# Progress Toward SRM 2372: Human DNA Quantitation Standard

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## Disclaimers

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**National Institute of Justice**
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## 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

## The issue:

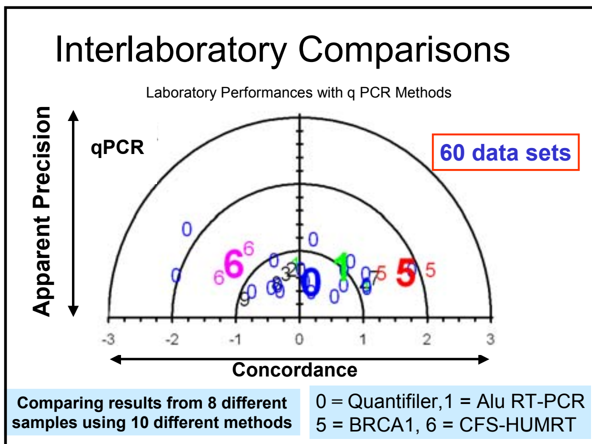
How can we reproducibly measure the concentration of extracted DNA?

## Interlaboratory Studies for DNA Quantitation

- NIST Mixed Stain Study #2 (1999)
  - [DNA] range 0.5 ng/μL to 5 ng/μL; ×1.8 variability
    - J Forensic Sci 2001;46:1199-1210
- NIST Mixed Stain Study #3 (2001)
  - [DNA] range 1 ng/μL to 4 ng/μL; ×1.7 variability
    - Anal Chem 2003;75:2463-2469
- NIST Quantitation Study 2004
  - [DNA] range 0.05 ng/μL to 1.5 ng/μL; ×1.8 variability
    - J Forensic Sci 2005;50:571-578

## Lessons Learned from the NIST QS04

- At low [DNA] 0.05 ng/μL labs recovered 73% more DNA from a Teflon tube than from a polypropylene tube.
- The “new” qPCR methods used in the study consistently provided quantitative results for samples with [DNA] 0.16 ng/μL and lower.
- The qPCR methods appeared to be biased to one another.

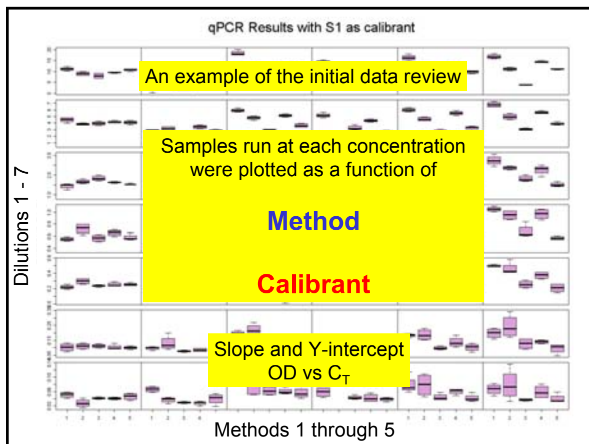


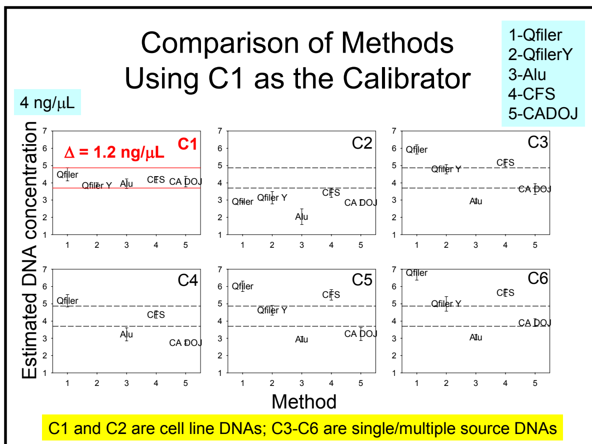

## Would a Quantitative DNA Standard Help?

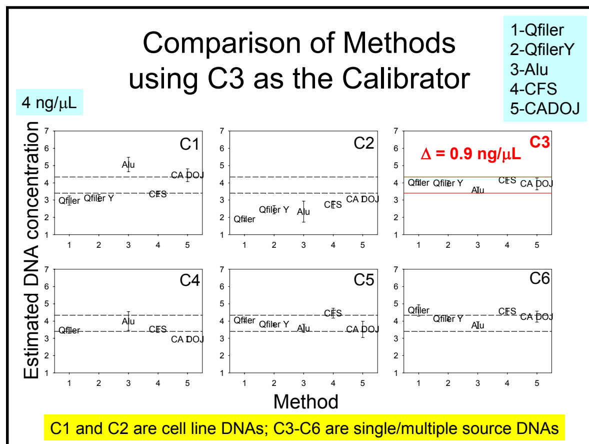
- Yes
  - If the bias seen among the QS04 qPCR methods is from the use different calibrators.
  - If the SRM material produced is fit for purpose.

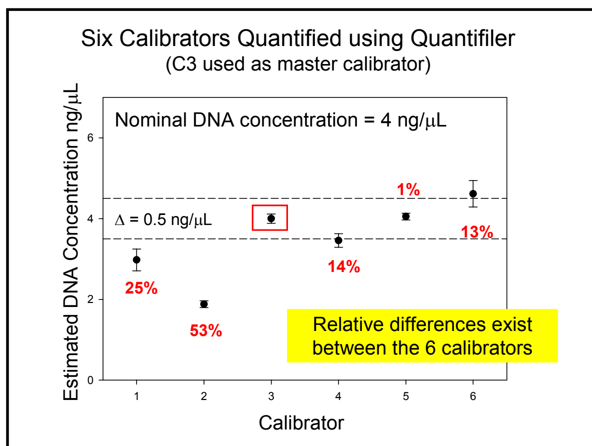
## Investigation of "bias" between qPCR Methods

- The experimental design:
  - 5 qPCR methods
    - **Quantifiler, Quantifiler Y, Alu, CFS, CADOJ**
  - 6 calibrators
    - **3 commercial sources**
    - **3 NIST extracted materials (1 Proteinase K treatment)**
  - 7 dilutions of each calibrator made daily
    - **10.0, 4.0, 1.6, 0.64, 0.26, 0.1, and 0.04 ng/μL**
  - Duplicate plates (run on the same day)






## Requirements for NIST SRM 2372 Human DNA Quantitation Standard

**Material must be fit for purpose:**

- **Homogeneity**
  - All tubes are the same
- **Stability**
  - Will withstand shipping and normal storage
- **Recoverability**
  - What went in the tubes comes out
- **Traceability**
  - Values assigned are traceable to the designated certification method.

## SRM 2372 Human DNA Quantitation Standard

**Anticipated 2006 issue**

Component A: Male
Component B: Female
Component C: Mixture

**Planned Amounts: Each component 110 µL of Human Genomic DNA with a concentration targeted @ 50 ng/µL.**

**The [DNA] for each component will be listed in the Certificate of Analysis.**

## Preparation of 2372 Components

- Female and male components isolated from Buffy coats at NIST using “salting out” extraction procedure
  - **Miller et al. Nucleic Acids Res. 1988, 16(3) 1215**
- Mixture material (multiple male and female donors) purchased as purified freeze-dried material.

## Component A: Male

- **Single Male donor** (Buffy coat).
- After initial extraction, EtOH ppt material was solubilized and treated with RNase.
- Following RNase treatment the material was retreated with Proteinase K and EtOH ppt.
- The ppt material was washed in 70% EtOH and allowed to dry.
- Final solubilization was in $TE^{-4}$ (10 mM Tris 0.1 mM EDTA, pH 8.0) buffer.

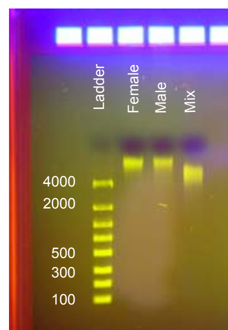
## Component B: Female

- **Multiple Female donors** (Buffy coats).
- After initial extraction, EtOH ppt material was solubilized and retreated with Proteinase K.
- The ppt material was washed in 70% EtOH and allowed to dry.
- Final solubilization was in $TE^{-4}$ buffer.

## Component C: Mixture

- **Multiple Male and Female donors**
  - (tissue extract, freeze-dried, Sigma #D7011)
- Solubilized in $TE^{-4}$ buffer.
- Allowed to equilibrate 2 weeks prior to measuring absorbance.
- Material has been used for stability testing.

## Gel Image Pilot DNA Components




All materials appear to be intact DNA.

The mobility of the commercially obtained mixture material appears slightly different than that of the NIST extracted material.

## Certification Plan

- Use a NIST National-Reference Spectrophotometer to **certify** (*value* and *uncertainty*) absorbance values at 230, 260, 270, 280, and 330 nm.
- This provides the traceability path… **so that future materials can have the same properties**.

## How do we get Traceability?

- **Photometry**
- Regular transmittance scale is maintained on the National Reference Spectrophotometer in the NIST Analytical Chemistry Division which is validated by the double-aperture method of light addition and benchmarked through **international intercomparisons** using optical filter artifact standards.
- Liquid or powder SRMs are assigned certified values for absorbance per unit pathlength or specific absorptivity at specified wavelengths.
- **NIST Special Publication 260-136**
  - **Definitions of Terms and Modes Used at NIST for Value-Assignment of Reference Materials for Chemical Measurements**

## Absorbance at 260 nm Facts:

- DNA, RNA, EDTA, and Phenol all absorb
- Absorption coefficients are affected by:
  - Ionic strength of the solution (needs to be low)
  - pH of the solution (needs to be controlled)
    - Beaven et al.,The nucleic acids, 1955 (1) 493 - 553;
    - Wilfinger et al. BioTechniques 1997 (22) 474 - 481
- Method reliable only in 5 ng/µL to 90 ng/µL range of DNA concentration.
- (you have to have light passing through the solution!)
    - Sambrook and Russell, Molecular Cloning, 3rd Ed 2001, A8.20

## Spectrophotometric Determination

- 260 nm & 280 nm readings
- 260 nm allows calculation of the conventional [DNA]
- OD =1 ≈ 50 µg/mL dsDNA
  ≈ 40 µg/mL ssDNA
  ≈ 33 µg/mL oligos
- 260 / 280 ratio ≈ 1.8 to 2.0

(Provides an estimate of contaminating protein)

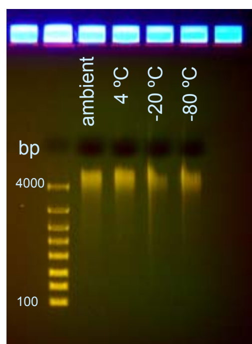
## Additional wavelengths:

- 230 nm Significant absorbance indicates:
  - Phenolate ion
  - Thiocyanates
  - And other organic compounds
- 270 nm
  - Water saturated with phenols absorbs
  - 260:270 ~ 1.2 indicates preparation free of phenol
    Stulnig and Amberger 1994 BioTechniques;16 :403-404
- 330 nm and higher absorbance
  - Caused by light scattering indicating presence of particulate matter

## Certification Plan continued

- Recoverability
- Stability
- Homogeneity
  - Use the Cary 100 Bio instrument (110 µL cell)
  - Made stable and accurate by virtue of the appropriate use of wavelength (SRM 2034) and absorbance standards (SRM 2031) before and after component measurements.
- Validity and commutability of conventional [DNA] with qPCR and other field methods.
  - qPCR methods preformed at NIST
  - Interlaboratory study

## Stability of a DNA Solution




A DNA solution was prepared in a sterile Teflon container Sept 2003. The material was aliquoted into sterile 5 mL Teflon vials and held at lab ambient temperature, 4 °C, -20 °C, and -80 °C.

After 33 months of storage there is no difference in the apparent quality of the material.

**Recommended Stored Temperature 4 °C**
(will be shipped on ice bricks NOT dry ice)

## Stability of [DNA] in Different Tubes

Can the end user get out what was put in?

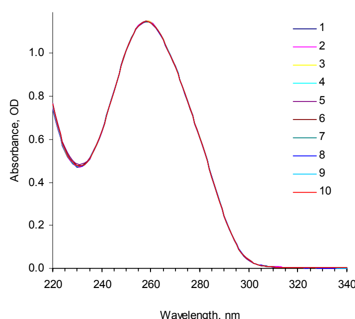
Five different tubes were evaluated at :
3 different storage temperatures
3 different [DNA]
Quantifiler used to evaluate the [DNA]
Duplicate tubes, duplicate qPCR runs
Duration 7 months : Averaged results for 5 time points

| [DNA] | A | B | C | D | E |
|---|---|---|---|---|---|
| 0.20 | 1.00 | 0.74 | 1.14 | 0.72 | 0.69 |
| 1.00 | 1.00 | 0.88 | 0.98 | 0.86 | 0.88 |
| 5.00 | 1.00 | 0.99 | 0.91 | 0.94 | 0.72 |

30 data points / tube type / [DNA]

[DNA] in ng/µL

## 10 weeks of UV measurements




**To make sure that *you* can retrieve what *we* put in the Sarstedt tubes.**

**Multiple aliquots of a preliminary material were tested for 10 weeks. The DNA did *not* change in that time period. The instrument required calibration with both wavelength (SRM 2034) and absorbance (SRM 2031) standards before and after measuring the DNA solution.**

## Absorbance of the Pilot Lots

| Dil | Female 260 | Female ratio | Male 260 | Male ratio | Mix 260 | Mix ratio |
|---|---|---|---|---|---|---|
| 1:2 | 1.299 | 2.6 | 0.580 | 1.2 | 1.401 | 2.8 |
| 1:5 | 0.518 | 2.6 | 0.235 | 1.2 | 0.556 | 2.8 |
| 1:10 | 0.263 | 2.6 | 0.114 | 1.1 | 0.279 | 2.8 |

| | Female | Male | Mix | |
|---|---|---|---|---|
| Mean [DNA] | 130.3 | 57.9 | 139.5 | ng/µL |
| sd | 0.89 | 0.83 | 0.55 | |
| rsd | 0.7 | 1.4 | 0.4 | % |
| 260 : 280 | 1.97 | 1.97 | 1.97 | |
| 260 : 270 | 1.2 | 1.2 | 1.2 | |

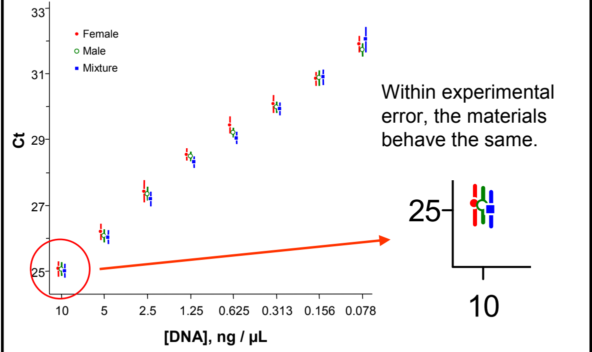
## Dilution scheme for qPCR

| Female 130.3 | µL DNA | 300 TE |
|---|---|---|
| 10 | 23.0 | 277.0 |
| 5 | 10 | 10 |
| 2.5 | 10 | 10 |
| 1.25 | 10 | 10 |
| 0.625 | 10 | 10 |
| 0.313 | 10 | 10 |
| 0.156 | 10 | 10 |
| 0.078 | 10 | 10 |

| Male 57.9 | µL DNA | 300 TE |
|---|---|---|
| 10 | 51.8 | 248.2 |
| 5 | 10 | 10 |
| 2.5 | 10 | 10 |
| 1.25 | 10 | 10 |
| 0.625 | 10 | 10 |
| 0.313 | 10 | 10 |
| 0.156 | 10 | 10 |
| 0.078 | 10 | 10 |

| Mix 139.5 | µL DNA | 300 TE |
|---|---|---|
| 10 | 21.5 | 278.5 |
| 5 | 10 | 10 |
| 2.5 | 10 | 10 |
| 1.25 | 10 | 10 |
| 0.625 | 10 | 10 |
| 0.313 | 10 | 10 |
| 0.156 | 10 | 10 |
| 0.078 | 10 | 10 |

## Quantifier Results for Preliminary Components of SRM 2372




## SRM 2372
## Human DNA Quantitation Standard

Components
A: Male/single donor/RNased/NIST
B: Female/multiple donors/NIST
C: Mixture/male & female/commercial

Quantities
110 µL of Human Genomic DNA
Absorbance of 1.0 OD
Conventional [DNA] 50 ng/µL.

Certification
Absorbance by US National Spectrophotometer
Homogeneity by Cary 100 Bio
**Validation of conventional [DNA] by Interlaboratory Study**

## What is Delaying Release?

- **Need to extract more DNA** in order to reach goal of producing >1,500 units (there is a great deal of interest in SRM 2372 outside of the forensic community—e.g., pharmaceutical industry)
  - ~30 units (3 mL) are required by the NIST National-Reference Spectrophotometer for its measurements
- **Additional studies to be performed**: interlaboratory (performed by multiple forensic labs), homogeneity (monitored by NIST statisticians), and continual stability testing for the life of the product

## An Interlaboratory Study Will Be Performed to Demonstrate Commutability of SRM 2372

You will have 3 weeks to return your data once we ship the final packaged material (≈August 2006).

Any Volunteers?

Contact: margaret.kline@nist.gov

## Thank you for your Attention!!

Acknowledgements

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