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

Introduction to Validation
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Outline

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

Steps of DNA Testing

Collection at Crime Scene → Sample Prep → DNA Extraction → Quantitation → PCR Amplification → Capillary Electrophoresis → Data Analysis & Interpretation → Reporting

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**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

**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

**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
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

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

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
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

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

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]
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]

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]

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
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