STANDING COMMITTEE
FOR QUALITY AND COMPETENCE (QCC)

With the financial support from the Prevention of and Fight against Crime Programme of the European Union
European Commission – Directorate-General Home Affairs

Guidelines for the single laboratory Validation of Instrumental and Human Based Methods in Forensic Science

DOCUMENT TYPE:	REF. CODE:	ISSUE NO:	ISSUE DATE:
GUIDANCE	QCC-VAL-002	001	10/11/2014

Supported by EU project ' General Programme on Security and Safeguarding Liberties/specific programme on prevention of and fight against crime (ISEC)' HOME/2009/ISEC/MO/4000000798. Theme: Sustainable quality with European Forensic Science (SQWEFS)

Project Team

Thierry De Baere (National Institute for criminalistics and criminology, Belgium), team-leader
Wiktor Dmitruk (Central Police Forensic Laboratory, Poland)
Bertil Magnusson* (SP Technical Research Institute of Sweden, Sweden)
Didier Meuwly (Netherlands Forensic Institute, Netherlands)
Geraldine O'Donnell (Forensic Science Laboratory, Ireland)
**Representing Eurachem, eurachem.org*

Preface

This guide was developed with support and funding from ENFSI and the European Union.
A substantial volume of literature is available to assist laboratories in the task of validating methods but little has been tailored to suit the diverse needs of forensic science. The purpose of the guide is:

- to clarify the requirements for performing validation exercises for instrumental and human based methods, here in after referred to as forensic methods.
- to highlight the similarities as well as the major differences in the validation strategies to be used in forensic methods.
- to illustrate, by examples, various ways of validating methods.
- to harmonize the validation procedures of forensic methods for accreditation to an international standard. As ENFSI recommends the use of ISO/IEC 17025 for forensic labs, reference to this standard is made in the document, however the recommendations are also applicable when other standards are used.

Contents

1 Scope

Forensic science encompasses a wide variety of disciplines, each of which may have traditionally had varied approaches to best practice in validation. The scope of this work is to setup guidelines towards best practice for both validation and re-validation of existing methods and present some examples.

The strategy for validation is to check that test results are fit for their intended purpose. The forensic practise consists of instrumental (qualitative and quantitative) and human-based methods. For instrumental methods the validation is more centred on checking instrumental performance. The more the methods are human based the more the strategy is centred on the demonstration of the competence of personnel.

Instrumental quantitative analysis can be exemplified by the determination of the concentration of blood alcohol or of cocaine in a bulk sample. Instrumental qualitative analysis can be exemplified by the identification of a constituent in blood using a colour test or by the identification of an accelerant in fire debris. Human based methods can be exemplified by the analysis and the comparison of patterns such as fingermarks and toolmarks. While the above are typical examples there can be others that fall into more than one category such as fibres examination.

Estimation of uncertainty is outside the scope even if in most cases there is an overlap between uncertainty and validation (reference to uncertainty is however covered in the example for the determination of cocaine in a powder, example 5.1.3). Also outside the scope of this project is the area of interpretation which will be dealt within a 2010 EU Monopoly funded project. In order to draw forensic conclusions interpretation will be needed and this process will need to be further validated.

The scope is mainly on the single laboratory validation and on the analytical part of the forensic process. However for human based methods the guide also covers the comparative process as well verification[1] of test results.

2 Introduction

2.1 Why validate

The objective of forensic methods is to obtain results with a measurement quality relevant for the criminal justice system. That those results must be consistent, reliable and accurate is without question. This means that during the process of the introduction or implementation of a new forensic method a specific step must be taken to prove in an objective way that the method is suitable for its intended use. This step is called validation.

The demand for validated methods has been driven by customers (e.g. police, prosecuting authorities), accreditation bodies (i.e. as part of ISO accreditation requirement), and forensic community. In this regard ENFSI has promoted quality assurance and the achievement of

[1] Note: The term verification here refers to the result. This term can also relate to the method see further section 2.2.

accreditation across the whole forensic process. This means that whereas validation is now an integral part in the development and implementation process of a new method that it is also a requirement for laboratories to re validate established methods to gain accreditation.

2.2 When to validate

According to ISO/IEC 17025[1] 5.4.5.2 *The laboratory shall validate non-standard methods, laboratory-designed/developed methods, standard methods used outside their intended scope, and amplifications and modifications of standard methods to confirm that the methods are fit for the intended use. The validation shall be as extensive as is necessary to meet the needs of the given application or field of application. The laboratory shall record the results obtained, the procedure used for the validation, and a statement as to whether the method is fit for the intended use.*

For standardised methods such as ISO, ASTM a full validation is not necessary but the laboratory needs to *verify* the in-house performance of the method as detailed in ISO/IEC 17025 5.4.2 …*The laboratory shall confirm that it can properly operate standard methods before introducing the tests or calibrations.* This is called verification according to VIM [2]. Also verification is required when there is an important change such as change of instrument, relocation of instrument etc.

The overall process of implementing a new method, starting with a particular analytical problem and ending with a method in use in the laboratory is described in Figure 1.

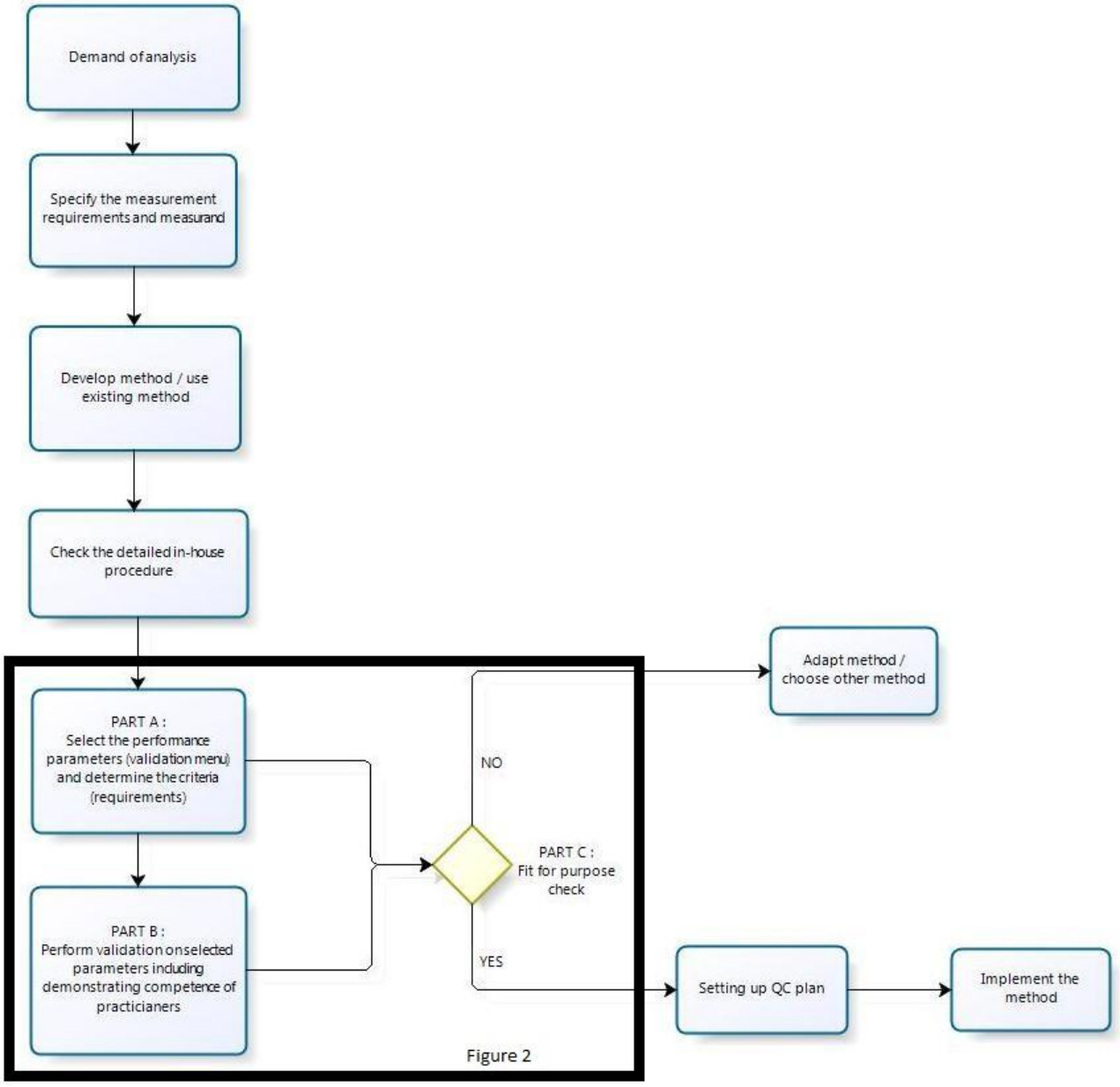

Figure 1 Overall process of implementing a method in the laboratory. The validation part of implementing a method is marked with a box (and presented in detail in figure 2)

While the process is self-evident from the figure, an important aspect for forensic methods is demonstrating the competence of practitioners, especially for human based methods where it can be challenging. It should also be noted that when the method has been in use for a period of time it can be useful to re-evaluate the method based on the experience gained from quality control and case work.

2.3 How to validate

Once the initial method development is finished the laboratory should document the measurement procedure in detail before starting the validation. An overview of the validation process in the laboratory is shown in the following figure 2.

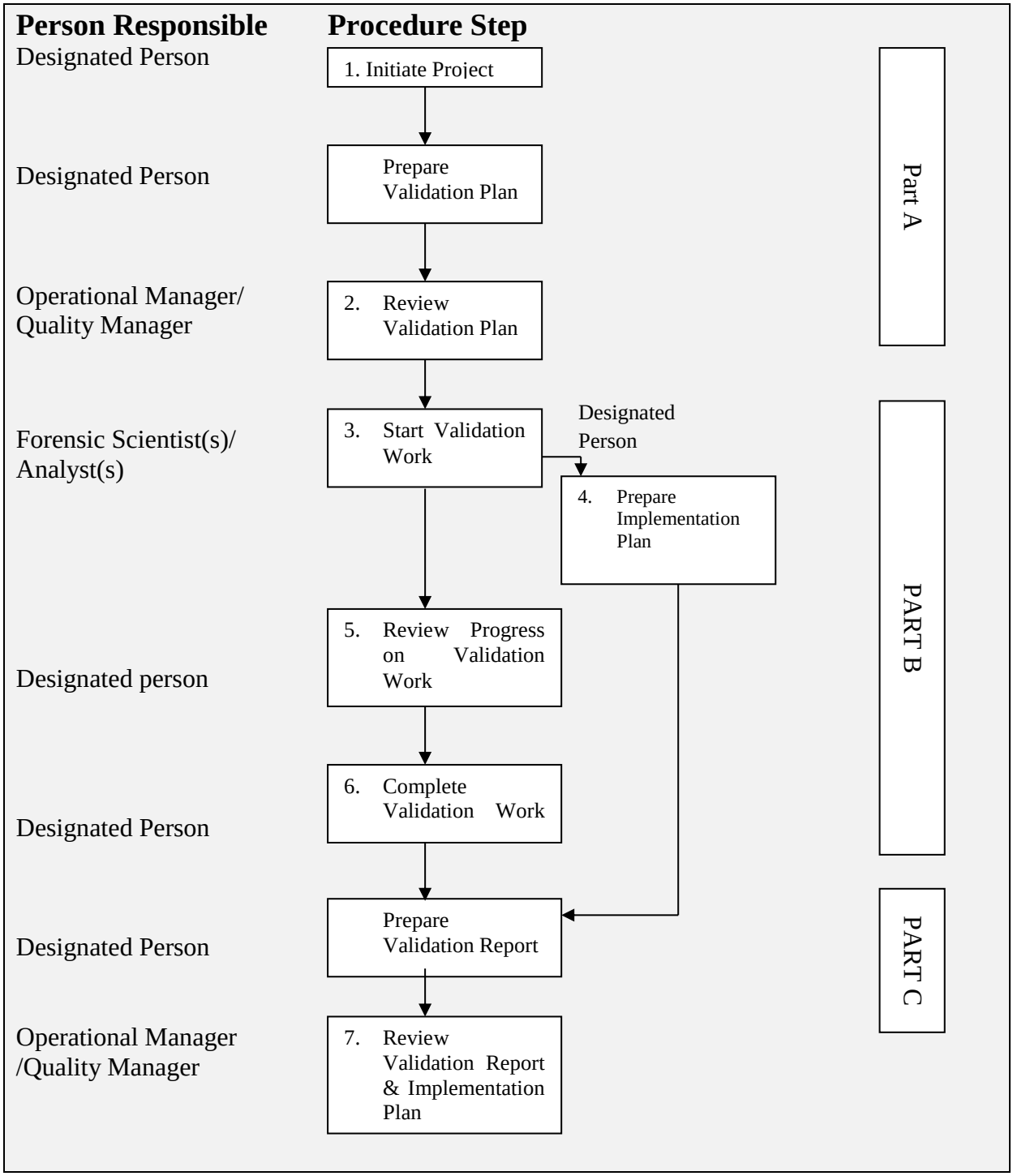

Figure 2 Overview of validation process

The detailed implementation of a validation project

The different steps (1 to 7 in the figure) of a validation project in a laboratory are described in detail.

Part A

1. Initiate project:

1.1 The validation project is initiated and a designated person is appointed to produce the validation plan.

1.2 The designated person will draw up the validation plan. This should contain the following four elements:

i The **laboratory and customer requirements,**

ii The performance parameters, that will need to be used to ensure that the outputs meet the laboratory and customer requirements. The selected parameters will be dependent on the technique or process under consideration, but should in general address, as appropriate:

- Sampling
- Precision
  - Repeatability
  - Within-lab reproducibility
- Bias
  - Matrix/substrate effects
  - Specificity
- Working range
  - Limit of detection/sensitivity
  - Linearity
- Robustness
  - Environmental susceptibility
- Competency of personnel

For the explanation of the different terms used in the validation menu we recommend consulting Chapter 4 in the Eurachem Guide Terminology in Analytical Measurements [3].

iii The Acceptance Criteria to be used to assess whether the performance parameters have been met.

Note: It is critical to the success of the validation that the acceptance criteria are set as specific as possible prior to the commencement of the validation work.

iv The **design of the validation tests** should also be considered at this stage to ensure that they are as objective as possible.

2. Review validation plan

The validation plan will be reviewed by the operational manager/quality manager to ensure that all the relevant technical and customer quality issues and any other relevant considerations are to be adequately addressed.

Part B

3. Start validation work

The validation tests should be carried out as soon as the relevant stage of development has been completed. The tests performed should be those specified in the validation plan. New or additional tests should not be introduced, or planned tests not performed, unless authorised by the operational manager for the designated area.

4. Prepare implementation plan

The designated person has to consider what needs to be in place before the new technique or process can be implemented and how the implementation will be carried out. **These considerations should be included as part of the final validation report.**

Where appropriate the following should be addressed:

- The staff training plan and the arrangements for competence assessment and proficiency testing
- The protocols for calibration, monitoring and maintenance of any equipment
- The supply and traceability of any standards/reference materials
- The supply and quality control of key materials and reagents
- The SOP documents for the technique or process and the assessment/interpretation/reporting of results
- Anti-contamination protocols
- Any special requirements associated with health and safety

5. Review progress on validation work

Regular review of the outcomes of the development work against the acceptance criteria should be carried out. Where the criteria are not met the designated person will advise what further tests should be carried out or what modifications to the technique or process are required.

If the results of the validation tests lead to a significant modification of the project, it may be necessary to re-design the validation plan.

PART C

6. Complete and prepare validation report

The designated person following completion of all aspects of the validation work will produce a validation report. It is important that this includes all the information needed to facilitate independent assessment of the fitness for purpose of the technique or process. A summary of the raw experimental data will normally suffice. But the raw data must be available. A statement of fitness for purpose regarding the method is added in the report.

7. Review validation report and implementation plan

The final validation report and implementation plan will be reviewed and approved at least by the operational manager for the designated area or the quality manager. The method, if necessary updated with findings learned during validation must be signed of formally as deemed fit for use.

3 Instrumental-Based Methods

Method validation is usually considered to be very closely tied to method development, indeed it is often not possible to determine exactly where method development finishes and validation begins. Many of the method performance parameters that are associated with method validation are in fact usually evaluated, at least somewhat, as part of method development. Once the initial method development is finished the laboratory should document the measurement procedure (SOP) in detail. Before the validation starts there should be a check of this detailed in-house procedure – see Figure 1

This section describes in a general manner the validation process for methods that are primarily instrumental based. As detailed in the introduction the validation process consists of mainly three parts; Part A) setting up a validation plan with criteria for accepting the performance of the method, Part B) carrying out the experimental work or collecting previous data from method development and evaluation of the results obtained Part C) a summary statement of the outcome of the validation –i.e. if the method is fit for the intended purpose. In the guide we introduce a validation report template (Appendix 1) for instrument based methods which consists of the above mentioned three parts. The examples are presented using this template.

3.1 Validation plan

It is implicit in the method validation process that the studies to determine method performance parameters are carried out using equipment that is within specification, working correctly, and adequately calibrated. Likewise the operator carrying out the studies must be competent in the field of work. The validation plan is set up with the template (Appendix 1). In order to illustrate the template, determination of cocaine (Example , 5.1.3) is used as an example below.

Specify the measurement procedure, analyte, measurand and units

The measurement procedure is the written document (Standard Operating Procedure, SOP) to be validated. The detailed definition of the measurand is important when setting up a validation plan when the analyte can exist in different forms such as: bound or unbound; dissolved or in particulate form, inorganic or organometallic; or different oxidation states.

Table 1 Specification of the measurement procedure - example determination of cocaine in street samples

The measurement procedure	Quantitative analysis of cocaine using GC-FID
Analyte	Cocaine
The measurand	Concentration of cocaine in powdered samples
Unit	Weight %

Specify the Scope

The scope specifies the sample types and concentration ranges that are to be covered by the method.

Table 2 Specification of the scope - example determination of cocaine in street samples

Sample type – matrix	Street drugs samples delivered to the laboratory
Measuring range	1 - 100 weight%
Intended use of the results	Court Reports and Intelligence reports to Police force

Requirement on the measurement procedure

Faced with a particular analytical problem, ideally the laboratory should firstly agree with the customer an analytical requirement which defines the performance parameters criteria that a method must meet to be deemed suitable for solving the analytical problem. In some cases regulatory bodies have published analytical requirements.

Table 3 Parameters to be validated - example determination of cocaine in street samples

Parameters to be validated			*Criteria - Value requested*
Precision	*Repeatability*	✔	RSD_r 5 %
	Within-lab reproducibility	✔	RSD_{Rw} 7 %
Trueness	*Test for bias*	✔	Bias < 10 % relative
	Selectivity	✔	Test for possible interference
Measurement range	*LOD or LOQ*	✔	LOQ 1 % Cocaine
	Linearity		Residual, max deviation 2 % relative
Ruggedness(Robustness)			Routine GC method – not necessary
Measurement uncertainty		✔	Expanded uncertainty < 20 % relative

Origin of the Measurement Procedure

The origin of the procedure will influence the extent of the validation plan. Where a method has been validated by a standards approving organisation, such as ISO, CEN or ASTM, the user will normally need only to verify published performance data and/or establish performance data for their own use of the method - verification. This approach, hence, reduces the planned workload in the validation for the laboratory using the method.

Table 4 Origin of the measurement procedure - example determination of cocaine in street samples

		VALIDATION/VERIFICATION
New in-house method	✔	*Full*
Modified standardised/in-house method		*Partial*
Standardised method		*Verification - "Simple validation"*

In the following sections section we discuss in detail the performance parameters for quantitative (3.2), qualitative (3.3) and DNA analysis (3.4).

3.2 Performance parameters - Quantitative methods

For single laboratory validation of quantitative methods we recommend the validation guidelines from IUPAC [4] and Eurachem [5] that can be downloaded for free. The Eurachem guide describes in detail how to select the different parameters for validation. Below is a short description of the different performance parameters.

In this Guide we use a template (Annex A) for setting up the validation and carrying out the validation work including reporting. For each parameter chosen to be validated the template has the following headings:

1) Description,
2) Demand (requirements)
3) Experiment (to be carried out)
4) Evaluation (of the validation results)
5) Results
6) Conclusion about fit for intended purpose

3.2.1 Precision

Precision is normally determined under specified measurement conditions. The two most common precision measures in a single laboratory validation are repeatability and within-laboratory reproducibility (also called intermediate precision). In most cases the most useful estimate is the within-laboratory reproducibility which is the precision measured with different analysts and over extended timescales, within a single laboratory. Note that a "single laboratory" can consist of many instruments and also different locations within an organisation. Precision is usually stated in terms of standard deviation or relative standard deviation (RSD). The precision is generally dependent on analyte concentration, and so should be determined at a number of concentrations and if relevant, the relationship between precision and analyte concentration should be established. Note that precision is only related to the spread in the results and is expressed in standard deviation.

3.2.2 Trueness – Bias

Trueness is an expression of how close the mean of a set of laboratory results is to the reference value. The reference value can come from a certified reference material (CRM), from a reference measurement procedure or in some cases from the assigned value in a proficiency test. Trueness is normally expressed in terms of measurement bias. The bias is estimated from the difference between the mean value of several measurement results preferable obtained under within-laboratory reproducibility conditions and a reference value**.** Trueness can also be investigated as selectivity e.g. by measuring bias at different levels of known interferences and in different matrices.

3.2.3 Measurement range

The measuring range is an interval of the concentration, which can be measured with a specified uncertainty using the method. Other terms commonly used for this term are measuring interval. The lower limit of the measuring range is often considered to be the limit of quantification, LOQ. Figure 3 illustrates the relationship between some of the key terms related to "measuring range". Note that this measurement range refers to the range of

concentrations in the sample coming into the laboratory, i.e. the laboratory sample (i.e. the sample as received in the laboratory). This may be in contrast with the instrument working range which refers to the analyte concentrations in the test portions or test solutions actually measured (e.g. a solution injected into a gas chromatograph).

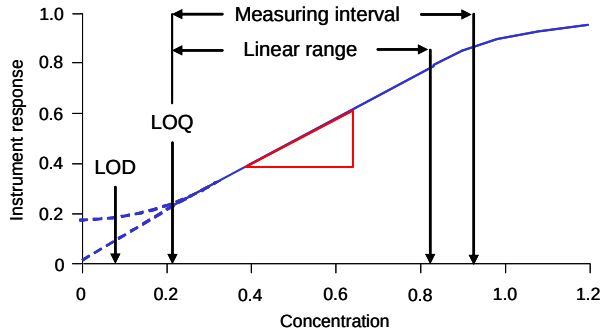

Figure 3 ***A calibration curve showing instrument response (indication) versus concentration; the measurement interval, linear range, LOQ and LOD are identified. The triangle illustrates the calculation of the sensitivity or the slope of the calibration curve (=Δindication/Δconcentration). Figure reproduced from ref [3] by permission of Eurachem.***

3.2.4 Ruggedness/Robustness

In any method there will be certain steps which, if not carried out sufficiently carefully, will have a severe effect on method performance. These steps should be identified and their influence on method performance can be evaluated using a ruggedness (robustness) test. This involves making deliberate variations to the method, and investigating the subsequent effect on performance. One general issue is cross-contamination

3.2.5 Measurement uncertainty

The expanded measurement uncertainty is normally what the laboratory reports to the customer. The expanded uncertainty provides an interval within which the value of the measurand is believed to lie with a high level of confidence (normally 95 %). If the laboratory has participated in proficiency testing the claimed uncertainty can be verified by comparing the difference between the result of the laboratory and the assigned value. This difference should be less than the expanded uncertainty at that concentration level in 19 cases out of 20.

3.3 Performance parameters – Qualitative methods

Qualitative analysis can be defined as *"Classification according to specified criteria"*. In every case, a decision can be made only if the properties of interest meet specified criteria. In analytical chemistry and related disciplines, the 'criteria' are understood to relate, in general, to information about the presence, composition and/or structure of materials. A qualitative method would normally give three possible results, positive, negative or inconclusive. The validation menu will therefore be different from a quantitative method. For single laboratory validation of qualitative methods we recommend the EU report MEQUALAN [6]. Below is given a short description of the different parameters on the validation menu.

3.3.1 Precision

For qualitative analysis the most useful estimate is the within-lab reproducibility which is the precision measured with different analysts, over extended timescales and, within a single laboratory. Precision for qualitative measurement can be stated in terms of a percentage of similar results obtained for test samples, e.g. 4 analyst analysing 25 samples the results was not the same for 2 samples where one analyst had a different result. In total 2 results out of 100 was different and the method performance in the laboratory would in 98 % of the cases give the same result when retested. The precision is generally dependent on analyte concentration, and so should be determined at different levels - see further measurement range.

3.3.2 Trueness – Bias

Trueness is an expression of how close the mean of a set of results is to the reference value. For qualitative analysis we can measure the false positive and false negative rates when we have prior information about the presence or absence of an analyte in a test sample.

For a given test method, the basic properties that need to be measured are the numbers of true positive (TP) and true negative (TN) results and the numbers of false positive (FP) and false negative (FN) results obtained on a range of samples. From these numbers, the fundamental measures of reliability *viz.* the *false positive* and *false negative* rates can be calculated.

Performance parameter	**Expression**
False positive rate	$\frac{FP}{TN + FP}$
False negative rate	$\frac{FN}{TP + FN}$

The false rates vary with the level of any analyte. For high levels of analyte, the likelihood of false negatives will be very low but at levels slightly above the threshold it will be relatively high. The false rates, both negative and positive, therefore depend upon the concentration of analyte in the population being sampled.

3.3.3 Measurement range

For a qualitative test there is a LOD or a threshold. Under this threshold the method will give negative results and over this threshold the method will give positive results. At the threshold the method will normally give inconclusive results. For further guidance see Eurachem Guide [5].

3.3.4 Ruggedness

Control of uncertainties in test parameters, such as times, temperatures, lengths etc, are vital for reliable qualitative testing. Typically, a laboratory is expected to control factors affecting the test result to within specified tolerances or in a validation demonstrate that the possible variation in individual test parameters have no significant influence on the outcome of the test.

3.3.5 Measurement uncertainty

Not directly applicable for a qualitative test.

3.4 Performance parameters – DNA analysis

For DNA analysis techniques we recommend the validation guidelines approved December 2012 from the scientific working Group on DNA Analysis Methods (SWGDAM) [7] that can be downloaded for free.

These guidelines set out and detail the two types of validation required to implement or modify technologies for forensic DNA analysis: 1) Validation and 2) Verification.

- Validation (developmental validation) is the demonstration of the accuracy (trueness and precision) of a procedure by the manufacturer, technical organisation, academic institution, government laboratory or other party. Developmental validation must precede the use of a novel methodology for forensic DNA analysis.
- Verification (internal validation) is carried out by each forensic DNA testing laboratory and is the in-house demonstration of the reliability and limitations of the procedure.

The recommended internal validation studies are summarized in the following table extracted from the SWGDAM guidelines.

	Extraction System[1]	Quantitation System[1]	Amplification System[1]/Reaction conditions	Detection system[1]
Known/Non probative samples	X	X	X	X
Precision and Accuracy: Repeatability	X	X	X	X
Precision and Accuracy: Reproducibility	X	X	X	X
Sensitivity studies	X	X	X	X
Stochastic Studies		X	X	X
Mixture Studies	X*	X*	X	X
Contamination assessment	X	X	X	X

[1]"System" includes methodology, chemistries, and instrumentation.

* Mixture studies will be required if the assay is intended to distinguish different contributors (male/female, major/minor, etc.).

For reference purposes two examples of the validation of DNA analysis techniques have been included as Example 5.1.1 Quantification of DNA Using Real-Time PCR and Example 5.1.5 DNA kit 17-loci STR PCR chemistry

A further source of information is the document developed by the ENFSI DNA WG "Recommended Minimum Criteria for the Validation of Various aspects of the DNA Profiling Process" issued November 2010 that can be downloaded for free [8]

4 Human-Based Methods

4.1 Introduction

Human based methods are used for forensic individualisation or association (see 7. glossary for the definitions). This section describes generic criteria to be fulfilled for a human-based method to be considered as validated for forensic casework.

Analysis, comparison and verification are three steps of a four-step procedure seeking forensic individualization or association: Analysis, Comparison, Evaluation and Verification[2] (ACE-V). The scope of this document applies to the validation of the three steps (AC and V, see figure 4). The requirements for the evaluation step are left outside of this document.

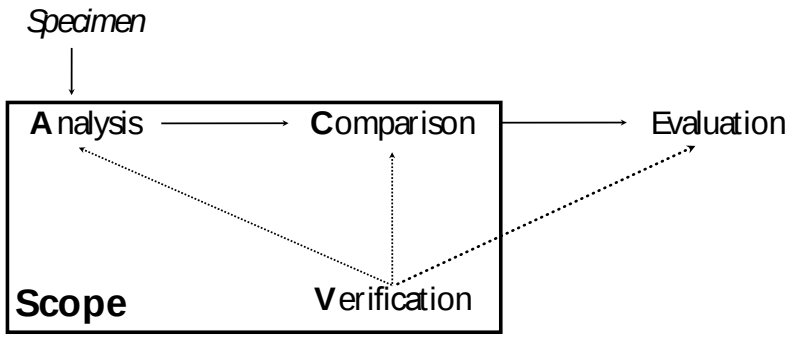

Figure 4: Scope of the document

The validation criteria can be taken and applied to forensic evaluation fields focusing on human individualization and association such as fingermark, earmark, bitemark, handwriting, speaker recognition, face recognition and hair or object individualization and association such as shoemark, toolmark, bullet and cartridge case or tyremark.
While this document will be confined to forensic individualization and association, some aspects of the validation criteria can be taken and also applied to identification (i.e. sperm head identification, identification of blood patterns), reconstruction (i.e. damage) and classification (i.e. it is a nylon fibre)

For all these forensic evaluation fields the test specimen is the one recovered from the crime scene and the reference specimen is the one collected from a suspected person (biometric data) or belonging (object). The method used in forensic casework for the analysis and comparison of trace and reference specimens are mainly instrumental, e.g. using analytical

[2] Note: the term verification here used for human-based method refers to the results and is not the same as used for instrumental based methods.

methods or mainly human-based, e.g. using the results of the observations[3] (feature extraction/analysis and feature comparison) of a forensic practitioner.

The validity of the analysis and comparison and the reliability of the results are highly dependent on the competence (theoretical knowledge, practical skills, professional attitude and controlled experience) of the practitioner, on the scientific methodology of the procedure chosen and on its proper use in casework.

4.2 Validation Process

Both human-based and instrumental methods involve method development, performance testing and the competence of the practitioner.
The key to understanding how to validate a human based method as against an instrumental method involves understanding what changes arise from substituting the instrument with the human.
For instrumental methods validation is achieved by direct testing of a method that has been fully developed and described. This is possible because the aspects of the method being tested are for the most part self-determining (i.e. retention time, mass spectrum). Typically standards are also available.
In contrast for human based methods the aspects of the method are not as self- determining (i.e. selection of critical features) and therefore method development is more demanding than that of instrumental. As there are no standards for human-based methods, before validation commences, it is necessary to make explicit and document the decisions/choices made for the selection of the critical features during the method development. These decisions and choices must be checked ahead of the performance testing of the method and this is referred to as the method development check.

4.2.1 Check of the detailed in-house procedure

The documentation of the feature (set) extracted/analysed from the specimens is crucial for human-based methods seeking individualisation and association, in order to demonstrate they are fit for purpose. Details on documentation of the feature (set) are covered in table 5. Also documented are the steps required for the method development check (table 6). For example for feature selection, description, classification and labelling, the method development check needs to show there is:
(1) documentation to cover justification of the features selected and their classification.
(2) documentation to show that the features have been adequately described and
(3) documentation to explain the labelling of the features (i.e. glossary)

The time frame for this phase will be dependent on steps such as the amount of scientific and peer-reviewed literature available relating to the empirical testing of the method, a structured training program, the existence of a Proficiency Testing and Collaborative Exercises programme (PT/CE) in the field and the amount of awareness about the results of PT/CE in the community. When these steps are in place the validation phase can be completed relatively quickly.
By way of contrast, for newer techniques where there is little literature, no training and no PT/CE program in place, this will be the most time consuming and challenging phase of the

[3] Observations can be visual, auditive, olfactory or sensitive but for sake of simplicity we will limit the examples to vision.

validation. It will require that practitioners establish the scientific foundation of the field, fostering the research and publication process in the laboratory and academic environment, setup a theoretical and practical training programme and a regular monitoring of the performance in form of PT/CE programmes reflecting operational conditions.

4.2.2 Performance testing of the method

For instrumental methods the performance parameters to be validated are for example precision, trueness, measurement range etc… as discussed in section 3 of this document
In contrast for human based methods performance testing involves validating the analysis, comparison and verification steps as discussed above at 4.1.
For performance testing of the human based method samples with **known** defined expectations must be used. The use of known samples as against case samples will ensure that the ground truth is known for the features in performance testing.

Analysis step

Validation of the analysis step requires demonstrating the accuracy including the precision (repeatability and reproducibility) of the feature extraction by the practitioners. This has to be tested in the absence of automation of the process. In the fingerprint field for example, the minutiae extraction from a reference specimen is automated, whereas the minutiae extraction from the test specimen remains largely human-based. During the analysis the features should be marked on the test specimen (lower quality) before the observation of the reference specimen (higher quality), in order to prevent the bias induced by the observation of the higher quality specimen during the analysis of the lower quality one.
Also explicit documentation for marking the features needs to be demonstrated during the validation process.

Comparison step

The comparison step consists in comparing the features extracted/analysed from the test and reference specimens. The validation of the comparison needs to demonstrate that the practitioners pair the same corresponding features, and that they describe and discuss consistently the correspondence-similarities and the difference-dissimilarities of the paired features. The focus needs to remain on the visual aspects of similarity and difference between the paired features, and not on the question if the specimens originate from the same or different source(s). When only common sense is sufficient to conclude on the basis of a quick comparison that the specimens obviously originate from different sources, a decision of exclusion without any extensive comparison is appropriate. On the other hand, when expert knowledge is required, the question of the evidential value of the similarities and difference and the question of the dependence of the features needs to be addressed in the evaluation phase.

Verification

Verification is defined as the independent examination by another qualified examiner resulting in the same conclusion [SWGFAST, 2002a p4 [10]].
One method of applying verification is having a second examiner carry out the analysis and comparison between the unknown and known specimens without indications of a previous conclusion by the original examiner. Reworking the case

with indications of decisions made by the original examiner is another method of applying verification.
There are other methods of applying the verification phase of an examination beyond these two examples. However the method of verification must be selected so that the verifier is not biased by the original examiner's observations and conclusions. The second examiner must be able to reach their own conclusion. The steps required for the validation of the performance of the analysis, comparison and verification are detailed below in table 7.

4.2.3 Demonstration of the competence of the practitioner

The competence of the practitioner is constituted of his/her theoretical knowledge (know), practical skills (know-how), professional attitude (know-how-to-be) and controlled experience. The assessment of the competence of the practitioner underpins the description of the method and the direct testing of the performance and the consistency of the results.
The demonstration of the competence of the practitioner includes the identification and the check of the necessary knowledge, skills, attitude and experience necessary to qualify in a forensic field. The assessment is performed through theoretical examination, practical work under supervision and independently, practical performance tests and monitoring of the experience through the participation to PT/CE . Setting up practical performance tests and monitoring the experience of practitioners on a regular basis will require both operational and managerial resources.
The steps required for the demonstration of the competence of the practitioner are detailed below in table 8.

Note: On completion of the validation process quality control measures must be put in place. These are set out in table 9.

The final step is a fit for purpose approval and the completion of the validation report with all essential data enabling an assessment of this technique.

Table 5 Documentation of the feature set

Feature	
Origin	The origin of the features can be **intrinsic**, like the general pattern of a human fingerprint, **manufactured**, like the pattern of the sole of a shoe or **acquired** like a scar on a fingerprint or damages on a shoe sole.
Distinctiveness/ discrimination	The features extracted from the trace and reference specimens from the same source (within-source variability or **intravariability**) should be **distinguishable** from the features extracted from the trace and reference specimens from different sources (between-source variability or **intervariability**). The set of features chosen need to maximise the discrimination between the intra- and intervariability. For example, the intravariability of a finger or shoe pattern includes the variation of characteristics like the clarity and distortion as well as damages like wear patterns or scares. The intervariability of a finger or shoe pattern includes the variation of intrinsic or manufactured characteristics like the size, the fingerprint general pattern or the shoe sole pattern.
Dependence/composition	The features composing the feature set need to show limited dependence, in order to optimize the evidential value. It should be made explicit if the feature set is **case-dependent** (e.g. handwriting) or **case-independent** (e. g. fingermarks).
Permanence	The features need to show permanence in time, to **remain exploitable over time** (the general pattern of the fingerprint remains permanent from foetal life until after death, whereas the pattern of a sole wears out with use from months to a few years).
Availability	The features need to be **available** on the vast **majority** of the trace and reference **specimens**.
Measurability	The extraction/measurement of the feature can be **automated**, and if not possible, at least **repeatable** for a practitioner and **reproducible** between practitioners.
Ruggedness/robustness	The feature should be largely **insensitive** to the external factors encountered in forensic casework, e.g. environmental (degradation of fingermarks due to aging) or human

	(imitation of handwriting).

Table 6 Steps required for the method development check

Human based methods	**Analogy to instrumental terminology**	**How to check**
Feature selection *Feature description* *Feature classification* *Feature labelling*	Measuring range	Literature/field studies about the feature (set) Documentation - Justification of the selection of the feature (set) - Description of the feature (set) and discussion of their properties: origin, distinctiveness/discrimination, independence/composition, permanence, availability, measurability, robustness) - Justification of the classification of the feature (set) - Explanation of the labelling of the feature (set) (for example through a glossary)
Feature availability *Feature measurability* *Specimens usability*	Selectivity	Demonstration that the features are present in a majority of the test samples Demonstration of the repeatability and reproducibility of the feature extraction Determination of the criteria for a specimen to be suitable for the comparison step
Feature (set) distinctiveness *Feature dependence*	Specificity	Empirical study showing that the features documented vary less within the same person/object (intra-variability) than between different persons/objects (intervariability) Empirical study of the dependence between the features
Permanence *Ruggedness/Robustness*	Ruggedness/robustness	Empirical studies showing that the features remain permanent in time on the specimens

Table 7 Steps required for the validation of the performance parameters of the analysis, comparison and verification

Performance testing	How to validate
Feature (set) analysis - Select, extract and mark the features - Determine the usability of the specimens for the comparison step **Feature (set) comparison** - Link and mark the paired features - Compare the features **Feature (set) verification** - Select method of verification and test **Specimens usability** - Determine the usability of the specimens for the evaluation step	A performance test is suitable to test all the aspects set out under testing performance. A performance test can be used to measure the accuracy (ground truth) of the practitioners using test specimens produced from **known** reference specimens (for example the material from older PT/CE) .

Table 8 Steps required for the demonstration of the competence of the practitioner

Competence assessment of the practitioner	How to validate
Feature set - Know the features, their structure and properties **Analysis** - Know how to recognize and select the feature (set) - Know how to extract and mark the features - Know how to assess the usability of specimens for comparison **Comparison** - Know how to link and mark pairs of features - Know how to describe and document the similarity and differences within the pair of features, from pattern recognition point of view - Know how to assess the usability of specimens for evaluation	**Perform** • Training - Under supervision - Doing simulated casework using portfolios of known cases - Doing real casework - Collaborative exercises and proficiency tests - Independently - Doing simulated casework using portfolios of known cases - Doing real casework - Collaborative exercises and proficiency tests • Theoretical examinations • Knowledge of the literature • Practical performance tests • Internal audits **Monitor** - The results of collaborative exercises and proficiency testing - The results of known cases - Differences between personnel at the shadowing stage

Table 9 Quality control measures

Quality control in place	Mechanism for trending, dealing with the differences arising between practitioners during the analysis/comparison/verification steps Mechanism for discussing the issues arising in the analysis/comparison/ verification steps involving all practitioners working in area and procedure for resolving and documenting the final decisions regarding these issues

4.3 Glossary : Important terms used in human based methods

Identification: Kirk and others emphasize the process of individualization, the reduction of a class of evidence to one. It is useful to take a step back and realize that identification, defining the physicochemical nature of the evidence, can be an end in itself. For some purposes, for example, the recognition of illegal drugs, the forensic process stops with identification. The criminal justice system is not necessarily concerned with the marijuana field or methamphetamine laboratory from which the drugs originated (although sometimes they may be); simple possession of the scheduled substance fulfils the criteria of illegality. The process of identification answers the case investigation question of what is it? [9]

Individualization: Identification may also occur as a step leading to individualization. To distinguish it from end-point identification as discussed in the above paragraph, we will refer to the intermediate process that may lead to individualization as classification. Several authors (DeForest et al., 1983; Tuthill, 1994; Saferstein, 1998; Cook et al., 1998a, b) have remarked on the special meaning of individualization in a forensic context as a conclusion of common source for two items. Any forensic analysis that proceeds on the path toward individualization relies on a comparison of at least two items. Physics and logic determine that any individual object is unique; this is not the question. The forensic question asks whether items share a common origin. There may be some disagreement about whether an item must be classified before it is individualized. We believe that, whether intentionally or not, the analyst will know what the item is by the time he concludes a common source. If ambiguity exists about the classification of an item, the individualization to a common source is also compromised. The process of individualization answers the questions of "which one is it?" or "whose is it?" depending on whether the item is animate or inanimate; it does this by inferring a common source or origin. [9]

Association: Although the word *association* is used freely in describing the results of a forensic examination, no clear definition seems to exist, at least not in published literature. We propose that *association* be defined as an inference of contact between the source of the evidence and a target. Such an inference is based on the detection of transfer of material or a physical match determined by complementary edges. The source and the target are relative operational terms defined by the structure of the case; if transfer is detected in both directions, for instance, each item is both a source and a target. The association process involves the evaluation of all of the evidence for and against the inference of common source; in other words, competing hypotheses are compared. The probability of the evidence under competing hypotheses is an expression of the likelihood of the evidence given that the target and source items were in physical contact, contrasted to the likelihood of the evidence given that the target was in contact with a different unrelated source. This process requires combining the strength of the evidence established during the individualization process with additional information (such as may be provided by manufacturers of materials and empirical studies), as well as assumptions made by the analyst. Others have commented on the complexity of determining the significance of an association, including Robertson and Vignaux (1995) and Cook et al. (1998a, b). To illustrate this concept, consider a fibre collected from the body of a deceased individual. The fibre is compared with a carpet from the floor of an automobile van. The evidence fibre from the body and the reference fibres from the van carpet are found to be the same type and to contain indistinguishable dye components. These similarities suggest

that the van carpet could be the source of the evidence fibre (alternatively, the van carpet cannot be eliminated as a possible source of the evidence fibre). Next, all possible sources of the evidence fibre are considered, including the carpet from the van, all of the carpet manufactured from the fibre, and any other items manufactured from that particular fibre, and any other fibre indistinguishable from the evidence fibre by the analysis performed. From the data obtained by the laboratory analyses, combined with real world information about the distribution of the fibre, an inference might be made that the deceased individual and the van carpet were in contact. Note the distinction between a conclusion of common source (the evidence and reference fibres are classified or individualized as sharing a common source) and an inference of contact between a source and a target (the carpet and the deceased are associated). [9]

5 Examples

The following examples have been chosen to illustrate some of the variety of methods used in forensic science.

5.1 Instrument-Based Examples

PRIMARILY QUANTITATIVE

5.1.1 Quantification of DNA Using Real-Time PCR

5.1.2 Quantification of Ethanol in Blood

5.1.3 Quantification of cocaine in powders

PRIMARILY QUALITATIVE

5.1.4 Qualitative Screening for Gun Shot Residues

5.1.5 DNA kit 17-loci STR PCR chemistry

5.1.6 Human salivary alfa-amylase detection by RSID^{TM}-saliva test

5.2 Human-Based Method Examples

5.2.1 Fingermark and fingerprint comparison

5.2.2 Bullet and cartridge case comparison

6 Bibliography

For a list of current references relating to validation in analytical measurement, please refer to the Eurachem *Reading List* located under *Publications* section on the Eurachem website, www.eurachem.org

[1] ISO/IEC 17025:2005 General requirements for the competence of testing and calibration laboratories, ISO/IEC (2005).

[2] BIPM, Report JCGM 200:2012, International vocabulary of metrology — Basic and general concepts and associated terms (VIM), www.bipm.org. Printed as ISO/IEC Guide 99:2007, ISO Geneva.

[3] V. J. Barwick, E. Prichard (eds.) Eurachem guide: Terminology in analytical measurement – Introduction to VIM 3 (2011), ISBN 978-0-948926-29-7, available from www.eurachem.org.

[4] M. Thompson, S.L.R. Ellison, R. Wood. Harmonized guidelines for single-laboratory validation of methods of analysis (IUPAC technical report), Pure Appl. Chem. 2002, **74**(5), 835

[5] The Fitness for Purpose of Analytical Methods; a Laboratory Guide to Method Validation and Related Topics, 1st Edition, Eurachem (1998), www.eurachem.org. Note 2nd Edition to be published in November 2014.

[6] M. Valcárcel, S. Cárdenas, D. Barceló et al.(2002), Metrology of Qualitative Chemical Analysis, report EUR 20605 EN, ISBN 92-894-5194-7, European Commission

[7] Validation guidelines for DNA analysis methods, SWGDAM (2012). http://swgdam.org/SWGDAM_Validation_Guidelines_APPROVED_Dec_2012.pdf

[8] Recommended minimum criteria for the validation of various aspects of the DNA profiling process, ENFSI DNA Worling Group (2010), http://www.enfsi.eu/sites/default/files/documents/minimum_validation_guidelines_in_dna_profiling_-_v2010_0.pdf

[9] Inman, K. and N. Rudin. Principles and practice of criminalistics: the profession of forensic science. CRC Press. (2001). ISBN number 0-8493-8127-4

[10]Quality assurance guidelines for latent print examiners, version 2.1, SWGFAST (2002). Printed on www. swgfast.org/

Appendix 1 **Template of the validation plan and report.**

IN-HOUSE VALIDATION
OF
MEASUREMENT PROCEDURES

VALIDATION PLAN

1. Specify the measurement procedure, analyte, measurand and units

The measurement procedure	
Analyte	
The measurand	
Unit	

2. Specify the Scope

Matrix	
Measuring range	
Intended use of the results	

3. Requirement on the measurement procedure

Parameters to be validated			*Value requested*
Precision	*Repeatability*	☑	
	Within-lab reproducibility	☑	
Trueness	*Selectivity*	☑	•
	Bias		
Measurement range	*LOD*	☑	
Ruggedness (Robustness)	*Cotton swabs*	☑	
	Forensic-like surfaces	☑	
High Dose Hook effect		☑	
STR analysis starting from the RSIDTM extraction buffer.	Genetic profile stability	☑	
Stability	Stability of the obtained test result	☑	

4. Origin of the Measurement Procedure

		VALIDATION
New In-House Method	☑	*Full*
Modified Validated/Standard Method		*Partial*
Official Standard Method		*"Simple"*

VALIDATION PLAN AND RESULTS

Parameter	Description
	Repeatability, Within.Lab Reproducibility,
	Demand
	Experiments
Precision	**DATA**
Results:	
Conclusions:	Fit for purpose

Parameter	Description
Trueness	Bias
	Demand
	DATA
Results:	
Conclusions:	Fit for purpose

Parameter	Description
	Ruggedness
	Demand
	-
	Experiment
Ruggedness	**DATA**
Results:	
Conclusions::	Fit for purpose

Conclusions

Summary	Laboratory/customer demands met	
Plan of internal quality control:		
Statement on fitness for intended purpose	**The method fulfils the demand**	**☐ The method does not fulfil the demand** **The following should be done….**