September 27, 2024

To: All Potential Respondents
From: Katelyn Howells, Purchasing Agent
Subject: 005-RFP-1285-2025 DNA Analyzer Instrument Validation

Addendum One

Please amend the subject RFP to include answers to the following timely received questions:

Q1. Please confirm whether reagents utilized for this project will be provided by the Iowa Criminalistics Laboratory or if vendor will need to obtain.

A1. The 3500 reagents and extraction/quant/amp reagents provided by DCI crime lab. Samples to be processed during the validation would be provided by vendor lab.

Q2. Will the vendor have access to run data on both 3.1 and 4.0 during the validation laboratory week? Is the software already purchased and installed at the laboratory? If not, what is the expected date of that installation?

A2. Yes, the vendor will have access to both 3.1 and 4.0 in DNA casework. DNA database is currently using DC 3.0 and the run data will be assessed between that version and 4.0. The software is already purchased and installed.

Q3. Specify what types of known samples are to be validated for the databasing lab.

A3. The majority would be saliva samples blotted onto color changing FTA filter paper cards. Blood on FTA cards, and cotton buccal swabs would also be evaluated in lesser quantity.

Q4. Vendor suggests that Iowa provide all mock and known databasing type samples required for this validation under Section 4.1. Vendor can supply collection cards/swabs if required. Alternatively, previously amplified known and mock probative samples could be used for capillary electrophoresis for this study if provided by Iowa.

A4. No.

Q5. Please clarify the number of 3500s in both the CW and DB labs.

A5. Three 3500's in CW and two 3500's in DB.

Q6. Please clarify what performance check is required for data collection 3.1 data stated in section Exhibit 8 of RFP? Has Iowa already validated and implemented STRmix 2.7?

A6. Iowa is currently validating STRmix 2.7 and anticipates having it implemented and in use in January 2025.

Casework performance check of 2 -3500 that aren't the primary validation instrument would be. We will run a subset of those samples on each 3500 to confirm they all perform similarly & confirm settings. This will be determined by DNA CW TL & successful vendor (around 40 samples) 2) STRmix Portion - We will need to run two known samples using a dilution series on the same instrument with DC 3.1 & DC 4.0 to use to determine if stutter & Model Maker will need to change.

DNA database would be the vendor will coordinate with the DNA Database TL to select validation a sample plate(s) that will be run on the validating instrument, the second 3500 instrument with DC 4.0 and the second instrument with the current DC 3.0.

Q7. Would Iowa agree to virtual teach back since the data collection 4 software is nearly identical in operation to the 3.1 software?

A7. Yes.

Q8. Regarding the competency tests for the 20 individuals, could you provide further insight into the expected balance between theoretical understanding and hands-on competency demonstration during the training process? Such as, should the training focus more on practical tasks, such as running the DC 4.0 software on the 3500 Genetic Analyzer and troubleshooting, or will there be an equally strong emphasis on theoretical knowledge, such as understanding the software's data analysis algorithms and compliance with FBI QAS standards?

A8. The training should focus on hands-on competency and practical aspects such as running DC 4.0 and CE troubleshooting.

Q9. Will the laboratory have a preference for a specific teaching format, such as in-person or virtual, for the "teach back" training sessions?

A9. In-person or virtual is acceptable.

Q10. Should the validation plan include provisions for recording these sessions to allow for future reference or refresher training? For instance, if virtual training is preferred, would interactive components or live demonstrations of DC 4.0 on the 3500 Genetic Analyzer be expected to enhance participant engagement?

A10. Yes.

Q11. Similarly, if recorded sessions are required, are there specific guidelines on how these should be structured to ensure they effectively support the long-term training needs of the laboratory staff?

A11. Respondents should include how they would propose to structure their training (either in-person or virtual) in their response to the RFP.

Q12. For the literature review component of the training, does the laboratory expect a focused emphasis on the FBI QAS 2020 updates, particularly regarding its impact on validation practices, or should the review extend to encompass a broader range of forensic DNA validation literature? For instance, should key papers addressing recent advancements in capillary electrophoresis, STR data analysis, or forensic software validation be included?

A12. We would recommend key papers addressing recent advancements in CE, STR data analysis, and forensic software validation.

Q13. Could you provide guidance on whether additional validation criteria, beyond those already specified, are required to ensure compliance with other applicable standards, such as HIPAA or GDPR, particularly in relation to the handling of human DNA data? For instance, are there specific privacy or data protection protocols that should be incorporated into the validation process to address the secure storage, transmission, or anonymization of sensitive genetic information?

A13. The vendor should ensure that no personal identification information is visible to any human DNA samples that they provide. It is suggested to use a numbering system to ensure privacy, with the key available to only key personnel from the vendor and the Iowa Criminalistics Laboratory.

Q14. Are there any preferred protocols or best practices the lab adheres to when analyzing and handling low-level DNA samples, particularly to ensure accuracy in collection and STR analysis on the 3500 Genetic Analyzer platform?

A14. Yes, the laboratory has validated procedures already in place for analysis and the handling of low-level DNA samples. These validated procedures will be provided to the awarded vendor.

Q15. Given the sensitivity of this instrument, are there specific procedures, such as adjustments to amplification cycles or enhanced contamination controls, that the lab recommends for these challenging sample types? For instance, would protocols involving reduced injection times or optimized buffer concentrations be expected, especially to prevent signal dropouts or excessive stochastic effects? Should such practices be consistently applied across both casework and databasing sections, or tailored depending on sample type and context?

A15. The laboratory already has validated procedures for amplification cycles and injection parameters, etc. Those would not change during, or for, the validation of DC 4.0. There would not be an expectation of changing injection times or optimizing buffer concentrations, etc. during this validation.

Q16. Given that software updates often present compatibility challenges, are there any known integration concerns between Genemapper ID-X v1.6 and DC 4.0, particularly in relation to handling prior data formats or parameters from earlier software versions? For example, are there specific issues the lab has encountered with data migration, such as the preservation of allele calling thresholds or settings for baseline adjustments, that might require attention during the upgrade?

A16. Not that the Iowa Criminalistics Laboratory is aware of.

Q17. Moreover, should validation efforts include checks for any potential discrepancies in data interpretation or result outputs when processing legacy data through the upgraded system?

A17. If any discrepancies are found between legacy data and data that is run for this validation then yes there should be checks and a resolution decided on by the lab and the vendor before this validation is completed.

Q18. How does the laboratory prefer that potential data loss scenarios be managed during the upgrade from DC 3.1 to DC 4.0? Are there specific protocols or redundancies already in place that should be preserved to mitigate such risks? For instance, should the upgrade process include automated data backups or recovery points at critical stages to ensure no interruption in ongoing workflows?

A18. The laboratory has data backup systems in place. The vendor will not have to do anything additional to ensure no interruption to ongoing workflows.

Q19. Should the validation plan include troubleshooting protocols specifically for the integration of DC 4.0 with other forensic DNA analysis software, such as STRmix, or will those workflows be managed separately by the laboratory? To explicate, are there anticipated challenges in data exchange, parameter alignment, or compatibility that should be proactively addressed during the validation, especially when transitioning between DC 4.0 and STRmix for probabilistic genotyping?

A19. While not required, it would be attractive for the successful vendor could prepare the small data set (2 sample dilution series) for use in STRmix for STRmix model maker & stutter variances and assist the DCI Crime Lab in the comparison of the two data sets after those have been performed in STRmix.

Q20. If integration is to be covered in the validation, are there specific processes or metrics that the laboratory prefers to focus on to ensure seamless interoperability between these systems?

A20. A subset of single source, partial single source, & mixtures with references for inclusion & elimination will be processed with STRmix to determine if similar conclusions are generated by both DC software sets (DC 3.1 and DC 4.0). Further discussion with DNA CW TL can be had after a success bid is awarded.

Q21. For the DNA sample collection during the validation process, could you specify if there are particular mock casework samples that should be prioritized based on their forensic significance? For instance, are there any preferences for working with trace evidence, degraded samples, or complex mixtures (e.g., sexual assault cases or low-template DNA) that may better represent real-world casework challenges?

A21. Yes, the vendor will work with the DNA casework technical leader to determine which mock samples would be needed and prioritized once this project is awarded. The samples will include blood, contact DNA, touch DNA, hairs, and sexual assault type samples.

Q22. Should samples with high forensic relevance, such as those involving multiple contributors or challenging extraction conditions, be given more focus during the validation to ensure robust performance across various casework scenarios?

A22. Yes, the vendor will work with the DNA casework technical leader to determine how much focus to give to challenging samples once this project is awarded.

Q23. Are there any particular environmental conditions under which DNA samples must be processed to ensure compliance with FBI QAS standards? For example, are there specific guidelines related to temperature controls, humidity, or storage duration that must be followed during the handling and processing of samples throughout the validation process?

A23. None that the Iowa Criminalistics Laboratory is aware of.

Q24. Are there any specific protocols for transitioning between storage environments (e.g., frozen to ambient) to prevent degradation, particularly for low-level or degraded samples, where environmental factors may have a more pronounced impact on data integrity and quality?

A24. No. That is outside the scope of this validation request.

Q25. When processing mixtures for validation, particularly sexual assault-type samples, are there any specific preferences regarding the ratio or complexity of the mixtures? For instance, should the validation include a range of major/minor contributor scenarios to reflect real-world forensic challenges, and if so, are there particular contributor ratios (e.g., 1:1, 1:4, or 1:10) that the laboratory prioritizes for testing?

A25. We would expect the following at the validation to cover a wide range of mixtures (2-5 person mixtures) with different donors including the ratios, but not limited to: 1:1, 3:1, 5:1, 10:1, 20:1, 1:1:1, 8:1:1, 5:5:1, 1:1:1:1, 4:4:1:1, & 1:1:1:1:1. This can be discussed further with DNA CW TL after success bid is awarded.

Q26. Should complex mixtures involving multiple contributors, or degraded samples with a minor contributor profile, be emphasized to assess the software's capability in handling such cases accurately?

A26. These types of complex samples should certainly be included in the validation plan, but do not necessarily have to be emphasized.

Q27. For the validation procedure of DC 4.0 on the 3500 Genetic Analyzer, could you clarify whether there are any specific automation requirements that you envision for streamlining the STR DNA collection and analysis process? In particular, are there preferences for certain stages of the validation, such as automated threshold setting or real-time monitoring of electropherogram results, that should be prioritized? For instance, in some labs, automated processes help standardize results and reduce manual intervention.

A27. There are no automation requirements envisioned for this validation.

Q28. Are similar efficiencies expected in this project, and if so, should the automation be implemented across all DNA sample types and processing phases?

A28. Yes, it is expected that DC 4.0 will work as well as our current data collection. There are no automation requirements envisioned for this validation.

Q29. Are there particular data points, such as allele calls or signal intensity variances, that need to be emphasized during these comparisons to avoid discrepancies in downstream analysis?

A29. Yes, both allele calls and signal intensity (RFU's on the 3500 instrument).

Q30. In accordance with FBI QAS (2020) standards, could you provide clarity on whether any predefined acceptance thresholds have been established for the precision and accuracy studies within the validation plan? Specifically, are there guidelines or benchmarks for factors such as error rates, reproducibility across different DNA sample types, or acceptable ranges for allelic dropouts and false positives? Let's say, precision studies in some validations prioritize reproducibility within 95% confidence intervals, while accuracy studies may focus on ensuring minimal deviation between expected and observed results. Should similar statistical criteria be applied here, or are there additional thresholds to consider based on the lab's forensic casework requirements?

A30. We are not thinking in terms of error rates. We would expect concordant profiles regardless of DNA sample types/DC software used. Allelic dropouts/false positives would be inherent to the strength of the input DNA but calculation of the significance of any differences between the dropout rates of profiles generated when using different instruments or data collection software would be warranted. There may be some additional data mining in terms of profiles that meet "passing" criteria based on analytical and stochastic standards set by our lab.

Base pair standard deviations of less than 0.15 base pair are desired for precision so that three times the standard deviation is less than the required 0.5 base pair size guideline.

Expect concordant profiles between 3500 instruments and 4.0 and 3.0/3.1 data collection software.

For DNA databasing the expectation would be concordant profiles when analyzed and quantitative metrics such as overall profile strength, average signal intensity, number of full profiles/passing loci.

For DNA Casework – We would have concordant profiles with similar average RFUs, average PHRs, profile completeness, mixture ratios, & interpretability. Assigned Iowa DCI Crime Lab staff can help with the interpretability assessment.

Q31. Could you clarify if there are any specific preferences or constraints when conducting performance checks across both the DNA Casework and Database sections simultaneously? Given that each section may have different instrumentation parameters, are there any expected methodologies or protocols for harmonizing these checks? For instance, in some setups, parameters like injection time or voltage settings differ between casework and database instruments, which could affect performance consistency.

A31. Our Casework and Database labs use different amplification kits from different manufacturer's. The 3500 parameters are separate and different between the two labs. There will not be a performance check between the casework and the database lab. There will be a performance check between multiple 3500 instruments in the casework lab. Separately, there will be a performance check between multiple 3500 instruments in the database lab.

Q32. Should the validation approach focus on maintaining uniformity across these parameters, or would separate performance benchmarks be required for each section to address their unique operational needs?

A32. Separate performance benchmarks. The casework lab and the database lab have different amplification kits and different instrument parameters.

Q33. In light of the DC 4.0 upgrade, have there been any pre-identified software components that are considered more critical for validation, particularly based on prior troubleshooting or operational feedback? For instance, features such as pull-up reduction and signal optimization often play a pivotal role in enhancing data quality and reducing artifacts in STR analysis. Are there any specific components that the lab has flagged as needing special attention or additional testing during the validation process?

A33. We will consider Spectral Pull-up reduction in DC4.0 for casework, but we will not be validating off-scale recovery. For Databasing since Promega's chemistry and dye sets are used therefore, this question is not applicable to databasing.

Q34. Are there any anticipated differences in DNA profile generation requirements between the caseworking and databasing sections, particularly when utilizing Globalfiler and PowerPlex Fusion chemistries in conjunction with the DC 4.0 software?

A34. Yes. Both chemistries have specific loci that are not present in the other chemistry. Furthermore, there could be primer binding site mutations that produce different allele calls at a given locus between the two chemistries. Since Globalfiler is only used in casework and Fusion is only used in database there is no expectation that profiles generated be exactly the same. The limit of detection, limit of quantitation (analytical thresholds), and stochastic threshold are different between both sections as well.

Q35. Given the distinct operational needs of casework (e.g., more complex mixtures) versus databasing (e.g., higher throughput and reference sample processing), are there unique parameters or thresholds that should be applied during the validation? Should the profile quality, allele peak heights, or stutter ratios be assessed differently depending on the section, or would a uniform standard be expected across both areas despite their differing analytical focuses?

A35. Profile quality, etc. should be assessed differently depending upon the section. There is no expectation of a uniform standard.

Q36. Could you confirm whether the laboratory expects specific quantitative metrics, such as signal intensity, off-scale event rates, or other key performance indicators, to be reported during the validation process to align with FBI QAS and ANAB AR 3125:2019 criteria? For instance, are there predetermined thresholds or acceptable ranges for these metrics that should be closely monitored to ensure compliance?

A36. Yes. With respect to average signal intensity comparison between instruments and data collection software versions. Ideally, determine a "sweet spot" range for optimal signal intensity with the system. A count of off scale event rates would be helpful for comparison of instruments/dc collection software versions. We would expect quantitative data on number of profiles meeting the laboratory-set thresholds. A KPI in casework is assessing profiles at varying concentrations and observing similar profile completeness between the older version of Data collection software and the new DC 4.0.

Q37. Are there specific reporting formats or data points the lab prefers to track (e.g., average peak height, baseline noise levels) to ensure that the validation results meet regulatory standards and are fully compatible with the lab's operational requirements?

A37. Yes, the vendor will work with the DNA casework technical leader and the DNA database technical leader to determine specific reporting formats once this project is awarded.

Q38. Are contact details for reference acceptable in lieu of actual letters?
A38. Yes.

Please acknowledge receipt of this addendum by signing in the space provided below, and <u>return this letter with your proposal (do not send back separately).</u>

I hereby acknowledge receipt of this addendum.

_____ _____

Signature Date

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Typed or Printed Name