Electronic supporting information

General methods for quantitative interpretation of results of digital variable-volume assays

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Verification of simulated volumes

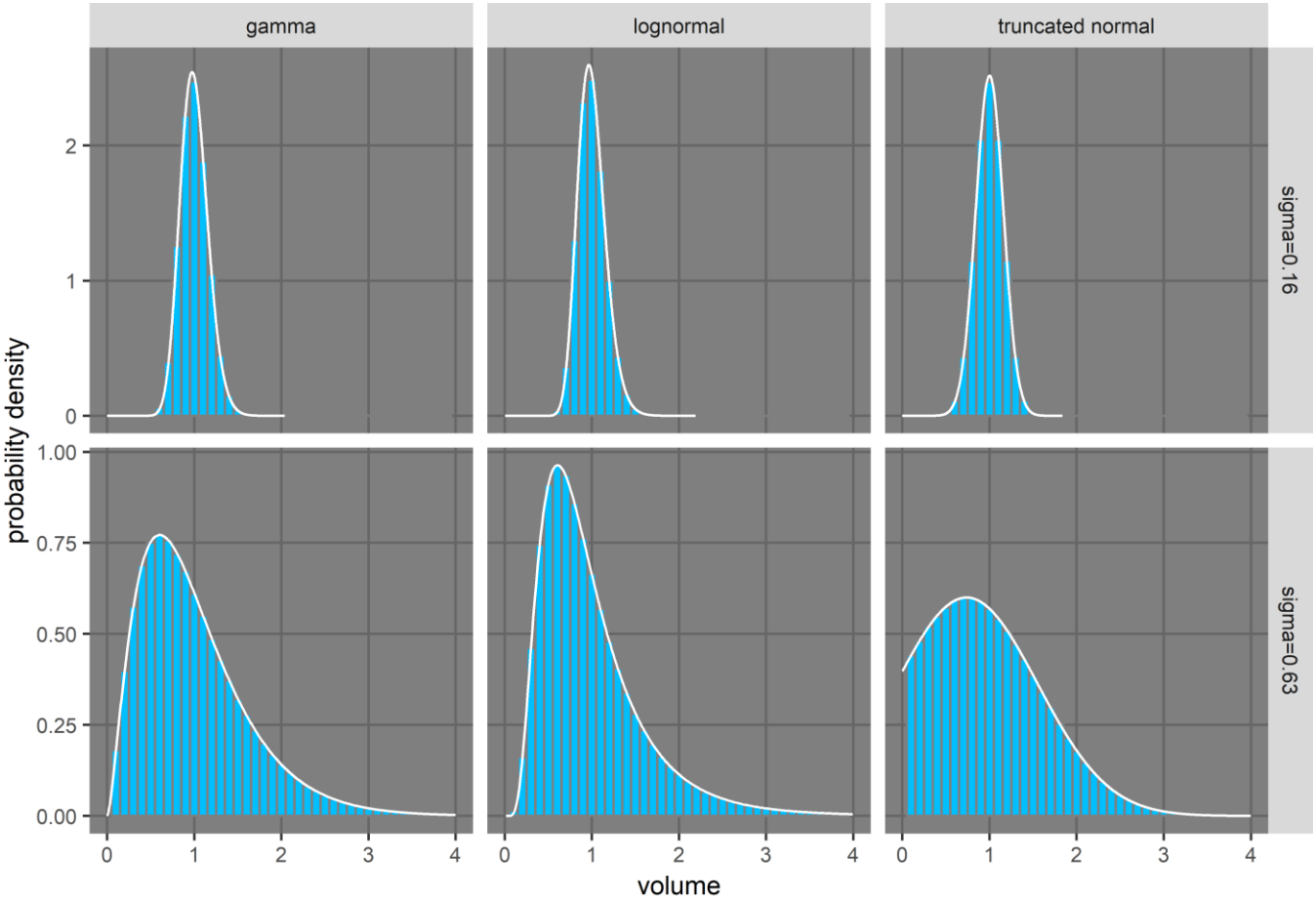

Fig. S1 Histograms of simulated volumes (blue bars) and the corresponding probability density functions (white lines). For each panel, 3e6 volume values were simulated.

Number of simulated assays

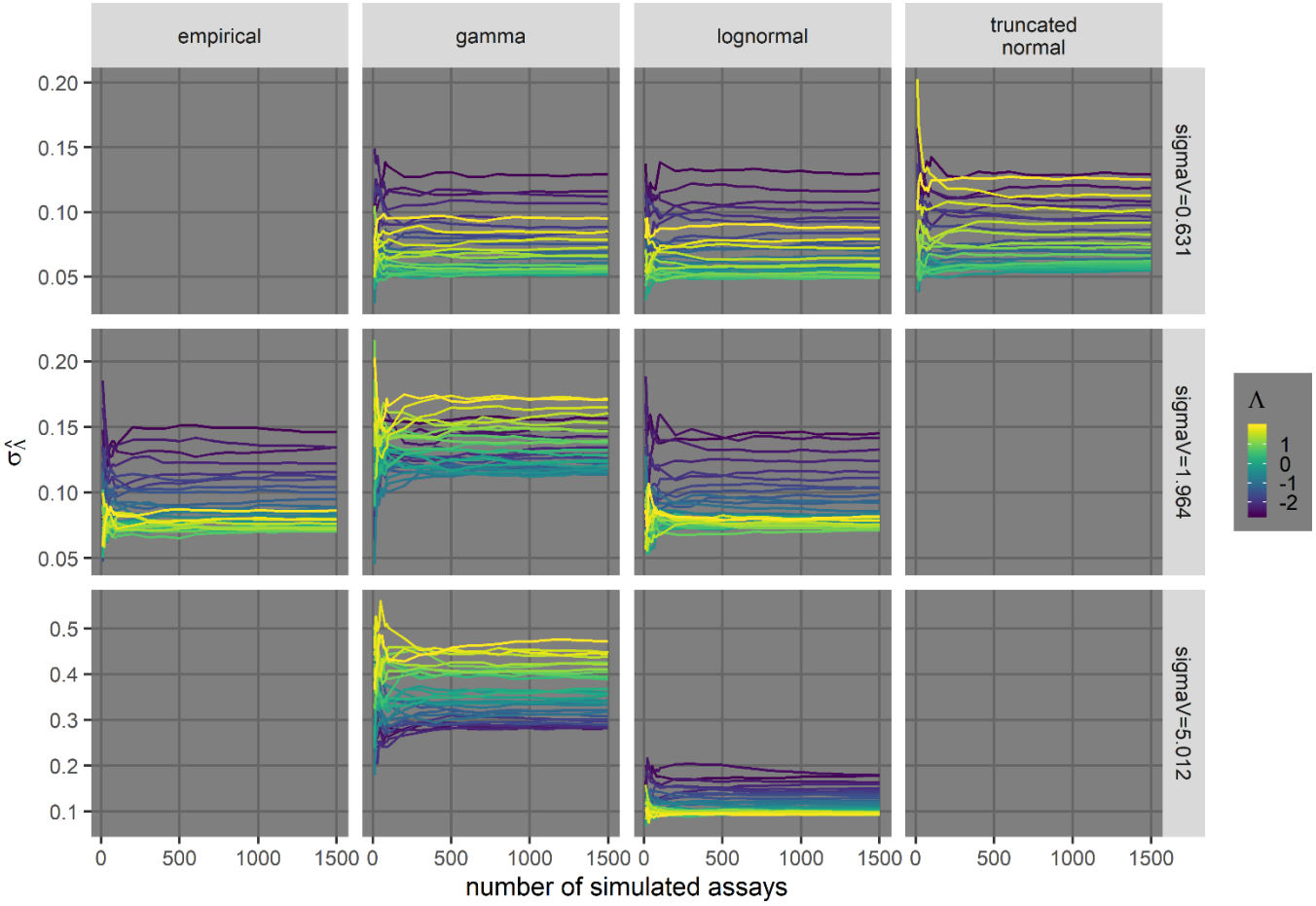

Fig. S2 Sample standard deviation of $\widehat{\Lambda}$ as a function of the number of simulated assays. The volumes followed the empirical distribution (same as the one used for Fig. 1 in the main text). The dvva method was used to calculate $\widehat{\Lambda}$ values. The number of pre-measured volumes, m, was 2000, and the number of compartments, n, in each assay was 1000.

Standard errors

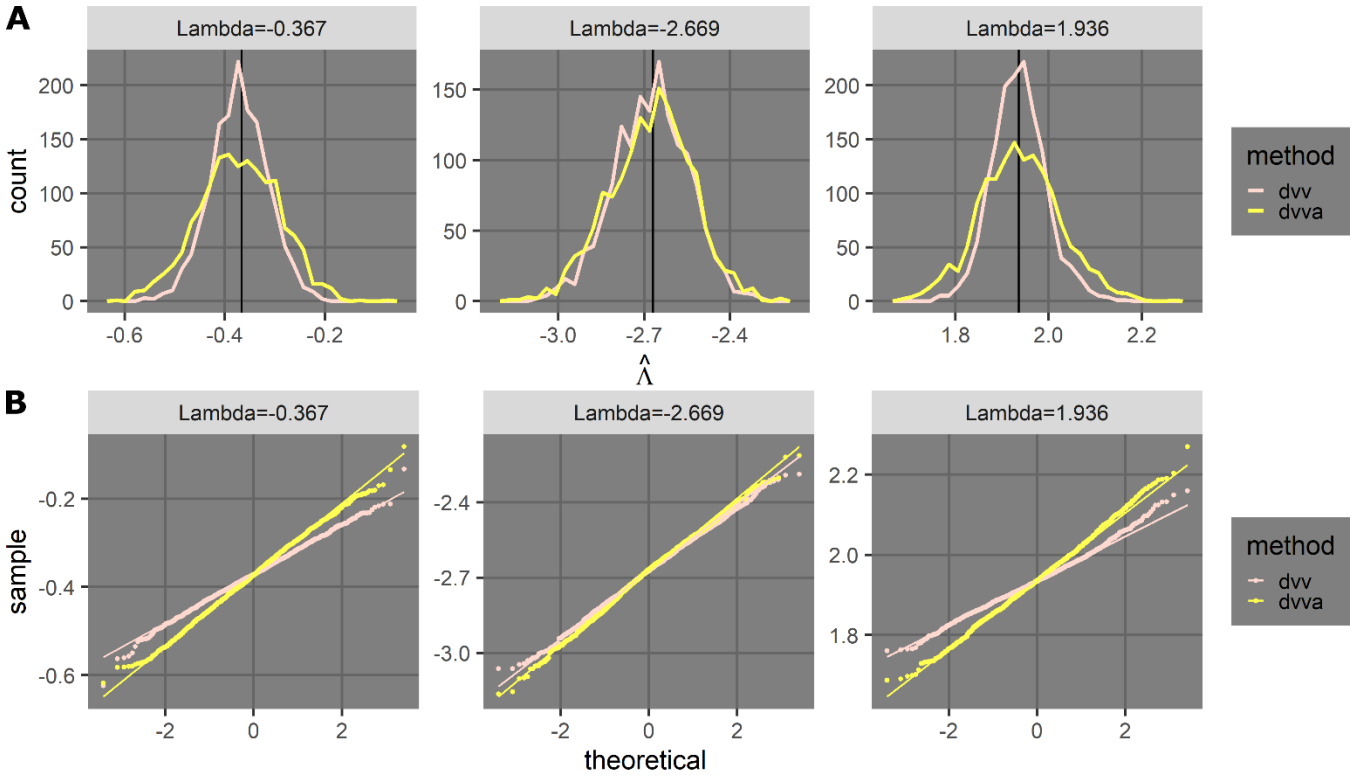

Fig. S3 Verification of normality of $\hat{\Lambda}$ through histograms (A) and q-q plots (B). The dataset used to make this figure is a subset of the dataset used to make Fig. 2 in the main text. The Λ choices are indicated in the panels' headers and the black vertical lines. The method choices are dvv and dvva, and volume distribution is the empirical distribution. The number of pre-measured volumes, m, was 2000, and the number of compartments, n, in each assay was 1000. For each condition, 1500 assays were simulated.

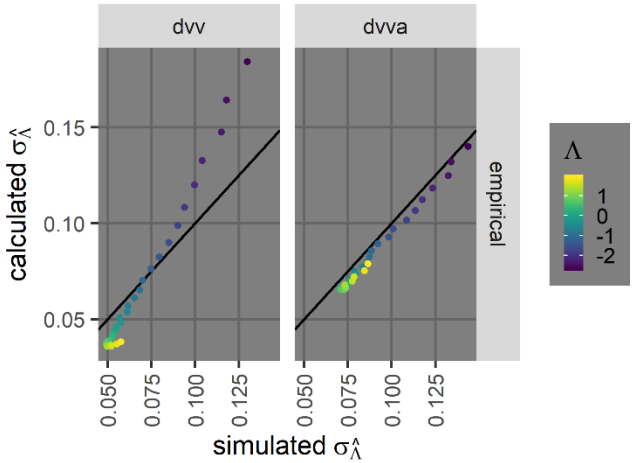

Fig. S4 Curves comparing standard errors of $\hat{\Lambda}$ calculated using eqn (10) (with the dvv method) and eqn (19) (with the dvva method) with those obtained from simulated $\hat{\Lambda}$ values. The black lines indicate parity, where the equations gave correct results. The volumes followed the empirical distribution. The number of pre-measured volumes, m, was 2000, and the number of compartments, n, in each assay was 1000. For each condition, 1500 assays were simulated.

Calculation time

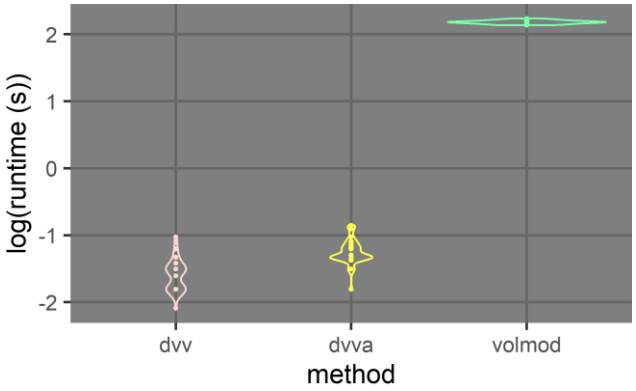

Fig. S5 Violin plots describing distributions of the log of runtimes (in seconds) during the calculation of $\hat{\Lambda}$ and the associated standard errors using the dvv, dvva, and volmod[1] methods. For each distribution, 54 points were used. Each point is the time needed to process 62 assay results, corresponding to 31 Λ values (from -2.67 to 1.94) with duplicates.

Simulation of limits of detection

As defined in the main text, the limit of detection is defined herein as the concentration at which 95% of assay runs (replicates) yield at least 1 positive compartment. For each assay setting (specified by the number of compartments, volume distribution type, and volume standard deviation, with volume mean of 1), the limit of detection was simulated by the following steps:

- Simulate assay results at 20 Λ values from -10 to 0, each with 1500 replicates. At each Λ value, calculate the fraction of the replicates with at least 1 positive compartment.
- Find the coarse lower bound, which is the highest Λ value with the fraction less than 95%
- Find the coarse upper bound, which is the lowest Λ value with the fraction not less than 95%
- Simulate assay results at 20 Λ values from the lower bound the upper bound found above, each with 1500 replicates. At each Λ value, calculate the fraction of the replicates with at least 1 positive compartment.
- Fit the curve of fraction versus Λ with a B-spline, and find the limit of detection as the Λ value that yields 95%. Prebuilt methods used for this step (scipy 1.2.1):
    - scipy.interpolate.splrep (s = 68, determined by inspection)
    - scipy.interpolate.sproot

Images from digital PCR experiments

Result images from digital PCR experiments can be downloaded using the following link.
https://drive.google.com/open?id=1HBczy7Dkyw-4oAFc2E0UuXZ6kb2zyVhq

References

1 M. Vynck and O. Thas, *Anal. Chem.*, 2018, **90**, 6540–6547.