Quality Division Use Only

Quality Tracking #:	2022-008	**Classification:**	Corrective Action
Non-Conformance Level:	Class II	**Section:**	Toxicology
Date of Discovery:	01/11/22	**Date of Incident:**	12/16/21

Forensic Case Number(s), if applicable:	Agency Case Number(s), if applicable:
2021-32716 2021-32717 2021-32718 2021-32818 2021-32868 2021-33041 2021-33048 2021-33202 2021-33245 2021-33251 2021-33391 2021-33394 2021-33461 2021-33505 2021-33509 2021-33675 2021-33680 2021-33683 2021-33732 2021-33863 2021-34012 2021-34092 2021-34157 2021-34166 2021-34254 2021-34257 2021-34324 2021-34374 2021-34376 2021-34383	140220821 139827421 139746821 140960721 141507721 143183821 142890021 144651321 143633121 144467421 146063721 146937821 146543821 148071421 145638221 150087221 149507621 150058421 150526521 152443021 153564721 152749221 155303621 136968921 156582221 155948621 156267021 157601921 156728421 159168321

Description of Non-conformance:

Two toxicology samples were inadvertently switched during the enzyme-linked immunosorbent assay (ELISA) drug screening analysis. The switch was discovered as part of the case review process and the affected results were not reported for these two samples.

Additional Information/Follow-Up:

During the routine toxicology analysis workflow of urine samples for sexual assault cases, samples are first analyzed by ELISA drug screening techniques. If the initial ELISA drug screen is negative, an additional basic drug screen is completed. If the ELISA drug screen is positive for any drugs or drug classes, additional screening tests are not required and the sample proceeds to the confirmatory testing part of the analysis workflow. Confirmatory testing is only performed by request in sexual assault cases.

After case 2021-33863 had undergone ELISA screening, the case technical reviewer noted that there was a significant discrepancy between the original and re-injection results compared to the re-analysis results. The original analysis and re-injection for this case were performed on December 8 and December 9, 2021, respectively where it screened positive for several drug groups but specifically for the cocaine assay for both analyses. Because the quality controls (QCs) failed to meet the acceptance criteria on both the original and re-injection analyses, the original urine sample was re-sampled and re-analyzed on December 16, 2021, by the same analyst. The result for this new analysis showed that the sample screened negative for the cocaine assay.

There is an associated inherent analytical variability for concentrations close to the cut-off calibrator (limit of detection) which can lead to either positive and/or negative results for samples. However, the original and re-injection positive results and the negative re-analyzed result both showed responses/absorbance levels that were significantly different and outside the cut-off calibrator threshold. This discrepant difference in results prompted further investigation from the section.

On January 18, 2022, an independent analyst screened this case specifically for the cocaine assay and it was found to be negative. After this analysis, it was concluded that a sample switch may have occurred with another case in the batch.

On January 25, 2022, all cases involved in the batch EIA_20211208U_JP were reanalyzed by a second independent analyst (except for 2021-32716 which had been outsourced for confirmatory analysis). Based on a review of the resulting data, it was determined that cases 2021-33863 and 2021-33683 had been switched in the initial analysis. The technical review step identified this discrepancy and prevented the affected results from being reported.

Both urine and blood samples that screen negative via ELISA undergo an additional screening step using the gas chromatography-mass spectrometry (GC/MS). GC/MS screening monitors additional drugs not covered by ELISA screening. This additional screening is not a verification step of the initial ELISA screening. Therefore, there is a possible risk in reporting false negative results for positive samples that only get screened. The reporting of false positive results is not a risk because samples that screen positive undergo additional qualitative and/or quantitative confirmation testing specific to the drug or drug classes screened positive.

HFSC acknowledges that due to the instability and degradation of drug analytes with time, it is impractical to go back, and re-test previously analyzed samples. To be able to conduct the re-analysis of samples, the section needs to complete this contemporaneously after the original analysis. Due to Toxicology's limited staff capacity, it is not able to introduce the re-testing of samples at this point in time.

As mentioned before, the absorbance and %-binding readings of samples with concentrations close to the cut-off calibrator concentration can lead to either positive and/or negative results due to the inherent analytical variability. This variability was taken into consideration during the validation of this method. Thus, repeated analysis of a sample containing drug analytes at this concentration range are statistically expected to vary, resulting in both positive and negative results due to the inherent variability observed during validation. The results for samples at the cut-off concentration range can be negative at the lower end of the calibrator variability range and positive at the higher end of the calibrator variability range. The following cases were affected by this variability because their absorbances were around the cut-off calibrator concentrations when comparing the original analysis with the results from the reanalysis.

Case 2021-33394 originally screened negative for cannabinoids but in the subsequent investigative re-analysis, it screened positive.

Case 2021-33461 originally screened positive for cannabinoids but in the subsequent investigative re-analysis, it screened negative.

Case 2021-32868 originally screened negative for methamphetamine but in the subsequent investigative re-analysis, it screened positive.

Case 2021-34383 originally screened negative for amphetamine but in the subsequent investigative re-analysis, it screened positive.

These four case samples will be considered as having a positive screen result (cannabinoids or methamphetamine/amphetamine), following the normal laboratory practice for such instances so that the screen results can proceed to confirmatory analysis upon request.

Case 2021-33683 when it was originally analyzed under the switched case number (2021-33863), screened negative for all analytes. In the subsequent investigative re-analysis, it screened positive for cannabinoids, cocaine, amphetamine, and methamphetamine.

Case 2021-33391: The initial analysis showed that it had screened positive for cannabinoids, but the subsequent investigative re-analysis showed that it was negative. Due to this, this case was re-analyzed a third time where it screened negative for cannabinoids, suggesting analyte instability in the sample. This case will be reported as having a negative cannabinoid screening result.

Case 2021-32716 initially screened positive for both cocaine and cannabinoids and was not re-analyzed because it had been outsourced for confirmatory analysis. The cocaine confirmation results were negative; however, this was not unexpected because the confirmation cutoff concentrations were much higher (benzoylecgonine at 150 ng/mL, cocaine at 200 ng/mL, and cocaethylene at 200 ng/mL) than the immunoassay cocaine/metabolites cutoff (50 ng/mL). The cannabinoids confirmation analysis was cancelled due to interfering substances.

Summary of Root Cause Analysis:
Note: Incidents are documented for tracking purposes and trend analysis. Root Cause Analysis is not required for incidents.

The root cause of this nonconformance was attributed to the analyst's visual verification process when aliquoting samples into labeled test tubes. The intent of this verification process is to visually check that the forensic case and item number matches both the evidence container and test tube. Because this verification process is done manually, it is vulnerable to the possibility of human error. HFSC is aware of this risk and had previously attempted to automate this process by implementing a barcode reader software prior and independently of this nonconformance but had difficulty implementing this.

Urine specimens are collected and submitted for analysis in a variety of containers that may differ in size and shape. Container types may include test tubes, collection cups and Nalgene bottles. Due to this difference, the evidence containers are typically placed within plastic shelf bins that may narrow the visibility of these case identifiers. This type of specimen is not commonly submitted as blood samples, and therefore is only analyzed approximately once a month. Since this was the first time this analyst performed ELISA screening on urine samples, she did not have a system set in place for how to best perform her verifications. She verified that the evidence containers were in the same order as her worklist but placed these containers back into the shelf bin after verification was complete. When aliquoting the samples into each corresponding test tube, she grabbed one

container at a time from the bin, verified the case identifiers corresponded to her test tube, but continued placing the containers back into the bin. By placing the containers back in the bin, she did not have a way of designating a visual cue as to what evidence container was next and limited the case identifier's visibility. This lack of visual factors in combination with the similarity in the case numbers for the switched cases (2021-33863 and 2021-33683) contributed to causing this nonconformance.

The analyst acknowledged that this was not a problem for blood specimens since the labels in this type of sample (all collected in test tubes) are fully displayed in a tube rocker. In addition, after each blood tube is verified and aliquoted, they are turned upside down to aid as a visual cue as to what blood tube is next. These engineering controls are not possible with the urine containers as they do not fit into tube rockers and inversion is not always possible, depending on the specific container.

Actions Taken:

During the section meeting on January 29, 2022, the analysts discussed the different ways they organize and visually verify case identifiers when analyzing urine samples. They all agreed that because this type of specimen is analyzed infrequently and due to the differing types of containers, an additional verification step would be helpful to prevent this from happening again. This verification step will consist of an independent person verifying that urine sample containers and the secondary test tube label identifiers are consistent for ELISA analysis, and both are in the same order prior to the analyst aliquoting their samples. This verification completed by an independent person will be documented by initialing the worklist.

The seven reports that were released prior to the reanalysis were amended and the 23 reports that had not yet been released will include a comment detailing that they had been re-analyzed due to an accidental sample switch and referenced the Quality Report for more information.

Section Manager: Dayong Lee **Date:** 06/21/22

Division Director: Amy Castillo **Date:** 07/18/22

Incidents or Corrective Actions that involve the Biology/DNA section are reviewed by the Technical Leader and CODIS Administrator.

Technical Leader: N/A **Date:** N/A

CODIS Administrator: N/A **Date:** N/A

Quality Director: Erika Ziemak **Date Closed:** 07/19/22