Recommended Minimum Criteria for the Validation of Various Aspects of the DNA Profiling Process			
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Aim :

One of the requirements of EN ISO/IEC 17025 is that methods used in testing laboratories should be validated. As EN ISO/IEC 17025 only determines a general standard it is the role of the experts in a given field to give more detailed recommendations.

The ENFSI DNA Working Group has agreed upon the minimum validation criteria as laid down in this document. This paper can only serve as a recommendation because each DNA testing laboratory has its own duties and workflows. There might be other approaches to validate a certain protocol or instrument. Whatever the criteria are to validate a system, they must give evidence that the procedures and instrument are suitable for the purpose they are used for according to EN ISO/IEC 17025. It is also an absolute necessity that the results are in concordance with the international standards to ensure that DNA profiles are comparable between laboratories.

These recommendations only apply to standard situations in a laboratory (internal validation). However, if a testing laboratory develops new methods or technologies, the validation efforts have to be far more extensive and considered as developmental validation (see below).

General prescriptions

Any change in technique (reagents, kits, apparatus ...) with a potential influence on the results, requires an internal validation (see below).

It is essential to show that profiles obtained using the new regime are of the same, or better, quality than those obtained under the previous regime.

All results could be statistically evaluated.

Environmental conditions can be taken into consideration.

The validation of any new method should examine inter-operator variation.

Proficiency tests samples should be part of the validation experiments (GEDNAP, other, …).

For the validation of a specific parameter (repeatability …) at least 5 samples have to be analysed (not including the negative control).

After running 5–10 replicate samples for a particular experiment, there are diminishing returns to obtaining additional results. The number five is already in use throughout the forensic DNA community[1].

When calibration or general performance of equipment is altered due to maintenance/repairs/relocation, verification of this equipment is necessary.

[1] When conducting an internal validation, the SWGDAM Revised Validation Guidelines recommend running a total of at least 50 samples—not 50 samples per experiment. (Debunking Some Urban Legends Surrounding Validation Within the Forensic DNA Community by John Butler National Institute of Standards and Technology, Gaithersburg, Maryland, USA – Promega, Profiles in DNA, September 2006)

Definitions (cfr. SWGDAM revised validation guidelines)

- Internal validation is conducted by each forensic DNA testing laboratory and is the in-house demonstration of the ***reliability*** and ***limitations*** of the procedure. Prior to using a procedure for forensic applications, a laboratory must conduct internal validation studies.

- Developmental validation is the demonstration of the accuracy, precision, and reproducibility of a procedure by the manufacturer, technical organization, academic institution, government laboratory, or other party. Developmental validation must precede the use of a novel methodology for forensic DNA analysis.

- Sensitivity : The range of DNA quantities able to produce reliable typing results must cover the range of DNA concentration encountered in the samples to be analysed using the technique.

- Specificity: indicates to what extend the test is likely to give positive results in the case that the tested substance is present and negative results in case of absence of the tested substance.

- Repeatability : is the variation in measurements obtained when one person measures the same unit with the same measuring equipment.

- Reproducibility : is the variation in average (mean) measurements obtained when two or more people measure the same parts or items using the same measuring technique.

- Linearity : indicates the range over which the output signal strength varies in direct proportion to the input signal.

- Profile quality assessment :
  - locus peak balance: check the peak balance of heterozygote alleles within a locus. Acceptable inter locus peak balance ratios are > 60% for good quality samples.

  - between loci peak balance : check the peak balance of alleles between all loci. Acceptable inter loci peak balance ratios are > 60% for good quality samples.

  - peak height/area: equivalent amounts of DNA should at least give equivalent peak height/area using the new regime as the previously used regime.

- presumptive test: is a test used to determine the biological origin of the trace sample (blood, saliva, semen …)

Presumptive tests

Due to the great variety of presumptive tests available, no specific indications are given in this document. Remember that the use of these tests in your laboratory should be validated or verified as appropriate.

Extractions

A new extraction method should be compared with the method already in use in the laboratory for all the parameters listed below.

The validation should include the sample types normally analysed in the laboratory or any new sample types intended to be processed with the new method.

As the DNA extraction is one of the most important steps for any downstream analysis consideration should be given to increasing the number of tested samples to reflect the degree of modification of the current method.

It should be verified that comparable quantities of DNA are extracted using both systems and that DNA profiles of the same, or better, quality are obtained using the new system.

Minimum parameters to be validated:

- Repeatability : 5 replicates of the same sample.
- Reproducibility : 5 replicates of the same sample (as in the repeatability test) extracted at another time by another person.
- Sensitivity (limit of detection): a series of 5 dilutions tested in three replicates.

Yield of DNA: use the sensitivity study to compare between replicates and with the previous technique in use.

In addition, the following parameters should be considered:

- Matrix-dependence : check the influence of the matrix in which the sample is present. Samples of known genotype are examined after contact with a variety of substrates commonly encountered in forensic cases.

- Different cell types (blood, semen, epithelial …) can be tested depending on the laboratory's common encountered samples.

DNA Quantification

The aim of the validation of a new DNA quantification system is to establish the optimum DNA concentration range, using the new system, to produce good quality DNA profiles.
Any commercially available and quantified human control DNA can be used to adjust a system although any quantification results obtained can only be calculated “referring to standard DNA xxx”.

When changing quantification systems, the new system should be compared with the previous system in order to see if amplification protocols should be modified.

Minimum parameters to be validated :

- Repeatability : 5 replicates of the standard DNA.
- Reproducibility : 5 replicates of the standard (as in the repeatability test) quantified by another person.
- Sensitivity (limit of detection) : a series of 5 dilutions tested in three replicates.
- Determination of the link between quantification results and the genetic profile (no quantification / DNA = no profile) due to a different sensitivity between quantification and PCR multiplexes or possible inhibition.

Other possible parameters:

- Sensitivity to inhibitors and degraded DNA.
- Detection of male/female components in mixtures when using human + human male DNA quantification kits.
- Conservation in time of standards and samples.

PCR Instruments

For a new thermal cycler model it is suggested that a number, adapted to the format of the machine, of samples previously profiled are repeated using the kits to be used on the machine. The quality of the resulting DNA profiles is to be equivalent or better than the profiles generated using the current thermal cycler. The selected samples will obviously allow examination of parameters such as reproducible results, including from mixtures and low DNA concentration samples.

For a new thermal cycler that is of the same model as others already in use, a certificate from the manufacturer detailing a technical performance check **done after installation in the laboratory and an internal sensitivity + homogeneity check would be sufficient.**

Minimum parameters to be validated :

- Sensitivity (limit of detection) : a series of dilutions tested in three replicates.
- Repeatability : the three replicates of the same sample, distributed over the entire heating block can be used to evaluate the repeatability.
- Reproducibility : 3 repetitions of the amplification reactions used in the sensitivity test.
- Homogeneity of heating block : temperature control of the heating block or a comparison of the replicates allows the evaluation of the homogeneity of the heating block.

New Multiplex Kit

If using a commercially manufactured kit there is no requirement for a developmental validation of the kit if the validation is published or done by a collaborative exercise (e.g. by ENFSI). A sensitivity and concordance study is recommended for samples that have previously given acceptable profiles, especially for samples with low amounts of DNA.

Minimum parameters to be validated :

- Repeatability: 5 replicates of the same sample.
- Reproducibility : 5 replicates of the same sample (as in the repeatability test) amplified at another time by another person(--> if manually processed).
- Sensitivity (limit of detection) : a series of 5 dilutions tested in three replicates.
- Mixture analysis (not necessary if only reference samples are processed with this kit) : a series of different laboratory defined mixture ratios should be tested in three replicates.
- Analysis of peak balance : check the peak balance of heterozygote alleles within a locus and of alleles between all loci. Acceptable peak balance ratios are > 60% for good quality samples.
- Check stutter ratios by calculating the ratio of the stutter peak height or area compared to the corresponding allele peak height or area. In general, stutter peaks have to be lower than the % of the allele peak height indicated by the manufacturer of the kit to be ignored as a biological artefact of the sample.
- Concordance study : a concordance study must be have been done using PCR products that have previously given full, balanced profiles.

Other possible parameters :

- Sensitivity to inhibitors and degraded DNA.
- Detection of male/female components in mixtures when using specific human male DNA amplification kits.

If deviations from the manufacturers' protocol are introduced, these deviations should be validated.

Electrophoresis Equipment

The sensitivity of the new instrument should be at least as good as the sensitivity of the old instrument and cover the PCR kit working range. The lowest concentration of DNA allowing the operator to obtain a full genetic profile on the new instrument, under the standard laboratory procedures, should be equal to or less than that obtained using the old instrument.

For a new capillary electrophoresis that is of the same model as others already in use a certificate from the manufacturer about a technical performance check **done after installation in the laboratory and an internal sensitivity check would be sufficient.**

Minimum parameters to be validated :

- Repeatability : 5 replicates of the same sample.
- Reproducibility : 5 replicates of the same sample (as in the repeatability test) run at another time.
- Sensitivity (limit of detection) : a series of 5 dilutions tested in three replicates.
- Mixture analysis : a series of lab defined mixture ratios should be tested in three replicates.
- Analysis of peak balance : check the peak balance of heterozygote alleles within a locus and of alleles between all loci. Acceptable peak balance ratios are > 60% for good quality samples.
- Check stutter ratios by calculating the ratio of the stutter peak height or area compared to the corresponding allele peak height or area. In general, stutter peaks have to be lower than the % of the allele peak height indicated by the manufacturer of the kit to be ignored as a biological artefact of the sample.
- Precision : the precision of the instrument should be such that all measured alleles fall within a ± 0.5 bp window around the measured size for the corresponding allele in the allelic ladder.
- Concordance study : a concordance study must be done using PCR products that have previously given full, balanced profiles.

Other possible parameters:

- Maximum number of runs with capillaries.
- Storage time of polymer.

New Software

Software eg. interpretation software sold by a company (or commercially available), should undergo a concordance study with the previously used method. Validation data of the software should be available from the manufacturer.

Every change in the system should be validated by a manual/visual inspection of the tested functionality and must be documented.

Home made software (like macros or calculation sheets) should be validated.

Parameters to be validated (can vary according to the nature of the software) should consider the following aspects :

- Sample tracking
- Data storage and alteration (traceability)
- Calculation method

Laboratory automation

For a pipetting robot that replaces manual pipetting for a single laboratory step, e.g. PCR or CE setup, the validation should consider the parameters listed above (see laboratory step that will be automated), together with the following:

The performance of different robots (i.e. extraction robots) should meet the requirements defined by the laboratory and yield enough DNA for their intended use (reference samples, crime scene samples…).

User programmed protocols, e.g. for pipetting channels or robotic arm displacements, should be tested with every change made to the protocol.

Minimum parameters to be validated:

- Reproducibility across the whole plate.
- Repeatability (dispensing volumes of each pipetting channel should be verified)
- Plate homogeneity.
- Cross contamination tests (i.e. droplet formation or spilling during liquid transfer).
- Traceability of sample tracking (e.g. sample dispatching protocols between source plate (tubes) and destination plate (tubes))
- Different cell types (blood, semen, epithelial …) can be tested depending on the laboratory's common encountered samples.