Validation of Forensic Science Techniques: Principles and Procedures

Introduction

"Validation" has become a hot topic in both the forensic science community and the legal community following the publication of the National Academy of Sciences report on the state of forensic science in 2009 ("NAS Report") [1]. Legal practitioners are struggling to understand validation and how a forensic science technique qualifies as "validated." Unfortunately, the term "validation" is commonly used within the forensic science community to describe several separate processes inherent in good scientific analysis, and legal practitioners have struggled to reconcile seemingly inconsistent references in their efforts to determine whether specific forensic science techniques qualify as "validated methods." Contributing to the confusion are legal publications that use "invalid" to refer to the lack of supporting empirical data [2] – a usage that is different from that of the scientific community, where "nonvalid" denotes the lack of supporting empirical data and "invalid" denotes the existence of disproving empirical data [3]. Essential to understanding validation is recognizing incongruous terminology and focusing on the purpose of validation.

Validation includes both the overall process of assessing the ability of a technique to achieve specified objectives (also referred to as "method validation" or "developmental validation") and the narrower process of demonstrating that validated methods perform as expected in a specific laboratory (also referred to as "performance check" or "internal validation"). Both processes – method validation and performance check – are part of best scientific practices. Validation is an essential component of a quality assurance program, along with laboratory accreditation (*see* **Accreditation: Laboratory**), industry standards, inspections/audits, and proficiency testing. A well-constructed and documented validation process can provide regulatory organizations and the courts with evidence that the forensic science technique is appropriate for its intended use and provides a basis for the development of interpretation guidelines. The validation process can also assist laboratories in improving efficiency and productivity as a part of the overall quality assurance program.

In the forensic science community, the term "validation" is commonly applied to the process of defining the capabilities and limitations of a technique. The NAS Report refers to validation as determining the "reliability under different conditions" and the "limitations" of methods [4]. The International Standard, ISO/IEC 17025, which is the basis for the American Society of Crime Laboratory Directors Laboratory Accreditation Board (ASCLD/LAB) International Accreditation Program, defines validation as "the confirmation by examination and the provision of objective evidence that the particular requirements for a specific intended use are fulfilled" [5]. The DNA Advisory Board Quality Assurance Standards for Forensic DNA Testing Laboratories define validation as "a process by which a procedure is evaluated to determine its efficacy and reliability for forensic casework analysis and includes . . . the determination of conditions and limitations . . . and demonstrat[ion] that established methods and procedures perform as expected in the laboratory" [6]. Another commonly cited laboratory guide defines validation as "[t]he process of establishing the performance characteristics and limitations of a method and the identification of the influences which may change these characteristics and to what extent" [7].

Using the fundamental concepts underlying these definitions, validation for a forensic science technique can be defined as the process of establishing

1. the performance characteristics (capabilities) of the technique;
2. the limitations of the technique;
3. the identification of the influences that may change the performance characteristics; and
4. the extent to which those influences change the performance characteristics.

It is important to know what validation is; it is equally important to understand what validation is not. Validation provides *an assurance of reliability* during normal use; it does not provide *a guarantee* that accurate results have been obtained in any particular case.[a] Validation is only the first step in implementing a forensic science technique and

only a part of the overall mechanism for ensuring accurate results (other components include accreditation of the laboratory, adherence to high quality standards and protocols, calibrated instrumentation, documented results, proficiency testing, audits, and qualified personnel). Even after a forensic science technique has been shown to be valid, the actual use of the technique must be standardized and acceptable protocols developed. The development of these protocols and the demonstration that they generate reproducible results are separate from and in addition to the fundamental validation research. Although the development of laboratory protocols is properly done in close coordination with personnel in the laboratories where the technique is to be applied, analysis on the fundamental questions of validity can be conducted in external laboratories and research facilities.

Examples of validation studies of forensic science techniques that have been conducted recently by the National Academy of Sciences (NAS) include the following:

1. At the request of the U.S. Department of Energy, the NAS investigated the scientific validity of polygraphs as an effective employee-screening method: *The Polygraph and Lie Detection*, Committee to Review the Scientific Evidence on the Polygraph, Board on Behavioral, Cognitive, and Sensory Sciences and Committee on National Statistics, Division of Behavioral and Social Sciences and Education, National Research Council, The National Academies Press, 2003.
2. At the request by the National Institute of Justice (NIJ), the NAS investigated the scientific validity of DNA identification: *DNA Technology in Forensic Science,* Committee on DNA Technology in Forensic Science, Board on Biology, Commission on Life Sciences, National Research Council, National Academy Press, 1992; and *The Evaluation of Forensic DNA Evidence,* Committee on DNA Forensic Science: An Update, Commission on DNA Forensic Science: An Update, National Research Council, National Academy Press, 1996.
3. At the request of the Federal Bureau of Investigation (FBI), the NAS examined bullet-lead analysis: *Forensic Analysis: Weighing Bullet Lead Evidence*, Committee on Scientific Assessment of Bullet Lead Elemental Composition Comparison, Board on Chemical Studies and Technology, Division of Earth and Life Studies, National Research Council, The National Academies Press, 2004.

An excellent example of a validation study of a more selective forensic science technique is "Validation of an Enzyme Immunoassay for Detection and Semiquantification of Cannabinoids in Oral Fluid" in *Clinical Chemistry* [8].

For legal practitioners, validation can loosely be analogized to court proceedings in which the plaintiff/prosecution theory of the case is challenged. The defense case involves assessing the reliability of the theory by changing conditions (for example, by blocking the admission of certain evidence, requiring limiting instructions, or introducing additional evidence) to determine the limitations of the plaintiff/prosecution proof. Judges and juries analyze the theory, looking for false assumptions, unsupported conclusions, and weaknesses in the evidence. The process of litigation is similar to the validation process of a forensic science technique. Similarly, although court proceedings are important to assure the reliability of litigation results, they do not guarantee that the correct result is reached in an individual case. Court proceedings are just a component of the overall justice system, along with competent counsel, impartial juries, equal access to evidence, and other factors important to ensuring reliable verdicts.

Specifics of Validation

Validation is a process. It is a documented program that provides a high degree of assurance that a specific technique will consistently produce a result within the defined specifications and quality parameters. During validation of a forensic science technique, the technique should be evaluated through-testing to demonstrate that reliability is maintained throughout the analysis and that the performance characteristics of the technique have not been compromised. The criteria for validation – the performance characteristics or analytical parameters of the technique – include the following:

Accuracy: how close the experimental results are to the true value

Limit of detection: the smallest amount of a substance that can be detected, but not necessarily quantified, with the technique
Precision: how close the experimental results are to each other – the degree of repeatability of the technique under the same conditions
Sensitivity: the smallest amount of substance in a sample that can be accurately measured by the technique – also referred to as the limit of quantitation
Specificity: the ability of the technique to measure or detect only what it is intended to measure or detect
Range: the interval between the lower and upper levels in which the technique has been demonstrated to produce accurate and precise results
Reproducibility: the ability of the technique to produce results that can be replicated, generally by another qualified analyst
Robustness: the capacity of the technique to remain unaffected by deliberate variations in the technique's other parameters
Ruggedness: the ability to use the technique in different laboratories or under different circumstances without the occurrence of unexpected differences in results

Many factors, such as the analyst, the equipment used, and the known standards, can affect the routine forensic analysis. In a validation assessment, these factors must be identified, defined, and then compromised to determine the significance that the factor will have on the entire analysis. Should any factor make a significant difference to the results, that factor must be controlled and procedures must be put into place to ensure oversight of the factor.

Validation processes can utilize historical data (retrospective validation) or casework data (concurrent validation), or use only experimental data (prospective validation) [9]. For many forensic science techniques and theories, most of the validation work will be retrospective – making use of historical data – but should be continuously reevaluated using casework (concurrent) data.

Stages of a Validation Process

Validation is conducted in a wide range of fields, from pharmacology to computer system design. All nonstandard techniques, laboratory-designed techniques, and standard techniques used outside their intended scope should be validated to confirm that they are fit for their intended use, to determine the capabilities and limitations of the technique, and to provide a basis for interpretation guidelines for use with the technique. Validation should also be conducted if quality control data indicate that the technique is not performing as intended or concerns are raised about the reliability and accuracy of the technique. The specific validation process necessary for any particular technique will be as extensive as necessary to meet the needs of the given application or field of application. The validation process must prove that the parameters of the technique meet the requirements of use.

Because analytical methods in the forensic science fields vary greatly, the validation process for a specific forensic science technique must be carefully tailored to the technique. In designing a validation process, the intended use of the technique guides what parameters need to be tested (e.g., a technique to determine the presence of human blood does not need a validation process for evaluating the limit of quantitation). Although some fields of forensic science such as forensic chemistry or forensic toxicology can utilize specific validation requirements derived from clinical laboratory work [10], most forensic science casework does not have correlated clinical standards. Although a generic protocol of validation is not appropriate for forensic science techniques, the general steps for validation processes are to

1. determine the analytical parameters;
2. plan the series of experiments to be conducted;
3. conduct the experiments;
4. evaluate the data produced to determine capabilities and limitations of the technique;
5. reassess parameters, change conditions, and repeat steps 2–4 as necessary until optimized performance is reached;
6. collect documentation of all steps and produce validation statement detailing scope of the technique.

The stages of the validation process can be summarized as follows:

Instrumentation Verification. The instrumentation used in the technique is assessed for proper installation, operation, and performance, including an assessment of the influence of external factors (such

as utility connections and temperature variations) on operation and performance. This instrumentation can include simple measurement devices – such as scales and graduated cylinders – or complex analytical devices – such as computers – that must be assessed for proper design, installation, operation, and performance. Manufacturer specifications must be obtained, followed, and documented for compliance. Maintenance – both initial and routine – must be performed and assessed for effects on operation and performance. This stage can frequently be minimized if the instrumentation used in the technique is not newly acquired, has previously been subjected to verification, and has met all necessary maintenance requirements.

Experimentation. The appropriate analytical parameters and goals of the technique are detailed and assessed through repeated experimentation. Statistically valid approaches should be used to evaluate data and make decisions to lessen the subjectivity of the validation process and the interpretation guidelines implemented as a result of the validation process. To establish validity, a sufficient number of samples should be analyzed to provide a statistically significant basis for any conclusions.[b]

Optimization. Analytical parameters of the technique are improved on the basis of the results obtained from repeated experimentation, in comparison to the predefined parameters. Given the need for forensic science techniques to be conducted by many different analysts in many different facilities, robustness and ruggedness should be among the first parameters assessed and refined.

System Suitability. Tests are conducted to verify that the entire system used in the technique (instrumentation, software, protocols, samples, etc.) is adequate for the analysis to be performed.

Documentation. Documentation of the validation process should include all results obtained, the procedures used for validation, and the specifications of the intended use of the technique. The documentation should be maintained in a form that is readily accessible to facilitate review. Documentation is necessary for internal reference, external review, legal proceedings, and accreditation and should be maintained for at least as long as the technique is in use [11]. As with validation in general, there is no single source of information for how to document validation, but at a minimum the documentation should include the following:

- Method: precise steps of the technique under review, experiment timeline, standards used, and parameters evaluated;
- Results: summary for each analytical parameter evaluated, instrument printouts or other result products, and appropriate statistical formulas;
- Conclusions: summary of validation (acceptance/rejection), documentation of parameters that were not met or were modified, explanations of modifications or deviations from validation protocol.

Validation Process Types

Although the specifics of the validation process will vary depending on the context, there are several common types of validation processes [12]:
Non-blind: A single analyst uses the technique with known samples.
Single-blind: One analyst prepares the samples and a second analyst uses the technique on the samples. The results are compiled and evaluated by the first analyst.
Double-blind: One analyst prepares the sample and a second analyst uses the technique. A third analyst compiles and evaluates the results.
Standard references: The technique is used in connection with standard reference materials. Analyst bias can be an issue if the analyst knows the identity or characteristics of the standard.
Collaborative studies: Samples are prepared, exchanged, and analyzed by cooperating laboratories.
Comparison with an accepted method: Results from the technique are compared with those obtained through a currently accepted method of analysis.

For forensic science analyses conducted in crime laboratories, laboratory accreditation standards provide guidelines for the validation process. The American Society of Crime Laboratory Directors (ASCLD) and its accrediting body (ASCLD/LAB: ASCLD–Laboratory Accreditation Board) have adopted an international standard for the International Accreditation Program [13]. This standard, ISO/IEC 17025 : 2005, was developed by the International Organization for Standardization (ISO) and the

International Electrotechnical Commission (IEC) as a guideline for testing laboratories.

ISO/IEC 17025: 2005 specifies the general requirements for competence to do testing using standard methods, nonstandard methods, and laboratory-developed methods. It is applicable to all organizations performing tests, including submitting laboratories, consulting laboratories, and laboratories where testing forms part of inspection and product certification. ISO/IEC 17025 : 2005 is applicable to all laboratories regardless of the number of personnel or the extent of the scope of testing activities. ISO/IEC 17025 : 2005 is for use by laboratories in the development of their management system for quality, administrative, and technical operations. Laboratory customers, regulatory authorities, and accreditation bodies may also use the standard in confirming or recognizing the competence of laboratories. Compliance with ISO/IEC 17025 : 2005, however, does not of itself demonstrate the competence of the laboratory to produce reliable and accurate results. It is merely a guideline, not the definitive checklist.

ISO/IEC 17025 : 2005 lists the following process types that can be used (often collectively) to validate a method (Section 5.4.5.1):

standard references
comparison with accepted methods
collaborative studies
systematic assessment of factors influencing the results and
assessment of uncertainty of results based on scientific understanding of the theoretical principles of the method and practical experience.

The last two types of validation can be accomplished using non-, single-, or double-blind experimentation.

Uncertainty Measurements

An important parameter of any forensic science technique that should be calculated through the validation process is the estimation of the uncertainty of the testing. Uncertainty acknowledges limitations of knowledge and the inability to control every influencing factor. Uncertainty analysis is necessary to measure the "meaningfulness" of a result to provide a basis for evaluating the weight to be attributed to the result in making decisions [14]. ISO/IEC 17025 : 2005 requires all laboratories to have and apply procedures for estimating the uncertainty of measurement [15]. The guideline recognizes that for some techniques (such as drug weight measurements), the calculation of the uncertainty can be rigorous and statistically valid. The nature of other techniques precludes such calculations, but the laboratories are required to identify all components of uncertainty and make a reasonable estimation on the basis of knowledge of the performance of the technique from validation data and prior results (the more the technique is used, the more refined the estimation should become). The laboratory must ensure that reporting does not give a false impression of the uncertainty.

ASCLD/LAB initially required compliance with the ISO/IEC 17025 estimated uncertainty of measurement requirements for all International Accreditation Program laboratories by December 31, 2008, but the deadline was rescinded after the organization determined that participating laboratories lacked sufficient awareness and understanding of the requirement [16]. The organization has implemented a graduated approach to requiring full compliance based on date of accreditation and to determining measurements of uncertainty on the basis of whether the measurement is included in the report or is another measurement impacting the accuracy of the results. Importantly, ASCLD/LAB currently only requires compliance with ISO/IEC 17025 standard for estimating uncertainty of measurement with respect to numerical values in quantitative analyses which are included in the written report of the analysis [17]. The exclusion of qualitative tests and unreported measurements severely limits the scope of the ASCLD/LAB requirement, excluding the common forensic science techniques used in identification (e.g., fingerprints, drug identifications, handwriting analysis for authorship, and impression comparisons).

Although not providing a specific means of estimating uncertainty of measurement, ASCLD/LAB does require the following to be included in the laboratory's estimation: identity of what is being measured, the measurement method, a list identifying all potential sources of uncertainty with their associated uncertainty estimations and excluding only potential sources of uncertainty that do not have significant impact based only on previous experience, sufficient measurement data, appropriate statistical formulas, and documentation of the estimation [18].

Uncertainty measurement requires the calculation of the combined effect of quantitative estimations of all sources of errors involved in the use of a technique and expresses the range of values possible on the basis of the measured result. Sources contributing to uncertainty include reference samples, instrumentation, environmental conditions, properties of the tested item, and the analyst (*see* **Interpretation: Observer Effects**). Any factors identified in validation that impact the results are sources contributing to uncertainty. To conduct an uncertainty estimation analysis, a laboratory must identify the errors that arise at every stage of the testing and then factor these errors into the overall uncertainty of the result. Error itself can be categorized as systematic or random. Both systematic and random errors must be considered in any overall estimations of uncertainty. Systematic error results from system factors such as miscalibrated equipment or from bias, and determines the accuracy of the result. Systematic error resulting from bias can be difficult to detect and minimize. Random error results from the differences in results under fixed conditions and determines the precision (repeatability) of the result. Both types of errors (systematic and random) should be minimized in an ideal technique. A technique that is highly repeatable may still be wrong as a result of a systematic error. Similarly, a technique that has low systematic error but poor repeatability is also of limited utility [19]. Under ISO guidelines, both random and systematic errors are assumed to follow a Gaussian distribution (the normal "bell-shaped curve") [20]. The overall uncertainty can then be characterized through the mean and standard deviation of the error.

Conclusion

Validation is important to ensure – to the extent possible – the integrity of the results of a scientific technique. Validation is conducted to establish that a technique is reliable over a specified range of conditions, and is a necessary part of any quality assurance program in any forensic science laboratory. Validation should not, however, be misinterpreted as guaranteeing the accuracy of results of a forensic science technique in any particular case. Laboratories perform validation processes to assure the reliability of results and to optimize forensic science techniques in the analysis of physical evidence.

Although validation processes vary depending on the forensic science technique being tested, the validation process can be evaluated for its rigor in assessing the applicable analytical parameters and in accounting for uncertainty. For the validation process to be broadly accepted, the validation should be conducted or endorsed by a scientific body recognized as authoritative and impartial, and the results should be published to permit reassessment, challenges, and further studies.

End Notes

[a.] The term "reliability" is used to denote accuracy, trustworthiness, and dependability. In other words, a method is reliable if it consistently produces correct results. The term is used in this article consistent with that definition. It should be noted, however, that "reliability" in scientific contexts often refers only to the extent to which a method produces the same results on repeated tests; accuracy (or correctness) is a separate concept.

Although validation evaluates more than the "reliability" of a method (*see* "Specifics of Validation" section), "reliability" is often the most important quality of a method. Publications frequently discuss validation only as a means of evaluating reliability. For example, the NAS Report referred to the need to validate new methods to "determine their reliability" and discussed the validation process as a means of determining whether the method can "reliably" support an evidentiary hypothesis (NAS Report, p.113). Validation, in addition to evaluating the reliability of a method, also contributes to the optimization of the performance of the method, improving laboratory efficiency and resource allocation.

[b.] The sample size and conditions of a validation process must be determined by reference to the technique being evaluated, the nature of the samples being tested, and the frequency of variables that may impact the results. For example, for a toxicology technique the prevalence in the population of a potentially interfering substance should be considered in determining the appropriate sample size for validation. If the potentially interfering substance is only found in 10% of the population, a random sample size of 6 would be insufficient to evaluate the technique with respect to this variable.

The limitations of a "small" sample size must be reflected, through appropriate statistical methods, in the uncertainty measurements associated with the technique. (*See* "Uncertainty Measurements" section).

References

[1] National Research Council of the National Academies, Committee on Identifying the Needs of the Forensic Science Community (2009). *Strengthening Forensic Science in the United States: A Path Forward*, The National Academies Press, Washington, DC.

[2] Garrett, B.L. & Neufeld, P.J. (2009). Invalid forensic science testimony and wrongful convictions, *Virginia Law Review* **95**(1), 1–97.

[3] Bohan, T.L. (2010). Strengthening forensic science: a way station on the journey to justice, *Journal of Forensic Sciences* **55**(1), 5–7.

[4] National Research Council of the National Academies, Committee on Identifying the Needs of the Forensic Science Community (2009). *Strengthening Forensic Science in the United States: A Path Forward*, The National Academies Press, p. 113.

[5] ISO (2005). *ISO/IEC 17025 : 2005(E) General Requirements for the Competence of Testing and Calibration Laboratories*, International Organization for Standardization, Geneva, Switzerland, section 5.4.5.1.

[6] DNA Advisory Board (1998). *Quality Assurance Standards for Forensic DNA Testing Laboratories*, Federal Bureau of Investigation, US Department of Justice, section 2.

[7] Eurachem Guide (1998). *The Fitness for Purpose of Analytical Methods. A Laboratory Guide to Method Validation and Related Topics*, LCG Ltd. Teddington, Middlesex, United Kingdom, p. 52 (A33.2).

[8] Schwope, D.M., Milman, G. & Huestis, M.A. (2010). Validation of an enzyme immunoassay for detection and semiquantification of cannabinoids in oral fluid, *Clinical Chemistry* **56**, 1007–1014.

[9] Gibson, W. & Powell-Evans, K. (2008). *Validation Fundamentals*, Informa Healthcare, New York, p. 28.

[10] Centers for Medicare and Medicaid Services, HHS; Clinical and Laboratory Standards Institute (CLSI) (2004). Clinical Laboratory Improvement Act, 42 CFR Ch. IV (10-1-04 Edition), Centers for Medicare and Medicaid Services, HHS; Clinical and Laboratory Standards Institute (CLSI) – www.clsi.org; International Association of Official Analytical Chemists (AOAC-International) – www.aoac.org.

[11] See American Board of Forensic Toxicology Laboratory Manual, (2010). available at: www.abft.org/files/ABFTLaboratoryManual.pdf.

[12] Swartz, M.E. & Krull, I.S. (1997). *Analytical Method Development and Validation*, Marcel Dekker, Inc., New York, pp. 35–38.

[13] www.ascld-lab.org/programs/programs_of_accreditation_index.html (2010).

[14] Brach, R.M. & Dunn, P.F. (2009). *Uncertainty Analysis for Forensic Science*. Lawyers & Judges Publishing Co., Inc. Tucson, Arizona, p. 1.

[15] ISO (2005). *ISO/IEC 17025 : 2005(E) General Requirements for the Competence of Testing and Calibration Laboratories*. International Organization for Standardization, Geneva, Switzerland, section 5.4.6.2.

[16] ASCDL/LAB (2008). *Updated Approach to Uncertainty of Measurement Requirements in the ASCLD/LAB-International Accreditation Program for Testing Laboratories*, available at www.ascld-lab.org.

[17] ASCLD/LAB (2007). *Estimating Uncertainty of Measurement Policy*, available at www.ascld-lab.org.

[18] ASCLD/LAB (2007). *Estimating Uncertainty of Measurement Policy*, available at www.ascld-lab.org, pp. 2–3.

[19] Brach, R.M. & Dunn, P.F. (2009). *Uncertainty Analysis for Forensic Science*. Lawyers & Judges Publishing Co., Inc. Tucson, Arizona.

[20] ISO 5725 (Parts 1–6) (1994). International Organization for Standardization, Geneva, Switzerland; and, ISO (1995). *Guide to the Expression of Uncertainty in Measurement*. International Organization for Standardization, Geneva, Switzerland.

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