine	Bottle 18 Down / Tight Cap 30 mL Neg Urine
Bottle 7 Up / Loose Cap 30 mL Neg Urine	Bottle 8 Down / Loose Cap 30 mL Neg Urine	Bottle 9 Up / Tight Cap 30 mL Neg Urine	Bottle 19 Up / Loose Cap 30 mL Neg Urine	Bottle 20 Down / Loose Cap 30 mL Neg Urine	Bottle 21 Up / Tight Cap 30 mL Neg Urine
Bottle 10 Down / Tight Cap 30 mL Neg Urine	Bottle 11 Up / Loose Cap 30 mL Neg Urine	Bottle 12 Down / Loose Cap 30 mL Neg Urine	Bottle 22 Down / Tight Cap 30 mL Neg Urine	Bottle 23 Up / Loose Cap 30 mL Neg Urine	Bottle 24 Down / Loose Cap 30 mL Neg Urine

Figure 2. Example of how BE spiked urine was applied to sample bottles in the boxes.



When AFDTL received the boxes, they were both soaked with urine. Upon opening, all the sample bottles were also found with varying degrees of wetness with urine, which per AFDTL policy would be assigned a BY untestable discrepancy code for Air Force samples and a BZ testable discrepancy code for all other service samples. The samples were set aside to dry before being handled further. After the liquid evaporated, the bottles were visually examined. Although dry, the labels showed evidence of having been wet. Also, all caps were still on the samples at the same level of tightness as when shipped as best can be determined. Most of the finger tight samples had leaked and 1 of the tightly capped bottles had leaked as well. AFDTL recorded the approximate volume of urine left in each sample. A volume of “trace” was recorded if the amount of urine appeared to be less than 5 mL but there was sufficient volume to test the sample. Where possible any urine that was contained inside the bottles was tested for presence of BE. In many cases an appropriate dilution with negative urine was used if there was not sufficient urine to meet the testing protocol requirement. Figure 3 shows the analysis results.

Figure 3. Observations and results for bottles shipped in the experiment. “Trace Urine” indicates that less than 5 mL remained in a bottle, but there was sufficient volume to test the sample.

Box 1, Shipment 1 No bag inside the box			Box 2, Shipment 1 – Inside bag inside the box		
Bottle 1 Trace Urine 29 ng/mL*	Bottle 2 30 mL Urine 16.87 ng/mL	Bottle 3 Trace Urine 67 ng/mL*	Bottle 13 30 mL Urine 0 ng/mL	Bottle 14 30 mL Urine 0 ng/mL	Bottle 15 10 mL Urine 7289 ng/mL
Bottle 4 Trace Urine 5363 ng/mL	Bottle 5 30 mL Urine 46 ng/mL	Bottle 6 30 mL Urine 16 ng/mL	Bottle 16 Trace Urine 5890 ng/mL	Bottle 17 30 mL Urine 24 ng/mL	Bottle 18 30 mL Urine 30 ng/mL
Bottle 7 Insufficient Volume to test	Bottle 8 Insufficient Volume to test	Bottle 9 30 mL Urine 12 ng/mL	Bottle 19 Trace Urine 2946 ng/mL	Bottle 20 Trace Urine 3774 ng/mL	Bottle 21 30 mL Urine 0 ng/mL
Bottle 10 30 mL Urine 0 ng/mL	Bottle 11 Trace Urine 143 ng/mL	Bottle 12 Trace Urine 5576 ng/mL	Bottle 22 30 mL Urine 0 ng/mL	Bottle 23 15 mL Urine 6684 ng/mL	Bottle 24 Insufficient Volume to test

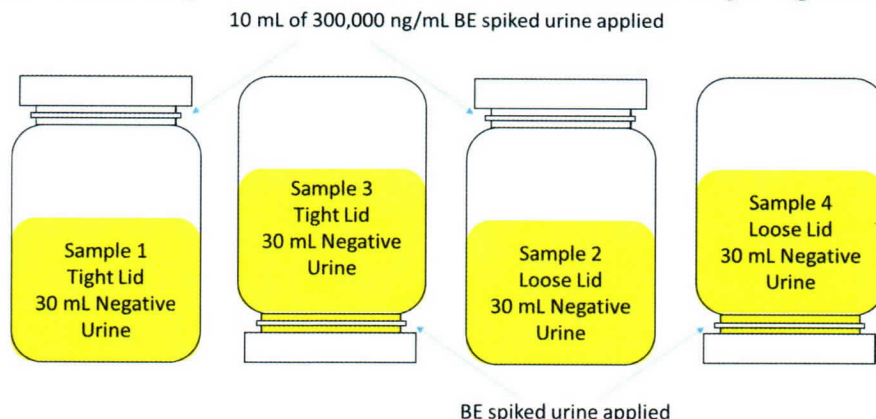
* These samples did not meet all criteria to officially be called a positive sample, but based on the data, AFDTL believes that BE is present in these samples.

The quantitative results for box 1 indicate that BE was detected meeting all acceptability criteria in 4 of 6 sample bottles with tight caps. BE was also detected meeting all acceptability criteria in 3 of 4 samples with finger tight caps (2 samples had insufficient volume to test). The quantitative results for box 2 indicate BE was detected meeting all acceptability criteria in 2 of 4 sample bottles with tight caps and 5 of 5 samples with finger tight caps (1 sample had insufficient volume to test). The control sample, which was spiked with 1 drop of the BE-spiked urine, quantitated at 159.54 ng/mL and 156.54 ng/mL in analysis of box 1 and box 2 respectively.

3. EXPERIMENT 2

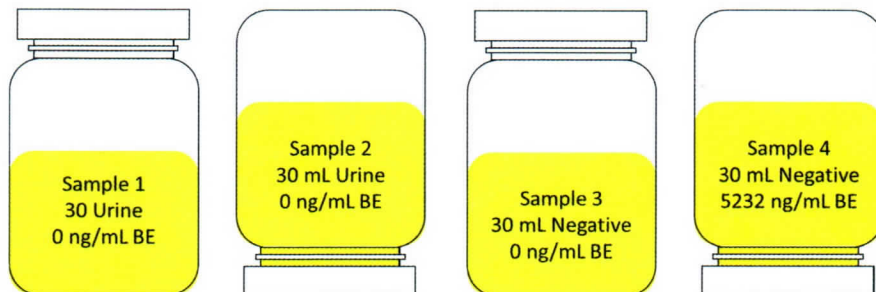
Spiked urine clearly infiltrated both tight and finger tight bottles in experiment 1; however, AFDTL wanted to evaluate if this was attributable to the actual shipping process, or due to the application of spiked urine around the neck of the sample bottle. As such, AFDTL conducted a second experiment where 4 sample bottles were filled with negative urine, and 10 mL of negative urine spiked with BE at 300,000 ng/mL was applied in a similar fashion to experiment 1. The bottles alternated between a right side up and upside down orientation and there were 2 samples with tight caps and 2 samples with finger tight caps. Figure 4 shows the experimental setup and indicates where the BE spiked urine was applied during the experiment.

Figure 4. Configuration of bottles that had urine spiked at 300,000 ng/mL at the neck of the bottle similar to experiment 1. “Tight Cap” means the bottles were tightened completely and “Loose Cap” means lids were screwed down to finger tightness.



After the BE spiked urine was applied, the samples were allowed to dry before being analyzed. This mimicked the procedure in experiment 1. None of the samples exhibited any appreciable change in the amount of urine in the bottle during the experiment. The results of the analysis are shown in Figure 5.

Figure 5. Observations and results for bottles that had BE-spiked urine applied at the necks.



Based on the results it appears that application of the spiked urine in experiment 1 possibly caused the infiltration of the BE into the upside down “finger tight” bottles, but does not appear to have caused the infiltration into the right side up “finger tight” bottles or the tight bottles regardless of orientation.

4. EXPERIMENT 3

The AFDTL recognized the conditions in experiment 1 and 2 were extreme and were not comparable to conditions in the field. In order to more closely mimic shipping conditions and possible risks associated with a leaking bottle as opposed to directly adding spiked urine into the box, AFDTL conducted another shipping experiment. It should be noted that as with the first 2 tests, experiment 3 was not modeled after any specific real world shipments received at the AFDTL. In this experiment, AFDTL prepared 2 identical standard sample boxes with 11 samples containing 30 mL of negative urine surrounding 1 sample with 30 mL of negative urine spiked with BE at a concentration of 300,000 ng/mL. The caps for the negative urine samples

were alternated between tight and finger tight similar to experiment 1. In box 1, all samples were placed in an upright orientation, and in box 2 all samples were placed in an upside down orientation. The cap for the spiked sample was tightened to finger tightness and then loosened back a quarter turn. Figure 6 shows the configuration of samples in both boxes.

Figure 6. Configuration of bottles in sample boxes for experiment 3. “Tight Cap” means the bottles were tightened completely and “Loose Cap” means caps were screwed down to finger tightness.

Bottle 1 Loose Cap 30 mL Neg Urine	Bottle 2 Tight Cap 30 mL Neg Urine	Bottle 3 Loose Cap 30 mL Neg Urine
Bottle 4 Tight Cap 30 mL Neg Urine	300,000 ng/mL BE Very loose Cap	Bottle 5 Loose Cap 30 mL Neg Urine
Bottle 6 Tight Cap 30 mL Neg Urine	Bottle 7 Loose Cap 30 mL Neg Urine	Bottle 8 Tight Cap 30 mL Neg Urine
Bottle 9 Loose Cap 30 mL Neg Urine	Bottle 10 Tight Cap 30 mL Neg Urine	Bottle 11 Loose Cap 30 mL Neg Urine

The boxes were placed inside a plastic sleeve in accordance with Air Force Instruction 90-507 prior to being placed inside another box, which was also taped shut. The standard boxes were placed in the second box such that their orientation remained the same as described above (1 box up and 1 box down). This enabled AFDTL to evaluate both possible configurations during shipment as AFDTL has no control over box orientation during shipment. On 14 May 2018 the boxes were shipped to NDSL Jacksonville via FedEx overnight delivery service. Representatives at NDSL Jacksonville then placed a return label on the boxes and shipped them back to the AFDTL via FedEx overnight delivery service. The boxes arrived to AFDTL on 16 May 2018.

When AFDTL received the boxes, box 2 (wherein the bottles were originally oriented down) was wet with urine, while box 1 was partly dry. Upon opening box 1, 6 bottles including the BE-spiked sample were wet with urine, while all the sample bottles in box 2 were wet with urine which per AFDTL policy would be assigned a BY untestable discrepancy code for Air Force samples and a BZ testable discrepancy code for all other service samples. All wet samples were set aside to dry before being handled further. After the liquid evaporated, the bottles were visually examined. In box 1, all the samples with negative urine appeared to have the same amount of urine as the bottles contained prior to the shipment. However, the BE-spiked sample had completely leaked out. In box 2, the BE-spiked sample had completely leaked out, and a majority of the urine in the finger tight bottles had leaked out as well. All of the bottles with tightened caps appeared to have the same amount of urine as there was prior to shipment.

AFDTL recorded the approximate volume of urine left in each sample for the boxes. A volume of “trace” was recorded if the amount of urine appeared to be less than 5 mL but there was sufficient volume to test the sample. Where possible any urine that was contained inside the bottles was tested for presence of BE. In many cases an appropriate dilution with negative urine was used if there was not sufficient urine to meet the testing protocol requirement. Figure 7 shows the analysis results.

Figure 7. Observations and results for bottles shipped in the experiment. “Trace Urine” indicates that less than 5 mL remained in a bottle, but there was sufficient volume to test the sample.

Box 1, Experiment 3, Box Shipped Upright – Results			Box 2, Shipment 2 – Box Shipped Upside Down		
Bottle 1 30 mL Urine 0 ng/mL	Bottle 2 30 mL Urine 0 ng/mL	Bottle 3 30 mL Urine 0 ng/mL	Bottle 13 Trace urine 2590 ng/mL	Bottle 14 30 mL Urine 0 ng/mL	Bottle 15 Trace urine 100 ng/mL
Bottle 4 30 mL Urine 0 ng/mL	300,000 ng/mL BE Empty	Bottle 5 30 mL Urine 0 ng/mL	Bottle 16 30 mL Urine 0 ng/mL	300,000 ng/mL BE Empty	Bottle 17 Trace urine 0 ng/mL
Bottle 6 30 mL Urine 0 ng/mL	Bottle 7 30 mL Urine 0 ng/mL	Bottle 8 30 mL Urine 0 ng/mL	Bottle 18 30 mL Urine 0 ng/mL	Bottle 19 Trace urine 0 ng/mL	Bottle 20 30 mL Urine 0 ng/mL
Bottle 9 30 mL Urine 0 ng/mL	Bottle 10 30 mL Urine 0 ng/mL	Bottle 11 30 mL Urine 0 ng/mL	Bottle 21 Trace urine 812 ng/mL	Bottle 22 30 mL Urine 0 ng/mL	Bottle 23 Trace urine 317 ng/mL

The quantitative results indicate that BE was not detected in any of the samples in box 1. Also, although the BE-spiked sample leaked out, since none of the finger tight samples leaked it is likely that this box remained in a mostly upright configuration during shipment. The quantitative results for box 2 indicate that BE was not detected in any of the tight capped bottles, but was detected in 4 of the 6 finger tight samples.

5. CONCLUSION

Based on the experiments conducted, it is possible for a negative urine specimen to result in detection of an analyte of interest after coming in contact with urine containing higher concentrations of that analyte. As such AFDTL believes that it is prudent to continue its practice of assigning a PY/BY untestable discrepancy code for Air Force samples that appear to be wet or have been wet upon arrival.