Validation of new assays for Genetic Diagnostics

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Overview

• Why validate?
• What is validation?
• When is validation required?
• How should it be done?
Why?

• To ensure tests can robustly provide correct and appropriate results for patients

• Regulatory requirement of laboratory accreditation
The Standard

- ISO 15189:2012 Medical laboratories -- Requirements for quality and competence
  - Section 5.5 deals with pre-implementation validation and verification
  - Section 5.6 deals with on-going quality assurance of tests and defining measurement uncertainty
For accreditation:

- You must validate your home-made tests
- You must (at least) verify all your imported tests, even well-established kits
- You must document the validations
- You must deal with measurement uncertainty, “where relevant” (it almost always is)
- You must plan and perform appropriate IQC and participate in inter-lab comparisons (e.g. EQA)

Required background

- Trained staff; validated material (instruments, reagents)
## Laboratory accreditation

<table>
<thead>
<tr>
<th>Country</th>
<th>Reference</th>
<th>Requirement</th>
</tr>
</thead>
<tbody>
<tr>
<td>UK</td>
<td>Clinical Pathology Accreditation (UK) Ltd (CPA) - Standards for the Medical Laboratory - Section F</td>
<td>No clear formal requirement Transition to ISO15189 by 2018 (<a href="http://www.cpa-uk.co.uk/">http://www.cpa-uk.co.uk/</a>)</td>
</tr>
<tr>
<td>France</td>
<td>Guide de bonne exécution des analyses de biologie médicale (GBEA)</td>
<td>Accreditation soon a legal requirement</td>
</tr>
<tr>
<td>Belgium</td>
<td>7 JUNE 2007 — Arrêté royal</td>
<td>Must be accredited to ISO 15189 or equivalent standard</td>
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<tr>
<td>Germany</td>
<td>Medizinprodukte-Betreiberverordnung (6.2.2)</td>
<td>The medical laboratory shall use only validated examinations</td>
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<tr>
<td>Switzerland</td>
<td>OAGH 2004 Art 15.</td>
<td>Appropriate system of quality management - ISO15189, 17025 or equivalent</td>
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Other references

- In-Vitro Diagnostic Directive (IVDD) 98/79/EC
- ISO/IEC 17025:2005 -- General requirements for the competence of testing and calibration laboratories
- ISO 9000:2005 -- Quality management systems — Fundamentals and vocabulary
- ISO 9001:2000 -- Quality management systems — Requirements
- ISO 31 (all parts) -- Quantities and units
What is validation?

- **Validation**: "Confirmation, through the provision of objective evidence, that the requirements for a specific intended use or application have been fulfilled" [CPA 3.37][ISO 9000:2000]

- **Verification**: "Confirmation, through the provision of objective evidence, that specified requirements have been fulfilled" [CPA 3.38][ISO 9000:2000]
What is validation?

- **Validation**: "Confirmation, through the provision of objective evidence, that the requirements for a specific intended use or application have been fulfilled" [CPA 3.37][ISO 9000:2000]

  Are we doing the correct test?

- **Verification**: "Confirmation, through the provision of objective evidence, that specified requirements have been fulfilled" [CPA 3.38][ISO 9000:2000]

  Are we doing test correctly?
Performance specification

Should comprise (at least):

- An estimate of the test **ACCURACY** including measurement uncertainty (eg, confidence limits)

- Limitations on **critical parameters** that will ensure the desired level of accuracy.

- Control measures required for monitoring routine maintenance of this level of accuracy
When do we need to validate?

ISO 15189 – Examination procedures

- Tests must be **VALIDATED** by manufacturer / method developer (define a *performance specification*).

- For ‘bought in’ tests, performance must be **VERIFIED** (against *performance specification*) before implementation -- Applicable to CE marked tests only.

- Any deviations from validated (CE marked) test must be fully **VALIDATED**.
New Test

Novel test
No performance spec

Existing test
Performance spec

Test development and assessment of utility

Validation
Define performance spec

Verification
Against performance spec

Implementation

On-going validation
Audit
Validation of Utility

- Applicability of measurements
- Selectivity
- Interferences
- Carry-over / cross contamination
How is validation performed?

By assessing the performance (ACCURACY) of the test in comparison with a ‘gold standard’ or reference.

Gold standard is a set of control samples that have mutational status assigned without error.
Components of Accuracy
Quantitative tests:

A key function of validation / verification is to estimate **ACCURACY**

- **Trueness**
  - describes how close the test result is to the true result (BIAS)

- **Precision**
  - Repeatability
  - Reproducability
  - [Robustness]
  - describes how scattered replicate test results are (SD)
Precision

Variation of result under different conditions

- **Repeatability** → same sample same conditions

- **Reproducibility** → different samples, operator, PCR machine, lab etc

- **Robustness** → stability of result under specific challenge e.g. Annealing temp, extraction method.
Components of Accuracy
Qualitative tests:

- Sensitivity describes how good the test is at detecting positives (mutants)
- Specificity describes how good the test is at detecting negatives (wild types)

NB: Sensitivity has other meanings - care is needed to avoid confusion
The validation must be as extensive as is necessary to meet the needs of the given application.

The laboratory must determine the uncertainty of results, where relevant and possible.

**sampling error** is incurred when the statistical characteristics of a population are estimated from a subset, or sample, of that population.
Rule of Three

For the 95% confidence interval

The probability of NOT seeing a FN in a validation of sample size $n$ $\approx \frac{3}{n}$

Maximum measurable accuracy for study size $n$ (as percentage) $\approx 100 - \frac{3}{n} \times 100$

This is true for any proportional value for $n \geq 20$
Sample size calculation

Accuracy ≥ 99% (95% CI)
Reportable accuracy

Options for reporting 95% accuracy (95% CI)

- All mutations detected
- 1 mutation missed
- 2 mutations missed
- 5 mutations missed

Graphs show the lower and upper 95% confidence limits.
General considerations

- Normally studies should be balanced
- Analyses should be blinded
- Data from >1 run
- Training set vs validation set
- Representative controls
  - Positives should reflect known strengths and weaknesses of the methodology
Not enough positive controls!

- Boost sample numbers:
  - Artificial controls
  - Inter-laboratory study
- Technology validation?

Most importantly results should be reported transparently:

Accuracy should **always** be given as a range of values with a confidence level for e.g.

\[ \geq 98\% \ (95\% \ CI) \]

\[ 85 \ - \ 95\% \ (95\% \ CI) \]
Requirements for documentation

a) purpose of the examination;
b) principle of the procedure used for examinations;
c) performance specifications (e.g. linearity, precision, accuracy expressed as uncertainty of measurement, detection limit, measuring interval, trueness of measurement, analytical sensitivity and analytical specificity);
d) primary sample system (e.g. plasma, serum, urine);
e) type of container and additives;
f) required equipment and reagents;
g) calibration procedures (metrological traceability);
h) procedural steps;
i) quality control procedures;
j) interferences (e.g. lipaemia, haemolysis, bilirubinemia) and cross reactions;
k) principle of procedure for calculating results, including measurement uncertainty;
l) biological reference intervals;
m) reportable interval of examination results;
n) alert/critical values, where appropriate;
...
SUPPLEMENTARY INFORMATION

FROM:
A standardized framework for the validation and verification of clinical molecular genetic tests
Christopher J Mattocks, Michael A Morris, Gert Matthijs, Elfriede Swinnen, Anniek Corveleyn, Els Dequeker, Clemens R Müller, Victoria Pratt and Andrew Wallace for the EuroGentest Validation Group

Supplementary Data (doc 124K)
1. Validation / Verification Details

<table>
<thead>
<tr>
<th>Text</th>
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1.1 Validation details

- Target Use of Application
- User Interface
- Functional Requirement
- Test Procedure
- Test Environment
- Data
- Test Data

1.2 Validation details

- Test Plan
- Test Procedure
- Test Environment
- Data
- Test Data

2. Validation of Utility

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- Applicability of Requirements
- Security
- Interoperability
- User Interface

3. Validation / Verification of Requirements

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3.1 Workplan

- Test Plan
- Test Procedure
- Test Environment
- Data
- Test Data

3.2 Test Plan

- Test Plan
- Test Procedure
- Test Environment
- Data
- Test Data

4. Validation / Verification Final Conclusions

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- Compliance
- Internal Quality Control
- External Quality Control
- Internal Quality Assurance
- External Quality Assurance

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- Experimental Results
- Interpretation
- Outcome / Limitations

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- Authorisation
- Name
- Signature
- Date
Summary

- Reasons to validate
- Standard – ISO15189
- Requirements for lab accreditation
- Validation vs Verification
- Quantitative vs Qualitative
- Sample size and other key considerations
- Documentation
Validation must be practical!

"FOLKS, WE'RE JUST NOT DOING THIS RIGHT."
A standardized framework for the validation and verification of clinical molecular genetic tests

Christopher J Mattocks*1,7, Michael A Morris2,7, Gert Matthijs3,7, Elfriede Swinnen3, Anniek Corveleyn3, Els Dequeker3, Clemens R Müller4, Victoria Pratt5 and Andrew Wallace6, for the EuroGentest Validation Group8


http://www.clsi.org/
Following steps of the E-course

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- Interactive
- Assignments/ statements by experts
- Possibility to ask and answer questions
- Open for 14 days after course

Quiz

- Test your knowledge!

On the course content page:
- Links to forum and quiz
- Links to slides, recorded webinar and course manual