MINIMUM REQUIREMENTS FOR DNA COLLECTION, ANALYSIS, AND INTERPRETATION

A document for emerging laboratories

International Forensic Strategic Alliance

Version 2

IFSA
International Forensic Strategic Alliance

INTERNATIONAL FORENSIC STRATEGIC ALLIANCE

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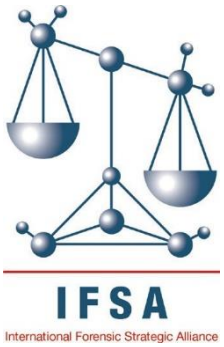
IFSA MRD 2



Version 1 of this document was first released October 2014. The document has been updated and is now released as Version 2.

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INTRODUCTION

The International Forensic Strategic Alliance (IFSA) has developed this document to be minimum requirements which will enable emerging forensic providers in developing countries to produce scientific services to the Criminal Justice System.

The purpose of this document is to establish a baseline or starting point that must be followed in order to achieve reliable results. Forensic providers should build on this foundation and strive to continually improve the quality of services provided.

This document describes the minimum requirements for DNA collection, analysis and interpretation. It addresses the following framework:

1. Competence of Personnel.
2. Equipment and Consumables.
3. Collection, Analysis, Interpretation, Reporting.
4. Procedures, Protocols, Validation.
5. Quality Management.

Note: This document does not apply to laboratories performing Rapid DNA Analysis or modified Rapid DNA Analysis. It is envisaged that future versions of the DNA Minimum Requirements Document will address emerging DNA technologies such as the aforementioned if and when they become more prevalent in the forensic DNA community.

FOREWORD

The International Forensic Strategic Alliance (IFSA) is a multilateral partnership between the six regional networks of operational forensic laboratories:

- the American Society of Crime Laboratory Directors (ASCLD)
- the European Network of Forensic Science Institutes (ENFSI)
- the National Institute of Forensic Science Australia New Zealand (NIFS ANZ)
- la Academia Iberoamericana de Criminalística y Estudios Forenses (AICEF)
- the Asian Forensic Sciences Network (AFSN)
- the Southern Africa Regional Forensic Science Network (SARFS).

IFSA works closely with its three strategic partners, Leverhulme Research Centre for Forensic Science, United Nations Office on Drugs and Crime (UNODC) and INTERPOL.

IFSA recognises the importance of a quality management framework in forensic laboratories to provide quality and standardised results, be it procedures undertaken in the field or in the laboratory.

In February 2012, at the special IFSA meeting hosted by UNODC and convened in Vienna to discuss the needs of the emerging forensic laboratories in developing countries, a decision was taken to create a set of minimum requirement documents (MRD) filling the gap in recommendations available for the current management of these laboratories.

In October 2014, the first series of three documents in the specific areas of identification of seized drugs, DNA analysis, and crime scene investigation were created. These documents have focused on the critical quality areas, using simple terms and illustrations All three MRDs have now undergone update and further review with version 2 of these documents published in December 2020. At the time of writing, a further three MRDs in the areas of digital and media evidence, document examination and latent fingerprint analysis are currently in development. A separate glossary document has also been created to guide the users through the important concepts of this documents.

These MRDs are meant to act as a start-up guide for emerging forensic laboratories to quickly establish their quality management system and scientific/technical capabilities. Once achieved, the laboratories should continue to build on this foundation and strive to continually improve the quality of services through undergoing accreditations to established standards.

In the drafting of these documents, scientific working groups and experts from the six regional forensic science networks, as well as IFSA strategic partners, made valuable contributions during the various rounds of consultation. The final MRDs presented in this series would not be possible without the involvement of all.

It is IFSA's hope that these documents will play an important role for emerging forensic laboratories in their journey towards building quality forensic services.

IFSA Board

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1 COMPETENCE OF PERSONNEL

All laboratory staff shall have a clear understanding of their duties and responsibilities and should fulfil these at all times according to a code of ethics/professional practice/conduct1(see the examples in the footnote below) adopted by the laboratory.

This section recommends minimum education and training required for laboratory staff to conduct DNA collection, analysis and interpretation[2].

1.1 EDUCATION

Laboratory staff shall have education, skills and abilities commensurate with their responsibilities.

Technician: Higher education requirements should be based on the nature and complexity of tasks to be performed.

Analyst: Staff issuing reports should have tertiary education with a strong emphasis in biological science and ideally including coursework in statistics. Coursework should include lectures and associated laboratory classes.

1.2 TRAINING

The laboratory should have a documented training plan for new staff or new tasks, documenting the required standards of performance, competency and assessment plan. The assessment can be done, for example, through fulfilled training plans or the analysis of unknown samples. The training should be delivered by experienced and competent staff.

The training program shall include a training manual covering all procedures that the analyst/technician will employ in the course of casework, as well as on the code of ethics. The training program shall teach and assess the technical skills and knowledge required to perform DNA collection, analysis and interpretation. Staff should be assessed as competent prior to assuming independent casework. A competency test will ensure proper skills and knowledge was acquired during training. Training may be augmented by participation in external courses or workshops.

A program for continued education should be established as an extension of credentialing and to ensure analysts stay abreast of scientific advancement and development in the analysis of DNA. The program may include conference/ seminar/course attendance, webinars, and review of scientific literature and other methods of self-learning.

Training and competency tests should be documented, and records retained according to guidelines established by the laboratory. All analyst/technician(s) shall participate in ongoing proficiency testing, and the results recorded.

[1] Examples of Code of Ethics adopted by regional forensic science networks:

- The American Society of Crime Laboratory Directors (ASCLD) – www.ascld.org
- The European Network of Forensic Science Institutes (ENFSI) – www.enfsi.eu
- The National Institute of Forensic Science Australia New Zealand (NIFS ANZ) – www.anzfss.org
- La Academia Iberoamericana de Criminalística y Estudios Forenses (AICEF) – www.aicef.net
- The Asian Forensic Sciences Network (AFSN) – www.asianforensic.net

[2] Extra information can be found in the ENFSI Guideline for the training of staff in DNA-Laboratories - www.enfsi.eu

2 EQUIPMENT AND CONSUMABLES

2.1 FACILITIES

Evidence receipt and storage shall be separated from the analytical areas.

The laboratory shall have appropriate utilities such as electricity, clean water, and adequate separated space and plumbing. More advanced laboratories working towards accreditation should include air conditioning, airtight windows and purified water, and may consider separate, pressure-controlled rooms for pre amplification processes (positively pressurised) and post amplification processes (negatively pressurised).

Biological samples shall be stored in an area protected from contamination, heat, and sunlight. Some biological samples may require refrigeration or freezing. Refrigerators and freezers' temperatures shall be monitored to prevent sample degradation and the laboratory shall specify an acceptable range of temperature for this equipment.

The facility shall be equipped with refrigerators and freezers dedicated to the storage of consumables. Biological samples shall not be stored with consumables. If the laboratory is unable to provide dedicated refrigerators and freezers, samples shall by physically separated from consumables using measures such as robust plastic bags, boxes or other physical separators.

Analytical, evidence and sample storage areas shall be secured, and access controlled.

2.2 EQUIPMENT

The laboratory shall use equipment that is suitable for the methods employed by the laboratory.

At a minimum, the laboratory shall have a procedure for conducting performance checks and calibration of all equipment deemed critical.

Examples of critical equipment include but are not limited to:

- thermal cyclers including quantitative Polymerase Chain Reaction (PCR);
- thermal cycler temperature verification systems;
- electrophoresis detection systems such as genetic analyzers;
- robotic systems; and
- mechanical pipettes

The laboratory shall have a schedule and follow a documented program to ensure that instruments and equipment are properly maintained, serviced, calibrated and verified. Performance of equipment shall be monitored, and records kept of performance checks.

Only trained staff shall operate the instruments. The manufacturer's operation manual and other relevant documentation, for example, standard operating procedures (SOP) for each equipment shall be readily available in the laboratory. Methods used on the equipment shall be validated prior to application on casework.

The laboratory shall have and follow a written procedure for monitoring, cleaning, and decontaminating facilities and equipment. It is the responsibility of laboratory management to design and implement appropriate cleaning techniques and protocols.

2.3 CONSUMABLES

The laboratory shall use reagents and consumables that are suitable for the methods employed by the laboratory. This includes but is not limited to: 'PCR grade', 'DNAse free', 'DNA free'.

The laboratory shall have written procedures for the preparation of reagents.

Commercial reagents shall be labeled with the identity of the reagent and the expiration date as provided by the manufacturer or as determined by the laboratory. It is good laboratory practice that commercial reagents should be dated and initialed when first opened.

All in-house prepared reagents shall be labeled with the identity of the reagent, the identity of the individual who prepared it and the date of preparation or lot number. Expiration date should also be available.

The laboratory shall identify critical reagents and evaluate them prior to use in the DNA analysis. These critical reagents shall include, but are not limited to, the following:

- test kits for performing DNA extraction, quantitative PCR and genetic typing; and
- proteinase, thermo-stable DNA polymerase, primer sets and allelic ladders, used for genetic analyses that are not tested as test kit components.

All consumables shall be stored at appropriate temperatures as recommended by manufacturer. Different reagents within the same kit may need to be stored at different temperatures. All reagents prepared in-house shall be stored at the appropriate temperature. Reagents shall be protected from direct sunlight.

3 COLLECTION, ANALYSIS, INTERPRETATION & REPORTING

3.1 COLLECTION

This section addresses collection of DNA evidence from items submitted to the laboratory. Collection of DNA evidence at crime scenes is covered under the Crime Scene Investigation Minimum Requirements publication and is applicable to a laboratory that also collects and processes crime scene evidence.

The laboratory shall have records of requests for analysis and the exhibits submitted. A unique identifier shall be assigned to each exhibit. Should there be significant discrepancy between the submission documentation and physical evidence, the client shall be informed as soon as possible and the discrepancy shall be recorded with the case notes.

A system to document a chain of custody for the evidence shall be established in the laboratory. Only authorized staff shall have access to exhibits.

Each exhibit shall be properly stored to maintain the integrity of the evidence. The exhibit shall be sealed upon submission to ensure integrity

The following should also be ensured:

- Individuals processing biological evidence shall wear proper personal protective equipment (PPE) such as lab coats, disposable gloves, and masks to limit the potential for contamination;
- Examination of evidence for the presence of biological fluids such as blood or semen should be conducted utilizing biochemical, microscopic or immunological techniques;
- Evidence items are examined in a room that is clean;
- Activity in this room is limited to examination of items;
- Utensils (e.g. forceps, scalpel, scissors) and surfaces are decontaminated with a 10% (v/v) household bleach solution or equivalent prior and after examination of each item of evidence;
- If possible, disposable bench paper is used to cover surfaces;
- Items are inventoried and marked with a unique identifier; and
- Examination is documented and notes are retained.

Evidence items shall be examined separately in time, space or examiner to avoid cross-contamination. If crime and reference samples are processed in the same area, the following shall apply to minimize the risk of contamination:

- Have designated separate benches and equipment for crime and reference sample testing;
- Crime and reference samples shall never be processed at the same time;
- Crime samples shall be processed first, before the reference samples; and
- All laboratory benches and equipment are to be thoroughly cleaned when switching from crime to reference sample testing, or vice versa.

3.2 ANALYSIS

DNA analysis is a complex process of sample extraction, quantitation (optional), amplification, electrophoresis, and interpretation.

DNA analysis utilizes the properties of electrophoresis which can be obtained by flat gel-based methods or capillary based methodologies.

Types of DNA analysis include:

- Autosomal STR markers;
- Y-STR markers;
- X-STR markers;
- Mitochondrial markers; and

Other markers used for ancestry and/or phenotypic characteristics.

Sample extraction

The laboratory shall have separated space for DNA extraction and utilize procedures for the isolation of DNA for forensic analysis. Extraction procedures shall include:

- Extraction methods for non-semen containing stains; and
- Differential extraction methods for semen containing stains (sexual assault related samples).

All extraction methods should contain a reagent blank control which is carried through the process of quantitation, amplification and interpretation.

Quantitation

DNA quantitation of human DNA should be performed on samples prior to amplification. This step may be skipped depending on the sample type, e.g. for reference samples (which may involve direct amplification of a set volume of liquid blood or a punch or cut-out sample of a dried stain).

All quantitation procedures will contain standards to determine the quantitative or qualitative value of the isolated DNA.

Amplification

All samples should be amplified utilizing developmentally validated commercial or in-house DNA typing kits. It is noted however that in-house kits shall be subjected to developmental validation procedures.

In order to utilize available forensic DNA databases, it is recommended that commercially available kits selected should contain at a minimum the recommended INTERPOL Standard Set of Loci (ISSOL)[2], CODIS Core Loci; or loci that are compatible to the database used in the region.

Positive and negative controls as well as a reagent blank shall be amplified with the evidence items.

All controls (amplification positive, negative and any reagent blanks) shall be carried out through analysis and interpretation.

The reagent blank shall be amplified in the most sensitive volume of the extraction set of samples.

The negative control shall be amplified in the highest volume allowable with the amplification kit.

Further analysis of a sample can be terminated based on a quantitation threshold with the notion that this sample will not yield an interpretable DNA profile. However, this assessment shall be supported by a validation study.

Pre and post amplification processes should be conducted in physically separate areas to avoid sample contamination.

Equipment such as pipettes should be dedicated to a specific area.

Electrophoresis

At least one allelic ladder shall be run with each set of samples or per plate.

The reagent blank and negative control shall be run under the most sensitive conditions, (i.e. injection time and/or voltage) of the set of samples. The amplicon volume of both controls shall also satisfy the most sensitive conditions.

Quality Control

The sensitivity of methods for DNA analysis requires the following safeguards against contamination:

- Pre and post amplification processes should be conducted in physically separate areas to avoid sample contamination.
- Equipment such as pipettes shall be dedicated to a specific area.
- Work surfaces and instruments used in the examination of items shall be cleaned before contact with evidence, between evidence items, and after evidence processing is complete.
- It is common practice for Glassine paper, Kimwipes®, butcher paper, or Benchkote® paper to be placed on the benchtop while processing evidence to act as a barrier. The paper shall be changed, and the benchtop cleaned between items.
- Centrifuges, thermal cycler, tube racks, pipettes and any other equipment deemed appropriate shall be cleaned before and after each use.
- Instruments such as forceps, scissors, scalpels, and tube openers shall be cleaned just prior to use. Some laboratories purchase sterile disposable instruments. These shall be opened just prior to sample processing and discarded after one use.
- Cleaning shall be done with a 10% (v/v) household bleach solution or a commercially available reagent such as Cidex® Plus which will minimize potential risks of DNA contamination.
- If an item is cleaned with bleach, it shall be rinsed with purified water or alcohol to prevent the build-up of sodium hypochlorite crystals. Instruments or equipment cleaned with bleach should also be cleaned with purified water or alcohol immediately afterwards to avoid corrosion.
- The bench and equipment shall be cleaned between the analysis of EVERY exhibit, even when analyzing related items (e.g. multiple items of clothing from the same person).
- The creation of a staff elimination database is highly recommended as an added quality assurance procedure to detect possible staff contamination.

3.3 INTERPRETATION[3]

The laboratory shall have and follow written guidelines for the interpretation of data to include all amplification positive and negative controls as well as reagent blanks.

Laboratories should have and follow written guidelines for DNA mixture interpretation that address major and minor contributors, inclusions, exclusions and policies for reporting results and statistics[3].

The statistical interpretation shall be based on:

- Ethnic Population Database. The laboratory should follow recommendations by expert groups such as the Scientific Working Group for DNA Analysis Methods (SWGDAM)[3] or the International Society for Forensic Genetics (ISFG)[4] for the minimum number of profiles to be included in the database. This number will vary depending on the type of marker analyzed.
- Statistical calculations derived from a relevant documented population database appropriate for the calculation.

[3] Extra information can be found in the ENFSI Guideline for Evaluative Reporting in Forensic Science - www.enfsi.eu

A laboratory performing genetic analysis such as Y-chromosomal[6] or mtDNA typing[7] shall have and follow documented statistical interpretation guidelines specific for such testing.

3.4 REPORTING

The laboratory shall have written procedures for recording observations and test results.

All efforts shall be directed to produce reports that are accurate, clear, objective and meet the requirements of the jurisdiction served. The laboratory shall maintain all analytical results used to support report conclusions. All analytical results used to support the conclusions in the report shall be retained.

Comprehensive documentation shall be maintained for peer review.

Reports shall include:

- Title of report;
- Date issued;
- Name and address of testing laboratory;
- Unique identification of the report on every page;
- Page number and total number of pages;
- Date of receipt of evidence;
- Descriptive list of submitted evidence (including items not examined)
- Sampling;
- Methodology used;
- Loci or amplification system;
- Results of analysis;
- Conclusions encompassing a quantitative or qualitative interpretation statement. The significance of a match should be associated to a statistical statement; and
- Identity of staff member issuing the report.

Reports may only be issued by personnel who are experienced, appropriately trained and have been authorized to do so.

Peer review

The laboratory shall determine a framework for a systemic review of reports by a reviewer competent in the testing/procedure being reviewed. This review helps ensure that all conclusions reached and supporting data are consistent with laboratory policy and guidelines.

Casework documentation shall contain sufficient information such that the reviewer is able to evaluate case notes and interpret data. Before a report is released it should go through a technical and administrative review.

In the event where the staff-in-charge of the case does not agree with the opinion of the reviewer, the matter will be referred to higher authority who is competent to determine the disputed issue.

Technical review shall include the following at a minimum:

- Case notes, worksheets and electronic data;
- DNA types (allele calls) to verify interpretation based on documented interpretation guidelines;
- All DNA profiles to ensure proper inclusions and exclusions;
- All inconclusive results;
- All controls, including internal lane standards and allelic ladders;
- Any statistical analysis if applicable;

- Chain of custody and disposition of exhibits; and
- Review of final report's content to ensure that all results and conclusions are supported by documented data.

Technical review shall be documented in the case record. Technical review shall be conducted by an individual qualified in the methodology used.

Administrative review shall include:

- Any clerical errors in the final report; and
- Compliance with section 3.4.

Case records

The laboratory shall have procedures for retention, control, confidentiality, and release of case records.

3.5 DATABASES

Numerous forensic databases have been established globally to solve cold cases and ensure 'safe' convictions. Since legislation/regulations pertaining to what data can be entered in a database differ between different countries, this document cannot address standards pertaining to DNA databases.

Recommendations and best practices have been published by the INTERPOL DNA Monitoring Expert Group for the establishment of a national DNA database[8]. The ENFSI DNA Working Group has published a document on the review and recommendations of DNA Database management[9].

4 PROCEDURES, PROTOCOLS AND VALIDATION

4.1 PROCEDURES AND PROTOCOLS

The laboratory shall have and follow analytical protocols and procedures. These procedures should include biological evidence identification, sample preparation, extraction methods, quantitation, amplification, analysis and interpretation.

Protocols and procedures shall be documented, tracked, and controlled. In-house developed procedures shall be validated or verified prior to application to demonstrate they are fit-for-purpose.

All protocols and procedures shall specify instruments, reagents and controls. Procedures should be a step-by-step process sufficiently detailed to ensure uniformity and consistency of testing and analysis of data/results.

If methods are changed at any time, the date that the change took place shall be recorded, so that for every sample, it is clear which method has been used in the processing of that sample.

4.2 VALIDATION [4]

All methods (published or developed in-house) used for exhibit analysis shall be validated to demonstrate that they are reliable and fit for its intended purpose. In-house equipment shall be used for validation studies. Validation should be performed by staff competent in the technologies used.

General guidelines:

- Select staff responsible for the validation study from beginning to end;
- Read peer-reviewed publications and manufacturer's recommendations;
- Draft a validation plan based on the afore mentioned. The plan shall include reagents, samples, instruments and equipment needed, the testing to be conducted and expected results. The validation plan shall be approved by the laboratory prior to commencing the validation;
- Select appropriate controls;
- Document the validation studies;
- Summarize results and approve prior to implementation;
- Draft SOP and interpretation guidelines based on validation results; and
- Draft a training manual and competency test for staff.

Staff shall be trained and pass a competency test prior to using the method on casework. The training and competency test shall be documented.

The following studies shall be performed for DNA analysis:

- Reproducibility (study using human DNA controls);
- Precision and accuracy (study using human DNA controls);
- Sensitivity; and
- Mixtures using sample ratios that represent what is encountered in casework.

[4] Extra information can be found in the ENFSI Guideline for Internal Validation of various aspects of the DNA Profiling Process - www.enfsi.eu

In addition, analytical thresholds (if applicable) shall be determined for the instrumentation used. This may include:

- Limit of detection;
- Dynamic range;
- Stochastic threshold; and
- Stutter range.

Contamination checks shall be performed with negative controls (blanks).

The laboratory shall have a documented relevant population distribution data which should include the distributions for the locus or loci obtained from relevant populations.

In-house developed databases should be tested for independence.

All documentation of validation processes shall be retained (hardcopy or electronically). Documentation shall include:

- Procedure of validation;
- Date of studies conducted;
- Data;
- Summary/conclusion of results; and
- Approval.

5 QUALITY MANAGEMENT[5]

The laboratory shall establish, follow and maintain a documented quality management system that is appropriate to the testing activities and is equivalent to what is required by these minimum requirements.

The laboratory shall document, maintain and follow a procedure regarding document retention that specifically addresses:

- Proficiency tests;
- Analytical results;
- Sample/exhibit continuity records/chain of custody;
- Sample receipt;
- Processing records;
- Sample retention;
- Corrective action;
- Audits;
- Training records;
- Continuing professional development;
- Court testimony monitoring; and
- Educational background (school, major etc.).

The quality system as applicable to DNA analysis shall be reviewed annually and documented.

This quality management program shall specify and document the responsibility, authority, and interrelation of all personnel who manage, perform or verify work affecting the validity of the DNA analysis.

[5] Extra information can be found in the ENFSI Quality Assurance Programme for DNA Laboratories - www.enfsi.eu

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CONTACT:

International Forensic Strategic Alliance: http://www.ifsa-forensics.org