The results of the NIST Quantitation Study 2004 and how it led to the production of SRM 2372 Human Quantitation Standard.

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Disclaimers

Funding: Interagency Agreements 2003-DN-R-121 and 2008-DN-R-121 between the National Institute of Justice and NIST Office of Law Enforcement Standards.

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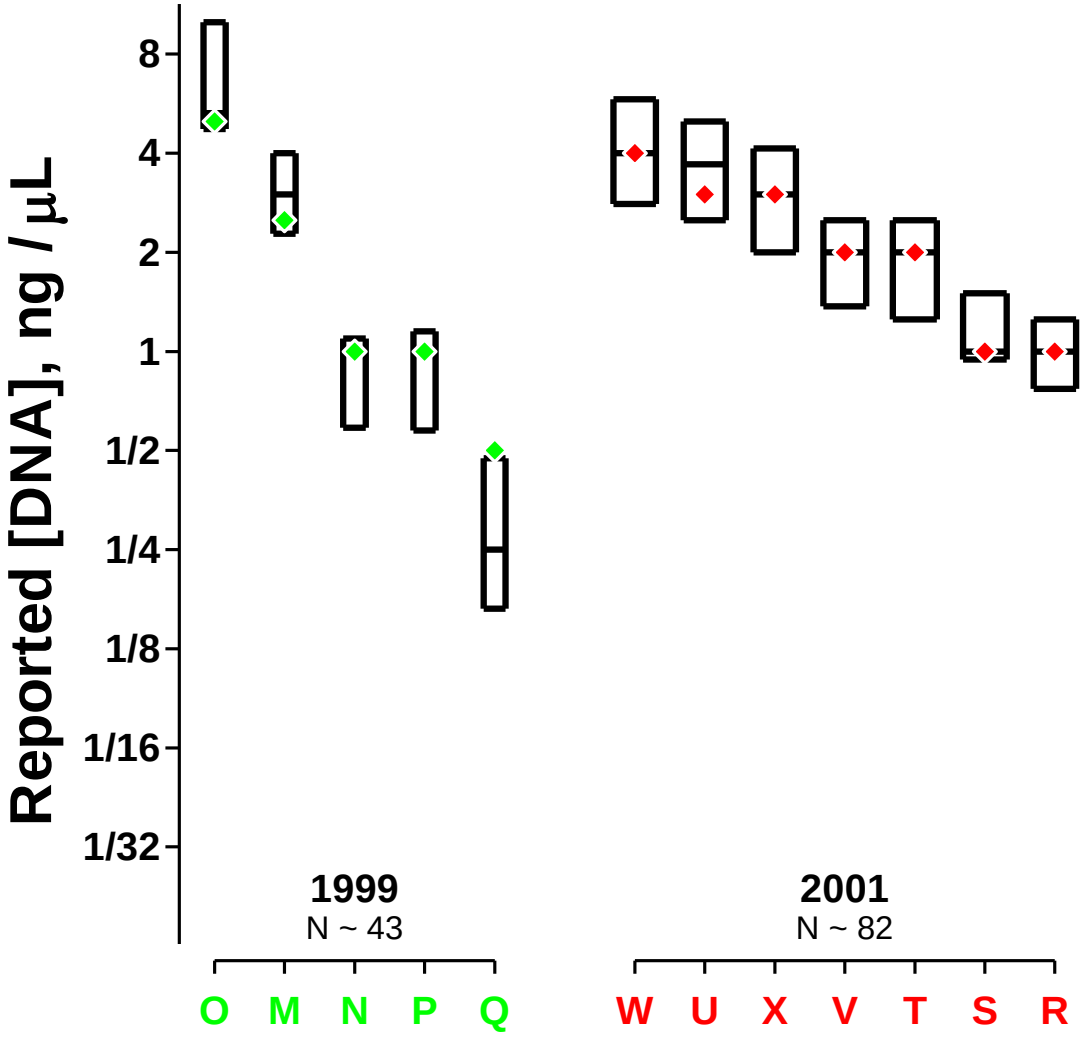
Background: Pre-QS04 Interlaboratory Quantitation Performance



Diamond symbols are the NIST [DNA] values.

Boxes enclose the central 50 % of the reported values.

The horizontal line within the box is the median value

The y-axis is scaled as factors of 2.

Most of the central 50 % values fell within a factor of 2 except the lowest [DNA] tested in the 1999 study.

Results of the 2001 study indicated an improvement of the [DNA] comparability.

Why QS04: Quantitation Study 2004?

- The STR genotyping kits were requiring a tighter controlled range of input DNA.
- Quantitative PCR (qPCR) methods were starting to be used the forensics labs along with the traditional Slot Blot methods.
- Lower [DNA] needed to be tested for comparability between labs.
- What happens with different tube materials?

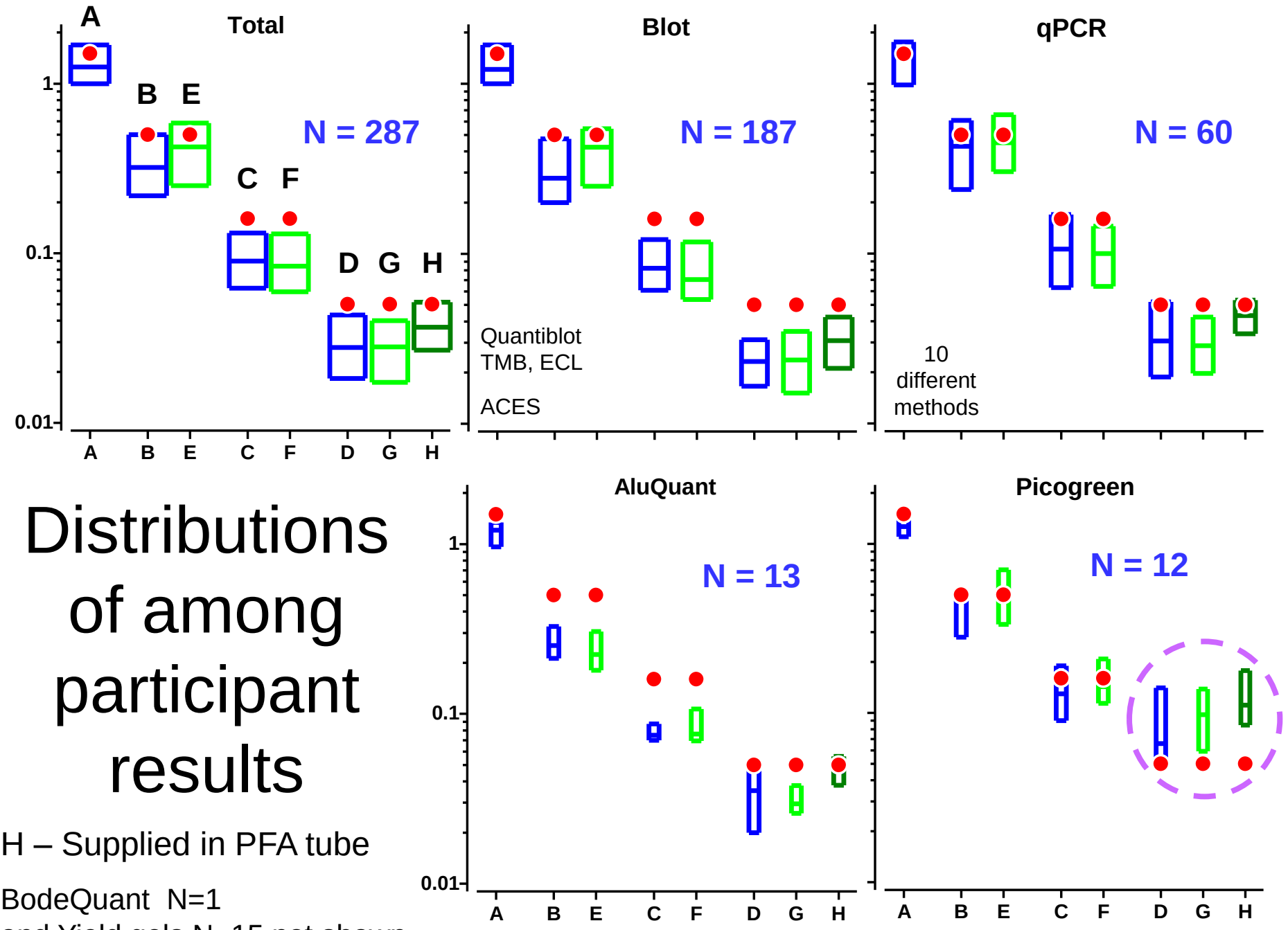
NIST Quantitation Study 04

Consisted of:

- 8 DNA extracts labeled A – H
  - (1.5 ng/µL, 0.5 ng/µL, 0.16 ng/µL, 0.05 ng/µL)
- A – D Dilutions of a multi-source lyophilized DNA
- E – H Dilutions of a single-source male DNA
- Shipped Dec 2003 –Jan 2004 to 84 laboratories
- Labs were requested to use multiple methods with multiple analysts

We received:

- Data from 80 Labs (95 % participation)
- **Total of 287 sets of data**
- Participants used 19 different quantification methods (primarily variations of Quantiblot and qPCR)

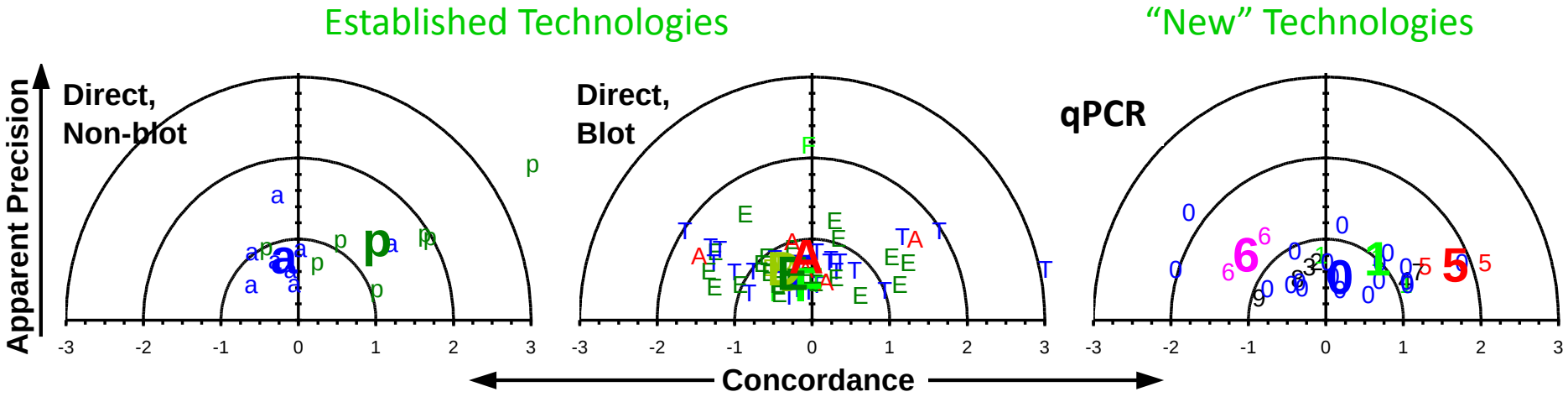


Distributions of among participant results

H – Supplied in PFA tube

BodeQuant N=1
and Yield gels N=15 not shown

QS04: Among-Participant Results



"Bold" characters represent the median performance of all results submitted for a particular method

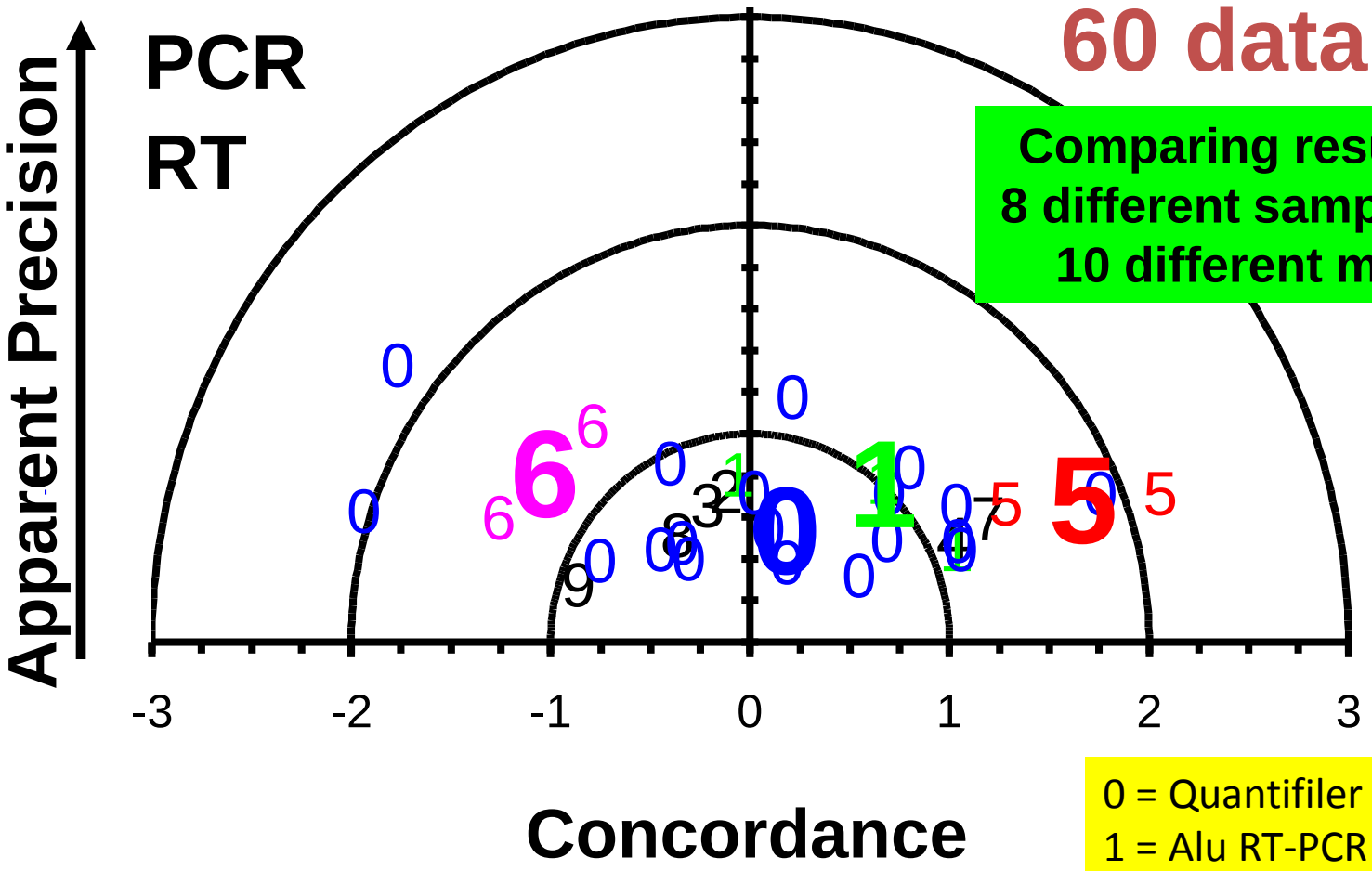
The 3 reference semi-circles:

- inner-most delimits a total comparability of 1 sd from perfect agreement with the consensus medians for all samples
- middle 2 sd
- outer 3 sd

a=agarose, p=Picogreen, A=ACES, T=QuantiblotTMB, E=QuantiblotECL

Interlaboratory Comparisons

Laboratory Performances with Real-Time PCR Methods



60 data sets

Comparing results from 8 different samples using 10 different methods

0 = Quantifiler
1 = Alu RT-PCR
5 = BRCA1
6 = CFS-HUMRT

Kline, *et al.* (2005) *J. Forensic Sci.* 50(3):571-578

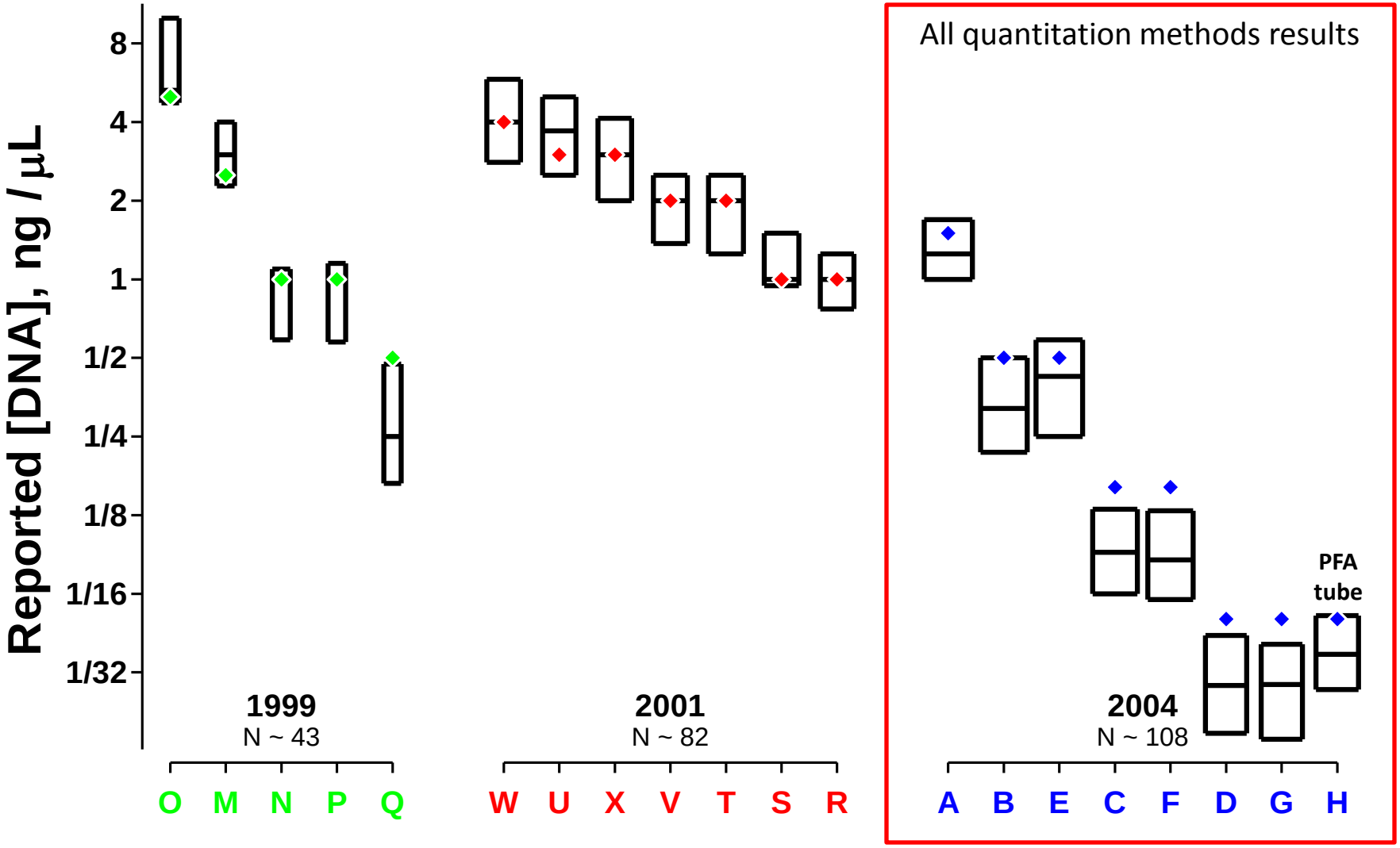
qPCR Facts

- qPCR is **RELATIVE** to the standards used to generate a calibration curve.
- qPCR instruments use a selected Cycle Theshold (C_T) for calculations.
- The premise is that at 100% PCR efficiency you have a doubling of the PCR product.
- Therefore 1 C_T = [DNA]/2 to [DNA] 2.

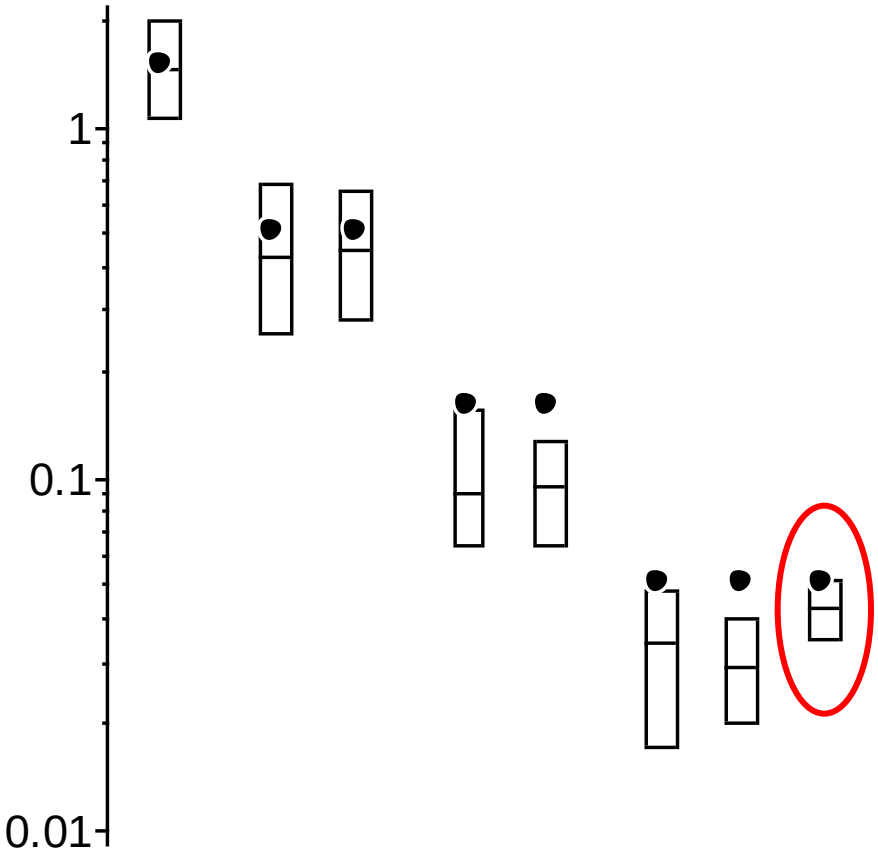
QS 04 Indicators

- Ten different qPCR methods were used to evaluate DNA samples distributed in the NIST Interlaboratory DNA Quantitation Study 2004 (QS04).
- These methods appeared to have some bias relative to each other.
- Is the bias method- or standard-based?

Interlaboratory Performance



QS04: Lesson Learned



Results for the sample in PFA (*ie*, Teflon) tubes were consistently close to the nominal DNA concentration of 50 pg/µL .

SRM 2372
Human DNA Quantitation Standard

Components

A: Male/single donor/RNased/NIST (52.5 ng/µL)
B: Female/multiple donors/NIST (53.6 ng/µL)
C: Mixture/male & female/commercial (54.3 ng/µL)

Quantities supplied:

110 µL of Human Genomic DNA

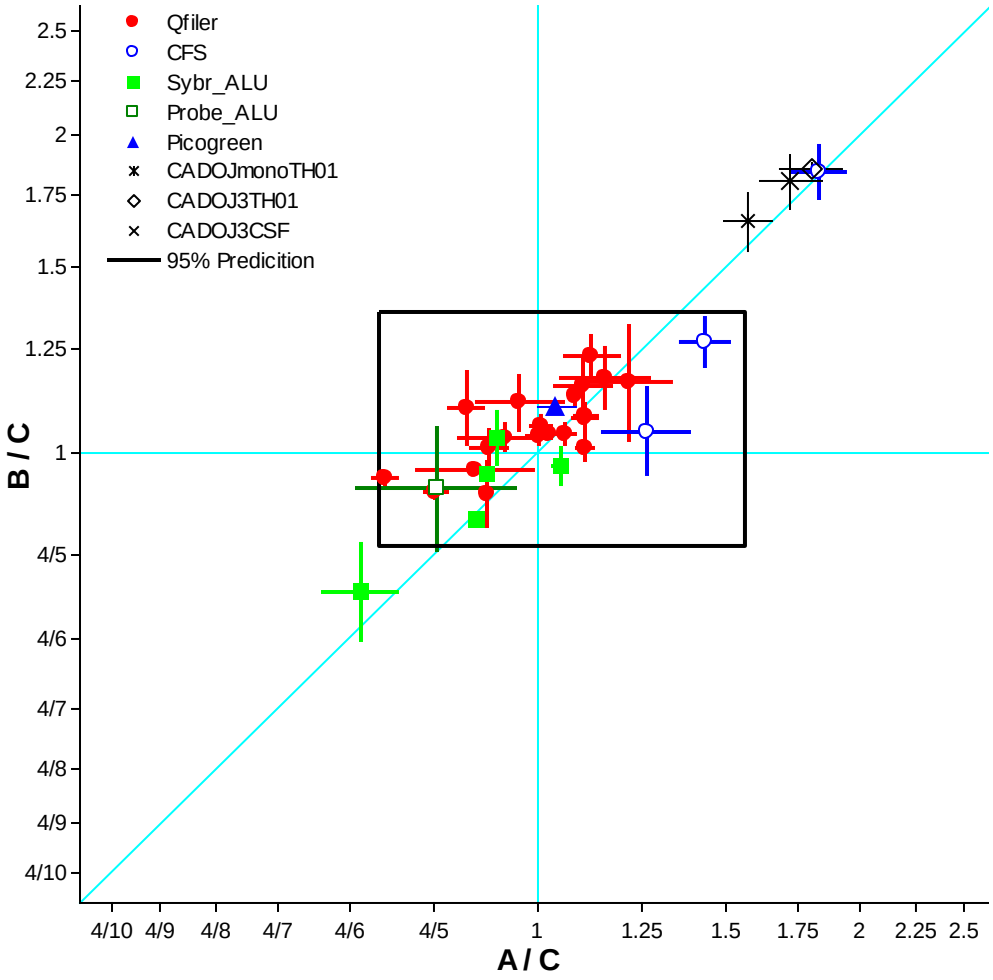
Certification

Decadic Attenuance **(Absorbance**) by a US National Reference Spectrophotometer
Homogeneity by a Cary 100 Bio Spectrophotometer
Validation of conventional [DNA] by Interlaboratory Study and NIST qPCR studies.

Interlaboratory Study

- 32 laboratories participated
- This limited study was advertised at the NIJ Grantees meeting, June of 2006
- All laboratories provided data (Thank You!)
- Net result of the study: the SRM materials are appropriate for use with different qPCR methods

Interlaboratory Data



Each symbol represents the average qPCR [DNA] results reported for a given method by one participating laboratory. The cross at each {A/C,B/C} pair represents approximate 95 % confidence intervals for the two ratios for the method as implemented at that laboratory. The diagonal line represents the expected behavior when measurements deviations from the consensus value are consistently biased.

Method dependent bias is present.

NIST Quantitation Interlaboratory Studies

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

1999 Duewer DL, Kline MC, Redman JW, Newall PJ, Reeder DJ.
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 2001;46(5):1199-1210**.

2001 Kline MC, Duewer DL, Redman JW, Butler JM.
NIST Mixed Stain Study #3: DNA Quantification Practice and its Influence on Short Tandem Repeat Multiplex Performance.
Anal Chem 2003;75(10):2463-2469.

2004 Kline MC, Duewer DL, Redman JW, Butler JM.
Results from the NIST 2004 DNA Quantitation Study.
J Forensic Sci, 50(3): 571-578.

2007 Kline, M.C., Duewer, D.L., Travis, J.C., Smith, M.V., Redman, J.W., Vallone, P.M., Decker, A.E., Butler, J.M. (2009) Production and certification of NIST Standard Reference Material 2372 Human DNA Quantitation Standard.
Anal. Bioanal. Chem. 394: 1183-1192.

Thanks for your attention

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