Emerging Efforts in Clinical Genetics by the Applied Genetics Group at the National Institute of Standards and Technology

Email: margaret.kline@nist.gov
Phone: 301-975-3134

Email: marcia.holden@nist.gov
Phone: 301-975-4162

Margaret C. Kline, Marcia J. Holden, Ross J. Haynes, Amy E. Decker*, Carolyn R. Hill, Janette W. Redman, Peter M. Vallone, and John M. Butler

National Institute of Standards and Technology (NIST), 100 Bureau Drive MS 8312, Gaithersburg, MD 20899-8312

*Current Address: Armed Forces DNA Identification Laboratory, Rockville, MD 20850

The Applied Genetics Group was established at the National Institute of Standards and Technology (NIST) in October 2008 to bring together expertise with DNA genotyping and sequence analysis and to leverage work performed over the past two decades with forensic DNA testing. NIST seeks to develop reference materials and evaluate technology that will aid measurements for specific applications. Reference materials with certified values for specific information enable traceability and confidence in comparisons of testing results between laboratories. Over the past two decades, NIST has worked closely with and learned a lot from the forensic DNA community. Experience has been gained in development of a human DNA quantitation standard that is widely used for quantitative PCR measurements. In addition, a clinical genetics website to standardize information on published assays used in various genetic tests is under development. This website will contain information similar to the Short Tandem Repeat DNA Database (STRBase) that is widely used for standard information on human identity testing DNA markers.

Currently NIST has a Fragile X Human DNA triplet repeat Standard Reference Material (SRM 2399) and has made progress in developing a Huntington Disease CAG repeat reference material (SRM 2393). Efforts are underway to develop a cytomegalovirus DNA standard certified for both quantity and sequence information at specific informative sites. **We are looking for partners to obtain clinical samples that can be characterized as well as recommendations on priorities for future reference material production.**

Poster #G47 at Association for Molecular Pathology 2009 Annual Meeting, Kissimmee, FL, November 19-22, 2009

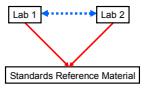
Applied Genetics Group

The National Institute of Standards and Technology (http://www.nist.gov) is a non-regulatory federal agency within the U.S. Department of Commerce. The mission of NIST is to promote U.S. innovation and industrial competitiveness by advancing measurement science, standards, and technology in ways that enhance economic security and improve our quality of life. NIST helps ensure compatible measurements by generating, certifying, and issuing Standards Reference Materials (SRMs). Information resources, including Standard Reference Data (SRDs), are also provided to support efforts in many fields.

Since the late 1980s, NIST has had scientists involved in DNA testing. Early concerns over measurement accuracy and issues with poor quality control of forensic DNA tests caused the Department of Justice to call upon NIST scientists to help with standards development and technology evaluation. For the past several years, our Human Identity Project Team has been part of the DNA Measurements Group of the Biochemical Science Division at NIST. Beginning October 1, 2008, a new Applied Genetics Group has been formed to focus on forensic, clinical, and agricultural biotech genetic measurements as well as DNA biometrics and genetic genealogy issues.

History of SRM Work and Certificates

There are three levels of values: certified, reference, and information. To be a "certified value", the measurement must be done at NIST using a primary method with confirmation by other methods or using two independent critically-evaluated methods.



Calibration with SRMs enables confidence in comparisons of results between laboratories

Effort that goes into SRM production

Homogeneity: single lot in a single container aliquoted to individual tubes packaged as components in each SRM unit

Purity (absence of significant impurities): single source DNA samples used; while it is not certified to be "mixture-free", foreign, contaminating alleles should not be seen; thus, the solutions can be considered >~90% pure (mixture detection limit ~10%)

Stability: generally certified for 5-6 years but likely stable much longer under appropriate storage conditions (refrigerated or frozen, out of sunlight)

Concentration: for genotyping reference materials, amount of DNA is not certified; some variability in amount of DNA present can be expected; samples generally supplied at near "ready-to-use" concentrations (~1-2 ng/µL)

For more information on NIST SRMs, see:
http://www.nist.gov/srm

Reference Materials

Current SRMs available related to various DNA needs:

Platform Testing
- SRM 2372 Human DNA Quantitation
- SRM 2392 mtDNA Sequencing

Clinical DNA Standards
- SRM 2399 Fragile X alleles (2007)
- SRM 2393 Huntington's alleles (expected 2010)
- SRM 2366 Cytomegalovirus (expected 2010)

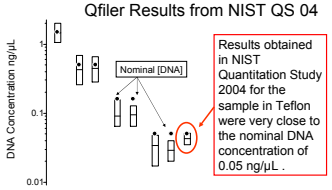
Reference materials are used for:
- Method validation
- Instrument validation
- Instrument calibration
- Production of a daily use NIST Traceable material

SRM → Assign value to → Everyday use calibrant

Use of SRM materials can reduce the Lab to Lab variability that can occur when labs use different calibration materials.

Measurement Issue

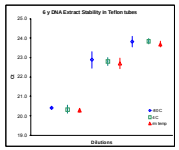
Qfiler Results from NIST QS 04



Results obtained in NIST Quantitation Study 2004 for the sample in Teflon were very close to the nominal DNA concentration of 0.05 ng/µL .

DNA stored in polypropylene tubes at concentrations of less than 5 ng/uL appeared less concentrated probably due to a portion of the DNA adhering to the tube walls. This was not seen with the 0.05 ng/uL material stored in PFA (Teflon) from Savillex Corp. (Minnetonka MN).

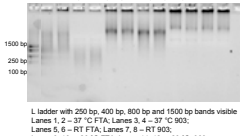
Storage



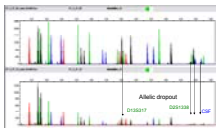
Data from DNA extracts stored in Teflon tubes at -80 °C, 4 °C, and room temperature for **6 years**. Each storage temperature had three DNA Concentrations: neat, 1→5, 1 →10. qPCR results using Quantifiler Human, triplicate aliquotes used from each sample are plotted. Error bars represent 2 standard deviations. There is no difference as a result of temperature storage.

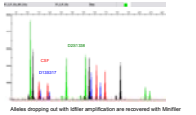
Blood Stain Stability

Blood collected in EDTA tubes was spotted on 903 and FTA papers. Dried stains were stored in sealed mylar bags, and stored at -20 °C, room temperature and +37 °C . Samples have been analyzed periodically with the most recent being 11 years post storage.



L ladder with 250 bp, 400 bp, 800 bp and 1500 bp bands visible
Lanes 1, 2 – 37 °C FTA; Lanes 3, 4 – 37 °C 903;
Lanes 5, 6 – RT FTA; Lanes 7, 8 – RT 903;
Lanes 9, 10 – -20 °C FTA; Lanes 11, 12 – -20 °C 903;



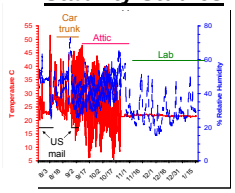


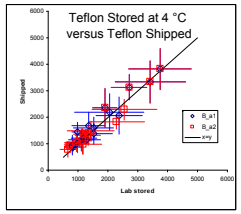
Alleles dropping out with Idfiler amplification are recovered with Minifiler

After 11 years of storage at 37 °C both FTA and 903 show signs of degradation, the FTA samples exhibit DNA with slightly higher molecular weight than the 903 samples.

+37 °C Samples typed with STR's show some allelic dropout due to degradation. Those same loci can be recovered using miniSTRs.

Stability Studies





Peak hts generated by Identifiler when amplifying 0.5 ng of DNA. Blue symbols are from 4 °C. Red Symbols from harsh ambient conditions. No real differences were seen.

DNA solution was placed in a Teflon vial and stressed at Harsh ambient temperatures for 147 days.

Aliquots of the "Stress" material was analyzed along with the same DNA material that had been stored at 4 ° C

Some Genetic Analysis Measurement Issues

Genetic markers used for clinical diagnostics involving genotyping data are generated using PCR amplification and electrophoretic sizing that involves an internal size standard with each sample.

PCR amplification is expected to generate consistent genotypes as long as primer positions are not changed between kits. Primer changes can result in allele dropout due to primer site mutations.

Occasionally new commercial kits are created with additional loci.

Genetic marker allele nomenclature rules have been established but do have some subjectivity in them permitting possible differences in how alleles can be named.

Information Resources

Under development

Clinical DNA Information Resource (CDIR)

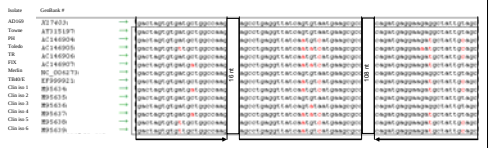
http://www.nist.gov/cstl/biochemical/genetics/clinical_dna.cfm

- "Although primer mismatching does not necessarily prevent PCR amplification, use of primers having a known high degree of sequence conservation…should reduce assay variability in clinical practice" (Chou 1992).
- Primer size and location of mismatch affect amplification efficiency which can effect clinical sensitivity and made defining a clinical threshold for treatment difficult.
- Database of sequence alignments will:
  - aid scientists designing and using primers and probes
  - save time and money in design & testing

•Example 1

	Tanaka 2000 (org pub)	Lengerova 2007 (review)
•Sensitivity:	76%	79%
•Specificity:	97%	not reported
•Number:	53	363
•Solution:	Use multiple assays	Don't use this assay.

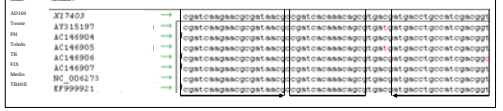
- False negative rate (21%) due to primer and probe mismatch



•Example 2 (Sanghavi 2008)

•Threshold:	50 copies/mL	500 copies/mL
•Sensitivity:	100%	92%
•Specificity:	94%	98%

- Number: 3422 anticoagulated peripheral blood samples
- Threshold of 500 copies/mL is clinically significant



- When performing statistical analysis after empirical testing with clinical samples your primary concern should be "what should be considered the clinical threshold for treatment?" and not "will this assay work well enough for diagnostic use?"
- When a PCR assay is designed on a highly polymorphic sequence – like in example 1 – some strains will yield false negative results. On the other hand example 2 shows a PCR assay on a well conserved sequence.

Poster available for download from NIST Applied Genetics website: http://www.nist.gov/cstl/biochemical/genetics/index.cfm