SCOPE OF SERVICES

Regulatory Agencies

***Note**: This is a scope of services document, not a proposal for a specific project. If you would like Digamma to generate a custom proposal for your project, please send a request to admin@digammaconsulting.com, and someone will follow up shortly.*

Thank you for your interest in Digamma Consulting's analytical laboratory audit solutions. Developing a comprehensive laboratory audit protocol is essential to ensure uniform auditing standards across laboratories. Laboratory audits often involve overlapping assessments, such as the prescriptive AOAC evaluation, which intersects with the ISO 17025:2017 assessment, and audits conducted by local and state regulatory authorities—particularly relevant for cannabis labs.

These laboratory audits can be performed either by state regulatory staff trained by Digamma Consulting or outsourced directly to our team of experts. It is also crucial to differentiate between using these audit protocols to regulate licensed third-party labs and enhancing the legal defensibility and precedent for data produced by a state-owned reference laboratory.

Digamma Consulting offers a range of audits tailored to assess the chemical analysis conducted by cannabis testing laboratories, ensuring the scientific accuracy and legal defensibility of the data generated. This service is invaluable for regulators, accreditors, inspectors, and any organization closely associated with cannabis testing labs, where verifying process accuracy and data integrity is paramount.

If you are interested in exploring Digamma's services, please contact us at your convenience, and we will schedule a time that suits you. We look forward to the opportunity to collaborate and assist in the growth of your organization.

Sincerely,

Marco Troiani
CEO
Digamma Consulting

Marc Whitlow
CFO
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APPENDIX A

CANNABIS LABORATORY CANNABINOID AUDITS

Executive Summary: Digamma Consulting uses a rigorous 10-step audit approach tailored for state regulatory agencies to ensure lab compliance. Each step is carefully crafted to guarantee accurate and reliable cannabis lab results, upholding the integrity of cannabinoid content reporting.

Outline of a Cannabinoid Analysis Laboratory Audit Steps

- Calibration / Reference Standard Manipulation
- Calibration Curve Manipulation
- Sampling
- Sampling Size, Homogenization, and Replication
- Correction Factors - Mass
- Correction Factors - Decarboxylation
- Chromatographic Co-Elution
- Detector Manipulation
- Data Analysis Manipulation
- Physical Instrument Parameter Manipulation

Digamma Consulting has identified critical components involved in the practice of cannabinoid inflation within the industry. These essential elements should be integrated into any audit protocol investigating this issue in suspected laboratories. Organized into specific categories, Digamma has precisely outlined the practices to be examined during an audit. The audit findings will be compiled into a comprehensive report for state regulatory agencies, providing detailed insights into the observed practices.

Calibration / Reference Standard Manipulation

Audit Deliverable Purpose: To investigate the manipulation of calibration standards through degradation, improper dilution, and sourcing of less-than-reputable concentration standards.

Recommended Audit Actions:

- Audit calibration standard storage and handling procedures to ensure a lack of degradation.
- Audit the calibration curve standard preparation from the stock material purchased by the lab.
- Review sources of calibration standards and their reliability for use as reference standards.
- Analyze unopened, stored, and diluted calibration standards for quantitative comparison of concentrations used by the laboratory (done by the lab's equipment or the state reference lab).

Note: ISO/IEC 17025:2017 already requires the use of accredited CRM when possible and requires verifications. However, the laboratory's internally developed method

determines the acceptance criteria (related to continued use after opening or expiry). Therefore, they could be a source of manipulation and should be prescribed by a regulator.

Calibration Curve Manipulation

Audit Deliverable Purpose: To investigate manipulations of calibration curves through extrapolated calibration curves, improper dilution steps in sample prep, and manipulations of the calibration curve.

Recommended Audit Actions:

- Audit of the procedure for quantifying unknown samples using Linear Dynamic Range (LDR), derivation of LDR, calibration curve standard concentrations, and LOQ and LOD values.
- Review and assess the matrix recovery values for compliance with accuracy and precision requirements.
- Audit the sample prep process to evaluate extraction efficiency, dilution procedure, and compliance with the method's declared LDR.

Sampling

Audit Deliverable Purpose: To investigate sampling procedure, including biased sampling of the batch or the laboratory representative sample, mis-weighing or mis-volume in sample prep steps, or contamination of samples during prep.

Recommended Audit Actions:

- Audit of procedure governing sampling of batches. *Note: This is examined in further detail in the next section on "Sampling Size, Homogenization, and Replication."*
- Audit the procedure governing the storage of samples.
- Audit the procedure governing the transportation of samples.
- Audit the procedure governing sub-sampling batch samples within the laboratory for individual analysis of each instrument. *Note: This is examined in further detail in the next section on "Sampling Size, Homogenization, and Replication."*
- Audit procedures governing sample weighing, scale calibration, and verification.
- Audit procedures governing pipette use, calibration, and verification.
- Audit procedures governing inventory record keeping.
- Audit procedures governing cross-contamination and adulteration prevention policies and practices.

Note: Calibration under ISO 17025:2017 for pipette balance 'inventory' is required. However, acceptance criteria for ongoing use & calibration schedule determined by the laboratory are outside the 'ISO/IEC 17025:2017 clause,' which would address an audit of product inventory.

Sampling Size, Homogenization, and Replication

Audit Deliverable Purpose: To investigate biased sub-sampling sizes using replicates in a method that allows for reporting of the highest observed value and any homogenization practice that would manipulate the final reported result, including contamination with target compounds.

Recommended Audit Actions:

- Audit of procedures outlining homogenization and sub-sampling of representative

samples of the production batch performed in the analytical laboratory, including examining the following items:
    - Sub-sampling mass size,
    - Replicate analysis policy and practices and their effect on reporting, and
    - Homogenization procedure used in the laboratory.
- Audit of the sampling size and its impact on replicate testing on the same batch of material indicates precision and repeatability, including standard deviation (STDev); significant variance combined with a policy of replicate testing by selecting a single or sub-section of results can easily give a higher-than-average value.

Note: Many laboratories do not consider the loss of volatiles such as terpenes and other VOCs statistically significant when reporting moisture in plant samples. Digamma's reviewed statistical data supports this claim, and the calculations supporting it can be verified for each audited laboratory.

Correction Factors - Mass

Audit Deliverable Purpose: To investigate the manipulation of mass-based correction factors, such as stem removal and moisture, and ensure that all correction factors are used accurately and uniformly and are not a source of errors or manipulation of reported results.

Recommended Audit Actions:

- Audit sample preparation and reporting procedures, focusing on the selective removal of plant tissue, such as stems.
- Examine correction factors for moisture content by mass, as inaccurate moisture values can manipulate cannabinoid results.
- Address issues with excessive heating of samples, which can lead to inflated moisture and cannabinoid content values after dry-weight correction.
- Investigate the moisture content procedure thoroughly, including auditing the process and reviewing all related quality and validation data.

Correction Factors - Decarboxylation

Audit Deliverable Purpose: To investigate improper use of molecular mass conversions, such as those between THCA and THC and other cannabinoids and their corresponding acid forms. It would also review the manipulation of reported results by improper summation of values such as unrelated or antagonistic cannabinoids such as THC and CBD.

Recommended Audit Actions:

- Evaluate the accuracy of correction factors, such as those used for cannabinoid acid decarboxylation.
- Assess the validity of reported equivalent concentrations post-conversion, including Total THC, CBD, and other compounds derived from theoretical calculations.
- Investigate claims of Total Active Cannabinoids (TAC), Total Potential Cannabinoids (TPC), and other potentially misleading statements on laboratory labels that lack supporting scientific evidence.

Note: To thoroughly assess these conversion factors, the precise mass conversion factors must be derived from regulatory guidelines or scientific literature, and the

policies regarding the reporting of combined, total, or potential cannabinoid concentrations must be carefully reviewed.

Chromatographic Co-Elution

Audit Deliverable Purpose: To investigate the mis-integration of non-target compounds by the analytical method, including other cannabinoids and UV-active compounds like waxes common in the cannabis plant. It includes intentional allowance of target compound carry-over from one sample analysis to the next in the same instrument, which inflates the final reported value relative to the amount present in the sample. Laboratories employing very short columns enable co-eluting compounds to artificially increase their reported values in matrix samples. This manipulation does not impact solvent standard calibrations, yielding compliant quality control sample data and Proficiency Testing (PT) results in some instances.

Recommended Audit Actions:

- Conduct an audit of chromatograms for target compounds to assess potential co-elution of other targets or matrix interferences that may affect the measured quantity of the target compound.
- Reviewing chromatogram procedures will be compared to the declared values and procedures outlined in the method's validation report.
- Assess the column length and maximum resolution. This issue can be examined by scrutinizing the data declared in the validation report on matrix interference studies and conducting an audit of routine quality samples that pertain to these components, including matrix blanks (MB) and matrix spike replicates (MSRs).
- Evaluate the instrument flush time and address carry-over contamination through solvent blanks, prep blanks, calibration blanks, and similar QC data points.
- Conduct a comprehensive matrix interference assessment, including a list of known interferences for a tested sample matrix.

Detector Manipulation

Audit Deliverable Purpose: To investigate the manipulation of detector settings, which may allow interfering compounds to be mis-integrated as target compounds.

Recommended Audit Actions:

- Audit instrument UV or visible light frequency used by the detector.
- Audit instrument the quantitation versus qualifier detector channels.
- Audit instrument any qualifying channel ratios derived from analytical standards.
- The audit will focus on known interferences declared in the analytical method's validation report and the probability of these interferences having a substantive impact on the final reported result of the target compound.

Data Analysis Manipulation

Audit Deliverable Purpose: To investigate the manipulation of data analysis procedure, emphasizing the mis-integration of target compounds, mis-integration, and manipulation of calibration standard integration.

Recommended Audit Actions:

- Conduct an audit of instrument chromatogram integration procedures, policies, and practices involving a comprehensive review of all manually integrated peaks from a randomly selected analytical batch conducted by the laboratory.

- The investigation will collect data on the amount and frequency of manual integrations versus auto-integrations per analytical batch for baseline consistency from peak to peak and the relationship of integration technique between calibration, quality, and client samples.
- The audit will focus on Gaussian integration parameters, including the following:
  - Auto-integration v. manual,
  - Baseline integration of noise,
  - Baseline up-shifting of integration area lowering final value (for LQC rather than direct inflation on client samples), and
  - Retention time variation and manipulation.

Physical Instrument Parameter Manipulation

Audit Deliverable Purpose: Investigate alterations in physical parameters on the analytical instrument by tracking logs to detect inconsistencies with the method's validation report and Proficiency Testing (PT) rounds. This includes identifying signs of manipulation, missing data, or alterations coinciding with periods of high reported values.

Recommended Audit Actions:

- Audit all instrument logs that verify the invariance of physical variable settings that impact the final reported value, including:
  - Injector volumes,
  - Flow rates,
  - Temperature settings,
  - Vacuum pressure,
  - Electrovoltaic parameters, and
  - Electromagnetic parameters (mass spec methodologies only).
- Traceability practices that show the physical parameters of the analytical method printed into each data packet by each analytical batch would make a step of the audit performable with document and data review only.
- If the laboratory in question does not adhere to standard traceability practices, on-site audits of current and established procedures will be essential to validate the uniformity of these physical instrument parameters.

APPENDIX B

CANNABIS LABORATORY PESTICIDE AUDIT

Executive Summary: Digamma offers a comprehensive audit protocol for pesticide analysis in cannabis laboratories, highlighting critical stages of analyte selection, matrix considerations, homogenization, extraction, and data integrity. Each step is meticulously detailed to ensure laboratory practices' accuracy, reliability, and defensibility, providing essential guidance for auditors in detecting and preventing inaccuracies or potential manipulations.

Outline of a Pesticide Analysis Laboratory Audit

- Analytes
- Matrix
- Homogenization
- Extraction
- Analysis
- Data

Digamma has provided insights into key components utilized in the pesticide analysis practiced in the industry. These crucial elements should be incorporated into any audit protocol designed to investigate this phenomenon in laboratories. These components have been organized into specific topics, and Digamma has concisely described the practices to be scrutinized during an audit. The resulting information will contribute to an audit report delivered to the state, presenting comprehensive findings on the observed practices.

Analytes

Audit Deliverable Purpose: To investigate the appropriate alignment of each analyte with the proper detection technique. This includes alignment with instrument components such as ionization sources, which are analyte-specific, as well as storage, solvent, and extraction conditions of said pesticide analytes.

Recommended Audit Actions:

- Audit the list of analytes on the analytical method and align with the proper detection technique, particularly with tandem mass spec (MS/MS) alignment of each analyte with a viable ionization source, which is particularly important to the accuracy of generated data.
- Review each analyte's storage conditions and solvent usage, including polarity, stability, pH, and cross-reactivity with other analytes.
- Review sources of calibration standards and their reliability for use as reference standards.
- Analyze unopened, stored, and diluted calibration standards for quantitative comparison of concentrations used by the laboratory (this can be done by the lab’s equipment or the state reference lab). Focus on the expiry management system for preparing pesticide solutions and managing their stability.

Matrix

Audit Deliverable Purpose: Across all matrices, four major interferences are typically observed: cannabinoids and terpenes, waxes and lipids, carbohydrates and amino acids, and polymers. Because each matrix class has varying ratios of the interfering compounds, matrix-matching the calibration is necessary to ensure consistent recoveries. This section examines the appropriateness of matrix choices in the methodology and their impact on reported data's accuracy.

Recommended Audit Actions:

- Assess the number and composition of the matrix classes that the analytical method used by the laboratory organizes for all received cannabis samples across all types. Key chemical components to monitor are:
  - Cannabinoids,
  - Terpenoids,
  - Waxes and other plant lipids,
  - Carbohydrates (simple and complex),
  - Amino acids and protein. and
  - Synthetic polymers and emulsifiers.
- Assess the appropriateness of matrix blank and other matrix sample proxies used in the method to sample type by examining the chemical composition of proxies and client samples.
- If matrix-matched calibration is used, the accuracy and precision of matrix-calibrated values must be assessed when compared to the same values in the solvent standard.
- If internal standard correction factors are used, the accuracy and precision of the corrected values in the matrix compared to corrected values in the solvent standard must be assessed.

Homogenization

Audit Deliverable Purpose: Proper homogenization of each sample tested is required for reproducibility of reported data. Pesticide distribution is often not uniform, so samples should be homogenized to fine particle sizes and well-mixed. A fine particle mesh also allows less acetonitrile to be sequestered in the plant matrix and a greater volume of acetonitrile to be collected after sample extraction.

Recommended Audit Actions:

- The homogenization technique's precision, accuracy, and repeatability were demonstrated through matrix spike replicates, either through validation data or with a CRM using the lab's method.
- Volume extraction recovery data showing inputted and recovered extraction volume from the homogenized sample matrix. If correction factors such as matrix-matched calibration or internal standard calibration, correction factors must be assessed for accuracy of recovered volume and analyte.

Extraction

Audit Deliverable Purpose: LC and GC systems have different vulnerabilities regarding matrix interferences and require extraction clean-up approaches that protect each instrument and allow for accurate and precise analyte quantitation. Extraction procedures must be validated for final recoveries, and any interactions with correction

factors (matrix-matched or internal standard) must be verified quantitatively.
Recommended Audit Actions:

- Review the appropriateness of the clean-up procedure for each analyte assigned to each instrument (see **Analytes** section above).
  - Major analyte loss at a theoretical chemistry level can be detected from these reviews (daminozide, captan, etc.) by cross-referencing chemical polarity with clean-up extraction procedures applied to each analyte at each instrument.
  - Major analyte loss theoretically predicted can be verified with existing laboratory records or low-resource additional analysis if it is already present.
- Review data logs to verify instrument sensitivity stability with analytes over time with extraction and clean-up recovery values to disprove an instrument drift or cumulative analyte loss in extraction efficiency.
- Review data logs to verify instrument sensitivity stability with interferences over time with extraction and clean-up recovery values to disprove an instrument drift or analyte accumulation in extraction. Ideal interferences to monitor cannabis products include:
  - THC,
  - CBD,
  - CBG, and
  - Plant cuticle waxes.
- Recoveries after clean-up must be assessed for repeatability in final reported values as measured by the following:
  - Relative Percent Difference (RPD),
  - Standard Deviation (STDev), and
  - Mean Accuracy ($\%R_{avg}$).

Analysis

Audit Deliverable Purpose: The analysis phase involves critical areas where errors or manipulations can occur, such as autosampler and inlet conditions, affecting accuracy in liquid (LC) and gas chromatography (GC). Thorough rinsing of the GC sampling needle is necessary to prevent jams, and matrix-matched or internal standard-corrected calibration curves are required to address hidden influences on instrument response. High-quality LC/MS-grade solvents are essential to avoid contamination, and additional chromatographic separation may be needed for each matrix class to ensure selectivity and accuracy.
Recommended Audit Actions:
Autosampler Section

- Review autosampler and inlet temperature for compatibility with analyte stability.
- Review of the rinse programs used by the instrument to ensure that there is no possibility of residue or degradation of analytical equipment.
- Replicate analysis data review to verify the lack of signal drift in the detector, accumulation, and decay.
- Needle dwell and fill times for each instrument and review of appropriate and repeatable instrument processes. An emphasis on the following points is recommended:
  - Oxidative loss of analyte in high-temperature environments/dwell times.
  - Un-repeatable fill volumes of LC loops or GC needles cause high

deviations.

Chromatography Section

- Review solvent standard instrument responses and matrix spike instrument responses to disprove the presence of dark interferences in the matrix for the method.
- Review the retention time of analytes in both solvent standard and matrix to disprove matrix shifts.
  - Review both deviation and mean accuracy of retention time for each analyte.

Mass Spectrometry Section

- Mass channel selectivity review based on responses in standard and matrix.
- Review adduct and fragment calculations to verify that signal responses authentically indicate the analyte's concentration.
  - Theory: Use molecular mass calculations.
  - Practice: Compare data sets, such as standard addition or matrix-matched calibration, to validate results.s such as standard addition or matrix-matched calibration
- Review signal-to-noise values in solvent standard and matrix to verify the method's stated LOD and LOQ values from validation.
- Review of isotopic channels, which help to validate signal-to-analyte association by revealing the presence of characteristic and sometimes unique atoms.
  - Theory: Based on the analyte's formula, calculate isotopic abundances at the A+1 and A+2 mass channels. Note: Errors here may necessitate changes in fundamental method development.
  - Practice: To confirm theoretical calculations, measure A+1 and A+2 mass channel relative abundance in solvent standards and matrix samples.

Data

Audit Deliverable Purpose: Data defensibility is the most critical part of any chemical analysis and is the underlying architecture of both a compliant method validation and a thorough laboratory audit. The validation metrics are verified by daily Quality Control (QC) samples whose data is kept in the QC log for records. These are outlined below by QC samples designed to measure and demonstrate their presence or absence in the data a method generates.

Recommended Audit Actions:

- Review QC logs for routine compliance with validation, accreditation, and licensing criteria.
  - Linearity as measured by:
    - Correlation coefficient (R^2 value),
    - Linear Dynamic Range (LDR), and
    - Calibration point residuals.
  - Precision as measured by:
    - Matrix Duplicates (MD),
    - Sample Duplicates (SD), and
    - Continuing Calibration Verification (CCV).
  - Accuracy as measured by:
    - Matrix Sikes (MS),

        - Lab Control Standards (LCS),
        - Independent Calibration Verifications (ICV), and
        - Continuing Calibration Verification (CCV).
    - Range as measured by:
        - Method Reporting Limit (MRL),
        - Limit of Detection (LOD), and
        - Limit of Quantitation (LOQ).
- Evaluate the Proficiency Testing (PT) Program participation and review associated performance data.
- Examine Certified Reference Material (CRM) data, which may be included in validation reports.
- Review key limits, such as Limits of Detection (LOD), Limits of Quantitation (LOQ), Action Limits (AL), and relevant Reporting Limits (RL).
    - Theory: Calculate these limits for key analytes to confirm the method’s detection capability.
    - Practice: Validate the method's detection ability by applying these calculations to real-world sample data, ensuring consistency with theoretical expectations.

APPENDIX C

CANNABIS LABORATORY MICROBIAL AUDIT

Executive Summary: Digamma has developed a comprehensive audit protocol to identify and mitigate potential manipulations in microbial analysis within the cannabis industry. By examining key stages such as sampling, extraction, analysis, and data handling, this protocol equips auditors with the necessary steps to uncover inaccuracies and ensure the integrity of laboratory microbial content reporting.

Outline of Microbial Analysis Laboratory Audit

- Sampling
- Extraction
- Analysis
- Data

Digamma has provided insights into key components utilized in the industry's microbial suppression practice. This practice is motivated by financial incentives driven by losses incurred when a batch fails a microbial test. These crucial elements should be incorporated into any audit protocol to investigate this phenomenon in suspected laboratories. These components have been organized into specific topics, and Digamma has concisely described the practices to be scrutinized during an audit. The resulting information will contribute to an audit report delivered to the state, presenting comprehensive findings on the observed practices.

Sampling

Audit Deliverable Purpose: Sampling is a major point of potential error and manipulation in microbiology. Because microbiology measures living organisms, sterile handling, and storage techniques are critical to the laboratory's generation of accurate and defensible results.

Recommended Audit Actions:

- Verify the scale weight log to verify that the total mass of the material being tested is being properly weighed and included in the final calculations.
- Random repeatable sampling shows conformity within pre-defined criteria comparable to microbiological results in food.
- Review standard operating procedures (SOPs) to ensure that recursive replicates (testing-until-you-pass) practices are now allowed, and review laboratory practices to demonstrate they are not occurring in the laboratory.
- Analyze unopened, stored, and diluted calibration standards for quantitative comparison of concentrations used by the laboratory (this can be done by the lab’s equipment or the state reference lab).
- Sterile handling techniques will be reviewed in SOP and verified by on-site observations. This includes the following:
  - Sterile handling and not introducing contaminants from the environment.
  - Cross contamination and not introducing contaminants from one sample to another.

        - Proper sample storage and microbial load preservation.
            - Under excessive sterilizing conditions, the microbial load is reduced relative to the original batch sampled.
            - Under excessive growth conditions, the microbial load is increased relative to the original batch sampled.
    - Appropriate homogenization steps are taken to extract and remove all microbial content being analyzed thoroughly, without excessive homogenization, which releases either antibiotic or reaction inhibitor compounds, which would affect the reported results for plating and PCR quantitative techniques, respectively.

Extraction

Audit Deliverable Purpose: Extraction is a critical step for accurate quantitation or detection, as the microbial load in the extraction suspension is highly susceptible to modification, often more so than in the original sample. Any errors in homogenization or preparation can significantly skew the final reported results, making it essential to independently verify and optimize these processes to ensure the highest precision and reproducibility in the analytical method.

Recommended Audit Actions:

- The time-sensitive nature of the extraction work-up is due to the potential for microbial fission (cell division) in this analytical method's extract suspension.
    - Theory: Review SOP steps to ensure batch analysis is performed promptly to minimize impacts on reported results.
    - Practice: Observe and verify on-site that practices align with the SOP to ensure timely and accurate execution.
- Review of extraction solutions used to generate microbial suspension and rule out any effects that may alter the reported value.
    - Review of pH and saline buffering elements required for microbial analytes.
    - The presence of carbohydrates and amino acids could promote microbial growth and increase final reported values.
    - The presence of antibiotics or other inhibitory compounds could compromise data accuracy.
- Review of volumes and homogenization techniques used in SOP to verify even distribution of microbial load from sample into suspension.

Analysis

Audit Deliverable Purpose: The analytical technique used greatly impacts the final reported value of a method. Although many methods exist, and many that will be invented in the future can be applied successfully to this audit, the most popular choices are agarose growth plate enumeration and quantitative Polymerase Chain Reaction (qPCR). For this reason, we will reference these techniques explicitly in the text below, but these audit actions can be applied to any microbial analysis technique.

Recommended Audit Actions:

- Agarose Petri Dish Enumeration qualifications are outlined in the four items below:
    - Dilution correlation - The dilutions CFU will be correlated to show agreement across the serial dilution series. Residuals can be calculated in the same way as a traditional calibration curve.
    - Significant figure verification - Verification ensures that the SOP and related

calculations correctly determine the number of significant figures the microbial method can accurately report for each analyte.
        - Proper spreader sterilization—When preparing a petri dish for enumeration, the spreader bar is a major source of cross-contamination (creating false positives) and sterilization (creating false negatives). Reviewing SOP and observing practice on-site can eliminate both possibilities.
        - Incubation Verification - Error or manipulation can occur if the samples are not incubated for the correct amount of time. Paper or, preferably, digital logs verifying the incubation time of each batch can disprove this practice in a laboratory.
    - Polymerase Chain Reaction (PCR):
        - Enrichment time—If the samples are not enriched for the correct amount of time, error or manipulation can occur. Paper or, preferably, digital logs verifying the enrichment time of each batch can disprove this practice in a laboratory.
        - Polymerase Kit (incl. storage) - Proper storage conditions of protein machinery found in PCR kits are critical for them to function at the proper reaction rate as calibrated for the analytical method and to have the correct concentration in the reaction vessel. Improper storage or handling can adversely affect these proteins and the final reported result.
        - Dilution Scheme - The dilutions Cp will be correlated to show agreement across the serial dilution series. Residuals can be calculated in the same way as a traditional calibration curve.
        - qCp correlation equation - Review and verify the accuracy of the Cp correlation equation used by the PCR analysis to generate a final CFU/g concentration of the microbial load being analyzed. This can be theoretically validated mathematically and verified with real-world data from the laboratory.
        - Inhibitor profile (matrix-specific) - This should be either made available by the instrument, method, consumables, or other manufacturer, but if the laboratory is developing their PCR analysis in-house or from base components in their reaction mixture, they are responsible for generating a known set of reaction inhibitors found in the sample matrix in question and apply these insights into developing a method which can produce reproducible data.

Data

Audit Deliverable Purpose: Processing raw data generated in the analysis and produced in parallel with Quality Control (QC) Samples is critical to generating an accurate and reproducible final result. This section addresses QMS qualification, final reported value calculations, compliance with reporting, and action limits.
Recommended Audit Actions:

- Final concentration calculator verification - This should often be presented in the final validation report, but a reference to an independent CRM, which was measured by the final analytical method, can verify all calculations and provide greater accuracy of the method overall.
- Replicate analysis—Replicate analysis is important to derive precision data, such as Relative Percent Difference (RPD) and Standard Deviation (STDev). Depending on the number of replicates performed, replicates can be performed on a spiked sample or a CRM.

- Global client trends - Global trends in reported client data can show bias around important concentration values, such as action limits. When a normal distribution shows such signs of manipulation near a value with a financial incentive, it strongly indicates some selective bias. It is a possible cause of intentional manipulation and fraud. This data can be reviewed to predict the laboratory's selective bias and subsequent conclusions.
- Qualitative (P/A) - Similar requirements as above are quantitative, but the specifically quantitative criteria are being removed or replaced with an appropriate metric of data accuracy.