Standards, Validation, and Evaluation Studies to Aid the Forensic DNA Community

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Presentation Outline

• Importance of Quality Results in DNA Testing
• Introduction to NIST and its Role
• Standard Reference Materials
• Validation Resources
• Evaluation and Interlaboratory Studies

Quality Is Essential in Forensic DNA Testing

- DNA results impact lives – the guilty can be implicated in a crime and the innocent can be exonerated
- Scientific attacks against the science behind DNA testing are rare in court now. Rather the focus is on demonstrating that quality results were obtained.
- DNA databases involve comparisons of DNA profiles analyzed at different times or in different locations

DNA Testing Requires a Reference Sample

A DNA profile by itself is fairly useless because it has no context...
DNA analysis for identity only works by comparison – you need a reference sample

Crime Scene Evidence compared to Suspect(s) (Forensic Case)
Child compared to Alleged Father (Paternity Case)
Victim’s Remains compared to Biological Relative (Mass Disaster ID)
Soldier’s Remains compared to Direct Reference Sample (Armed Forces ID)

Elements for Guaranteeing Quality Results in Forensic DNA Testing

- Accepted Standards and Guidelines for Operation
- Laboratory Accreditation
- Proficiency Testing of Analysts
- Standard Operating Procedures
- Validated Methods
- Calibrated Instrumentation
- Documented Results
- Laboratory Audits
- Trustworthy Individuals

Our publications and presentations are made available at:
http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm
Many Labs Are Moving to ISO 17025 as Part of Their Laboratory Accreditation

ISO/IEC 17025

5.6 Measurement traceability

5.6.1 General

All equipment used for tests and/or calibrations, including equipment for subsidiary measurements (e.g., for environmental conditions) having a significant effect on the accuracy or validity of the result of the test, calibration or sampling shall be calibrated before being put into service. The laboratory shall have an established programme and procedure for the calibration of its equipment.

NIST History and Mission

- National Institute of Standards and Technology (NIST) was created in 1901 as the National Bureau of Standards (NBS). The name was changed to NIST in 1988.
- NIST is part of the U.S. Department of Commerce with a mission to develop and promote measurement, standards, and technology to enhance productivity, facilitate trade, and improve the quality of life.
- NIST is the National Metrology Institute for the U.S.
- NIST supplies over 1,300 Standard Reference Materials (SRMs) for industry, academia, and government use in calibration of measurements.

Overview of NIST Organization

http://www.cstl.nist.gov/biotech/DNA Technologies/Human_Identity.htm
NIST Human Identity Project Team

John Butler (Project Leader) Margaret Kline Pete Vallone Mike Coble

Dave Duewer Jan Redman Amy Decker Becky Hill Chris DeAngelis

Funding: Interagency Agreement 2003-IJ-R-029 between National Institute of Justice (NIJ) and NIST Office of Law Enforcement Standards (OLES)

Congress Passed the DNA Identification Act of 1994 (Public Law 103-322)

FBI’s DNA Advisory Board

Quality Assurance Standards for Forensic DNA Testing Laboratories (October 1, 1998)

STANDARD 9.5

The laboratory shall check its DNA procedures annually or whenever substantial changes are made to the protocol(s) against an appropriate and available NIST standard reference material or standard traceable to a NIST standard.

Standard Reference Materials (SRMs)

• Relevant Forensic DNA SRMs
  – SRM 2391b (DNA profiling – STRs, D1S80, DQA1/PM)
  – SRM 2392-I (mtDNA, Cell line HL-60)
  – SRM 2395 (Y-chromosome)
  – SRM 2372 (Human DNA quantitation); in development

• Provides national/international traceability and compatibility (aids in ISO 17025 compliance)

http://www.nist.gov/srm

Forensic DNA Timeline with NIST SRM Development


1995: SRM 2391 PCR-based DNA Profiling Standard

- D1S80 Locus
- Variable Number Tandem Repeat (VNTR)
- 480 bp
- Silver stained gels for STR monoplexes
- Samples originally selected to possess all DQA1 types

1990: SRM 2380 PCR-based DNA Profiling Standard

- D16S74 Locus
- Short Tandem Repeat (STR)
- 240 bp
- Silver stained gels for STR monoplexes
- Samples originally selected to possess all DQA1 types

1995: SRM 2391 PCR-based DNA Profiling Standard

- D1S80 Locus
- Variable Number Tandem Repeat (VNTR)
- 480 bp
- Silver stained gels for STR monoplexes
- Samples originally selected to possess all DQA1 types

http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm
2003: SRM 2391b released
- 22 STR Loci
- D1S80 and DQA1/PM (still certified)
- Many labs using 16plex STR kits

Identifiler STR kit
PowerPlex 16 STR kit

Standard Reference Material® (SRM)
A NIST SRM is prepared and used for three main purposes:
1) to help develop accurate methods of analysis;
2) to calibrate measurement systems used to facilitate exchange of goods, institute quality control, determine performance characteristics, or measure a property at the state-of-the-art limit;
3) to ensure the long-term adequacy and integrity of measurement quality assurance programs.

The Current Task:
SRM 2372: Human DNA Quantitation Standard
Challenge: What is a nanogram of genomic DNA?
From interlaboratory studies we know there is a factor of 1.6 in the measurement systems currently in use. But the range is 20 fold.

SRM 2372
Human DNA Quantitation Standard
Anticipated 2006 issue
Component A: Male
Component B: Female
Component C: Mixture

Planned Amounts: Each component 50 µL of Human Genomic DNA with a concentration targeted @ 50 ng/µL. The [DNA] for each component will be listed in the materials Certificate of Analysis.

Some of the Information Resources on the NIST STRBase Website
http://www.cstl.nist.gov/biotech/strbase
- ../str_fact.htm STR Fact Sheets on Core Loci
- ../multiplex.htm Multiplex STR Kit Information
- ../y_strs.htm Y-Chromosome Information
- ../var_ref.htm Variant Alleles Reported
- ../mutation.htm Mutation Rates for Common STRs
- ../str_ref.htm Reference List with ~2,500 Papers
- ../training.htm Downloadable PowerPoints for Training
- ../validation.htm Validation Information
- ../miniSTR.htm miniSTR Information
- ../address.htm Addresses for Scientists
- ../NISTpub.htm Publications & Presentations from NIST

http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm
Validation Resources

- Survey initiated at June 2004 NIJ meeting and conducted last summer resulted in 53 responses
- Talk at Promega meeting Oct 2004
- Validation summary sheets
- Validation website on STRBase
- Soliciting Information on Studies Performed by the Community
- President’s DNA Initiative
- EDNAP/ENFSI European studies
- ISFG (Sept 2005), Promega (Sept 2005)

We invite submission of your internal validation studies for inclusion in the NIST validation website.

http://www.cstl.nist.gov/biotech/strbase/validation.htm

New Validation Homepage on STRBase

http://www.cstl.nist.gov/biotech/strbase/validation.htm

Laboratory Internal Validation Summaries

- We have supplied information to assist U.S. companies in assay development: Orchid Cellmark (validation of autosomal SNPs); Bode Technology Group (miniSTR assays)
- We are regularly participating in EDNAP/ENFSI European studies regarding Y-STRs, SNPs (FSS 21plex and SNP FDDX), and degraded DNA samples
- We have supplied information to assist U.S. companies in assay development: Orchid Cellmark (validation of autosomal SNPs); Bode Technology Group (miniSTR assays)

Movements by Our Project Team

Our project team has assisted in beta-testing numerous products:
- Applied Biosystems (Foster City, CA)
- GeneMapper ID, Yfiler kit
- Invader Y-SNP kit
- Promega Corporation (Madison, WI)
- PowerPlex Y kit
- ReliaGene Technologies, Inc. (New Orleans, LA)
- Y-PLEX 5, Y-PLEX 12 kits
- Roche Molecular Systems (Alameda, CA)
- mSDNA HIV/HIV LINEAR ARRAY

Other information and conclusions

Other information and conclusions

NIST Initiated Interlaboratory Studies

- Evaluation of CSF1PO, TPOX, and TH01 (CTT)
- Mixed Stain Studies #1 and #2 (Apr–Nov 1997 and Jan–May 1999)
- Mixed Stain Study #3 (Oct 2000–May 2001)
- DNA Quantitation Study (Jan–Mar 2004)
- Mixture Interpretation Study (Jan–Mar 2005)

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<th>Studies involving STRs</th>
<th># Labs</th>
<th>Publications</th>
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<tr>
<td>Mixture Interpretation Study (Jan–Mar 2005)</td>
<td>69</td>
<td>Data analysis currently on-going</td>
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http://www.cstl.nist.gov/biotech/strbase/interlab.htm

http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm

http://www.cstl.nist.gov/biotech/strbase/validation.htm

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Quantitation Issue from Mixed Stain 2 (1999)

Target plot of replicate samples N and P.

J. Forensic Sci. 46: 1199-1210

NMSS#3 Reported DNA concentrations of the samples, ng/µL

Among Participate Results for QS04

Concordance – is a direct multi-material analogue of bias
Apparent precision – is analogous to precision but also incorporates sample-specific measurement differences or “matrix effects”.

“Bold” characters represent the median performance for all results submitted for a particular method
The semi-circles delimit 1, 2 and 3 standard deviations of total comparability.

Interlaboratory Comparisons

Laboratory Performances with Real-Time PCR Methods

Comparing results from 6 different samples using 10 different methods

Quantifier Results
1.6 ng of genomic DNA; n=4

Variations in C_T due to the DNA standard used area apparent

ΔC_T = 1.13 between STDs 2 & 6 (factor of 2.2)
Acknowledgments

Funding from interagency agreement 2003-IJ-R-029 between NIJ and the NIST Office of Law Enforcement Standards

NIST Human Identity Project Team

This presentation available as pdf file from http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm

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