Validation Studies in ISO 17025 Accredited Laboratories

INTERNATIONAL ASSOCIATION FOR IDENTIFICATION
Cincinnati, OH

Robert Ramotowski

All references pertaining to manufacturers and their products do not imply endorsement by the United States Secret Service or the authors.

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Nomenclature

- Level I Validation
  - Used for novel techniques (or major modifications of an existing technique) or pieces of equipment. Requires extensive testing of most of the key elements and documentation.
- Level II Validation
  - Used for minor modifications to existing techniques; software modifications; evaluation of COTS equipment. Requires approximately 50-100 samples and documentation.
- Level III Validation (modified function/performance test)
  - Used for equipment that takes no measurements or collects any analytical data (e.g., cameras, imaging systems, light sources). Requires only 10-25 samples and documentation.

Key Elements Validation Level I

Key Elements – Level I

- The following elements should be considered (but not all of them need be addressed):
  - Accuracy – agreement between accepted and obtained values
  - Precision – consistency of measurements
  - Range – upper/lower limits of detection (e.g., split depletion prints)
  - Repeatability – intra-assay precision
  - Reproducibility – replication of data by another examiner
  - Robustness – efficacy of method to small variations in parameters
  - Specificity – ability to detect analyte in presence of other components

For additional, more detailed information see “Supplemental Information”

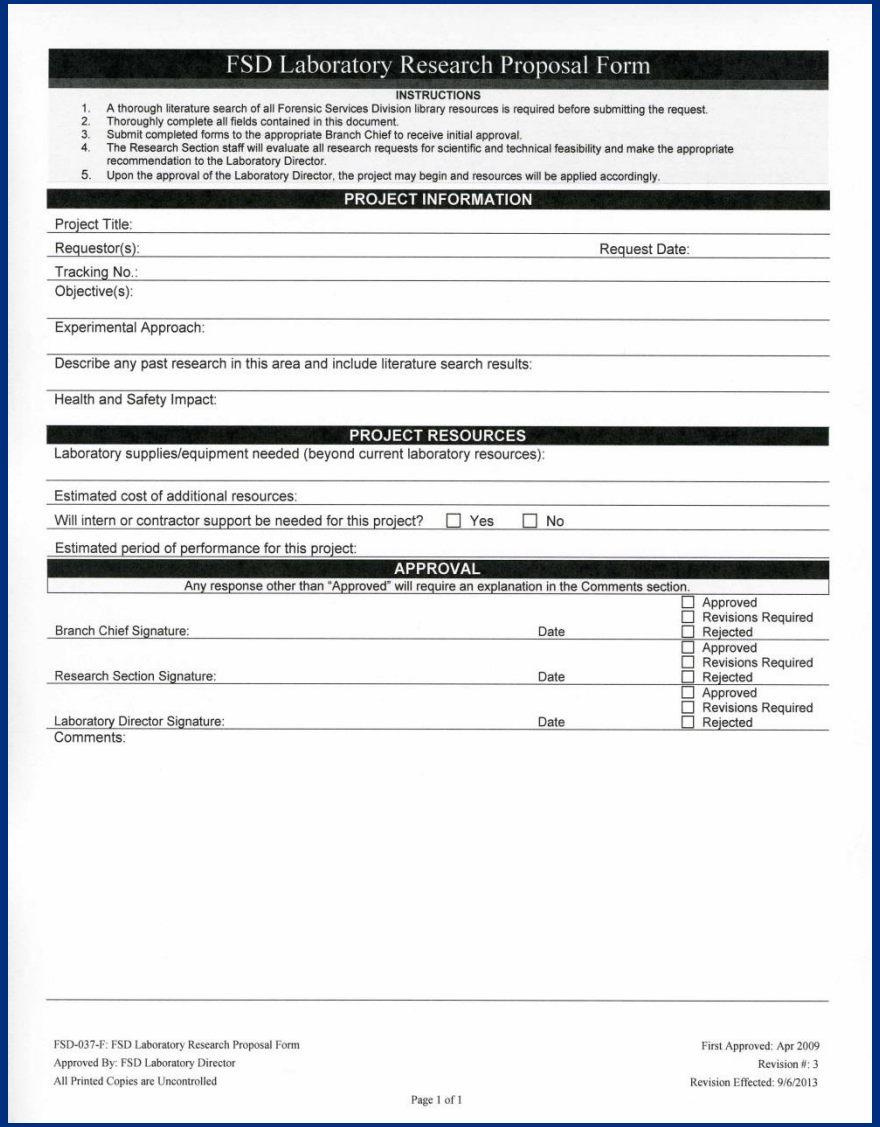
Key Elements
Validation Levels II/III

Key Elements – Levels II/III

- Remember – Validation Levels II/III ≠ Empirical Research
  - Previously tested methods or pieces of equipment (i.e., COTS) that have been validated/tested or published in peer reviewed publications do not require extensive testing/experimentation.
  - These validation tests can focus primarily on repeatability testing; however, in rare cases (e.g., satellite laboratories), reproducibility would also have to be addressed.
  - Where applicable, the use of stock “test sets” to test software (e.g., ULW, FISH) can significantly increase efficiency when conducting these types of validation tests.

Documentation

- The Laboratory Research Proposal Form formally initiates the research process.
- Accompanied by: 1) design of experiment(s), 2) detailed cost estimate, and 3) literature review.
- The Method/Equipment Validation Form completes the formal process with approvals and impact on laboratory SOPs.



FSD Laboratory Research Proposal Form

INSTRUCTIONS

1. A thorough literature search of all Forensic Services Division library resources is required before submitting the request.
2. Thoroughly complete all fields contained in this document.
3. Submit completed forms to the appropriate Branch Chief to receive initial approval.
4. The Research Section staff will evaluate all research requests for scientific and technical feasibility and make the appropriate recommendation to the Laboratory Director.
5. Upon the approval of the Laboratory Director, the project may begin and resources will be applied accordingly.

PROJECT INFORMATION

Project Title:

Requestor(s): Request Date:

Tracking No.:

Objective(s):

Experimental Approach:

Describe any past research in this area and include literature search results:

Health and Safety Impact:

PROJECT RESOURCES

Laboratory supplies/equipment needed (beyond current laboratory resources):

Estimated cost of additional resources:

Will intern or contractor support be needed for this project? ☐ Yes ☐ No

Estimated period of performance for this project:

APPROVAL

Any response other than "Approved" will require an explanation in the Comments section.

Branch Chief Signature: Date ☐ Approved ☐ Revisions Required ☐ Rejected

Research Section Signature: Date ☐ Approved ☐ Revisions Required ☐ Rejected

Laboratory Director Signature: Date ☐ Approved ☐ Revisions Required ☐ Rejected

Comments:

FSD-037-F: FSD Laboratory Research Proposal Form
Approved By: FSD Laboratory Director
All Printed Copies are Uncontrolled

First Approved: Apr 2009
Revision #: 3
Revision Effected: 9/6/2013

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Sample Plan with Cost Estimate

1) **Analytical Procedure**
Thermal Ribbon Analysis Platform (TRAP) Operating Manual Draft.

2) **Specificity**
N/A; analyte identity is not evaluated.

3) **Accuracy**
Scan at least three types of thermal ribbons using the TRAP and a desktop scanner. Crosscheck the results for accuracy. (Ribbons # 1, 2, 3)

4) **Precision**
   a. **Repeatability**
      i. Have a single examiner scan the same thermal ribbon once a week for four weeks. (Ribbon # 4)
   b. **Intermediate Precision**
      i. Have no less than four examiners independently scan the same thermal ribbon. (Ribbon #5)
   c. **Reproducibility**
      N/A; no other laboratories have such a system.

5) **Detection Limits**
N/A; no quantitation conducted.

6) **Quantitation Limits**
N/A; no quantitation conducted.

7) **Linearity**
N/A; no quantitation conducted.

8) **Range**
Ribbons of various widths and lengths will be tested under Robustness section.

9) **Robustness**
Use the system to scan a variety of ribbons to demonstrate robustness.
Include at least:
- A foil ribbon (Ribbon # 6)
- A label maker thermal ribbon (Ribbon # 7)
- A CR80 thermal ribbon (Ribbon # 11)
- A letter size thermal ribbon (Ribbon # 8)
- A CMYK thermal ribbon (Ribbon # 9)
- A CMYKO thermal ribbon (Ribbon # 10)

10) **Published Literature and Standards**
Relevant literature will be pulled mostly from the FSD library and askSam database of scientific articles.

Table 1: Paper types used in this study

Paper Type	Characteristics	Manufacturer Information
White photocopy paper	20#, recycled	
White Lined paper		
Yellow legal pad paper		

Table 2: Latent print processes used in this study

Chemical Process(es)
Indanedione-zinc (acetone/PE formulations)
Ninhydrin (acetone/PE formulations)
Physical developer
Indanedione-zinc (acetone formula) + physical developer
Ninhydrin (acetone formula) + physical developer

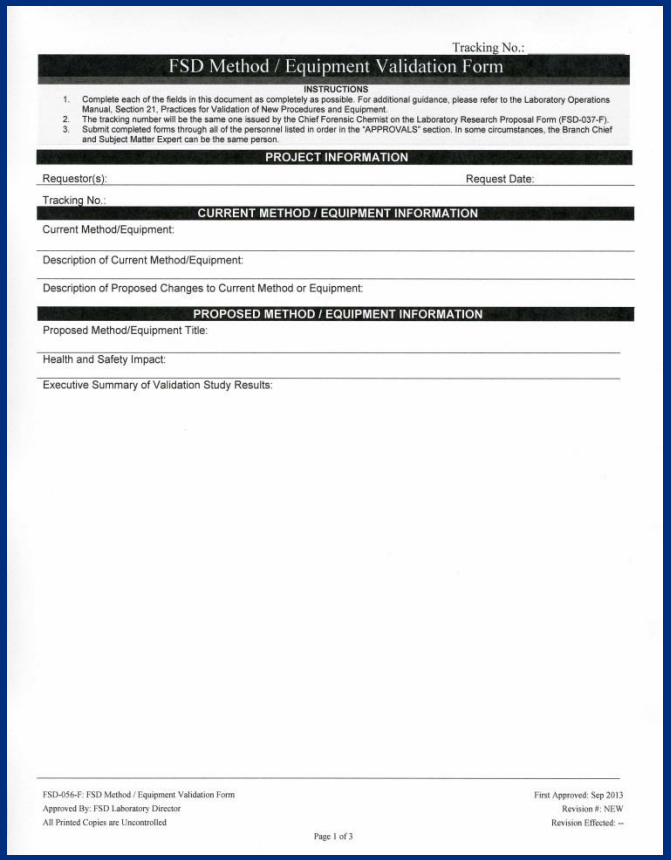
Table 3: Supplies needed for this study

Supplies (for ~400 samples)	Number Needed	Cost
Replacement solvents[1] (acetone, PE, ethanol, acetic acid)		$192.47
Replacement reagents (zinc chloride)	< 1 g	nominal
PD reagent[2]	3 L	$165.00
Magnetic Powder[3]	1	$41.00
Hair Spray (AquaNet)	12	$42.00
Total Cost		$440.47

Method/Equipment Validation Form

- Ensure that the results are summarized and that each participant and reviewer of the work product signs and dates the validation form.
- Reviewers should consist of (but not potentially be limited to):
  - Section/Unit supervisor
  - Subject matter experts (SMEs)
  - Laboratory Director
  - Chief/Senior Scientist (or similar position)
  - Alternates/Designees (as needed)

Documentation



Tracking No.:

FSD Method / Equipment Validation Form

INSTRUCTIONS

1. Complete each of the fields in this document as completely as possible. For additional guidance, please refer to the Laboratory Operations Manual, Section 21, Practices for Validation of New Procedures and Equipment.
2. The tracking number will be the same one issued by the Chief Forensic Chemist on the Laboratory Research Proposal Form (FSD-037-F).
3. Submit completed forms through all of the personnel listed in order in the "APPROVALS" section. In some circumstances, the Branch Chief and Subject Matter Expert can be the same person.

PROJECT INFORMATION

Requestor(s): Request Date:

Tracking No.:

CURRENT METHOD / EQUIPMENT INFORMATION

Current Method/Equipment:

Description of Current Method/Equipment:

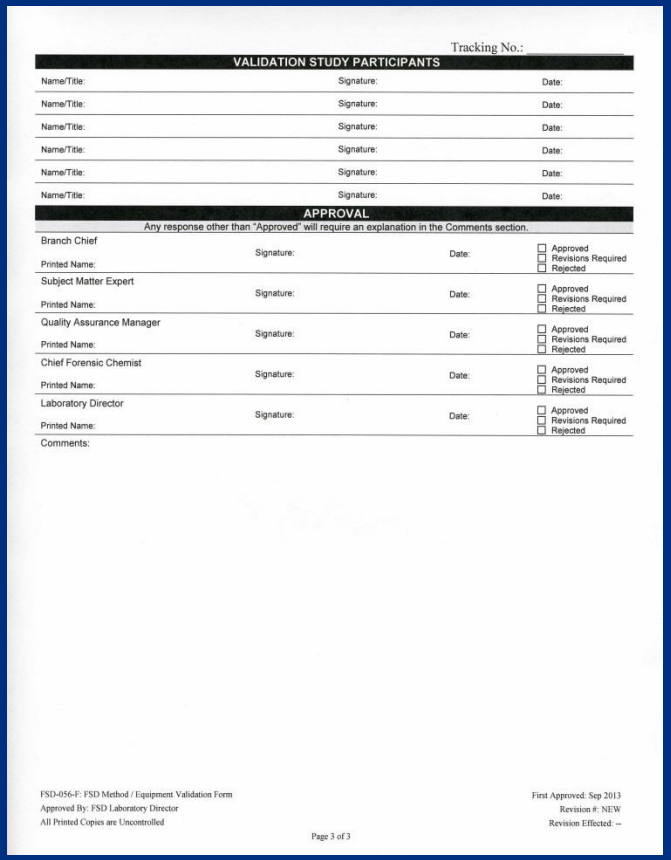
Description of Proposed Changes to Current Method or Equipment:

PROPOSED METHOD / EQUIPMENT INFORMATION

Proposed Method/Equipment Title:

Health and Safety Impact:

Executive Summary of Validation Study Results:



Tracking No.:

VALIDATION STUDY PARTICIPANTS

Name/Title:	Signature:	Date:
Name/Title:	Signature:	Date:
Name/Title:	Signature:	Date:
Name/Title:	Signature:	Date:
Name/Title:	Signature:	Date:
Name/Title:	Signature:	Date:

APPROVAL

Any response other than "Approved" will require an explanation in the Comments section.

Branch Chief Printed Name:	Signature:	Date:	☐ Approved ☐ Revisions Required ☐ Rejected
Subject Matter Expert Printed Name:	Signature:	Date:	☐ Approved ☐ Revisions Required ☐ Rejected
Quality Assurance Manager Printed Name:	Signature:	Date:	☐ Approved ☐ Revisions Required ☐ Rejected
Chief Forensic Chemist Printed Name:	Signature:	Date:	☐ Approved ☐ Revisions Required ☐ Rejected
Laboratory Director Printed Name:	Signature:	Date:	☐ Approved ☐ Revisions Required ☐ Rejected

Comments:

Example – Level I Validation

- Determine the ability of 1,2-indanedione to develop latent prints and how it compares to existing methods.
- 1,2-indanedione was a novel reagent – first used for LP development in 1996.
- Several peer-reviewed articles on this reagent were published between 1997-2008 (complicated by addition of $ZnCl_2$).
- 10s of donors; 1000s of samples; 10s of evaluators; many environmental factors studied
- It was accepted into USSS SOPs in 2009.

J Forensic Sci, September 2008, Vol. 53, No. 5
doi: 10.1111/j.1556-4029.2008.00826.x
Available online at: www.blackwell-synergy.com

Danna E. Bicknell,[1] M.S.F.S. and Robert S. Ramotowski,[1] M.S.

Use of an Optimized 1,2-Indanedione Process for the Development of Latent Prints*

ABSTRACT: 1,2-Indanedione belongs to a class of compounds which have demonstrated great potential in the processing of latent prints, particularly in the area of fluorescence. However, variability in results achieved worldwide has precluded it from being used extensively. In order to isolate the cause of this variability, various components of the formulation were analyzed, including purity level of the indanedione, type of carrier solvent, and the use of $ZnCl_2$ both as a secondary application and incorporated into the reagent. Using a resultant optimized formulation (Ind-Zn), performance comparisons were then made in the areas of visible color development, fluorescence, and degree of substrate staining with those of 1,8-diazafluoren-9-one (DFO) for both fresh and aged prints. Moisture content of the paper substrates on which the prints had been deposited was measured and a correlation found with percentage ambient relative humidity (% RH). Determination of visible color and fluorescence as it corresponded to percentage moisture content allowed for defining critical threshold levels necessary for achieving optimal results. Correlating these values with % RH then allowed for the development of standard operating procedures for obtaining best possible print development. Through this work, it was determined that a 7.4% v/v formulation of Ind-Zn having petroleum ether as a carrier solvent yielded the most optimal results when processing methods optimized for % RH in the laboratory were utilized. Both initial color development and fluorescence were superior to that of DFO; prints developed with Ind-Zn were a minimum of 6.5 units dE* darker and more red than with DFO for all substrates tested. Processing with Ind-Zn on the majority of the substrates examined yielded fluorescence intensity values approximately four times greater than with DFO.

KEYWORDS: forensic science, fingerprints, indanedione, DFO, relative humidity, moisture, fluorescence, visible color

Two of the most sensitive and widely used reagents for visualizing latent prints on paper are ninhydrin (1) and 1,8-diazafluoren-9-one (DFO) (2). The former compound is considered the standard for visible color detection of latent prints, while the latter is the standard for fluorescence detection. Ninhydrin offers many advantages as a reagent, including low cost and good solubility in a range of solvents. Its major drawback is that its reaction product with amino acids, Ruhemann's purple, is not fluorescent, which could limit its ability to aid in the detection of weak prints. DFO produces a weakly colored reaction product with latent print residue that has the advantage of exhibiting strong fluorescence without additional treatment. However, it has several disadvantages, including high cost and poor solubility.

The quest for improving the color and fluorescence obtained from the reaction of ninhydrin and amino acid residue in a latent print began with the work of Almog, et al. in the early 1980s (3). This effort focused on improving the fluorescence intensity of ninhydrin compounds without the need for liquid nitrogen or postreatment with zinc salt solutions. In the mid-1980s, the U.S. Secret Service began its research program to investigate these new ninhydrin analogs. At that time, a partnership was established with Dr. Madeleine Joullie's research group at the University of Pennsylvania's (UPENN) Department of Chemistry. Over the next 10 years, the Research Section of the Forensic Services Division evaluated nearly 100 compounds synthesized by that group (4). Although some of these compounds showed promise for latent print visualization, their commercial viability was limited by cost.

Although the synthesis of 1,2-indanediones (Ind) had been published before (5,6), these compounds had never before been tested on latent prints. In December of 1995, the first novel Ind compound was received for evaluation from the UPENN. Application of this compound, 6-methylthio-1,2-indanedione, to latent prints produced pale orange color ridge detail that fluoresced moderately. The fluorescence of this new compound was comparable to the best ninhydrin analogs. This fluorescence was significantly enhanced by the subsequent application of a zinc nitrate solution. In early September 1996, the parent compound, Ind, was submitted for evaluation. Application of this compound to both amino acid spots and latent prints on paper produced pale pink initial color ridge detail with moderate fluorescence. Once again, the subsequent treatment of these spots and prints with zinc nitrate resulted in not only enhancement of fluorescence, but also visible color. Given its structural simplicity and its relatively easy synthesis, Ind became one of the most commercially viable of all of the ninhydrin analogs produced up to that time.

Research began to focus on the optimization of the Ind reagent. Initial studies reported that the application of zinc salt solutions significantly enhanced the intensity of the fluorescent reaction product, making it comparable and in some cases better than DFO (7–9). Others reported that the application of zinc chloride had little or no effect on the fluorescence intensity (10,11). The fluorescence of some of the compounds evaluated was found to be superior to that of DFO even without subsequent zinc salt treatment. Other studies found that the performance of DFO was superior to Ind (12–14). Another publication reported that when deciding with which reagent to process porous items (ninhydrin or 1,2-indanedione), the

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*Presented, in part, in poster format at the Spring 2007 Educational Conference, Chesapeake Bay Division of the International Association for Identification, Cumberland, MD, March 30, 2007.

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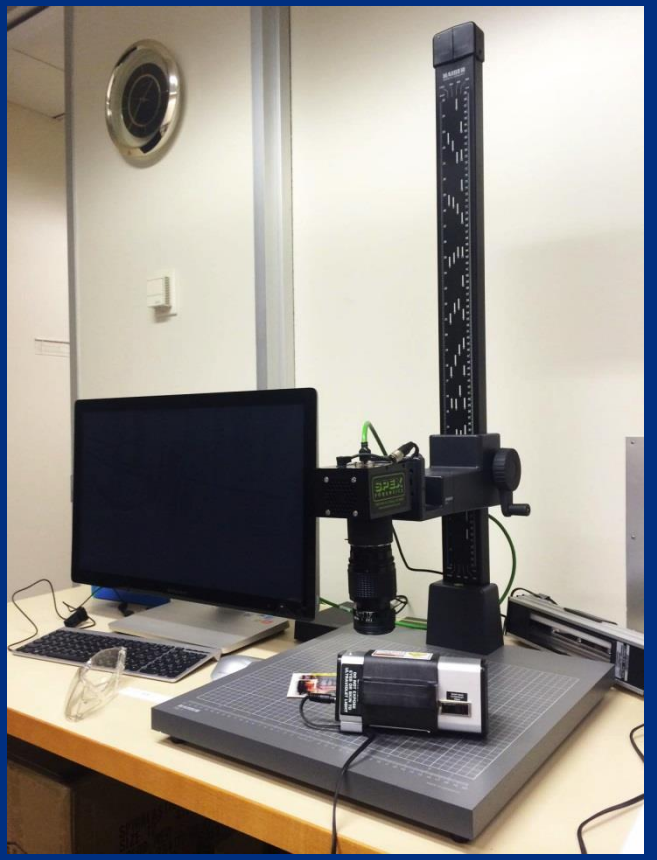
Example – Level II Validation

- Determine the impact of changing the grade/purity of a particular chemical.
- In this case, determine how two less pure silver nitrate grades affect the success of the physical developer process.
- X donors; 202 samples total (101 each comparison); 4 evaluators
- No significant operational impact was found regardless of silver nitrate grade.

Example – Level II/III Validation

- Since only imaging is involved, the evaluation of a new RUVIS can be classified as a level III test.
- Level III testing requires only modified function/performance testing to prove that this piece of equipment is “fit for purpose”.
- 2 donors; ~50 samples total; 2 evaluators (1-2 weeks total time)
- Documentation is still required (as are approvals and technical reviews).



Health and Safety Impact

- Have health and safety personnel in your organization review the impact of the new equipment or method.
- Assess the impact of any new potentially hazardous chemicals.
- Assess the impact of the new chemicals on waste disposal.
- Health and safety officer should sign off on the final documentation to confirm that this review took place.

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Supplemental Information

ISO/IEC 17025:2005(E) Requirements

ISO/IEC 17025:2005(E)

- **Section 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.

ISO/IEC 17025:2005(E)

- **Section 5.4.5.2 (NOTE 2)**

The techniques used for the determination of the performance of a method should be one of, or a combination of, the following:

- Calibration using reference standards or reference materials
- Comparison of results achieved with other methods
- Inter-laboratory comparisons;
- Systematic assessment of the factors influencing the result;
- Assessment of the uncertainty of the results based on scientific understanding of the theoretical principles of the method and practical experience.

ISO/IEC 17025:2005(E)

- **Section 5.4.5.2 (NOTE 4)**
  - Validation studies can be conducted by the scientific community (as in the case of standard or published methods) or by the forensic laboratory itself (as in the case of methods developed in-house or where significant modifications are made to previously validated methods).

ISO/IEC 17025:2005(E)

- **Section 5.4.5.4**
  - Prior to implementation of a validated method new to the laboratory, the reliability of the method shall be demonstrated in-house against documented performance characteristics of that method.
  - Records of performance shall be maintained for future reference.

ISO/IEC 17025:2005(E)

- **Section 5.4.7.2**

When computers or automated equipment are used for the acquisition, processing, recording, reporting, storage or retrieval of test or calibration data, the laboratory shall ensure that:

a) *Computer software developed by the user is documented in sufficient detail and is suitably validated as being adequate for use*;

NOTE: Commercial off-the-shelf software (e.g., word processing, database and statistical programs) in general use within their designed application range may be considered to be sufficiently validated. *However, laboratory software configuration/modifications should be validated as in 5.4.7.2a*.

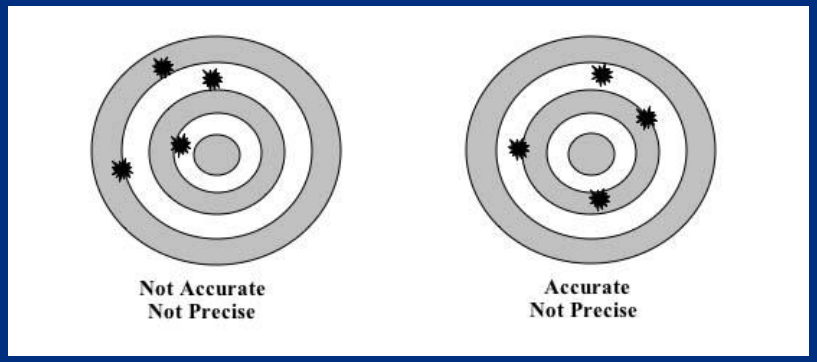
Key Elements

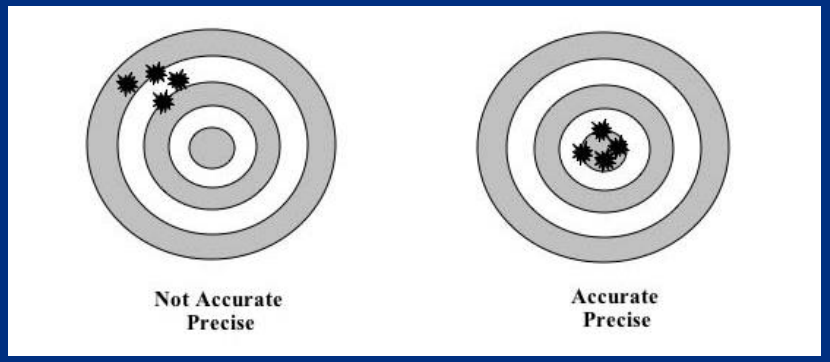
Validation Level I

Accuracy/Precision

- Accuracy is the agreement between the accepted and the obtained value.

- Precision is the ability of a measurement to be consistently reproduced.

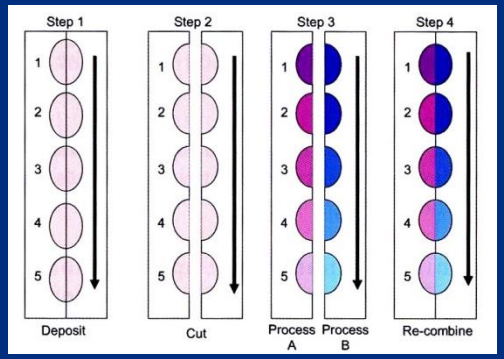


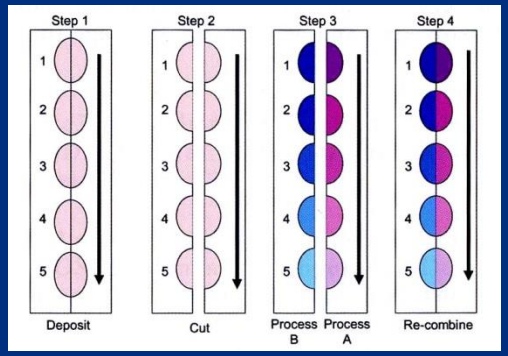


http://celebrating200years.noaa.gov/magazine/tct/accuracy_vs_precision.html (accessed 6/27/14)

Range

- Range covers the upper and lower values of a particular analyte in a sample capable of being detected by a method.
  - (e.g., the use of split depletion samples to create a range of amino acids or lipid concentrations to test amino acid or lipid reagents).





From: Lee JL, Bleay SM, Sears VG, Mehmet S, Croxton R. Evaluation of the Dimethylamino-cinnamaldehyde Contact Transfer Process and its Application to Fingerprint Development on thermal Papers. *J Forensic Ident.* 2009;59(5):551.

Repeatability/Reproducibility

- Repeatability – intra-assay precision; measurements by one person or instrument on the same item (and over a short time interval).
  - Can one examiner using a particular instrument (e.g., GC-MS) or method (e.g., ninhydrin) process the same sets of samples on different days and obtain the same (or similar) results that are acceptable?
- Reproducibility is the ability of a result to be replicated by someone else independently.
  - Can multiple examiners using a particular instrument (e.g., GC-MS) or method (e.g., ninhydrin) process the same sets of samples and obtain the same (or similar) results that are acceptable?
  - Can the technique be reproduced by a competent practitioner in another laboratory with the same equipment and resources?

Robustness

- Robustness – The resistance to small variations in method parameters.
  - Use of multiple substrate types
  - Use of multiple donors/samples
  - Changes in environmental conditions (e.g., temperature, %RH)
  - Changes in concentrations of certain components of a method (e.g., changing the concentration of ferric nitrate in PD to see if it changes the expected result)

Specificity

- Specificity – The ability to assess an analyte in the presence of other components.
  - Does the method successfully develop the latent print without developing the background substrate as well (e.g., using powder suspensions on methacrylate-based adhesives)?
  - Does the presence of interfering species cause the reagent to become less effective or even ineffective (e.g., the presence of calcium ions on paper causes the reagent physical developer to bind indiscriminately)?