



Quality Division Use Only

Quality Tracking #:	2021-004	Classification:	Incident
Non-Conformance Level:	N/A	Section:	Biology/DNA
Date of Discovery:	04/16/21	Date of Incident:	06/04/21

Forensic Case Number(s), if applicable:	Agency Case Number(s), if applicable:
2021-20198	019102421
2021-20677	025357721
2021-21968	033273721
2020-17054	164081320
2020-17603	162406820

Description of Non-conformance:

A Hamilton instrument extraction reagent blank (RB2-HA21-0036) exhibited allelic activity consistent with a reference sample (2021-21968_3.1).

Additional Information/Follow-Up:

Twelve peaks were observed in RB2_HA21-0036 below the analytical threshold. Six peaks were assigned an allele call when the reagent blank was analyzed at the peak detection threshold. The technical leader determined that RB2-HA21-0036 was consistent with another sample in the tray (2021-21968_3.1- reference). This sample was positioned after RB2_HA21-0036.

The technicians that performed the extraction process using the Hamilton instrument were interviewed.

When interviewed, the first technician stated that her role in the extraction was to open all the elution tubes and then close them all once the samples were processed by the Hamilton instrument. She noted that she always closes the elution tubes in the order written in the worksheet (sequentially from 1-85), therefore the elution tube containing RB2_HA21-0036 was closed before the elution tube containing 2021-21968_3.1, making contamination at this process step highly unlikely. The technician explained her usual decontamination procedure and noted that she would note any discrepancy in the documentation and would elevate notification to a supervisor if any samples could be affected.

When interviewed, the second technician (who loaded the samples onto the instrument) examined a grid of a plate to assess if an instrument error or manual handling could have caused the contamination. She hypothesized that because the reagent blank was manually handled before sample 2021-21968_3.1, it is highly unlikely the contamination occurred at this step. She noted that in the processing plate RB2_HA21-0036 and 2021-21968_3.1 were not far apart. This technician noted that when an instrument fails, (whether it aborts or stops) she notes it in



the instrument log. But if the error affects the samples, it should be written on the worksheet and depending on the issue, in addition to the worksheet note, it will be elevated to notification of the technician supervisor. The technician who performed the quantification, amplification, and load procedures was also interviewed. There was a note in the quantification worksheet documenting that water from the Tecan instrument had splashed on the reagent block during daily set up, before any samples were loaded. When interviewed the analyst noted that she thoroughly cleaned the splash, moved to another instrument to process the samples, and no samples were affected. Since she was off the day the 3500 instrument run finished, the data was reviewed by another DNA analyst. It was at this point when the potential contamination was noticed, and forensic biology management was notified.

Summary of Root Cause Analysis:

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

N/A

Actions Taken:

To determine if the Hamilton instrument was responsible for the observed contamination the Operations Coordinator conducted a test with 48 samples and 48 reagent blanks in a checkboard pattern. The quantification results of the samples were less than expected. This test run was being used as a QC run for new reagents. To help determine the cause of the quantification results a smaller run with only 8 samples and 8 reagent blanks was run with the reagents again. The results of this run were as expected. During the second run it was determined that the unexpected results from the first run were due to operator error. Wash buffer A was used for all three washes, instead of only the first and second washes.

A second test run with 48 samples and 48 reagent blanks in a checkboard pattern was performed on the instrument. The results for the samples were concordant with expected profiles. All reagent blanks were DNA free at the quantitation step. Four reagent blanks spread throughout the checkerboard pattern were taken through amplification and were free of DNA.

The following factors were investigated in the casework run: type of samples and whether the samples were amplified below or at the same volume as the associated reagent blank. The associated samples were all high-level. The majority of the samples were single-source. Nineteen samples were amplified below the input volume of RB2_HA21-0036 and 2 samples were amplified at the same volume of RB2_HA21-0036. Of these two samples, one sample was single-source and one was low-level and uninterpretable. The technical leader reviewed the data and determined that the activity seen in the associated reagent blank was not detectable in any of the samples and therefore there was no concern regarding the appropriate interpretation of the samples.

Although the instrument performed as expected, the technical leader could not determine with certainty the contamination of RB2-HA21-0036 which was consistent with 2021-21968_3.1 (reference) was caused by the instrument or by any of the technicians involved in the processing of the sample.



The technical leader approved reagent blank RB2-HA21-0036 for use in casework. A memo documenting this approval was created and uploaded to the associated case records and no quality report statement was added to the associated reports.

Courtney Head 09/12/22
Section Manager: _____ Date: _____
Division Director: Amy Castillo _____ Date: 09/20/22

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

Technical Leader: Cheron Maxwell _____ Date: 09/01/2022
CODIS Administrator: Jennifer Clay _____ Date: 09/12/2022
Quality Director: Erika Ziemak _____ Date Closed: 10/22/22