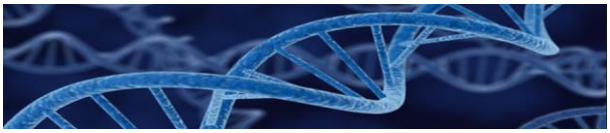


Bias in qPCR: Does it matter for forensic applications?

Erica Romsos, Steven Lund, and Peter Vallone

American Academy of Forensic Science
February 23, 2019
Baltimore, MD

APPLIED GENETICS

AAFS 71st Annual Scientific Meeting — Baltimore, Maryland 2019

Disclaimer

I will mention commercial platforms and chemistry, but am in no way attempting to endorse any specific product.

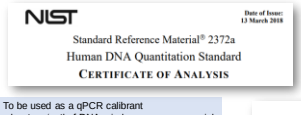
NIST Disclaimer: Certain commercial equipment, instruments and materials are identified in order to specify experimental procedures as completely as possible. In no case does such identification imply a recommendation or it imply that any of the materials, instruments or equipment identified are necessarily the best available for the purpose.

Information presented does not necessarily represent the official position of the National Institute of Standards and Technology

Outline

- Brief overview of SRM 2372a: Human DNA Quantitation Standard
- Lessons learned from development
  - Performance with commercial qPCR chemistries
  - Variance across commercial qPCR chemistries
- Understanding bias in qPCR
  - Does it matter?

SRM 2372a: Human DNA Quantitation Standard


NIST

Date of Issue: 13 March 2018

Standard Reference Material® 2372a
Human DNA Quantitation Standard
CERTIFICATE OF ANALYSIS

To be used as a qPCR calibrant
OR to assign a value to a 'pot' of DNA – in house or commercial

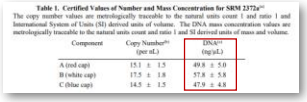


Table 1. Certified Values of Number and Mass Concentration for SRM 2372a[a]

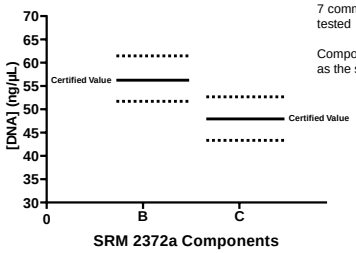
Component	Copy Number[b] (per nL)	DNA[c] (ng/µL)
A (red cap)	15.1 ± 1.5	49.8 ± 5.0
B (white cap)	17.5 ± 1.8	57.8 ± 5.8
C (blue cap)	14.5 ± 1.5	47.9 ± 4.8


NIST Special Publication 260-189

Certification of Standard Reference Material® 2372a
Human DNA Quantitation Standard

SRM 2372a became available for purchase March 2018

SRM 2372a qPCR Performance

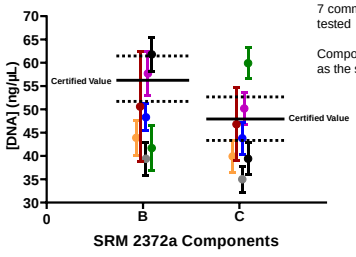


7 commercial qPCR chemistries tested

Component A of SRM 2372a used as the standard curve calibrant

- Trio
- HP
- Duo
- Qfiler
- PQ
- Qplex Pro
- Qplex HY

SRM 2372a qPCR Performance



7 commercial qPCR chemistries tested

Component A of SRM 2372a used as the standard curve calibrant

- Trio
- HP
- Duo
- Qfiler
- PQ
- Qplex Pro
- Qplex HY

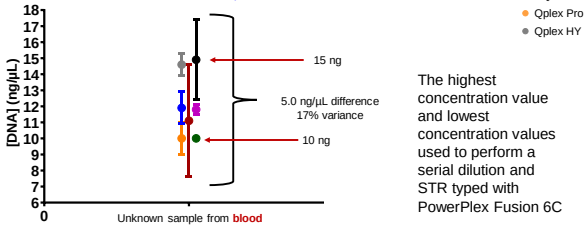
Observed a 17% variance across qPCR kits

Possible Sources of Bias and Variance

- Multiple sources of bias when performing qPCR
- Standard bias
  - DNA standard for standard curve
  - Known bias with cell lines (hTERT)
- Individual sample bias
  - Type of sample (cell line vs. human)
- Assay bias
  - Chemistry being employed
  - Target within assay
    - Single target vs. multicopy
- Human/Robotic bias
  - Pipette calibration
  - Ability to reproducibly serial dilute samples

Assay Bias

7 independent qPCR chemistries
SRM 2372a Component A used as standard curve calibrant

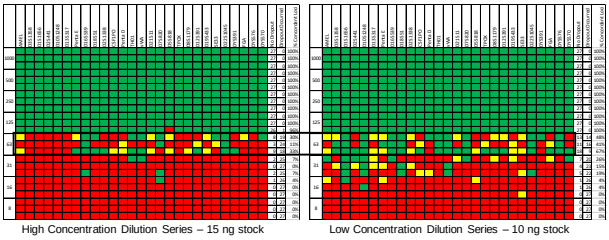


- Trio
- HP
- Duo
- Qfiler
- PQ
- Qplex Pro
- Qplex HY

The highest concentration value and lowest concentration values used to perform a serial dilution and STR typed with PowerPlex Fusion 6C

PowerPlex Fusion 6C
Dilution Series

Practical Effects of Assay Bias



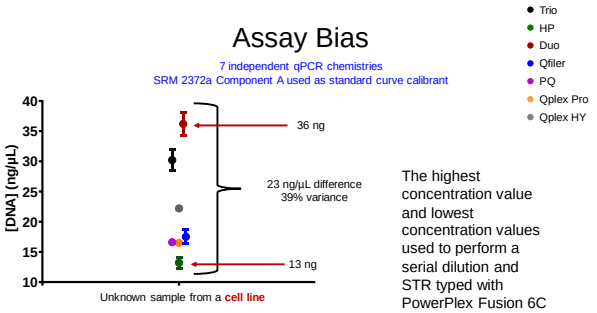
High Concentration Dilution Series – 15 ng stock

Low Concentration Dilution Series – 10 ng stock

Dropout predominantly observed beginning at 63 pg of input DNA for both dilution series

Assay Bias

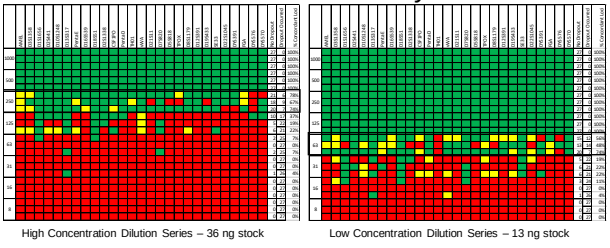
7 independent qPCR chemistries
SRM 2372a Component A used as standard curve calibrant



The highest concentration value and lowest concentration values used to perform a serial dilution and STR typed with PowerPlex Fusion 6C

PowerPlex Fusion 6C
Dilution Series

Practical Effects of Assay Bias



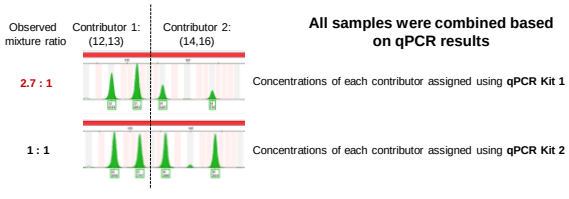
High Concentration Dilution Series – 36 ng stock

Low Concentration Dilution Series – 13 ng stock

Dropout observed beginning at 250 pg of input DNA for the high concentration dilution data

Practical Effects of Assay Bias

Preparing a 1:1 mixture ratio



All samples were combined based on qPCR results

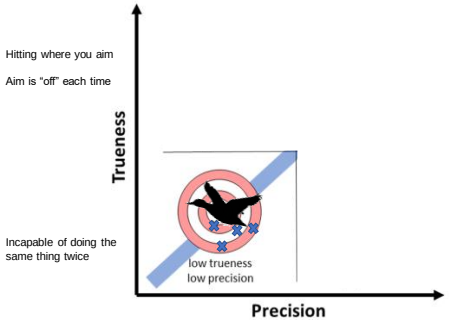
Concentrations of each contributor assigned using **qPCR Kit 1**

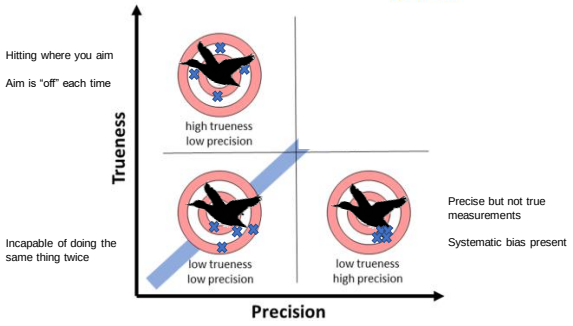
Concentrations of each contributor assigned using **qPCR Kit 2**

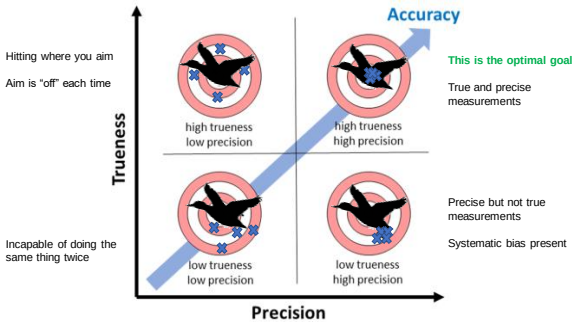
Bias with the target of Contributor 2 using qPCR kit 1

Potential Remediation of Bias

Does it really matter? Can SRM 2372a help?






Accuracy
Hitting where you aim
Aim is "off" each time
Incapable of doing the same thing twice
This is the optimal goal
True and precise measurements
Precise but not true measurements
Systematic bias present
high trueness low precision
high trueness high precision
low trueness low precision
low trueness high precision
Trueness
Precision

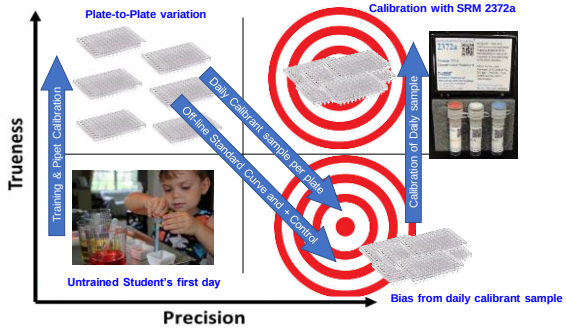


Plate-to-Plate variation
Calibration with SRM 2372a
Training & Pipet Calibration
Daily Calibrant sample per plate
Off-line Standard Curve and + Control
Calibration of Daily sample
Untrained Student's first day
Bias from daily calibrant sample
Trueness
Precision

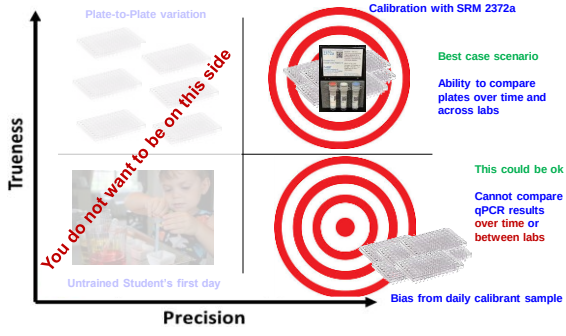


Plate-to-Plate variation
Calibration with SRM 2372a
You do not want to be on this side
Best case scenario
Ability to compare plates over time and across labs
This could be ok
Cannot compare qPCR results over time or between labs
Untrained Student's first day
Bias from daily calibrant sample
Trueness
Precision

Conclusions

- It is important to understand where your bias is coming from
- Multiple sources of bias exist in qPCR, some of which cannot be remediated
- Day-to-day or plate-to-plate variation may be corrected with an normalization sample run on each plate
- **Artificial standard curves** from validation data run with a **positive control** ***may help*** normalize plate-to-plate variation
- **Systematic bias** from **commercial standards** ***may be corrected*** with calibration to SRM 2372a
- Bias from commercial DNA standards **can be remediated** with calibration to SRM 2372a

Acknowledgements

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Peter Vallone


Steven Lund

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