VALIDATION SUMMARY	
Procedure Name	**TOX-438-01 Cocaine and Metabolites Confirmation**
Validation Summary	The TOX-438-00 technical procedure was successfully implemented in September 2023. This validation revision covers the following topics: • Incorporation of a second Waters Acquity UPLC/Thermo ID-X mass spectrometer system to increase redundancy • Evaluation of an additional ultra-high pressure liquid chromatography (UPLC) column to verify robust chromatographic conditions • Cross-instrument data handling • Cross-instrument decision criteria • Enabling within-run internal mass calibration to aid in maintaining high mass accuracy • Use of bench-top supplied deionized water as well as liquid chromatography/mass spectrometry (LC/MS) grade deionized water to reduce operating costs Additionally, competency testing was provided to analysts along with the validation activities **Results:** The TOX-438-01 validation was successful. The second Tribrid Thermo-ID-X system is fit for purpose. The qualitative and quantitative data generated between the two instrument systems was highly accurate, precise and comparable. There were inconsequential differences between two UPLC columns that demonstrated the procedure's robustness. Internal mass calibration was successful, generally increased mass accuracy and will be enabled in the TOX-438-01 revision. While there was a significant difference demonstrated for the responses generated by the Tribrid-2 system, this was found not to impact the analyses in any significant way. There are minor adjustments recommended to further improve the streamlined data analysis and reporting functions pioneered by this method. There are few decision criteria adjustments recommended to ensure comparable cross-instrument analysis. A full method validation report is also available for review. Notes: 1. Due to a delay in the exchange of deionized water filters, evaluation of Millipore bench-top supplied water versus bottled LC/MS grade water was not performed during this validation. 2. Low and High Controls were evaluated; Mid-level controls were not evaluated since the aim of the validation was to demonstrate Tribrid-2 fitness for use, and the Low/High controls spanned the concentration range

	covered by the Mid-Level. Mid-level controls will not be routinely analyzed in casework. 3. In the first batch of analyses, d3-benzoylecgonine (instead of d8-benzoylecgonine) was used as the internal standard for benzoylecgonine. The second batch used d8-BZE. However, this had no impact to the qualitative and quantitative performance of the method; Tribrid-1 and Tribrid-2 results were highly comparable regardless of benzoylecgonine internal standard.

APPROVALS			
Technical Approval	Redacted	Date	1/9/24
Unit Chief Approval		Date	1/10/2024