New Technology Integration: Benefits of Interlaboratory Testing in DNA Forensics

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Chemical Science and Technology Laboratory

Biochemical Science Division (~80)

Applied Genetics Group (9)

Human Identity Project (7)

National Institute of Justice
The Research, Development, and Evaluation Agency of the U.S. Department of Justice

Current Areas of NIST Effort with Forensic DNA

- **Standards**
  - Standard Reference Materials
  - Standard Information Resources (STRBase website)
  - **Interlaboratory Studies**
- **Technology**
  - Research programs in SNPs, miniSTRs, Y-STRs, mtDNA, qPCR
  - Assay and software development
- **Training Materials**
  - Review articles and workshops on STRs, CE, validation
  - PowerPoint and pdf files available for download

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

Human Identity (DNA) Testing Applications

- Forensic cases: matching suspect with evidence
- Paternity testing: identifying father
- Missing persons investigations
- Military DNA "dog tag"
- Convicted felon DNA databases
- Mass disasters: putting pieces back together
- Historical investigations
- Genetic genealogy

>3 million tests performed per year

Purpose of an Interlaboratory Study

- Interlaboratory studies (ILS) are a way for multiple laboratories to compare results and demonstrate that the methods or instrument platforms used in one's own laboratory are reproducible in another laboratory

The lifecycle of a method of analysis

Feinberg et al. (2004) Anal. Bioanal. Chem. 380: 502-514

Human ID Project Team Experience

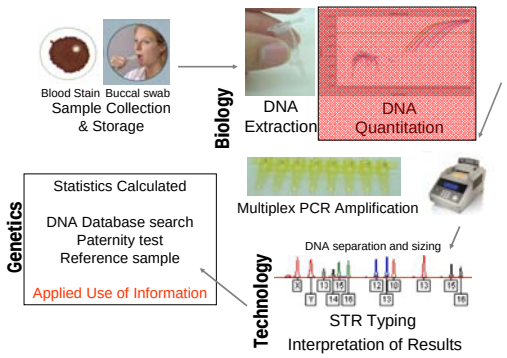
- Coordinated 6 interlaboratory studies over the last 15 years
- Participated in 17 national and/or international interlaboratory studies

NIST Initiated Interlaboratory Studies

Studies involving STRs	# Labs	Publications
Evaluation of CSF1PO, TPOX, and TH01	34	Kline MC, Duewer DL, Newall P, Redman JW, Reeder DJ, Richard M. (1997) Interlaboratory evaluation of STR triplex CTT. *J. Forensic Sci.* 42: 897-906
Mixed Stain Studies #1 and #2 (Apr–Nov 1997 and Jan–May 1999)	45	Duewer DL, Kline MC, Redman JW, Newall PJ, Reeder DJ. (2001) NIST Mixed Stain Studies #1 and #2: interlaboratory comparison of DNA quantification practice and short tandem repeat multiplex performance with multiple-source samples. *J. Forensic Sci.* 46: 1199-1210
Mixed Stain Study #3 (Oct 2000-May 2001)	74	Kline, M.C., Duewer, D.L., Redman, J.W., Butler, J.M. (2003) NIST mixed stain study 3: DNA quantitation accuracy and its influence on short tandem repeat multiplex signal intensity. *Anal. Chem.* 75: 2463-2469. Duewer, D.L., Kline, M.C., Redman, J.W., Butler, J.M. (2004) NIST Mixed Stain Study #3: signal intensity balance in commercial short tandem repeat multiplexes, *Anal. Chem.* 76: 6928-6934.
DNA Quantitation Study (Jan-Mar 2004)	80	Kline, M.C., Duewer, D.L., Redman, J.W., Butler, J.M. (2005) Results from the NIST 2004 DNA Quantitation Study, *J. Forensic Sci.* 50(3):571-578
Mixture Interpretation Study (Jan - Aug 2005)	69	Poster at Promega meeting (2005); available on STRBase

Steps in Forensic DNA Analysis

Usually 1-2 day process (a minimum of ~8 hours)



DNA Quantitation

- Interlaboratory study to help assess the accuracy of DNA quantitation in forensic DNA laboratories
- Quantitation of human genomic DNA

DNA Quantitation

- Four primary purposes
  1. to examine concentration effects and to probe performance at the lower DNA concentration levels that are frequently seen in forensic casework
  2. to examine consistency with various methodologies across multiple laboratories
  3. to examine single versus multiple source samples
  4. to study DNA stability over time and through shipping in two types of storage tubes

Kline, M.C., Duewer, D.L., Redman, J.W., Butler, J.M. (2005) Results from the NIST 2004 DNA Quantitation Study, J. Forensic Sci. 50(3):571-578

Material used in an ILS

- Well characterized for the intended analysis
  - Homogeneous so all participants are analyzing the same material
  - Stable to shipping methods used, unless this is part of the study
- Made in sufficient quantities
  - So additional material can be resent or reanalyzed
- In a similar matrix to what the participants are used to analyzing
- Relevant concentration range and volumes

Prior to sending out samples

- Experimental design
  - – Exact experiments to run
  - – How analysis should be performed
  - – A worksheet to store data, parameters, notes, etc was provided
  - – Set a final date for receiving data

NIST Quantitation Study 2004 (QS04)

Consisted of:
•8 DNA extracts labeled A – H
•Shipped Dec 2003 –Jan 2004 to 84 laboratories for quantification; data received back by April 2004
•Labs were requested to use multiple methods / multiple analysts

We received data from 80 Labs (95%)
Total of 287 sets of data
Participants used 19 different quantification methods
21% were obtained using newly available quantitative real-time PCR (Q-PCR) techniques

TABLE 1—*Quantitation Methods Used in QS04. Key for detection and instrument codes is listed below. There are 34 categories and 19 unique "codes" or quantitation methods examined.*

Signal	Instrument	Code	Labs	Set	Ref
Probe	TLA	a	4	9	23
"	TD20/20	a	3	3	"
"	TR	a	1	1	"
Picogreen	FA	p	2	3	24
"	LF320	p	1	1	"
"	PBC4000	p	1	1	"
"	PK_FL	p	1	2	"
"	SMAX	p	2	3	"
"	"	p	1	2	"
EtBr	visual	y	3	14	18
Solulink	visual	A	4	10	21
KI	"	A	1	4	"
ECL	visual	E	18	40	20
"	CCDBIO	E	2	11	"
KI	"	E	3	7	"
SSWD	visual	D	3	11	"
SSWF	visual	F	4	6	"
TMB	visual	T	31	98	"
Colorimetry					
Endpoint PCR	BodeQuant	Fluorescence	Picogreen	CF4000	—	1	1	32
Real Time PCR	Quantifiler	Fluorescence	Probe	ABI7000	0	16	32	25
"	"	"	"	ABI7700	0	1	2	"
"	"	"	"	ABI7900	0	2	3	"
"	Alu Q-PCR	"	Sybr	RG3000	1	2	4	26
"	"	"	"	ABI7000	1	1	1	"
"	Alu_2Sults	"	"	i-Cycler	2	1	2	27
					3	1	1	—
					4	1	1	—
					5	1	2	28
					5	1	1	"
					6	1	1	29
					6	1	3	"
					7	1	2	—
					8	1	3	30
					9	1	2	31
						118	287	

80 unique labs participated
287 data sets

Multiple…
detection assays
instrument platforms
laboratories
analysts

Samples shipped December 2003 though January 2004
Results came in April 5, 2004
A large amount of data!
Multiple questions and trends can be investigated
A statistician is needed (Dr. David Duewer)

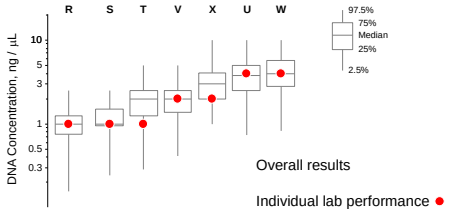
Data Analysis

- Request from the start that the results are submitted in a certain way, specific units used
- After transcribing the submitted data ask the participant to verify this data
  - – Typographical errors are found early this way
- Give each participant a feel for how they did compared to others

Always keep the results blind to the other participants

Individual Performance in an Interlaboratory Study

DNA Quantitation



Modified plot from Kline, M.C., *et al.* (2003) *Anal. Chem.* 75: 2463-2469

Results…

- Quantitation Methods and Frequency of Use
- Interpretation of Semi-Quantitative Data
- Method Sensitivities
- Combining Within-Analyst Replicates and Within-laboratory Duplicates
- Distributions of the Among-laboratory Results
- Measurement Variability
- Measurement Performance Characteristics
- Consensus Values and Variability as Functions of [DNA]
- Blot-Based Vs. Q-PCR Methods
- Single-Source Vs. Multiple-Source Materials
- Polypropylene Vs. Teflon Sample Containers

Design

- The ILS should be designed to answer a specific question(s)
- The ILS is focused on a (validated?) method currently performed in the community
- Through coordinating multiple studies we learn what to do better next time

Use of ILS data

- Value assignment of a material
  - Determine a consensus value for a material to be used as a reference (control material) when a suitable higher order standard is not available
  - All methods used should be previously validated
- Comparability of different analysis methods/instrumentation used on the same material
- Comparability of the same analysis methods/instrumentation used on the same material

Steps in Field Analysis for Biothreat Detection


Sample Sent to PHL (LRN)

Question:
What aspect of the workflow do you wish to learn more about?

- Sample Collection? (methods – personnel)
- Sample storage? (stability, recovery yield)
- Instrument performance?
- Data Interpretation?
- Other?

The ILS can be properly designed to answer the correct question

What is needed to test a new Technology?

- Platform(s) common to the community
- A foundation of validation within the community
- For Biothreat detection - surrogate materials need to be developed for the ILS

How can NIST help?

- Assistance in experimental design
- Analysis of data that results from ILS
  - Independent analysis & reporting

Informatics and statistical expertise

Acknowledgements

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