Source Molecular QA/QC Summary

Special Training/Certification

Individuals appointed to MST projects hold at minimum a Bachelor's degree and have a sound knowledge in genetics and molecular biology. Individuals must have had 1 year of previous hands-on qPCR experience at another laboratory. Trainees undergo supervised hands-on training by the Laboratory Manager, which typically lasts 1-4 months depending on experience. An initial demonstration of technical capability is required before personnel are permitted to work independently on client projects. This involves:

- Successfully preparing five, 5-point standard curves that satisfy accuracy and precision criteria; and
- A side-by-side comparison test in which the trainee's results are compared to a qualified individual's results after both independently prepare and analyze the same randomly selected client samples (5 batches up to 100 samples).

Training records are documented by the Laboratory Manager and hard copies are kept on file in the company's office.

Quality Objectives and Criteria

Quality control procedures are utilized to monitor the validity of test results. Source Molecular ensures that only valid results are reported to the client by continuously monitoring and reviewing the performance of tests. Key performance acceptance criteria and Data Quality Indicators are described below.

Data Quality Indicators and QC Requirements for MST Tests

Data Quality Indicators	**QC Item/Activity Used to Assess Measurement Performance**	**Purpose**	**Frequency**	**Measurement Performance Criteria**
Accuracy/Bias	Extraction blank	Evaluates contamination during DNA extraction/purification	Once every week samples are extracted	No detection or detection at least 3 C_T units above sample C_T values
Accuracy/bias	Diluted sample	Monitors for sample matrix inhibition affects	Every sample analyzed	C_T value must be greater than that of unknown sample
Accuracy/bias	Positive control	Monitors for false negatives	One reaction for every sample analyzed	C_T value below 35. No false negatives

Accuracy/bias	Negative control	Monitors for false positives	Three reactions for every sample analyzed	No detection or detection at least 3 C_T units above sample C_T values
Accuracy/bias	Standard Curve	Monitors overall reaction performance and efficiency -Ensures confidence and comparability between sample data -Sets linear dynamic range to accurately quantify samples	One curve in duplicate for every sample analyzed and requiring quantification	R^2: ≥0.98 Efficiency: 80-110% Slope: -3.0 - -4.0 Analytical Limit of Quantification (copies). Sample unknown within the linear dynamic range limits
Precision/ Comparability	qPCR duplicates	Ensures precision and confidence in data	Every sample analyzed	± 1 standard deviation unless CT value ≥33

Analytical Methods

Test methods meet the needs of the project and are appropriate for the tests undertaken. The microbial source tracking tests aim to identify potential animal host sources of fecal contamination in water samples. Currently, no standard methods exist for microbial source tracking. Genetic markers used for microbial source tracking tests are adopted by the Source Molecular laboratory from published, peer-reviewed scientific texts or journals whenever possible. Tests have been validated internally and/or externally in the microbial source tracking research community. If possible, reference methods published as international, national or regional standards are used. The laboratory ensures that the latest edition of a standard is used unless it is not appropriate or possible to do so.

Quality Control

Quality control procedures are utilized to monitor the validity of test results. Source Molecular ensures that only valid results are reported to the client by continuously monitoring and reviewing the performance of tests. All QC criteria must be met for the results to be considered valid and reported to client.

Statistical calculations are calculated automatically by the qPCR software. These include qPCR replicate standard deviations, replicate means and standard curve efficiency, slope, y-intercept and coefficient of linear regression (R^2).

QC Item/Activity	Data Quality Indicator	Frequency
Extraction blank	Accuracy/Bias - Evaluates contamination during DNA extraction/purification	Once every week samples are extracted
qPCR duplicates	Precision, Comparability -ensures precision and confidence in data	Every sample analyzed
Diluted sample	Accuracy/Bias -Monitors for sample matrix inhibition affects	Every sample analyzed
Positive control	Accuracy/Bias -Monitors for false negatives	One reaction for every sample analyzed
Negative control	Accuracy/Bias -Monitors for false positives	Three reactions for every sample analyzed
Standard Curve	Accuracy/Bias, Comparability, Sensitivity -Monitors overall reaction performance and efficiency -Ensures confidence and comparability between sample data -Sets linear dynamic range to accurately quantify samples	One curve in duplicate for every sample analyzed and requiring quantification

Instrument/Equipment Testing, Inspection, and Maintenance

Access to laboratory equipment is controlled to ensure that only authorized personnel use the equipment. Instructions on the use and maintenance of equipment are readily accessible by authorized personnel.

Generally, the handling, transport, storage, use and maintenance of equipment are outlined in the manufacturer's manual. Manuals are located in the laboratory at all times. Specific requirements, if any, are outlined in the test method standard operating procedures.

The manufacturer's manual is critical in describing the safe handling requirements of the equipment, to avoid any damage, alteration, contamination, deterioration, change of integrity or reliability and condition of the equipment (or samples). The manufacturer's manual also provides guidance for suitable environmental conditions for the calibrations, inspections, measurements and tests performed. These guidelines should be followed at all times unless specified otherwise in standard operating procedures.

Routine test work is completely discontinued on equipment that shows minor non-conformances. Not only do we do this for ethical reasons in support of our customer, but minor non-conformances are often indicative of major breakdowns in expensive equipment. These breakdowns need to be avoided wherever possible. Out of service equipment is clearly marked with an "out of service" label.

General Equipment

General service equipment is maintained by cleaning and performing safety checks as necessary. Calibrations or performance checks will be necessary where the setting can significantly affect the test or analytical result (e.g., the temperature of a water bath). Instructions on the use and maintenance of general equipment are located in the laboratory at all times.

Volumetric Equipment

The correct use of volumetric equipment is critical to analytical measurements. Volumetric equipment are suitably maintained and calibrated as specified in the Equipment Records and Inventory datasheet located in the web-based storage system.

Attention is paid to the possibility of contamination arising from the equipment or cross-contamination from previous use. The type used, cleaning, storage and segregation of volumetric equipment are critical. Volumetric equipment should be sterilized with 10% bleach solution and 70% ethanol, DNA Away, or autoclaved as appropriate. Instructions on the use and maintenance of volumetric equipment are located in the laboratory at all times.

Measuring Equipment

Measuring equipment, which include the real-time qPCR instrument, must be used correctly, with care and requires stringent calibration and maintenance plans to ensure adequate performance. Such equipment shall not be used for measurement of customer test items if they go out of calibration. If this occurs, items must be re-measurement once the equipment has been re-calibrated. Operating instructions for the Applied Biosystems StepOnePlus Real-Time qPCR System are located in the laboratory office and also in the StepOnePlus Software Instrument Maintenance Manager.

Instrument/Equipment Calibration and Frequency

All measurement and test equipment having an effect on the accuracy or validity of tests are calibrated and/or verified before being put into service. Calibration records for these equipment, including calibration dates and due dates, are maintained in the Source Molecular web-based storage system. Equipment may be calibrated internally or externally. External calibration

services must be conducted by a calibration laboratory that demonstrates competence by being accredited and demonstrating measurement capability and traceability. The frequency of calibration depends on the accuracy requirements of the test, the stability of the instrument and manufacturer recommendations. It is crucial that calibration measurements are traceable to the International System of Units (SI) whenever possible. Calibration reports, traceability certificates and certificates of analysis are maintained in the Calibration Certificates binder that is kept in the laboratory office. Records are retained for 5 years or more at the discretion of the laboratory.

The procedures for checking newly received equipment are as determined by manufacturers' specification and/or those determined by the laboratory during procurement.

After repair, equipment must be calibrated, when appropriate, and verified to perform correctly by following procedures in the manufacturer's manual and/or by comparing pre-nonconformance and post-repair tests.

Anytime the equipment goes outside the direct control of the laboratory, the function and calibration status must be verified before the equipment can be returned to service. This is done by ensuring that calibration stickers and calibration reports are correct, calibration values are within a specified range (if applicable) and that all components of the instrument are functioning properly. This, along with other key information, is recorded and documentation is stored for 5 years or more at the discretion of the laboratory. When verification of the calibration status and functionality of the equipment is not possible, the equipment must be re-calibrated and serviced, respectively.

Generally, spare parts do not have to be kept on hand in the laboratory. Any parts that are needed as part of equipment servicing are provided and installed by the manufacturer or service contractor.

Inspection/Acceptance of Supplies and Consumables

For all test methods, only services and supplies of the required quality and grade are used. If the specified reagent or material is discontinued by the manufacturer, an alternative from a different manufacturer may be purchased as long as the grade and specifications are identical to the discontinued item. The Laboratory Manager verifies and approves the alternate items and the change is made in the appropriate SOP. Supplies, materials and consumables to be purchased are determined by the Laboratory Manager and entered into an electronic "order list" that includes a description of the item, the name of the vendor, the item catalogue number, the quantity and the cost and is stored for a minimum of 5 years.

Shipments are received at the receiving area and brought to the laboratory. The Laboratory Manager or other authorized personnel is responsible for checking shipments for accuracy. Packing slips are checked against package content labels and matched with the electronic order list. Certificates of analysis (COA) are verified (when applicable) to ensure the received item

meets minimum specifications. All standards, reagents, filters, and other consumable supplies are purchased from manufacturers with performance guarantees and industry recognition, and are inspected upon receipt for damage, missing parts, expiration date, and storage and handling requirements. Labels on reagents, chemicals, and standards are examined to ensure they are of appropriate quality. Reagents are marked with the "date received". Primers and plasmid DNA standards are quantitated and aliquoted for storage at -80˚C.

Once the materials are verified, the appropriate box is checked next to the item in the order list and an electronic signature is created. If a discrepancy is found that could affect the quality of laboratory output, the supplier is contacted and the material is replaced.

All supplies will be stored as per manufacturer labeling and discarded past expiration date. Long term storage of nucleic acids is in a -80˚C feezer. Whenever possible, consumables and reagents that come into contact with test samples are received pre-sterilized and disposable (e.g. filtering funnels). They are used once and not re-used. Specific information of supply and consumable vendors are specified in individual Test Method SOPs' materials list.