
An Application of the Kipling Method to DNA Validation in the 21st Century

**Introduction to Validation**

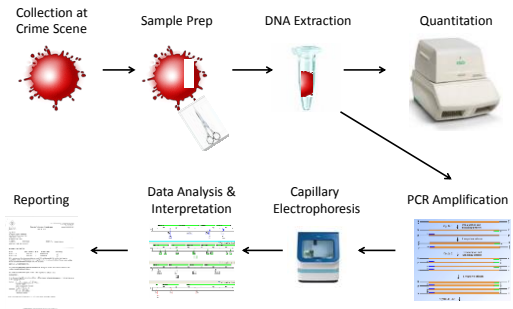
Charlotte J Word, Ph.D.

## Outline

- Overview of DNA Process
- Overview of Validation Needs at Each Step
- Categories of Validations
- Factors Affecting Validation



## Steps of DNA Testing



## Validation Needs

### DNA Extraction

- Introduce new extraction procedure
  - Chelex, organic, Qiagen columns, etc.
  - High pressure
- Manual to automated
- Aim for improved recovery
  - More DNA
  - Less inhibitors
- Area needing more research

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## Validation Needs

### Quantitation

- Introduce new quantitation kit or thermal cycler
- Manual to automated
- Aim for improved assessment of extracted DNA
  - How much total human? How much male vs. female? Degraded?
  - Inhibitors present

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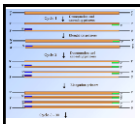

## Validation Needs

### Amplification

- Introduce new amplification kit or thermal cycler
- Manual to automated
- Aim for improved amplification and/or more data
  - Overcome inhibitors
  - Increased Sensitivity (less DNA needed)
  - Modification to developmental validation studies
  - Post-amplification clean-up

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## Validation Needs

### Capillary Electrophoresis

- Introduce new Genetic Analyzer
- Manual to automated sample preparation
- Aim for more data within range for interpretation
  - Increased injection time, voltage, sample in prep tube

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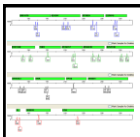

## Validation Needs

### Data Analysis & Interpretation

- Introduce new data analysis and/or interpretation software
- Aim for improved data analysis and interpretation
  - Detection of artifacts vs. true data
  - Statistical calculations
  - Automated interpretation and comparison to known individuals

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## Categories of Validation

- Required
  - CODIS expansion to 20 loci by January 1, 2017
    - Fusion or GlobalFiler amplification kits
    - Qiagen kits in summer of 2015
  - New or upgraded instrumentation
    - e.g., 3500 Genetic Analyzer or 3130 upgrade
      - To support new kits (GlobalFiler)
      - Loss of support of prior models

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## Categories of Validation

- Optional – existing system
  - New STR amplification kit (e.g., PowerPlex 16 to PowerPlex 16 HS; add Minifiler; add other new kit)
  - New quantitation kit (e.g., Quantifiler to Quant Trio)
  - Switch from GeneMapper *ID* to *ID-X* or GeneMarker
  - Automation/robotics
  - **New software for likelihood ratio calculations/ probabilistic genotyping**

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## Categories of Validation

- Optional – Expanded capabilities/New system/New service provided
  - Y STRs
  - Rapid DNA
  - mtDNA
  - SNPs
    - Phenotyping
    - Ancestry
    - Facial construction
  - "Next Generation" Sequencing
  - Familial Searching

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## Types of Validation

- Developmental Validation
  - For new systems
  - Not generally done in crime laboratory
  - Generally done by commercial manufacturer/ distributor

***Developmental validation*** is the acquisition of test data and determination of conditions and limitations of a new or novel DNA methodology for use on forensic and/or casework reference samples. [Definition from 2011 QAS for Forensic DNA Testing Laboratories]

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## Types of Validation

- Internal Validation
  - What we do in crime laboratories
  - After developmental validation is done

***Internal validation*** is the accumulation of test data within the laboratory to demonstrate that established methods and procedures perform as expected in the laboratory.

[Definition from 2011 QAS for Forensic DNA Testing Laboratories]

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## Validation vs. Performance Check

- Performance Check on duplicate instrumentation if have already validated the same model previously (e.g., RT-PCR thermal cyclers; Genetic Analyzers)
- Much more limited evaluations needed
  - Basically demonstrate new instrument performs in similar manner to existing instrument

***Performance check*** is a quality assurance measure to assess the functionality of laboratory instruments and equipment that affect the accuracy and/or validity of forensic sample analysis. [Definition from 2011 QAS for Forensic DNA Testing Laboratories]

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## Factors Affecting Validation

- Databasing vs. Casework laboratory
  - Single source vs. unknown & mixtures
- Portion of the process vs. the whole process
  - If portion, generally only the downstream part is affected
- Modifications to existing system vs. new system
  - mtDNA & sequencing
  - Experience

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## Factors Affecting Validation

- Many labs on line vs. first laboratory
  - Publications
  - Networking
  - Training
  - Understanding of strengths and limitations
  - Court – admissibility hearings?



## Steps of Validation

- Planning
  - Designing experiments
  - Getting equipment, reagents, etc.
  - Construction?
  - Personnel
- Doing the experiments, Data collection
- Data evaluation
- SOP development
- Summary write-ups



## Goals of Validation

- Test limit of system
- Find optimal range for generation of data
- Develop SOPs for bringing system on line