Bias in qPCR: Does it matter for forensic applications?

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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.

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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

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

Certified Value

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. multicy
- 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

5.0 ng/µL difference
17% variance

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

Practical Effects of Assay Bias

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

Practical Effects of Assay Bias

Preparing a 1:1 mixture ratio

All samples were combined based on qPCR results

<table>
<thead>
<tr>
<th>Observed mixture ratio</th>
<th>Contributor 1: (12,13)</th>
<th>Contributor 2: (14,16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.7 : 1</td>
<td></td>
<td></td>
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<tr>
<td>1 : 1</td>
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</tbody>
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Bias with the target of Contributor 2 using qPCR kit 1
Potential Remediation of Bias

Does it really matter? Can SRM 2372a help?

- Incapable of doing the same thing twice
- Hitting where you aim
- Aim is "off" each time
- This is the optimal goal
- True and precise measurements
- Precise but not true measurements
- Systematic bias present
- Incapable of doing the same thing twice
- Hitting where you aim
- Aim is "off" each time
This is the optimal goal
True and precise measurements

Precise but not true measurements
Systematic bias present

Incapable of doing the same thing twice

Hitting where you aim
Aim is “off” each time

Plate-to-Plate variation
Calibration with SRM 2372a

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

Bias from daily calibrant sample

Bias from daily calibrant sample

Untrained Student’s first day
Plate – to – Plate variation

Training & Pipet Calibration
Calibration of Daily sample
Bias from daily calibrant sample

Can’t compare qPCR results over time or between labs
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

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