General Guidelines for Forensic Biology and DNA Casework		
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1 Laboratory organization

1.1 To minimize the potential for carry-over contamination, the laboratory is organized so that the areas for DNA extraction, PCR set-up, and handling amplified DNA are physically isolated from each other.

1.1.1 Rapid DNA instruments are field-deployable. If operated in-house, a Rapid DNA instrument used for processing casework reference samples shall be maintained in rooms outside of evidence examination areas or those containing amplified DNA.

1.2 Based on need, microcentrifuge tube racks and trays have been placed in sample handling areas. These racks and trays should only leave their designated area to transport samples to the next designated area. Immediately after transporting samples, the racks and trays should be cleaned and returned to their designated area.

1.3 Dedicated equipment such as pipettors should not leave their designated areas. Only the samples in designated racks should move between areas.

1.4 Analysts in each work area must wear appropriate personal protective equipment (PPE). Contamination preventive equipment (CPE) must be worn where available. All PPE and CPE shall be donned in the bio-vestibules.

1.5 Required PPE and CPE for each laboratory are posted conspicuously in each bio-vestibule.

1.6 In order to maintain the negative air pressure in the labs necessary to prevent contamination, laboratory doors must be closed securely after entering the lab and upon leaving the lab.

1.7 Optimal laboratory temperature is between 65- 80 degrees Fahrenheit. Any time the temperatures fall below or above that range the Quality Assurance Team should be contacted so that facilities may be alerted and the determination as to whether to cease work in those labs may be made.

2 Workplace Preparation

2.1 Apply 10% bleach followed by water and/or 70% Ethanol to the entire work surface, cap opener, pipettes, and computer keyboard/mouse (when appropriate) before and after each procedure. Use disposable bench paper to prevent the accumulation of human DNA on permanent work surfaces.

2.2 Obtain clean racks and cap openers, and tubes, from storage. **Arrange workplace to minimize crossover.**

2.3 Position gloves nearby with 10% Bleach/70% Ethanol/water in order to facilitate frequent glove changes and cleaning of equipment.

3 Microcentrifuge tube and pipette handling

3.1 All tubes used in pre-amplification areas, except serology, must be irradiated prior to use (see QC 243).

3.2 Avoid splashes and aerosols. Centrifuge all liquid to the bottom of a closed microcentrifuge tube before opening it.

3.3 Avoid touching the inside surface of the tube caps with pipettors, gloves, or lab coat sleeves.

3.4 Use the correct pipettor for the volume to be pipetted. For pipettors with a maximum volume of 20µL or over, the range begins at 10% of its maximum volume (i.e., a 100µL pipette can be used for volumes of 10-100µL). For pipettors with a maximum volume of 10µL or under, the range begins at 5% of its maximum volume (i.e., a 10µL pipette can be used for volumes of 0.5-10µL).

3.4.1 Both the P2 and P10 pipettors are able to accurately dispense volumes less than 1µL, however it is strongly recommended that a P2 be used for these small amounts.

3.5 Filter pipette tips must be used when pipetting DNA and they should be used, whenever possible, for other reagents. Use the appropriate size filter tips for the different pipettors; the tip of the pipette should never touch the filter.

3.6 Always change pipette tips between handling each sample.

3.7 Never “blow out” the last bit of sample from a pipette. Blowing out increases the potential for aerosols, this may contaminate a sample with DNA from other samples. The accuracy of liquid volume delivered is not critical enough to justify blowing out.

3.8 Discard pipette tips if they accidentally touch the bench paper or any other surface.

3.9 Wipe the outside of the pipette with 10% bleach solution followed by a 70% ethanol solution if the barrel goes inside a tube.

4 Reagent and Sample handling

4.1 Reagents are available within the laboratories and are quality control checked before use, where possible.

4.2 Check the volume of each reagent before performing a test batch to confirm that enough is available. Each reagent within a test batch should come from one lot number unless otherwise indicated within the specific protocol.

4.3 Samples that have not yet been amplified should never come in contact with equipment in the amplified DNA work area. Samples that have been amplified should never come in contact with equipment in the unamplified work area.

4.4 The DNA extraction and PCR setup of evidence samples should be performed at a separate time from the DNA extraction and PCR setup of exemplars. This precaution helps to prevent potential cross-contamination between evidence samples and exemplars.

4.5 Limit the quantity of samples handled in a single run to a manageable number. This precaution will reduce the risk of sample mix-up and the potential for sample-to-sample contamination.

4.6 Change gloves frequently to avoid sample-to-sample contamination. Change them whenever they might have been contaminated with DNA and whenever exiting a sample handling area.

4.7 Make sure the necessary documentation is completely filled out, and that the analyst's ID is properly associated with the notations.

5 DNA Extraction Guidelines

5.1 Slightly different extraction procedures may be required for each type of specimen. Due to the varied nature of evidence samples, the user may need to modify procedures.

5.2 Tube set-ups must be witnessed/confirmed as outlined in each specified procedure.

5.3 Use lint free wipes or a tube opener to open tubes containing samples; only one tube should be uncapped at a time.

5.4 Be aware of small particles of fabric, which may cling to the outside of tubes.

5.5 An extraction negative must be included with each batch of extractions to demonstrate extraction integrity.

5.5.1 The extraction negative control contains all solutions used in the extraction process but no biological fluid or sample.

5.6 Samples that cannot be amplified may be re-extracted, reported as containing insufficient DNA, or in some instances concentrated using a Microcon (see The Microcon DNA Fast Flow Procedure of the STR manual). The interpreting analyst shall consult with a supervisor to determine how to proceed. Other DNA samples may also be concentrated and purified using a Microcon if the DNA is suspected of being degraded or shows inhibition or background fluorescence during quantitation.

5.7 After extraction, the tubes containing the unamplified DNA should be transferred to a box and stored in the appropriate refrigerator or freezer. The tubes should not be stored in the extraction racks.

5.8 All tubes must have a LIMS label and/or the complete case number and sample identifier. This includes aliquots submitted for quantitation.

6 Amplification guidelines and controls for PCR analysis

6.1 If a sample is found to contain less than the minimum DNA required for a particular amplification system, then the sample must not be amplified in that system.

6.2 The following controls must be processed alongside the sample analysis:

6.2.1 A positive control is a DNA sample where the STR alleles for the relevant STR loci are known. The positive control tests the success and the specificity of the amplification, and during the detection and analysis stage the correct allele calling by the software.

6.2.2 An extraction negative control consists of all reagents used in the extraction process and is necessary to detect DNA contamination of these reagents. For samples that will be amplified ensure the extraction negative passes based on the current Quantitation protocol.

6.2.3 **Note:** Since the Y STR system only detects male DNA, one cannot infer from a clean Y STR extraction negative the absence of female DNA. Therefore, an extraction negative control originally typed in Y STRs must be retested if the samples are amplified in Fusion.

6.2.4 Samples that were extracted together should all be amplified together, if practicable, so that every sample is run parallel to its associated extraction negative control.

6.2.5 An amplification negative control consists of only amplification reagents without the addition of DNA, and is used to detect DNA contamination of the amplification reagents.

6.3 Failure of any of the controls does not automatically invalidate the test. Under certain circumstances it is acceptable to retest negative and positive controls. Refer to the appropriate amplification system Interpretation manual for further information.

6.4 Technical Leader approval is required for re-typing of evidence samples previously typed in a legacy kit.

7 Sample and DNA storage

7.1 Store evidence and unamplified DNA in a separate refrigerator or freezer from the amplified DNA.

7.2 During analysis, all evidence, unamplified DNA, and amplified DNA should be stored refrigerated or frozen.

7.3 Refer to the Evidence Control procedure for further information regarding the retention and storage conditions of evidence and work product.

7.4 If an extract retrieved from storage appears to have evaporated, it may be reconstituted using TE^{-4}. Refer to the Reconstitution of Evaporated Extracts Procedure. The extract tracking information should be used to estimate the remaining volume.

8 Concordant analyses and "duplicate rule"

8.1 Typing results must be evaluated to identify possible sample mix-up or any typing results that are in conflict with the context of the case. If either of those situations are encountered, it may be necessary to perform additional testing. Most often, that additional testing will take the form of repeating the DNA testing for a particular sample with a separate extraction and/or aliquot, amplification, and electrophoresis plate.

8.2 The reporting analyst will evaluate any DNA typing results in a case and determine if the results make logical sense in the context of the case. In many instances, this is demonstrated by concordance of typing results. For example:

- a series of bloodstains from a homicide scene match the victim
- mixtures in a sexual assault case are consistent with the victim and the same male donor(s)
- a set of samples from a gun show similar alleles in the mixtures

8.2.1 Some evidence types may not be expected to have concordant typing results. For example:

- samples collected from a variety of locations at a burglary scene
- numerous beverage containers collected from a bar
- samples taken from a variety of discarded clothing items from a shooting scene

8.3 EVIDENCE SAMPLES

8.3.1 Evaluate typing results and any match, inclusion or exclusion within the case. Any additional testing takes the form of a separate aliquot, amplification and electrophoresis run. The following guidelines apply:

8.3.1.1 Identical single source DNA profiles among at least two items (two evidence samples or one evidence sample plus an exemplar) within a case are considered internally concordant results.

8.3.1.2 If an autosomal result does not match any other sample in the case and does not make sense in the context of the case, it must be repeated with a second amplification, either in autosomal or Y-STRs.

8.3.1.3 If a Y-STR result does not match any other sample in the case and does not make sense in the context of the case, it must be repeated in the Y system.

8.4 MIXTURE SAMPLES

8.4.1 If the sample consists of a **mixture of DNA**, several scenarios must be considered. Further analysis steps have to be decided based on the nature of each case. Consult with your supervisor if you encounter a situation that is not represented in the following examples:

8.4.1.1 If the alleles in a mixture are consistent with coming from any of the known or unknown samples in the case, e.g. a victim and a semen source, no further testing is needed.

8.4.1.2 If two or more mixtures in a case are consistent with each other and display substantially the same allele combinations, they are considered concordant.

8.4.1.3 If mixtures are found within a case that are consistent with the context of the case, it is acceptable for them not to be concordant. For example:

8.4.1.3.1 Mixtures of DNA found on touched items in a home invasion.

8.4.1.3.2 Discarded items in any kind of case.

8.4.2 Inconclusive samples and minor components of mixed samples do not require additional testing.

8.5 EXEMPLAR SAMPLES

8.5.1 Evaluate typing results and any match, inclusion or exclusion within the case, or between a suspect and an evidence case. Any additional testing takes the form of a separate extraction, aliquot, amplification and electrophoresis run. The additional testing must start with a second independent extraction, with the exemplar cut and submitted for extraction at a different time. If there is no additional exemplar material available for extraction, the additional testing may begin at the amplification stage.

8.6 ELIMINATION SAMPLES

8.6.1 Non-victim elimination exemplars (such as consensual partners, homeowners, business employees) do not require additional testing.

8.7 VICTIM EXEMPLAR

8.7.1 Additional testing of **victim's exemplar** is required if it does not match any of the DNA profiles of evidence samples in the case, including mixtures, and the case did not meet any of the criteria listed above.

8.7.2 Additional testing of a victim's exemplar is not required in the following situations:

8.7.2.1 A case which contains only samples that are insufficient for amplification and/or had no DNA alleles detected in evidence samples.

8.7.2.2 A case which contains only samples which are inconclusive/not suitable for comparison.

8.7.2.3 There is no reasonable expectation to detect the victim's DNA on an item of evidence, e.g. a crime where a hat was seen being dropped by fleeing suspect.

8.7.2.4 A case with a female victim where the samples processed yielded only male DNA profiles, or a case with a male victim where the samples yielded only female DNA profiles.

8.7.2.5 If the DNA profile of a **victim's exemplar** matches any of the DNA profiles of evidence in the case or is present in a mixture.

8.8 SUSPECT EXEMPLAR

8.8.1 Additional testing of a suspect's exemplar is not required. If a comparison results in a DNA match/matches, carefully evaluate the information in the matching case(s) to see if additional testing is warranted. See Verifying and Reporting DNA Matches.

8.9 ABANDONMENT SAMPLES

8.9.1 Abandonment samples do not require additional testing. If a comparison results in a DNA match/matches, carefully evaluate the information in the matching case(s) to see if additional testing is warranted. See Verifying and Reporting DNA Matches.

8.9.1.1 Detection of a mixture on an abandonment sample makes the sample inconclusive; no further testing is required.

8.9.1.2 If multiple abandonment items are received from a single individual, and testing generates different DNA profile results between the items, all results are to be deemed inconclusive.

8.10 Y-STR TESTING

8.10.1 Additional Y-STR testing should be performed at the discretion of the reporting analyst in the following situations:

8.10.1.1 If there is concern that a sample mix-up occurred.

8.10.1.2 If there is possible contamination from a source in or out of the laboratory.

8.10.1.3 If typing results do not make sense in the context of the case.

8.10.2 Additional Y-STR testing is not required in the following situations:

8.10.2.1 If the concordance policy has been met with Y-STR typing results.

8.10.2.2 If the Y-STR results are concordant with the autosomal typing results: agreeing with an inclusion or exclusion, confirming the presence of male DNA, and/or confirming the number of male donors.

9 Sample contamination policy

9.1 Contamination is defined as the addition of exogenous DNA/biological fluid to evidence items, samples, or controls subsequent to the incident. Sources of contamination could be first responders, EMT's, crime scene technicians, MLI's, ME's, ADA's, NYPD personnel, or laboratory personnel.

NOTE: If the circumstances of the incident are such that the presence of DNA from someone other than a victim, witness, or perpetrator may be reasonably expected, that is not considered contamination. An example is the DNA of a police officer on a weapon that she/he removed from a suspect with bare hands during arrest. An exemplar received from such a person is considered as a routine elimination sample, not a sample submitted specifically to investigate potential contamination.

9.2 Contamination is identified in a variety of ways:

9.2.1 When a DNA profile from an evidence sample is compared to the laboratory's LabTypes database in LDIS and a match is found to an individual represented there.

9.2.2 When a DNA profile from an evidence sample is compared to the forensic databases in LDIS and a match is found there and subsequent examination of the case records reveal that it is possible that the evidence was collected by the same NYPD personnel, processed by the same NYPD laboratory criminalist, or the samples were processed together during DNA testing.

9.2.3 When a DNA profile, or alleles, are seen in a control sample. If a negative or positive control contains exogenous DNA, all the associated samples are deemed inconclusive.

9.2.3.1 Follow the procedures in the appropriate STR Results Interpretation procedure to evaluate negative or positive controls.

9.2.3.2 Any associated samples should be re-extracted or re-amplified, if possible.

9.2.3.3 If re-extraction or re-amplification of the sample is not possible, then use the appropriate statements in the template report to report the results of the associated samples.

9.2.3.4 The Control of Non-Conforming Work procedure in the Quality Assurance/ Quality Control Manual should be followed to document the incident.

9.3 The source of any exogenous DNA should be identified so that samples can be properly interpreted. It may be possible to identify the source by:

9.3.1 Examining other samples from the same batch for similar occurrences.

9.3.2 Examining samples from different batches handled or processed at approximately the same time for possible similar occurrences (such as from dirty equipment or surfaces).

9.3.3 Processing samples submitted specifically to investigate potential contamination in specific case(s), e.g. from NYPD personnel.

9.4 If contamination within a sample has been discovered, the first step is to try to find an alternate sample.

9.4.1 As appropriate, a new extraction, amplification, or electrophoresis of the same sample can serve as an alternate for the affected sample.

9.4.1.1 All case notes related to the discovery of exogenous DNA are retained in the case record. A Not Suitable for Comparison/Inconclusive Form is added to the case record that identifies the source of the exogenous DNA by Lab Type ID Number, if known, and stating which samples were affected.

9.4.1.2 For this type of alternate sample, the discovery of exogenous DNA is not noted in the report.

9.4.1.3 The Control of Non-Conforming Work procedure in the Quality Assurance/ Quality Control Manual should be followed to document the incident.

9.4.2 If there are other samples from the incident which would serve the same purpose, they could be used as an alternate sample. For example, in a blood trail or a blood spatter, another sample from the same trail/spatter should be used. Another swab or underwear cutting should be used for a sexual assault.

9.4.2.1 All case notes related to the event are retained in the case record. A Not Suitable for Comparison/Inconclusive Form is created that identifies the source of the exogenous DNA by Lab Type ID Number, if known, and stating which samples were affected.

9.4.2.2 For this type of alternate sample, the discovery of the exogenous DNA is noted in the report for the contaminated sample(s). Use the appropriate statements in the template report to report the results of the contaminated sample(s). No names for the possible source(s) of the exogenous DNA are listed in the report.

9.4.2.3 The Control of Non-Conforming Work procedure in the Quality Assurance/ Quality Control Manual should be followed.

9.5 If an alternate sample cannot be found, then as a last resort, the samples containing the contaminant DNA can be interpreted, but must be done so carefully. An analysis first must be done to support the evaluation that the contaminant DNA profile is a part of the mixture.

9.5.1 For Fusion, the contaminant DNA profile may be used as a conditioned sample in a STRmix™ analysis to aid in the mixture deconvolution in accordance with the following procedure:

9.5.1.1 A STRmix™ LR calculation for the contaminant DNA profile must be performed before conditioning.

9.5.1.2 If the resulting LR > 1,000, the contaminant DNA profile can be used as a conditioned profile in a subsequent STRmix™ deconvolution. The discovery of the exogenous DNA is noted in the report for the contaminated sample(s).

9.5.1.2.1 The original deconvolution should be noted on a STRmix™ "Not Interpreted/Reported" form in the case file. The results and conclusions must be reported using the template report wording and indicate that the contaminant DNA profile was used as a conditioned profile to obtain results.

9.5.1.3 If the resulting STRmix™ LR is ≥0.001 but less than ≤ 1,000, this STRmix™ analysis should be noted on a "Not Interpreted/Reported" form in the case file. The sample must not be conditioned upon and the samples cannot be interpreted any further.

9.5.1.4 If the resulting STRmix™ LR is <0.001, this is indicative of support for exclusion of the contaminant DNA profile. The sample may be interpreted after consultation with a supervisor.

9.5.2 For cases with testing in kits other than Fusion, if exogenous DNA is suspected to be a part of the evidence sample, the samples cannot be interpreted. The discovery of the exogenous DNA should be noted in the report for any contaminated sample(s).

10 Technical Deviations

10.1 Technical Deviations must be requested when standard courses of actions will not be followed (aka, a "planned deviation") or when standard operating procedures were not followed and the resulting data will be used in casework (aka, an "unplanned deviation"). The impact of the deviation must be thoroughly evaluated.

Examples:

- Incorrect elution volume selected for an automated extraction run but did not affect the DNA extracted from the sample. Analyst would like to send the sample for further testing.
- Incorrect RPM's on a centrifuge set and used for a microcon assay but the eluant still filtered through the microcon. The analyst would like to send the sample for further testing.

- Incorrect length of time for an incubation step in an extraction assay but it was determined that the assay still extracted DNA from the substrate. Analyst would like to send the sample for further testing.

10.2 Technical deviations should be a rarity, and are not intended to be a general occurrence. Where possible, the analysis should be re-done. If it has been determined that a deviation is necessary, the proposed deviation should be discussed with a supervisor and/or manager first to determine if re-testing or submitting a deviation request is the best course. Deviation requests are submitted to the appropriate technical leader for approval. Such requests must be accompanied by a sound scientific justification as to why, even though the technical procedure was not followed, it is acceptable to use the resulting data.

10.3 If a technical procedure was not followed, or an instrument or assay had a failure, and you are not intending to use the data for interpretation or for a subsequent assay, then a technical deviation is not necessary. A note in the batch or other documentation within the case file is sufficient.

Examples:

- Instrument error results in a failed automated extraction batch; samples are recut.
- Failed negative control from an amplification; samples are re-amplified.

10.4 The mechanism to submit a technical deviation is through the LIMS deviations tram stop. This tram stop is also used for evidence discrepancy forms as well as STR electrophoresis batch failures, neither of which is considered a technical deviation.

10.5 Technical deviations can only be approved by the relevant Technical Leader or Assistant Technical Leader. If the relevant Technical Leader and the Assistant Technical Leader are out of the office, approval of a technical deviation will have to wait until their return.

10.6 Depending on the complexity of the technical deviation, it may be necessary for the analyst requesting the deviation to meet with their supervisor, their manager and the relevant technical leader in order to discuss the planned deviation. The potential end result of the deviation and its impact on the case and/or reported results must be considered. Several meetings may be necessary during the implementation of the deviation in order to assess the results of the deviation, before proceeding to the next stage of testing. The need to meet, and the number of meetings needed, is entirely based on the complexity of the deviation.

10.7 **Technical deviations** are different than **non-conforming work**. Refer to the Control of Non-Conforming Work section of the Quality Assurance/Quality Control Procedures Manual for details on when to submit a non-conforming work form.