Quality Division Use Only

Quality Tracking #:	2017-014	**Date Quality Division Notified:**	3/15/2017
Non-Conformance Level:	Class I	**Date Submitted to Management for Review:**	4/5/2017
Date Submitted to Quality for Review:	5/31/2017	**Dated Closed:**	5/31/2017

Date of Discovery:	3/8/2017	**Division:**	Comparative & Analytical Division
Date of Incident:	2/23/2017	**Section:**	Toxicology

Forensic Case Number(s), if applicable:	Agency Case Number(s), if applicable:
2016-12109 2016-14497 2016-13510	065198716 065966116 088938216

Description of Discrepancy/Non-conformance. Do not include analysts' names unless otherwise instructed by the Section Manager or Division Director(s):

d/l-methamphetamine was used in the Enzyme-Linked Immuosorbent Assay (ELISA) blood validation. Toxicology SOP version 2.7, in effect on February 23, 2017, when this nonconformance was noted, required the use of d-methamphetamine. SOP v2.8 was changed to reflect the current validation parameters and both the ELISA Blood Validation and SOP v2.8 were issued on March 1, 2017.

Associated documents: Blood batches: 20160707_ASG, 20160708_ASG, 20160713_ASG, 20160714_ASG, 20160719/20_ASG, 20160726/27_ASG, 20160728_ASG, 20160729_ASG, 20160804_ASG2, 20160805_ASG, 20160816_ASG2, 20160825_ASG, 20160902_ASG, 20160909_ASG, 20160916B_ASG, 20160922B_ASG, 20160929B_ASG, 20161011B_ASG, 20161012B_ASG, 20161013B_ASG, 20161026B_ASG, 20161027B_ASG, 20161107B_ASG, 20161108B_ASG, and 20161109B_AAJ.

Urine batches: 20160921/22/30U_AAJ, 20161006/28U_AAJ, 20161101U_AAJ, 20161129U_AAJ, 20161130U_ASG, 20161209U_ASG, 20170111U_AAJ, 20170119U_ASG, EIA_20170124U_ASG_Tecan, EIA_20170131U_ASG_Tecan, EIA_20170207U_ASG_Tecan, and EIA_20170217U_ASG_Tecan.

LAB-27 (Working Stock/Standard Preparation Logs Involved): Blood stocks, sub-stocks, calibrators, and controls: 061616C-C-MXA, 061616C-C-MXB, 061616C-C-MXC, 061616L-Q-MXD, 061616L-Q-MXE, 061616L-Q-MXF, 061616L-Q-MXA, 061616L-Q-MXB, 061616L-Q-MXC, 092116B-SS-MXH, and 092116B-MXH. Urine stocks, sub-stocks, calibrators, and controls: 071216C-CMX1, 071216C-CMX2, 071216-C-CMX3, 121216C-CMX2, 121216C-CMX3, 081216U-ST-H, 081216U-W-H, 081216U-MXH, 081216U-ST-N, 081216U-W-N, 081216U-MXN, 121216U-W-H, 121216U-MXH, 121216U-W-N, and 121216U-MXN.

Verifications Involved: Blood: Method Verification for Qualitative Test Methods signed July 6, 2016 Urine: Method Verification for Qualitative Test Methods signed September 8, 2016 and Method Verification for Qualitative Test Methods signed December 21, 2016.

Actions Taken:

After researching the potential effect of this nonconformance on casework, the section manager determined that using a mixture of d/l-methamphetamine artificially lowered the target cut-off value for detecting d-methamphetamine from 20 ng/mL to approximately 13 ng/mL. This prompted a review of all ELISA methamphetamine documents, including Working Stock/Standard Preparation Logs, data, SOPs, verifications, and validations since the 2015 Blood validation (effective October 19, 2015) and the urine ELISA validation (October 27, 2015).

After determining when the use of d/l-methamphetamine first occurred, all subsequent blood and urine batches were evaluated for methamphetamine to determine the effect, if any, on reported test results. The review of these documents indicated that three cases could have been affected. To ensure the accuracy of the results reported by HFSC, these three cases were recalled from the HPD Property Room and sent to an external laboratory for analysis. The Toxicology section also revalidated the urine and blood ELISA methods using d-methamphetamine for calibrators and controls.

Reanalysis results: 2016-14497 Item 1.1 was screened by an external laboratory for the presence of methamphetamine using ELISA at the cutoff 20 ng/mL. The results from the external laboratory were none detected. The results produced from the external laboratory will be added to the original outsource report with the following comment on the cover page:

“HFSC issued this report (Report No. 5) to amend Toxicology Report No. 4, dated February 6, 2017. Item 1.1 was externally re-analyzed for methamphetamine because the HFSC Toxicology Section used d/l-methamphetamine to make the immunoassay calibrators and controls used to screen this item. The SOP requires the use of d-methamphetamine. Please see Corrective Action Report 2017-014 for further details.”

2016-13510 Item 2.1.3 was analyzed by the external laboratory for the presence of methamphetamine using the Enzyme Multiplied Immunoassay Technique (EMIT®), with an amphetamine kit at a cutoff 500 ng/mL. The results from the external laboratory were none detected. These negative results do not aid HFSC in determining the acceptance of our cutoff using the d/l-methamphetamine mixture verses d-methamphetamine.

The Toxicology section analyzed Item 2.1.3 again using the revalidated Urine ELISA method to determine the accuracy of our original results. The results from the revalidated Urine ELISA screen were negative. The toxicology screen report for this case (Report No. 3) was amended to indicate Item 2.1.3 was negative for all drugs screened by ELISA with the following comment:

"HFSC issued this report (Report No. 4) to amend Toxicology Report No. 3, dated January 5, 2017. Item 2.1.3 was re-analyzed for methamphetamine because the HFSC Toxicology Section used d/l-methamphetamine to make the immunoassay calibrators and controls used to screen this item. The SOP requires the use of d-methamphetamine. Please see Corrective Action Report 2017-014 for further details."

2016-12109 Item 1.1 was analyzed by the external laboratory for the presence of methamphetamine using ELISA. The results were presumptively positive for methamphetamine. Item 1.1 was then analyzed for methamphetamine by High Performance Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS). The external laboratory reported 15 ± 3 ng/mL of methamphetamine. The reanalysis results were consistent with the original results. The results produced from the external laboratory will be added to the original outsource report with the following comment on the cover page:

"HFSC issued this report (Report No. 4) to amend Toxicology Report No. 2, dated September 19, 2016. Item 1.1 was externally re-analyzed for methamphetamine because the HFSC Toxicology Section used d/l-methamphetamine to make the immunoassay calibrators and controls used to screen this item. The SOP requires the use of d-methamphetamine. The reanalysis result of methamphetamine was consistent with the original result. Please see Corrective Action Report 2017-014 for further details."

Summary of Root Cause Analysis:

This nonconformance occurred because the Toxicology SOP was not clear and concise, leading to confusion in how to prepare stock solutions. To address the confusion, the section created Supplemental Worksheets instead of revising the SOP. These worksheets, rather than the SOP, were used as a reference when making the stock solutions documented in this corrective action. The worksheets did not specify whether to use d-methamphetamine or d/l-methamphetamine nor were the worksheets controlled documents. The Toxicology SOP has since been revised and the Supplemental Worksheets are no longer used.

Another cause of this nonconformance was the lack of documentation in the historical training process. Prior to August 2015, the section did not have a well-documented training program. All analysts trained since this time have been trained under the revised program. However, to ensure all analysts received adequate training in the ELISA process, a three-part training series was taught section wide on March 17, March 27, and April 12, 2017. The training included: understanding the differences between d/l, D/L, R/S, and +/- isomers; reading and discussing the ELISA SOP and the ELISA-related questions in the Training Manual; reviewing the proper preparation and verification of calibrators, controls, and reagents; and reviewing the manufacturer's ELISA test kit product inserts.

Additional Information/Follow-Up:

The blood calibrator (lot 0616C-C-MXA) and control (lots 061616L-Q-MXA and 061616L-Q-MXD) stock solutions were also made using d/l-methamphetamine. These stock solutions were subsequently used in the preparation of the blood calibrator (lot 061616C-C-MXC) and controls (lots 061616L-Q-MXC and 061616L-Q-MXF) used in casework. These controls and the calibrator underwent a performance verification that met all acceptance criteria necessary to be used in casework.

Blood samples from 483 cases were analyzed with the d/l-methamphetamine mixture. Of those cases, 38 cases screened presumptively positive for methamphetamine. The 38 cases were evaluated based on their relative % binding (B/B0) values in comparison to the cut-off and high controls. As discussed above, the cut-off per our SOP was 20 ng/mL. However, by using d/l-methamphetamine instead of d-methamphetamine, the cut-off calibrator was artificially lowered to approximately 13 ng/mL for d-methamphetamine. Additionally, because d/l-methamphetamine was also used to prepare the high control, the level of the high control was artificially lowered from 40 ng/mL to approximately 26 ng/mL for d-methamphetamine.

The theoretical values of 13 ng/mL and 26 ng/mL were determined based on calculations using the methamphetamine ELISA kit inserts cross-reactivity data. Therefore, evaluating case samples based on their B/B0 values with respect to the cut-off and the high control made it possible to determine any case samples that may have produced a negative instead of positive screening result had d-methamphetamine been used to prepare the calibrator and controls.

Among the 38 cases that indicated a presumptive positive and thus were sent to an external laboratory for confirmation, 10 cases had a B/B0 value that fell between the cut-off and the high control. Only two of the 10 cases produced positive confirmation results. Since the external laboratories do not differentiate between d-methamphetamine and l-methamphetamine in their confirmation methods, these two cases were recalled and re-analyzed to see if the sample would screen positive at a target concentration of 20 ng/mL using d-methamphetamine only.

Although urine results are reported only qualitatively, methamphetamine was evaluated as well for urine samples to ensure preliminary results were accurate at our target concentration of 200 ng/mL. After reviewing all urine documents, it was noticed that on July 12, 2016 the urine calibrator stock solution (lot 071216C-CMX1) was prepared using d/l-methamphetamine, which was subsequently used to prepare the urine calibrators (lots 071216-C-CMX3 and 121216C-CMX3) used in casework. The urine control stock solutions (lots 081216U-St-H and 081216U-St-N) were also prepared using d/l-methamphetamine. These solutions were subsequently used to make urine controls (lots 081216U-MXH, 081216U-MXN, 121216U-MXH, and 121216U-MXN) that were used in casework. These urine calibrators and controls were performance verified and met all necessary requirements to be used in casework. The verification data can be reviewed in Method Verification for Qualitative Test Methods effective September 8, 2016 and December 21, 2016.

After determining when the use of d/l-methamphetamine first occurred, all subsequent urine batches were evaluated for methamphetamine to determine the effect, if any, on reported results. Urine samples from 244 cases were analyzed with drug standards containing the d/l-methamphetamine mixture. Of those cases, 28 cases screened presumptive positive for methamphetamine. These 28 cases were evaluated based on their B/B0 values in

comparison to the cut-off and high control as done in the evaluation of blood batches. Any case sample with a B/B0 falling between the cut-off and the high control may have produced a negative screening result if d-methamphetamine was used to prepare the calibrators and controls.

From the 28 cases that indicated a presumptive positive, one case had a B/B0 value that fell between the cut-off and the high control. A report was released stating that the "preliminary results indicate the presence of one or more drugs." This case was recalled and re-analyzed to see if the sample would screen positive at a target concentration of 200 ng/mL using d-methamphetamine only.

Section Manager: Dayong Lee **Date:** 5/30/2017

Division Director: Peter Stout **Date:** 5/31/2017

Quality Director: Lori Wilson **Date:** 5/31/2017