Validation in ISO 17025 Accredited Laboratories – Policy Guidance and a Recent Example of a Validation Study

INTERNATIONAL ASSOCIATION FOR IDENTIFICATION
Atlanta, GA

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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 5.4.5.4

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.
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.
Nomenclature

- Exemptions
  - It should be noted that certain equipment, like purely optical devices (e.g., magnifiers, stereomicroscopes), do not require validation testing or documentation. This would also apply to other equipment like scanners, optical microscopes, and commercial-off-the-shelf software packages (e.g., Microsoft Office, Photoshop). This type of equipment need only be tested to ensure that it is in good working order and no formal documentation of performance is required.
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 depleted LP).
  - **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.
Accuracy/Precision

- **Accuracy** is the agreement between the accepted and the obtained value.
- **Precision** is the ability of a measurement to be consistently reproduced.

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).

Repeatability

- **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

- **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)?
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.
Sample Plan with Cost Estimate

1) Analytical Procedure

2) Specificity
N/A; analytic 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. (Ribbons # 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 CMYK0 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.

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Table 1: Paper types used in this study

<table>
<thead>
<tr>
<th>Paper Type</th>
<th>Characteristics</th>
<th>Manufacturer Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>White photocopied paper</td>
<td>20#, recycled</td>
<td></td>
</tr>
<tr>
<td>White lined paper</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yellow legal pad paper</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Latent print processes used in this study

<table>
<thead>
<tr>
<th>Chemical Process(es)</th>
</tr>
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<tbody>
<tr>
<td>Indanedione-zinc (acetone/PE formulations)</td>
</tr>
<tr>
<td>Ninhydrin (acetone/PE formulations)</td>
</tr>
<tr>
<td>Physical developer</td>
</tr>
<tr>
<td>Indanedione-zinc (acetone formula) + physical developer</td>
</tr>
<tr>
<td>Ninhydrin (acetone formula) + physical developer</td>
</tr>
</tbody>
</table>

Table 3: Supplies needed for this study

<table>
<thead>
<tr>
<th>Supplies (for ~400 samples)</th>
<th>Number Needed</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replacement solvents¹ (acetone, Pf, ethanol, acetic acid)</td>
<td>&lt; 1 g nominal</td>
<td>$152.47</td>
</tr>
<tr>
<td>Replacement reagents (zinc chloride)</td>
<td>3 L $165.00</td>
<td></td>
</tr>
<tr>
<td>PD reagent²</td>
<td>1</td>
<td>$41.00</td>
</tr>
<tr>
<td>Hair Spray (Aquashot)</td>
<td>12</td>
<td>$42.00</td>
</tr>
<tr>
<td>Total Cost</td>
<td></td>
<td>$440.47</td>
</tr>
</tbody>
</table>
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/Desigenees (as needed)
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.
21 Practices for Validation of Procedures and Equipment

This document provides the general practices for conducting validation studies prior to the use of technical procedures or instrumentation in the FSD Laboratory. These practices shall apply to both routine and non-routine procedures. Validation is the process by which the scientific community acquires the information necessary to assess a procedure's capability for obtaining reliable and reproducible results.

21.1 Scope

These practices shall apply to all FSD laboratory personnel who are involved in validation studies of new or novel methods and/or equipment, whether the method was developed internally or externally. It is important to note that regardless of whether the new or modified technique and/or equipment was validated externally or published in a peer-reviewed scientific journal, an internal validation study is still required before it can be applied to casework.

The scope of the validation study shall depend on whether or not the method/equipment is novel or if it involves a mature technology or procedure. Established methods or technologies that have been published in peer-reviewed scientific journals would require a more limited validation study whereas a truly novel method or piece of equipment would require a more extensive validation. The impact of the changes will dictate the scope of the validation study. Validation testing has been broken down into different levels based on what testing is required. These different levels (as well as exemptions) are explained in the subsections below. Note that all testing should be properly documented (see also QAM 5.5 Equipment or 5.5.2 and LOM 7.5 Equipment Calibration and Maintenance).

21.1.1 Level I Validation

A level I validation is a comprehensive examination of the technical and scientific parameters of a truly novel technique or instrument. This level of validation would require extensive sample testing to fully examine repeatability and reproducibility (or other factors listed in section 21.3.1.2). Examples of what would qualify for a level I validation study would include the Thermal Ribbon Analysis Platform (TRAP) system (a completely new and unique instrument) and the original introduction of 1,2-indanedione-dnc (a completely new and unique method). For guidance in developing a project plan for level I validation studies, the appropriate peer reviewed guidelines should be consulted (e.g., Sears VG, Bleay SM, Bandey HL, Bowman VJ. A Methodology for Finger Mark Research. Sci. Just. 2012, 52 (3), 145-160; International Fingerprint Research Group. Guidelines for the Assessment of Fingermark Detection Techniques. J Forensic Ident 2014;64(2):174-200).

21.1.2 Level II Validation

A level II validation is a more limited-scale examination of the technical or scientific aspects of a well-established technique or instrument. Such an evaluation should consist of approximately 50-100 samples, require as few as two participants (to investigate repeatability and reproducibility), and be able to be completed within 1-2 weeks (depending on the complexity of the sample preparation). Examples of instruments or techniques that would require level II validation would include the replacement of the RO/DI purification system; replacement of standard, commercially available instruments (e.g., FTIR, SEM, XRF); and changing the purity or manufacture of a chemical used for processing latent prints or in a particular analytical technique. With regard to instrumentation, it would be beneficial to incorporate (if possible) a written statement from the company technician installing the equipment (along with his/her signature) that certifies that the equipment has been calibrated and is working
Silver Nitrate Validation Study
Justification

- The international standard ISO/IEC 17025:2005(E), section 5.4.5.2, specifies that “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.”

- When substituting for a chemical in any reagent, including changes in reagent grade/purity and/or manufacturer, a validation study must be conducted to ensure no loss of process efficiency will occur.

- The performance of a new chemical must be compared directly against the effectiveness of the current, validated method.
Background

- A chemical that meets the requirements of the U.S. Pharmacopeia and is acceptable for drug, medicinal, food, and laboratory use is labeled as USP grade.

- Chemicals labeled as technical grade are typically less pure than the other two grades. Technical grade chemicals are acceptable for industrial or commercial use, but they are not pure enough for drug, medicinal, or food use.
Background

- The price of silver nitrate in recent years has increased significantly, making the cost of the overall PD reagent more expensive.

- The use of a lower grade of silver nitrate could be more cost-effective; however, the potentially adverse effects of using the lower grade chemical and its potential impurities are not known.
FSD Laboratory Research Proposal Form

INSTRUCTIONS
1. A thorough literature search of all Forensic Sciences Division Library resources is required before submitting the request.
2. Completely fill out 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: Physical Developer Evaluation
Requestor(s): Ramotowski
Request Date: 4/12/11

Objective(s):
The objective of this project is to evaluate whether or not different grades of silver nitrate can be used to prepare physical developer. The effect of silver nitrate purity will be evaluated with respect to reagent performance.

Experimental Approach:
The experimental approach will generally follow the experimental procedure outlined in: Kent T. Standardizing Protocols for Fingerprint Reagent Testing. J Forensic Ident. 2010;50(3):371-379. Many different donors, substrates, and reagents will be used to test the various hypotheses described above. In addition, split depletion prints will be used to isolate variables for comparative evaluation.

Describe any past research in this area (include literature search results as an attachment to this request):

n/a

CONTINUED ON BACK

Project Title: Physical Developer Evaluation
Requestor(s): Ramotowski
Request Date: 4/12/11

PROJECT RESOURCES

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

n/a

Estimated cost of additional resources: n/a

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

Estimated period of performance for this project: 1 month

APPROVAL

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

Branch Chief Signature: [Signature]
Date: 4/12/11

Research Section Signature: [Signature]
Date: 4/12/11

Laboratory Director Signature: [Signature]
Date: 4/12/11
Methodology

- Experiment 1: 4 females/5 males.
- 101 depletion strips (6 per strip).
- Experiment 2: 4 females/6 males.
- 101 depletion strips (6 per strip).
- Latent prints were aged from 2-12 months prior to processing.
- Evaluators were: a non-expert; 2nd year trainee; and three IAI certified examiners with 13, 23, and 30 years of experience.
Methodology


Materials

- Substrate A: Premium white copy paper (Hammermill; 28#, 100)
- Substrate B: White photocopy paper (Xerox; 20#, 92)
- Substrate C: Steno notebook paper (Quill, 6” x 9”, Gregg ruled)
- Substrate D: Newsprint (Washington Post Express)
- Substrate E: Newsprint (Washington Post)
- Substrate F: Manila envelope paper (Quill, 28#)
- Substrate G: Brown Kraft paper (Uline)
### Evaluation/Scoring

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>+2</td>
<td>Left side shows significantly better development</td>
</tr>
<tr>
<td>+1</td>
<td>Left side shows slightly better development</td>
</tr>
<tr>
<td>0</td>
<td>No difference in development</td>
</tr>
<tr>
<td>-1</td>
<td>Right side shows slightly better development</td>
</tr>
<tr>
<td>-2</td>
<td>Right side shows significantly better development</td>
</tr>
</tbody>
</table>

PD Working Solution (Current SOP)

Redox Solution
- 30 g ferric nitrate
- 80 g ferrous ammonium sulfate
- 20 g citric acid
- 900 mL RO/DI water

Detergent Solution
- 3 g n-dodecylamine acetate
- 3 mL Tween 20
- 1 L RO/DI water

Silver Nitrate Solution
- 10 g silver nitrate
- 50 mL RO/DI water

Malic Acid Solution
- 25 g malic acid
- 1 L RO/DI water

PD Working Solution
- 900 mL Redox Solution
- 40 mL Detergent Solution
- 50 mL Silver Nitrate Solution
Results – ACS vs. USP
Results

- (l) An image of the first three depletions in the series of a sample from Experiment 1 in which the majority of evaluators selected the right side as being superior (the PD containing the ACS grade silver nitrate).

- (r) An image of the first three depletions in the series of a sample in which the majority of evaluators selected the right side as being superior (the PD containing the USP grade silver nitrate).
Results

- An image of a sample from Experiment 1 that resulted in mixed voting.

- In this sample, the PD containing the ACS grade silver nitrate processed sample is on the left and the PD containing the USP grade silver nitrate processed sample is on the right.

- There was one vote for 0 and two votes each for +1 and -1.
Results – ACS vs. Technical

![Bar Chart]

- Evaluator 1
- Evaluator 2
- Evaluator 3
- Evaluator 4
- Evaluator 5
- Consensus

Comparison categories:
- No Difference
- Technical
- ACS
Results

- (l) An image of the first three depletions in the series of a sample from Experiment 2 in which the majority selected the right side as being superior (the PD containing the ACS grade silver nitrate).

- (r) An image of the first three depletions in the series of a sample in which the majority selected the right side as being superior (the PD containing the technical grade silver nitrate).
Results

- An image of a sample from Experiment 2 that resulted in mixed voting.
- In this sample, the PD containing the ACS grade silver nitrate processed sample is on the left and the PD containing the technical grade silver nitrate processed sample is on the right.
- There was one vote for 0, two for +1, and one each for -1 and -2.
Discussion

[Ag⁺]/[Ag⁺]_{fresh}

0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0

Good Quality Marks

Development Time ≤ 20 min

No Marks Developed

Poor Quality Marks

Development Time ≥ 20 min

[Fe²⁺]/[Fe²⁺]_{fresh}
for identification purposes. Thus, even though the current ACS grade silver nitrate was chosen to produce “superior” prints 7% (versus USP) and 12% (versus technical) of the time, the overall impact of switching to either grade of these silver nitrate would be negligible. One primary reason for this is that there is a considerable excess of silver nitrate present in the PD working solution. Small changes in silver nitrate purity would have no significant impact in changing the quantity of silver ions available in solution to form the colloidal silver particles that would deposit on the latent print ridges.

The overall recommendation is that both the technical and USP grades of silver nitrate would be acceptable substitutes for the current ACS grade.
VALIDATION STUDY PARTICIPANTS

| Name/Title: Allison Fuchs/Student Intern | Signature: See Email to Ramotowski | Date: 8/26/17 |
| Name/Title: Robert Ramotowski/Chief Forensic Chemist | Signature: | Date: 8/26/17 |
| Name/Title: Brian Jones/Fingerprint Specialist | Signature: | Date: 8/26/17 |
| Name/Title: Michael Mann/Fingerprint Specialist | Signature: | Date: 8/26/17 |
| Name/Title: Esther Chervinsky/Fingerprint Specialist | Signature: | Date: 8/26/17 |
| Name/Title: Kim Smith/Fingerprint Specialist | Signature: | Date: 8/26/17 |

APPROVAL

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

Branch Chief
Printed Name: Brian Jones
Signature: Brian Jones
Date: 4/7/17

Subject Matter Expert
Printed Name: Brian Jones
Signature: Brian Jones
Date: 4/7/17

Quality Assurance Manager
Printed Name: Kelli Lewis
Signature: Kelli Lewis
Date: 4/16/17

Chief Forensic Chemist
Printed Name: Robert Ramotowski
Signature: Robert Ramotowski
Date: 11/3/17

Laboratory Director
Printed Name: Robert Ramotowski
Signature: Robert Ramotowski
Date: 11/3/17

Comments:

Approved, Revision Requested

brian Jones 4/7/17

BC 04/03/17

Approved with Revisions

First Approval: Sep 2013
Revision #: NEW
Revision Effected: ---

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Conclusions

- Raw data is backed up on agency network.

- The PD working solution prepared using the USP grade was found to produce better results in 27% of the samples compared to 5% of the time for the ACS grade.

- The PD working solution prepared using the technical grade was found to produce better results in only 17% of the samples compared to 10% of the time for the ACS grade.

- There was a tendency at the lower end of the experience scale to determine that the two halves of a sample were equal (i.e., a grade of 0). The three IAI certified examiners were more likely to choose one side as being of better overall quality.
Conclusions

- Based on these results, a lower grade of silver nitrate (e.g., USP, technical) could be used in the physical developer working solution and that it would be more cost effective to forensic laboratories without sacrificing the overall quality of fingerprint development.
Contact Information

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