# TG 42-03

## TECHNICAL GUIDELINES FOR FORENSIC DNA TESTING LABORATORIES

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## 1. Purpose and Scope

The standards describe the quality assurance requirements that a laboratory, which is defined as a facility in which DNA testing is performed, should conform in order to ensure the quality and integrity of the data and competency of the laboratory. These standards do not preclude a laboratory, either on its own or in conjunction with others, from carrying out research and development, or from using procedures that have not yet been validated.

## 2. Definitions and References

As used in these standards, the following terms shall have the meanings specified:

3.1 **Administrative review.** The evaluation of a case report and the supporting documentation for consistency with laboratory policies and editorial correctness.

3.2 **Amplification blank.** A control consisting of only amplification reagents without the addition of sample DNA that has been subjected to thermal cycling under the same conditions as amplification reagents containing sample DNA. This control is used to detect DNA contamination of the amplification reagents.

3.3 **Analytical procedure.** An orderly step-by-step procedure designed to ensure operational uniformity and to minimize analytical drift.

3.4 **Audit.** An inspection used to evaluate, confirm, or verify activity related to quality and the effectiveness of the quality system.

3.5 **Calibration.** The set of operations carried out on an instrument or device that ensures its functioning according to specified criteria.

3.6 **Critical reagents.** Reagents that have been found by empirical studies or routine practice, to require testing before being used on evidentiary samples.

3.7 **Critical Instruments**. Instruments that are used in the analysis process that require maintenance programs and/or regular verification and/or calibration, to ensure that the function properly and yield accurate results.

3.8 **Commercial test kit.** A validated pre-assembled kit that allows the user to conduct a specific forensic DNA test.

3.9 **Examiner/analyst** (or equivalent role, position or title as designated by the laboratory). An individual who conducts and/or directs the analysis of forensic case samples, interpret data and reaches conclusions.

3.10 **Forensic DNA testing.** The identification and characterization of biological evidence for court purposes using DNA technologies.

3.11 **International Calibrator Positive Control (IC positive)** The IC positive is a DNA sample of which the DNA profile has been established and independently verified by an internationally accredited laboratory or manufacturer of DNA typing kits. It is used to establish the accuracy and reliability of DNA typing for a set of loci used by the laboratory.

3.12 **Known samples.** Biological materials whose identity or type has been established.

3.13 **Laboratory.** A facility in which forensic DNA testing is performed.

3.14 **Laboratory support personnel.** Individuals who perform laboratory duties but do not analyse evidence samples.

3.15 **Non-probative evidence samples.** Evidentiary samples excluding samples of limited size.

Not test samples.

3.16 **Polymerase Chain Reaction (PCR).** An enzymatic process by which a specific region of DNA is replicated during repetitive cycles which consist of:
(i) Denaturation of the template;
(ii) Annealing of primers to a complementary template at an empirically determined temperature; and
(iii) Extension of the annealed primers by a DNA polymerase.

3.17 **Proficiency test sample**. A sample of biological material whose DNA type has been Characterized or will be characterized by consensus, and which is used to monitor the quality performance of a laboratory or an individual. A known sample could be used for proficiency testing.

3.18 **Proficiency testing**. A quality assurance measure used to monitor performance and identify areas in which improvement may be needed. Proficiency tests may be classified as:

3.18.1 *Internal proficiency tests*: Tests prepared and administered by the laboratory.

3.18.2 *External proficiency tests*: Open or blind proficiency tests set by a second agency (an entity or organization external to and independent of the laboratory and which performs forensic DNA analysis).

3.19 **Quality assurance**. The systematic actions necessary to ensure that a product or service meets specified requirements for quality.

3.20 **Quality manual**. A document describing the quality policy, quality system and quality practises of an organisation.

3.21 **Quality system**. The organizational structure, responsibilities, procedures, processes and resources for implementing quality management.

3.22 **Reagent blank control**. A solution containing all of the reagents used in the test process without any sample. This is to be used to detect DNA contamination of the analytic agents.

3.23 **Reference material** (certified or standard). Material that has been characterized by a technically valid procedure, and which is accompanied by documentation that makes it possible to recover a certificate or other documentation from the certifying body attesting to its established nature.

3.24 **Reporting officer** (or equivalent role, position or title as designated by the laboratory). The person responsible for interpreting the results and compiling the report on the DNA findings pertaining to a case, and who is normally responsible for giving evidence in courts of law.

3.25 **Restriction Fragment Length Polymorphism (RFLP)**. A genetic polymorphism that manifests as alternatively sized DNA fragments produced by digestion with specific restriction endonucleases.

3.26 **Review**. An evaluation of the documentation pertaining to a case in terms of consistency and accuracy.

3.27 **SANAS**: An acronym for the South African National Accreditation System.

3.28 **Secure area**. A locked space (for example, cabinet, vault or room) to which access is restricted to authorized personnel.

3.29 **Should**. Used before a verb in the infinitive to indicate an order, a requirement or an obligation.

3.30 **Subcontractor**. An individual or entity that has a transactional relationship with a laboratory.

3.31 **Technical leader**/ **support** (or equivalent role, position or title as designated by the laboratory). A person who is responsible for correct application of the DNA technology used in a laboratory. The person does not necessarily have to be the manager in charge of the

DNA operations but should be well conversant and experienced in molecular biology as applied in the forensic/paternity laboratory.

3.32 **Technical review**. An evaluation of reports, notes, data and other documents pertaining to a case to ensure that an appropriate and sufficient basis for the scientific conclusions has been established.

3.33 **Technician** (or equivalent role, position or title as designated by the laboratory). An individual who uses analytical techniques or has received training and has been proven competent to do presumptive tests on evidence samples independently or under supervision of a qualified examiner/analyst/reporting officer and/or performs DNA analysis on samples for inclusion on a database. Technicians do not evaluate or reach conclusions on typing results or prepare final reports.

3.34 **Traceability.** The property of a result of a measurement whereby it can be related to appropriate standards, generally international or national standards, through an unbroken chain of comparisons.

3.35 **Validation.** A process by which a procedure is evaluated to determine its efficacy and reliability for forensic casework analysis and includes:

3.35.1 **Developmental validation**. The accumulation of test data to establish the validity of a novel method/test and the conditions and limitations of its application on forensic samples. Developmental validation is normally performed by the manufacturers of forensic DNA testing kits.

3.35.2 **Internal validation**. The demonstration that established methods and procedures perform as they should in the laboratory through the use of accumulated test data generated in the same laboratory. Internal validation is normally only performed by the forensic testing laboratory. The participation in proficiency testing is recognised as the minimum that a laboratory can do to ensure compliance to internal validation.

## 3. Introduction

This document consists of definitions and standards; and is used in conjunction with the requirements of ISO/IEC 17025:2017 “General requirements for the competence of testing and calibration laboratories”. The standards are quality assurance measures that place specific requirements on the forensic DNA laboratory. The document must be read in conjunction with the SANAS document TG 01 “Criteria for laboratory accreditation in the field of forensics”.

## 4. List of General Requirements

All assessments are done in accordance with the relevant ISO/IEC Standards and SANAS requirements. SANAS documents are available from SANAS and are made available on receipt of the application for Accreditation. Additional copies of SANAS documentation may be purchased from the office.

## 5. Management System requirements (ISO/IEC 17025 Section 8)

The laboratory should establish and maintain a documented quality system that is appropriate to its testing activities.

5.1 The quality documentation should address at a minimum:

a) Goals and objectives
b) Organization and management
c) Personnel Qualifications, Continuing Education and Training
d) Facilities
e) Sample control
f) Validation

g) Analytical procedures
h) Calibration and maintenance
i) Proficiency testing
j) Corrective action
k) Reports
l) Review
m) Audits
n) Monitoring court testimony

*Discussion:*

*The DNA laboratory quality manual should address each of the above listed criteria. Individual sections that deal with subject areas that are defined through laboratory-wide policies or procedures guidelines (such as evidence control) may be referenced in the DNA quality manual or procedures. If such sections have been supplemented by DNA laboratory-specific practices, the DNA quality manual should reflect such additions.*

*An annual review of the quality system is important for ensuring that measures are being taken by the laboratory to continually provide the highest quality service. This review is typically directed to the quality manual and standard operating procedures used by the laboratory. Audit reports, with recommendations for improvements, may identify areas in need of attention and provide the basis for changes to the quality system. Such changes may include new or improved quality control activities for monitoring the quality of the laboratory work product. Additionally, significant modifications of forensic DNA testing, such as the incorporation of a new technology, may necessitate a review of the quality system. The annual review should be documented.*

## 6. Structural requirements (ISO/IEC 17025 Section 5)

6.1 The laboratory should:

Have a managerial staff with the authority and resources needed to discharge their duties and meet the requirements of the standards in this document.

Have a DNA technical leader who is responsible for correct application of DNA technology to casework.

Specify and document the responsibility, authority, and interrelation of all personnel who manage, perform or verify work affecting the flow of the DNA analysis.

*Discussion*
*As a tool in the assessment of the organizational standards, laboratories should maintain a current organizational chart, referencing the various members of the laboratory with their specific position assignments (laboratory head, technical manager, etc). Additionally, current job descriptions/or duty sheets /or competency profiles/or performance enhancement process documents must be available for all laboratory personnel, accurately defining the technical and/or administrative responsibilities associated with each position.*

## 7. Personnel (ISO/IEC 17025 Section 6)

7.1 Laboratory personnel should have the education, training and experience commensurate with the type of examination and testimony required.

7.1.1 The laboratory should:

i) Have written job descriptions for personnel who are involved with casework which include authorities, responsibilities, duties and skills.
ii) Have training programmes for qualifying all scientific technical laboratory personnel.
iii) Ensure that scientific technical qualifications are maintained through continuing education (encourage reading of articles, attending workshops, conferences, and mock courts).

iv) Maintain records on the relevant qualifications, training, skills and experience and proficiency testing of the personnel involved with casework.

7.2 The DNA technical leader should have the following:

7.2.1 Degree requirements:

The DNA technical leader of a laboratory should have at a minimum NQF 7 level qualification or equivalent qualification/degree/diploma in biology-, chemistry- or a forensic science- related area and have successfully completed a combination of undergraduate and in-house training programmes covering the subject areas of biochemistry, genetics and molecular biology (molecular genetics, recombinant DNA technology), or other subjects which provide a basic understanding of the foundation of forensic DNA analysis.

7.2.2 Experience requirements:

The DNA technical leader must have a minimum of three years of forensic DNA laboratory experience.

7.2.3 Duty requirements:

i) General: responsible for providing technical support in DNA analysis and ensuring that DNA technology is correctly applied in casework.

ii) Specific duties:
Responsible for evaluating methods used by the laboratory and for proposing new or modified analytical procedures to be used by examiners.

Responsible for technical problem solving of analytical methods and be directly involved with making recommendations for training, quality assurance, safety and proficiency testing and future application in the laboratory.

Responsible for ensuring that quality control standards are correctly applied in the DNA laboratory and ensuring that validation studies are satisfactorily concluded before implementing a new technique.

The DNA technical leader should be accessible to the examiners and reporting officers.

7.3 Examiners and Reporting Officers should have:

7.3.1 At a minimum a NQF 6 level qualification or equivalent qualification in biology, chemistry or a forensic science related area and must have successfully completed a combination of undergraduate and in-house training covering the relevant subject areas of biochemistry, genetics and molecular biology (molecular genetics, recombinant DNA technology) or other subjects which provide a basic understanding of the foundation of forensic DNA analysis.

7.3.2 Mentorship

Reporting officers should after being assessed as competent after completing an internal in-service training programme or assessed for Recognition of Prior Learning (RPL) participate in a mentorship programme, under the supervision of an experienced DNA examiner. A reporting officer is not required to participate in a mentorship programme for each part of the DNA analysis process, provided that he/she does not perform testing in the DNA process.

Examiners performing a specific test in the DNA analysis process (e.g. PCR or electrophoresis or allele designation or application of an expert system for allele designation and confirmation) should participate in a relevant mentor programme prior to beginning independent casework analysis in the specific DNA process.

Examiners must participate in a mentorship programme as defined by the quality assurance programme of the specific forensic laboratory before becoming an independent signatory.

7.3.3 Successfully completed a qualifying test (written and/or oral assessment and/or practical assessment and may include a proficiency test) before beginning independent casework responsibilities.

7.4 Technicians/laboratory assistants should have:

7.4.1 On the job training specific to their job function(s) which is commensurate with the tests that they should perform.

7.4.2 Successfully completed a competency test before assuming casework examination responsibilities.

7.5 Laboratory support personnel (administrative) should have:

Training, education and experience commensurate with their responsibilities as outlined in their job description.

## 8. Facilities and Environmental Conditions (ISO/IEC 17025 Section 6.3)

8.1 The laboratory should have a facility that is designed to provide adequate security and minimize contamination. The laboratory should ensure that:

8.1.1 Access to the laboratory is controlled and limited.

8.1.2 Amplified DNA product is generated, processed and maintained in a distinct area, separate from that used for presumptive testing on exhibits; DNA extractions and PCR set-up.

8.1.3 Procedures for monitoring, cleaning and decontamination of facilities and equipment are followed.

## 9. Evidence Control (ISO/IEC 17025 Section 7.4)

9.1 The laboratory should have and follow an evidence control system to ensure the integrity of physical evidence. This system should ensure that:

9.1.1 Evidence is marked for identification (e.g. laboratory and sample identifier or bar code reference).

9.1.2 Chain of custody of all evidence is maintained (e.g. seals and transfer notes or work lists and barcoding)

9.1.3 Procedures that minimize loss, contamination, and/or deleterious change of evidence are followed.

9.1.4 There are secure areas for evidence storage (e.g. areas with electronic access control).

9.2 Where possible, the laboratory should retain or return a portion of the evidence sample or extract.

The laboratory should have a procedure for storing such evidence sample/extract(s) that minimizes degradation and allows for easy retrieval.

*Discussion*
*It is recognised that forensic biological stains are often of a limited size and cannot be divided into different portions or retained for further analysis. The stain/scrapings/extract of body fluid may then be directly submitted for DNA isolation and then split for further analysis (duplication by extraction split sample). Although duplication of analysis is not prescribed, it is recommended where the size of the stain/scrapings/extract/sample permit, that a portion thereof is kept for independent re-analysis by the same laboratory or external laboratory or by the defence.*

## 10. Selection, verification and validation of methods (ISO/IEC 17025 Section 7.2)

10.1 The laboratory should use validated methods and procedures for forensic casework analyses.

Novel forensic DNA methodologies should undergo developmental validation to ensure their accuracy, precision and reproducibility. Developmental validation is normally performed by the manufacturer or developer of DNA testing kits for use in forensics. The developmental validation should include documentation on:

10.1.1 Species specificity, locus specificity, sensitivity and stability.

10.1.2 Population data:

a) Details of allele and genotype frequency distributions in the relevant population groups.
b) Where appropriate, the results of tests for Hardy-Weinberg equilibrium.

10.1.3 Internal validation:

a) The procedure should be tested using known and/or non-probative evidence samples. The laboratory should monitor and document the reproducibility and precision of the procedure using one or more DNA controls.

b) The laboratory should establish match criteria based on empirical data.

c) Before the introduction of a procedure into forensic casework, the analyst or examination team should successfully complete a qualifying test.

d) Significant modifications made to analytical procedures should be documented and subject to validation testing.

10.1.4 Where methods are not specified, the laboratory should, wherever possible, select methods that have been published by appropriate technical organizations or that have appeared in relevant scientific texts or journals.

The intensive participation in proficiency testing or use of an international calibrator or analysis of ten samples from different individuals should be a minimum criterion by which a DNA method may be validated.

## 11. Analytical Procedures (ISO/IEC 17025 Section 7.2, 6.6 and 7.7)

11.1 The laboratory should have and follow written analytical procedures approved by the laboratory management / specialist support analysts / reporting officers / examiners / technical leader, where applicable.

11.1.1 The laboratory should have a standard operating protocol for each technique used.

11.1.2 The procedures should include details regarding reagents, sample preparation, extraction, equipment, and controls that are standard for DNA analysis and data interpretation.

11.2 The laboratory should use reagents that are suitable for the methods employed.

11.2.1 The laboratory should have written procedures for documenting commercial supplies and for the in-house formulation of reagents.

11.2.2 Reagents should be labelled with the identity of the reagent, the date of preparation or expiration and the identity of the individual who prepared the reagent.

The laboratory should identify critical reagents and ensure the application of appropriate quality control such as visual inspection. These critical reagents include but are not limited to:

11.2.2.1 Restriction enzymes
11.2.2.2 Commercial kits for performing genetic typing
11.2.2.3 Agarose for analytical RFLP gels
11.2.2.4 Membranes for Southern blotting
11.2.2.5 Molecular weight markers used for RFLP sizing
11.2.2.6 Primer sets
11.2.2.7 Internal lane standards

11.3 The laboratory should have and follow a strategy to ensure that they are working within the optimal parameters for a particular procedure that was validated using appropriate methodology.

*Discussion:*

*Reference blood samples do not have to be quantified before PCR, if the Quantiblot is used provided that the laboratory has validated the procedure for the DNA analysis of these samples. The efficiency of PCR amplification is influenced by the quality (degree of degradation), purity, and total quantity of DNA in a sample. Lack of amplification is usually due to highly degraded DNA, the presence of PCR inhibitors, insufficient DNA quantity, or any combination of these factors. Although it is important to quantify a sample before PCR, a laboratory must understand the shortcomings of the particular quantification method employed. (For example, the Quantiblot method often underestimates the quantity of DNA. A negative quantification result may result even if sufficient DNA is available. Hence samples with negative quantification results may be submitted for STR PCR when using the Quantiblot method. It is then more useful and is acceptable to determine an appropriate minimum peak height threshold in the STR electropherogram based on the laboratory's own results using low amounts of input DNA. It is acceptable to evaluate the electropherogram and deduce the quality of a sample submitted for STR PCR analysis). Hence it is acceptable to perform STR PCR analysis on samples without a positive quantification value and without running an agarose gel.*

*There are currently no quantification kits for animal DNA commercially available. Standard methods of DNA quantification require a relatively large quantity of sample that may not be available for forensic samples. Validation using a modified Taguchi method provided guidelines with regard to sample quantity (optimum, minimum and maximum number of hairs, uL of blood, size of blood stain, etc.) of a particular quality that should be extracted for DNA profiling in order to obtain reliable results. Using the electropherogram minimum peak height threshold, the quality of a sample submitted for STR PCR analysis can be evaluated.*

11.4 The laboratory should monitor the analytical procedures using appropriate controls and standards.

11.4.1 The following controls should be used in RFLP casework:

a) Quantification standards for estimating the amount of DNA recovered by extractions.

b) Molecular weight size markers that bracket known and evidence samples with appropriate size fragments between the smallest and largest fragments.

c) Procedures to monitor the completeness of restriction enzyme digestion.

d) The concentration of high molecular weight DNA on crime samples must be determined.

11.4.2 The following controls should be used for PCR casework analysis:

a) Quantification standards for estimating the amount of nuclear DNA recovered from samples (excluding reference blood samples).

b) Positive and negative amplification controls/reagent blanks.

c) Allelic ladders and/or size markers for allelic identification when available.

d) Internal lane standards where appropriate.

11.5 The laboratory should review its DNA procedures annually or whenever substantial changes are made to the protocol(s) against appropriate and available national standard reference material or standard material traceable to a national or international standard or against an international calibrator. This reference sample is obtained from an individual whose DNA profile has been typed and verified by a national or international accredited laboratory and/or the manufacturer of the DNA kit.

The laboratory should have and follow general guidelines for the interpretation of data.

11.5.1 The laboratory should verify that all control results are within established tolerance limits.

11.5.2 Where appropriate, visual matches should be supported by numerical match criteria.

11.5.3 For a given population(s) and/or hypothesis of relatedness, a statistical evaluation should be made using a population database appropriate to the calculation. Calculations for all four major population groups (racial) to which possible suspects can belong should be made.

*Discussion:*

*Laboratories should be encouraged to retain a portion of the sample (where sample size permits) for independent re-testing (either by another member or by another laboratory) or retain the extract where the sample size was limited. Acceptable duplication includes a split of an extract sample (where the sample is limited).*

*When statistical independence of alleles at the loci tested by the laboratory exists, the frequency of a multi-locus DNA profile can be estimated by multiplying the genotype frequencies at each locus. A 95% Confidence Interval is applied to this answer and the most conservative value reported.*

*If the possible contributors of the evidence sample include relatives of the suspect, DNA profiles of these relatives should be obtained. If these profiles cannot be obtained, the probability of finding the evidence profile in those relatives should be calculated as described in appendix B.*

## 12. Equipment (ISO/IEC 17025 Section 6.4)

12.1 The laboratory should use equipment suitable for the methods employed.

12.2 The laboratory should have a documented programme for the calibration of instruments and equipment.

12.2.1 Where available and appropriate, standards traceable to national or international standards should be used for calibration.

12.2.1.1 Where traceability to national standards of measurement is not applicable, the laboratory should provide satisfactory evidence of correlation of results with such standards.

12.2.1.2 The frequency of calibration should be documented for each instrument requiring calibration.

12.2.1.3 The calibration criteria as described in the attached Appendix A will be the minimum criteria for the calibration of critical equipment.

12.3 The laboratory should have and follow a programme to ensure that instruments and equipment are properly maintained.

12.3.1 Critical new instruments and equipment, or instruments and equipment that have undergone repair or maintenance, should be verified for accuracy and/or calibrated before being used in casework analysis.

12.3.2 Written records or logs should be maintained for maintenance and service performed on critical instruments and equipment.

## 13. Reporting the Results (ISO/IEC 17025 Section 7.8)

13.1 The laboratory should have and follow written procedures for taking and maintaining case notes to support the conclusions drawn in laboratory reports.

13.1.1 The laboratory should maintain, in a case record or on an electronic system, descriptions of exhibits received and examined, cuttings or electronic format of relevant markings where possible, all correspondence and reports issued, and final results. The raw data and final results may be kept in either hard copy in a separate file or in the case file or in electronic format.

13.1.2 Reports according to written guidelines should include:

13.1.2.1 A case identifier

13.1.2.2 A description of evidence examined

13.1.2.3 A brief description of the methodology

13.1.2.4 The names of loci typed

13.1.2.5 The results and/or conclusions

13.1.2.6 An interpretative statement (either quantitative or qualitative)

13.1.2.7 The date of issue

13.1.3 A signature and title, or equivalent identification, of the person(s) accepting responsibility for the content of the report.

13.1.4 The laboratory should have procedures for the release of case report information.

13.1.5 A tracking record of the case report should be maintained (electronically or paper trail).

## 14. Review (ISO/IEC 17025 Section 8.7)

14.1 The laboratory should conduct administrative and technical reviews of case files and reports to ensure that conclusions and supporting data are reasonable and within the constraints of scientific knowledge.

14.1.1 The laboratory should establish a policy on the number of cases to be reviewed and this may vary according to the competency level of the particular examiner.

14.1.2 The laboratory should have a mechanism in place to address unresolved discrepant conclusions between analysts and reviewer(s).

14.2 The laboratory should have and follow a programme that documents the monitoring of the testimony of each examiner and laboratory assistant.

## 15. Proficiency Testing (ISO/IEC 17025 Section 7.7)

15.1 Examiners and other personnel designated by the technical support analyst who are actively engaged in DNA analysis should undergo, at regular intervals not exceeding two years (2) years, external proficiency testing in the tests normally performed in their day-to-day activities. Reporting Officers should be required to participate as signatories in proficiency tests and are not required to do actual analysis testing provided that they do not perform any tests on the samples.

15.1.1 The laboratory should maintain the following proficiency test records:

15.1.1.1 The test set identifier.

15.1.1.2 The identity of the examiner.

15.1.1.3 The date of analysis and completion.

15.1.1.4 Copies of all data and notes supporting the conclusions.

15.1.1.5 The proficiency test results.

15.1.1.6 Any discrepancies noted.

15.1.1.7 The corrective actions taken.

15.1.2 The laboratory should establish at a minimum the following criteria for the evaluation of proficiency tests:

15.1.2.1 All reported inclusions are correct or incorrect.

15.1.2.2 All reported exclusions are correct or incorrect.

15.1.2.3 All reported genotypes and/or phenotypes are correct or incorrect according to consensus genotypes/phenotypes or within established empirically determined ranges.

15.1.2.4 All results reported as inconclusive or un-interpretable are consistent with written laboratory guidelines. The basis for inconclusive interpretations in proficiency tests must be documented.

15.1.2.5 All discrepancies/errors and subsequent corrective actions must be documented.

15.1.2.6 All final reports must be graded as satisfactory or unsatisfactory. A satisfactory grade is attained when there are no analytical errors for the DNA profile typing data. Administrative errors should be documented and corrective actions taken to minimize such error in the future.

15.1.2.7 All proficiency test participants should be informed of the final test results.

*Discussion:*

*Although it is important to participate in regular proficiency tests, the rationale behind participation in proficiency testing is to add value (scientifically and financially). It should not just be employed for the sake of participating in proficiency tests within the minimum prescribed time frames or to ensure that personnel participate in the tests that are employed in the laboratory. It is also not a requirement that whenever minor changes on tests (to which similar principles apply) or the use of a new substrate (for example the*

*introduction of the FTA method which requires purification steps) that each examiner participate in a proficiency test before employing the adapted or new method, provided that: i) they are conversant with the methodology; and ii) validation studies have demonstrated that the method is performed as prescribed and results obtained by the laboratory are within the accepted parameters of the test.*

## 16. Corrective Action (ISO/IEC 17025 Section 8.7)

16.1 The laboratory should establish and follow procedures for corrective action whenever proficiency testing discrepancies and/or casework errors are detected.

16.2 The laboratory should maintain documentation of the corrective action taken.

## 17. Internal Audits (ISO/IEC 17025 Section 8.8)

17.1 The laboratory should conduct audits annually in accordance with the standards outlined herein.

17.1.1 Audit procedures should address at a minimum:

(a) Quality assurance programme
(b) Organization and management
(c) Personnel
(d) Facilities
(e) Evidence control
(f) Validation
(g) Analytical procedures
(h) Calibration and maintenance
(i) Proficiency testing
(j) Corrective action
(k) Reports
(l) Review
(m) Previous audits

17.1.2 The laboratory should retain all documentation pertaining to audits in accordance with legal and agency requirements.

## 18. Safety

The laboratory should provide a safe working environment.

## 19. Externally provided products and services (ISO/IEC 17025 Section 6.6)

19.1 A laboratory operating under the scope of these standards will require certification of compliance with these standards when a subcontractor performs forensic DNA analyses for the laboratory.

19.1.1 The laboratory should establish and use appropriate review procedures to verify the integrity of the data received from the subcontractor.

## Appendix A: Verification and/or Calibration of Critical Instruments

The following instruments/items of equipment are considered to be critical in DNA analysis and will be verified/calibrated according to an appropriate schedule:

**Analytical Balances:**

Annually – Perform calibration checks using calibrated weights traceable to a national standard.

Verify using own calibrated as needed – Service and calibration by a trained professional engineer.

**Autoclave:**

As Needed – Clean. Have serviced by a trained service engineer.

**Biological Safety Hoods:**

Biannually – Have authorized vendor service and calibrate.

Each Day of Use – Before and after each use, wipe down inside of hood with chlorinated bleach.

**Centrifuges:**

As Needed – Clean and replace brushes. Have serviced and calibrated by a trained service engineer.

**Electrophoresis Tanks**

Each Day of Use – Rinse with distilled water, and air dry.

As Needed – Wash with mild soap.

**Fume Hoods:**

As needed – Have serviced and calibrated by a trained service engineer.

**Freezers/Refrigerators:**

Weekly – Check temperatures. Where appropriate, change temperature charts.

**Heat Blocks:**

Weekly – Check and record temperatures.

**Hot Shaker Water Bath:**

Weekly – check for cleanliness and water level. Check and record temperature.

**Hybridisation Incubator:**

Each Day of Use – Check temperature with independent thermometer.

**Incubators:**

Weekly – Check and record temperatures.

**Orbital Shakers:**

As Needed – Lubricate and check brushes.

**Ovens:**

Weekly – Check and record temperatures.

**pH meter:**

Each Day of Use – Check and calibrate.

As Needed – Check solution in probe and top-up or replace.

**Pipettes/ Micropipettes:**

Annually – Check performance and calibrate.

**Stopwatch:**

Annually – Calibration traceable to a national standard.

**Thermal Cycler Verification Unit:**

Annually – Calibration traceable to a national standard.

**Thermal Cycler:**

Bi-annually – Run, where available, programme containing diagnostic test. If test fails, contact licensed service support personnel.

Annually – Perform and record temperature calibration and uniformity tests. If test fails, contact licensed service support personnel for service.

**Thermometers:**

Annually – Check calibration of each thermometer in use against a national standard.
Check calibration prior to putting into service.

**Type 1 Water System:**

Annually – Have cleaned and inspected by a trained service engineer.

Before use – Visually check conductivity.

**Vacuum Pumps:**

As Needed – Clean and flush.

**DNA Sequencers:**

Gel-based systems – Sensitivity test as part of an annual service.
Capillary systems – Annual verification and calibration with a baseline sensitivity test for capillary systems

Annually – Check optical alignment and do matrix run

**Spectrophotometer:**

Annually- Check optical readings

***NOTE**: Refer to the manufacturer's instrument/equipment operating manual for maintenance and calibration of each piece of equipment, unless directed to do otherwise. If an instrument/item of equipment is out of calibration according to the manufacturer's specifications, the instrument/item of equipment will immediately be taken off-line for repair.*

**Liquid handlers**

Annually – Check and calibrate

**Real-time PCR Instrumentation**

Bi-annually – Run, where available, programme containing diagnostic test. If test fails, contact licensed service support personnel.

Annually – Perform and record temperature calibration and uniformity tests. If test fails, contact licensed service support personnel for service.

## Appendix B : Population Statistics

**Recommandation 1 : Population group:**

Forensic and Paternity laboratories have defined the following four major population groups: Asian, Caucasian, Black and Coloured.
Calculations for all four major population groups to which possible suspects can belong are stated when reporting match probabilities. In parentage calculations only the one population group to which the involved parties belong, is stated.

**Recommendation 2: Heterozygote and Homozygote profiles:**

The following Hardy Weinberg formulas are applied to the following loci:
Heterozygote profiles : $p^2$
Homozygote profiles. 2pq
Where p and q are the frequencies of the respective alleles.

**Recommendation 3: Multiple locus profiles:**

Given the statistical independence of alleles at these loci in the NDSD, the frequency of a multi-locus genotype can be estimated by multiplying the genotype frequencies at each locus (product rule). A 95% Confidence Interval is applied to this answer and the upper limit (most conservative value) reported on.

**Recommendation 4: Minimum allele frequencies:**

Where alleles occur at a low frequency (less than five times in a population database), a minimum frequency of 5 divided by 2N, where N equals the number of individuals in the database, is assigned to these alleles.

**Recommendation 5: Biological relationships, where requested:**

If the possible contributors of the evidence sample include relatives of the suspect, DNA profiles of those relatives should be obtained. If these profiles cannot be obtained, the probability of finding the evidence profile in those relatives should only be calculated if requested by court.
The Forensic Science Laboratory, for example, applies the following formulas:

Genotype of suspect Probability of same genotype in a relative
Homozygote: AiAi pi2 + 4pi (1 – pi) F,
Heterozygote: AiAj 2pipj + 2(pi + pj – 4pipj) F.
For parent and offspring, F = ¼; for half-siblings, 1/8; for uncle and nephew, 1/8; for first cousins, 1/16.
For full siblings, the following formulas should be used:
Homozygote: AiAi (1 + 2pi + pi2))/4,

Heterozygote: AiAj (1 + pi + pj + 2pipj)/4.

**Appendix C: Re-testing of Samples and acceptance of DNA Results by Courts of Law**

It is recommended that where the size of the stain/scrapings/extract/sample permit, a portion thereof is kept for independent re-testing by the same laboratory or external laboratory or by the defence.

It is recognised that forensic biological stains are often of a limited size (e.g. swabs and smears etc), and cannot be divided into different portions or retained for further analysis. The stain/scrapings/extract of body fluid may then be directly submitted for DNA isolation and then split for further analysis (duplication by extraction split sample). The DNA isolated (if quantity thereof permits) should be available for re-testing.

No matter what the history of the laboratory or the participation record of the laboratory in a proficiency testing or the successful implementation of a Quality System, this will not guarantee that a DNA result is correct.

The best method of determining the accuracy of a DNA result is to subject the samples for re-testing.

Whenever DNA results are disputed or the competence of a laboratory is questioned, the defence or the Courts of Law should be encouraged to request re-testing of the samples by an independent laboratory.

## ADDENDUM 1: Amendment Record

| Proposed By: | Section | Change |
|---|---|---|
| STC | Whole document | Aligned the document to the new ISO/IEC 17025:2017. |