Uncertainty evaluation in chemistry and molecular biology:
From Reproducibility to Bayes

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Science
for a safer world

Introduction

- Uncertainty evaluation in chemistry
  - Propagation, Reproducibility and Bayes
- Molecular biology
  - Recent examples of uncertainty evaluation
- Summary
  - Current practice and future directions

Chemistry

i) Basic approaches

Uncertainty evolution in Chemical measurement

Pre-1978 Random/systematic error; Error propagation in chemistry (Eckschlager 1961); Collaborative study

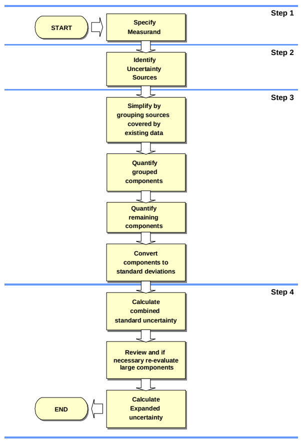
1980	• BIPM INC-1 (1980) – Type A / Type B – Combine as variances	
1982		• AOAC Stats manual *(Development/validation)*
1986		• ISO 5725:1986 *(Collab trial)*
1993	• **ISO Guide**	
1995	• EURACHEM Guide 1st ed	• ISO 5725:1994 *(Adds trueness)*
2000	• EURACHEM Guide 2nd ed (QUAM:2000	
2010	• GUM Supplement 1 (MCS)	• *ISO 21748 – Uncertainty from collab study data*

2012 • **3rd Edition EURACHEM/CITAC guide published**

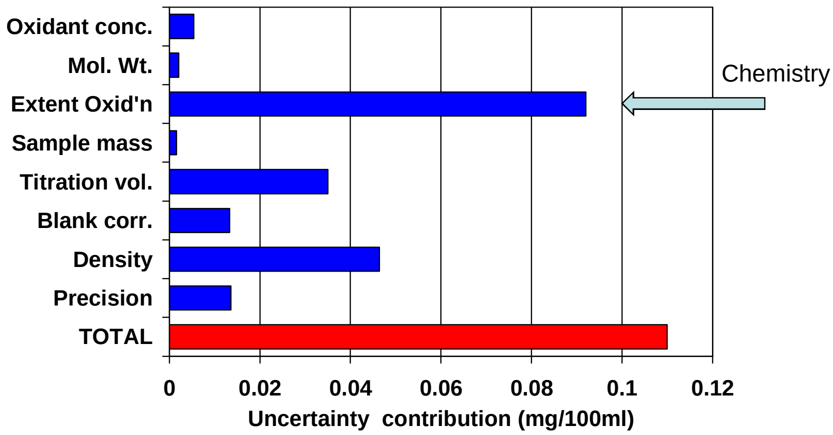
What next?

The Process of Measurement Uncertainty Estimation

- Specify measurand
- Identify Sources
- Group and quantify
- Combine



Example 'GUM' approach: Forensic alcohol standard titration



Uncertainty evaluation approaches

"Well characterised"
quantified effects,
differentiable, continuous,
traceable

Poorly characterised;
Unpredictable effects;
Input quantitiesunclear

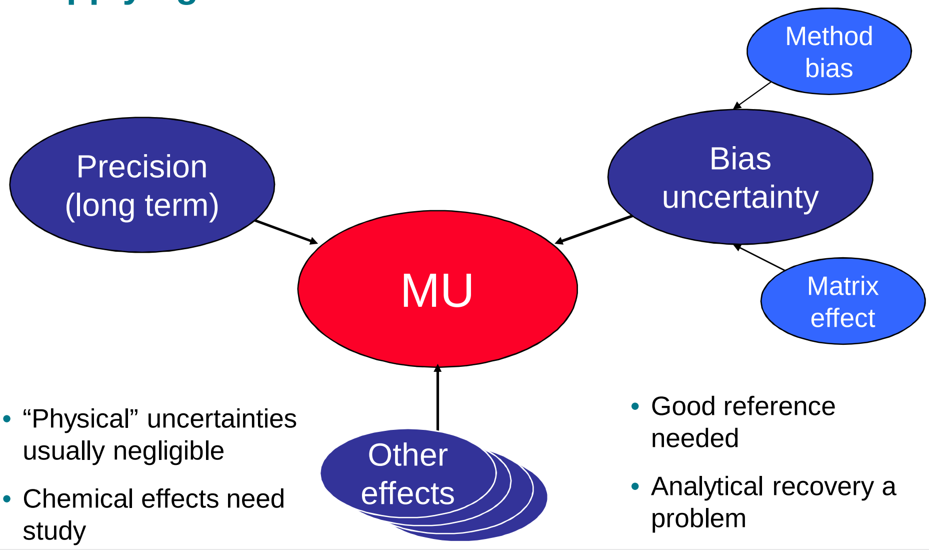
WELL ← **Measurement model applies** → **POORLY**

POORLY ← **Whole method study applies** → **WELL**

Quantifying Uncertainty in chemical measurement: Eurachem Guide options

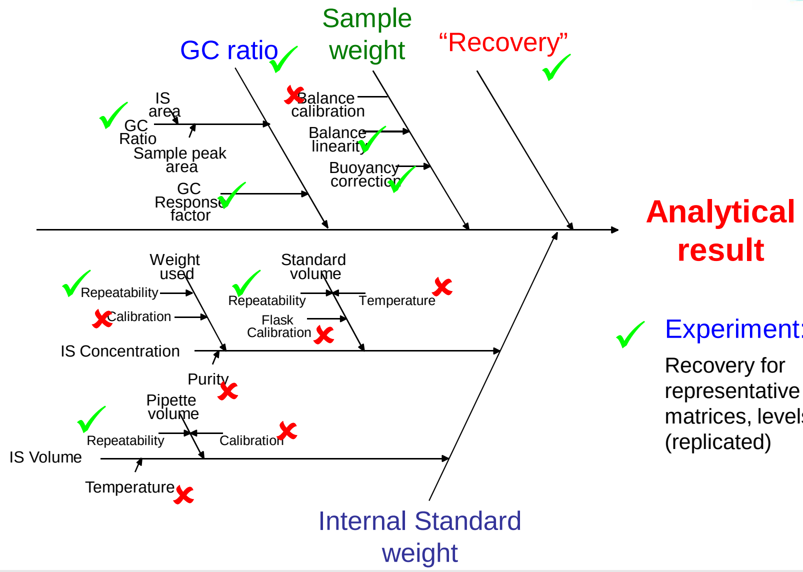
- Evaluating uncertainty by quantification of individual components
- Closely matched certified reference materials
- Uncertainty estimation using prior collaborative method development and validation study data
- Uncertainty estimation using in-house development and validation studies
- Data from proficiency testing
- Empirical and ad-hoc methods

Principle: Applying in-house validation data



- “Physical” uncertainties usually negligible
- Chemical effects need study
- Good reference needed
- Analytical recovery a problem

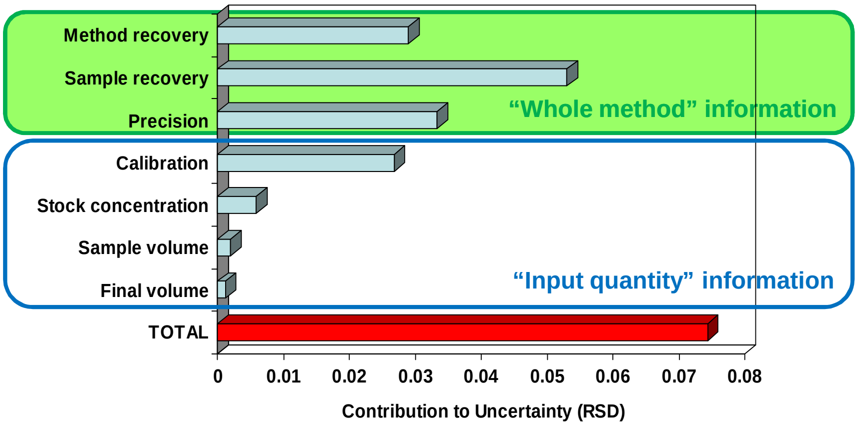
Cause and effect analysis



Experiment:
Recovery for representative matrices, levels (replicated)

Top-down approach:

Determination of Acesulfam-K



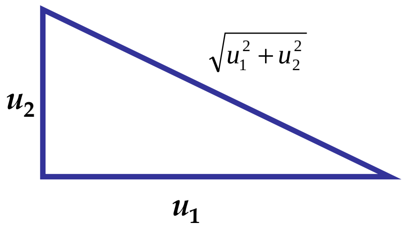
Most of the uncertainty comes from poorly understood effects

Chemistry

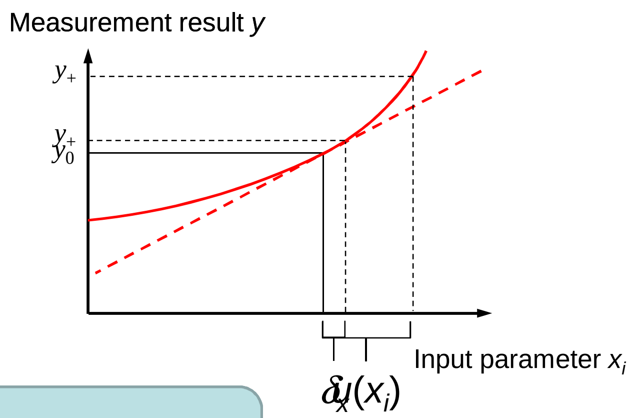
ii) Combining uncertainties

Combining uncertainties for chemistry

- The basic GUM theory
- Simple spreadsheet methods
- Simulation methods



Kragten's spreadsheet method



$$u_i(y) \approx y_+ - y_0$$

Why use a simplistic estimate?

- Exact only for linear examples
- Does not reproduce 1st order GUM

- Usually adequate for mild nonlinearity
- May be **better** for highly non-linear cases

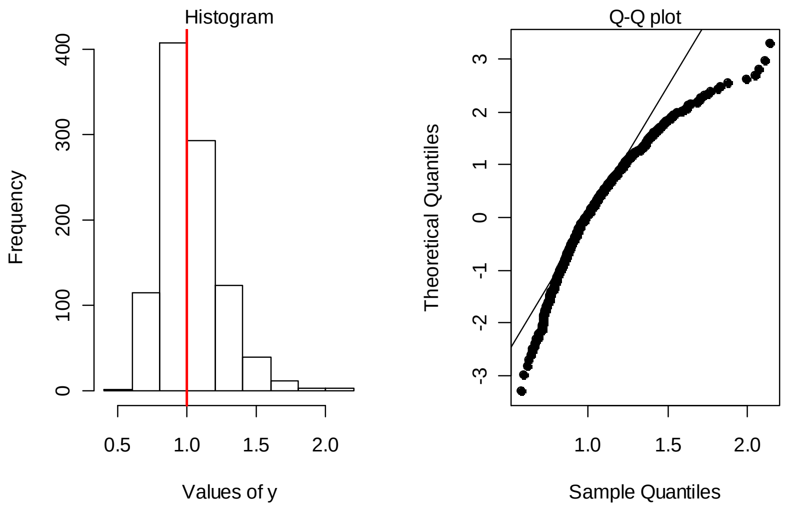
Much simpler than manual differentiation

GUM Supplement 1 ‘Propagation of distributions’ using MCS

- Starts from observed *x* and *u*
- Assumes distributions appropriate to input quantities
- Samples from each (“Monte Carlo simulation”)
  - calculates *y* for each sample
- Calculates $u(y)$ from ‘observed’ distribution
- Can calculate quantiles to provide coverage interval
  - May be asymmetric

- Only corresponds to distribution for the true value under some assumptions

MCS example
y = *a*/(*b*-*c*) (999 replicates)



Calculations carried out using metRology 0.9-4 (http://sourceforge.net/projects/metrology/)

Compare GUM and MCS

GUM

Expression: a/(b - c)

Uncertainty budget:

	x	u	c	u.c
a	1	0.05	1	0.05
b	3	0.15	-1	-0.15
c	2	0.10	1	0.10

y: 1
u(y):0.1870829
y = 1 ± 0.37 (*k*=2)

MCS

Expression: a/(b - c)

Uncertainty budget:

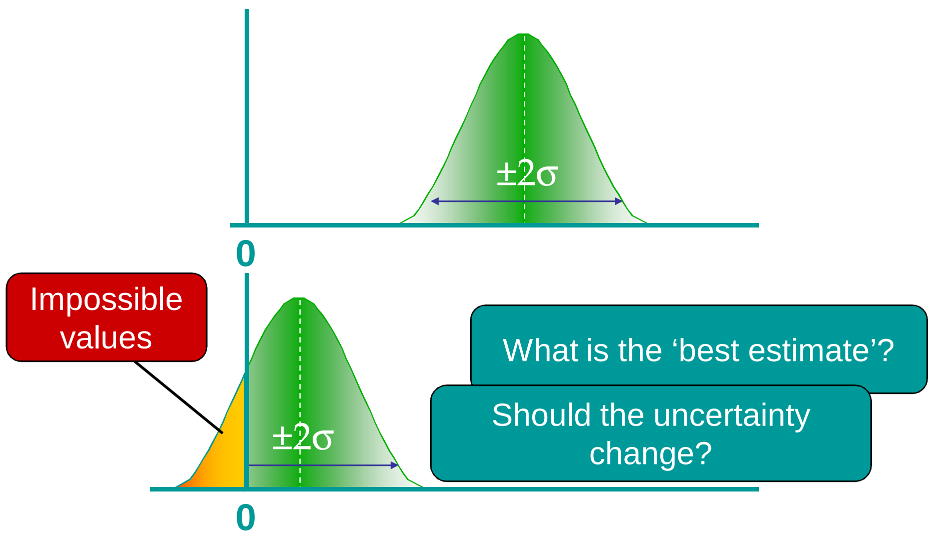
	x	u	c	u.c
a	1	0.05	1.08	0.054
b	3	0.15	-1.09	-0.16
c	2	0.10	1.06	0.11

y: 1
u(y): 0.221
y = 0.718 to 1.535

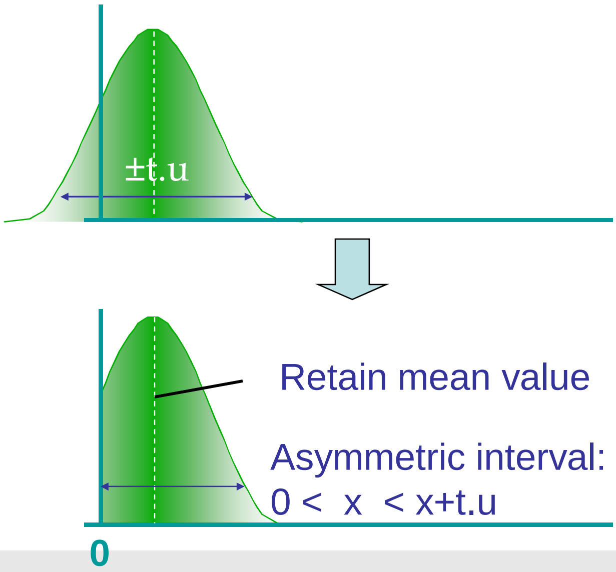
Chemistry

iii) Uncertainty near natural limits:
A Bayesian approach helps

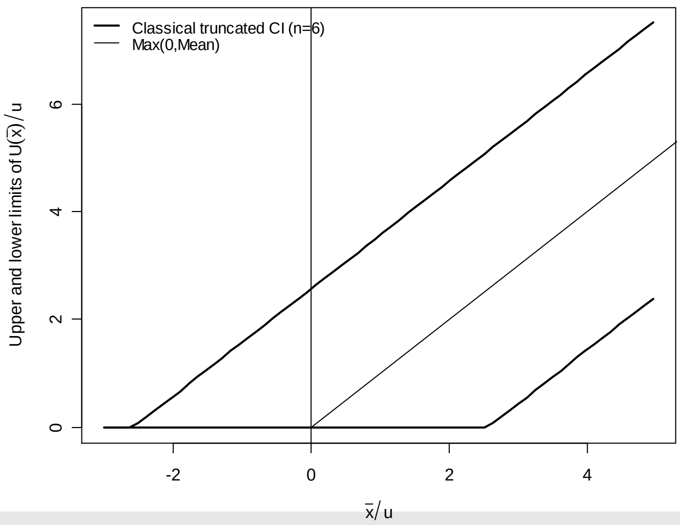
Uncertainty near zero/100%



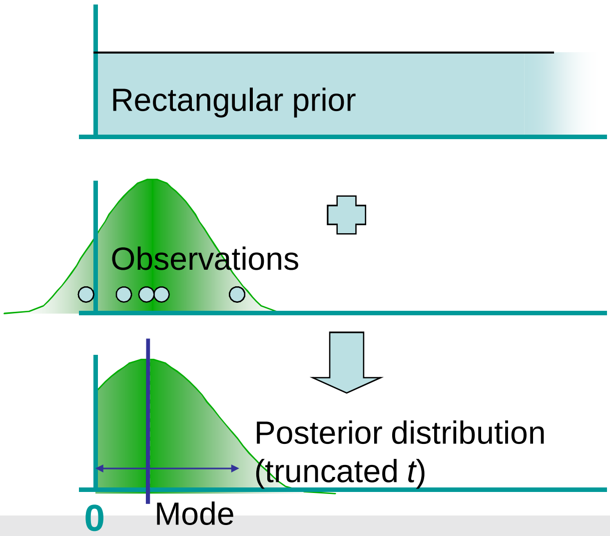
Truncation provides accurate coverage



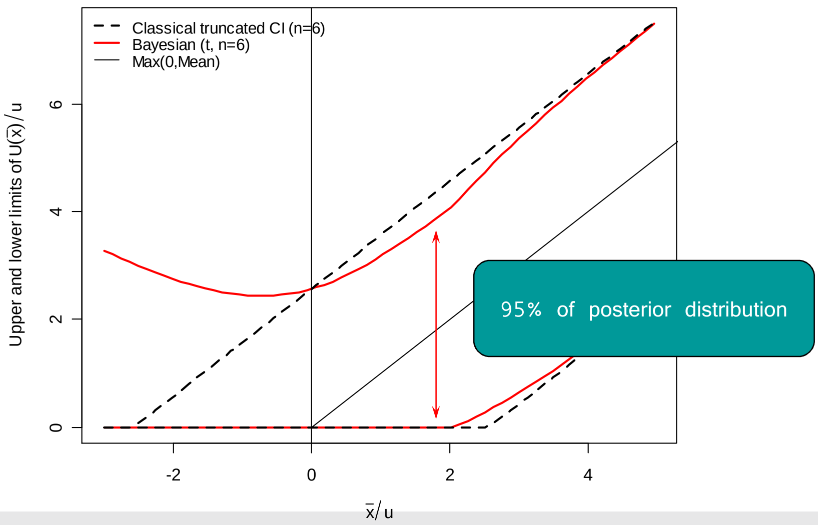
Truncated interval near zero



Bayesian approach



Bayesian interval



Uncertainty on values near zero

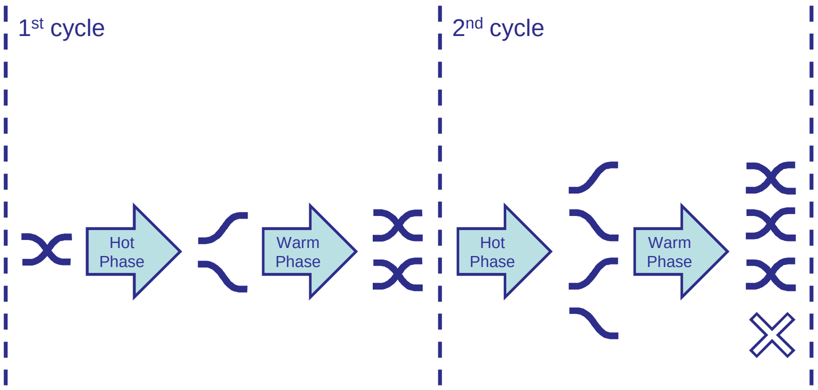
- Truncated interval retains exact coverage properties
  - Standard uncertainty unchanged
  - Minimally biased mean
  - Convergence to zero width implies probable measurement failure
- Correct Bayesian interval more general but more complex to calculate
- Essential to truncate **AFTER ALL OTHER CALCULATIONS**
  - Truncating interim values leads to increased bias

"Handling undetected and low-level components in purity determination".
S Cowen, S L R Ellison, Accred. Qual. Assur. ***12****, 323-328 (2007)*

Biology

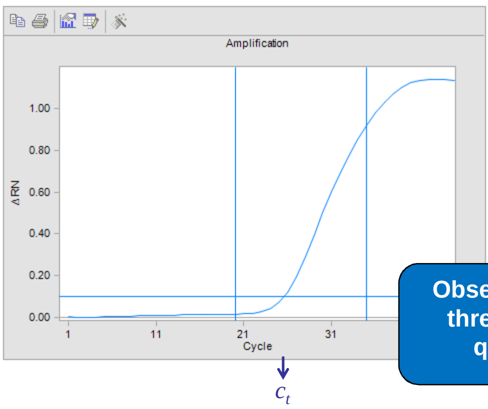
DNA measurement using Real-Time PCR

The Polymerase Chain Reaction (PCR)



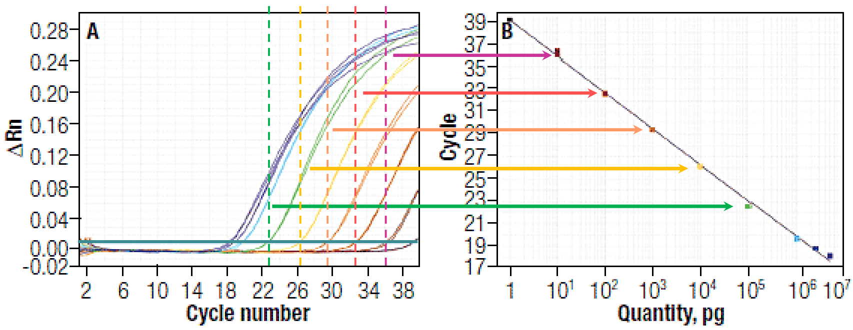
PCR Threshold Cycle

Threshold cycle c_t is the fractional cycle at which the amplification curve crosses a chosen threshold



Observing count to threshold allows quantitation

qPCR Calibration



Calibration regresses C_q on $\log_{10}(c)$

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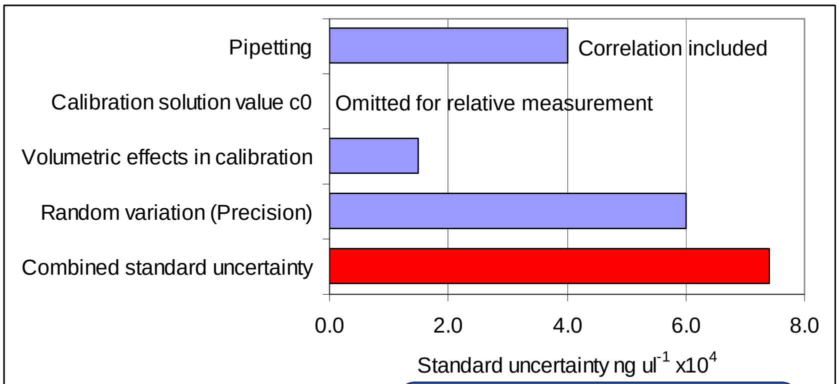
Uncertainties arising from "systematic" effects

- Reference value uncertainty
- Uncertainties in calibration material dilution volumes
- Uncertainties in test material aliquot volume
- Uncertainty in calibration material aliquot volume
- Differences in amplification efficiency

Uncertainty contributions
Matched calibration standard



Does not fully account for long-term variations or interlaboratory dispersion

Uncertainties arising from “systematic” effects

- Reference value uncertainty
- Uncertainties in calibration material dilution volumes
- Uncertainties in test material aliquot volume
- Uncertainty in calibration material aliquot volume
- Differences in amplification efficiency

2% difference in efficiency leads to 80% error in result (30 cycles)

Biology 2

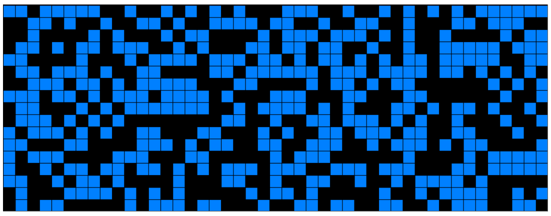
Digital PCR

Digital PCR

- Perform PCR process in large number of wells
- Choose dilution so that some wells have no molecules
- Run for 30-40 cycles

Data

Count number of positive wells *k*



Positive wells contain(ed) ≥ 1 molecule

Average number λ estimated from $p(0)$ in Poisson distribution:

$$p(0) = \exp(-\lambda) \quad \Rightarrow \quad \lambda = -\ln\left(\frac{N-k}{N}\right)$$

Assumptions

Assumptions about the distribution of the number of molecules in the wells:

- Independent
- Identical (requires same volume per well)
- Poisson distribution

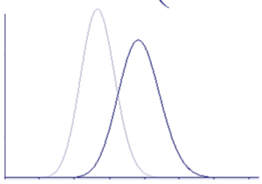
Bayesian modelling allows us to build a more complete model for uncertainty evaluation

Variable volume likelihood

- If we assume the well volume is Gamma distributed with relative standard deviation ω, then the distribution of molecules is **Gamma-Poisson** with the likelihood

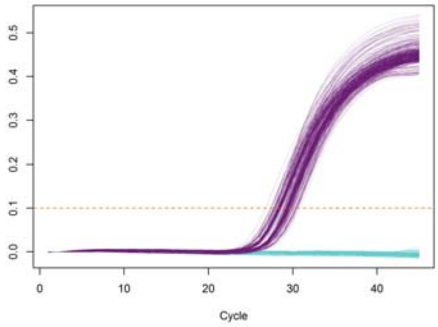
$$L(\lambda;k,n) \propto \left(1-\left(1+\lambda\omega^2\right)^{-\frac{1}{\omega^2}}\right)^k \left(1+\lambda\omega^2\right)^{-\frac{n-k}{\omega^2}}$$

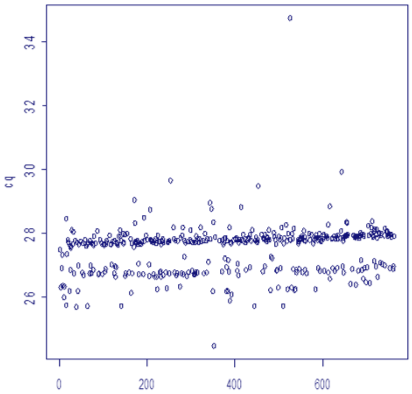
$$k \sim \mathrm{Binom}\left(n, 1-\left(1+\lambda\omega^2\right)^{-\frac{1}{\omega^2}}\right)$$



Volume *variation* causes a bias as well as increased uncertainty

Using C_q data to allow for departure from Poisson distribution





Cq data shows distinct groups for different initial copy number

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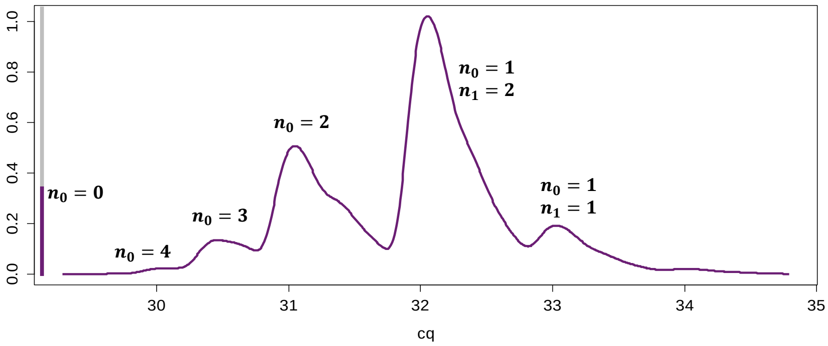
Model for c_q data



Main Parameters:

μ – mean molecules per partition
E – efficiency
A – Fluorescence per molecule

Optional Parameters:

ν – dispersion parameter
E_0 – cycle 1 efficiency
b_x, b_y – trends

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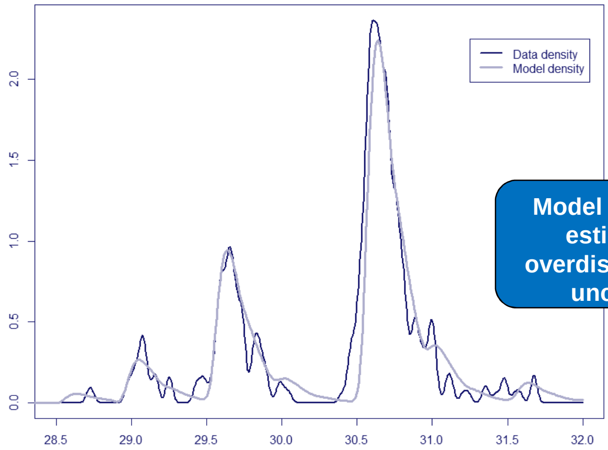
Approximate Likelihood given threshold cycle

$$L(E,\mathbf{p}_0;c,h) \approx \delta^{-1} \sum_{i=1}^{2^{c0}} p(i,c \mid \mathbf{p}_0) \Big[\Phi\Big(h, Ai(1+E)^{c-c_0}, A^2 i(1-E)(1+E)^{c-c_0}\big((1+E)^{c-c_0}-1\big)+\sigma^2\Big) - \Phi\Big(h, Ai(1+E)^{c+\delta-c_0}, A^2 i(1-E)(1+E)^{c+\delta-c_0}\big((1+E)^{c+\delta-c_0}-1\big)+\sigma^2\Big) \Big]$$

Comparison of c_t data to model



Model fit provides estimated λ, overdispersion and uncertainty

Summary of current practice

- Chemistry
  - Most chemical testing labs rely on reproducibility data for uncertainty evaluation
    - Often more realistic because the most important effects can not be well understood
  - Some reference measurements allow input-based models
  - Numerical methods of combination rare outside NMIs
  - Some advanced treatment appearing in a few NMIs
- Biological measurement
  - More heavily dominated by variability
  - Wider range of distributions
  - Somewhat more statistical awareness
  - Some advanced treatments appearing

Looking forward

Looking forward

- Testing laboratories appear content with uncertainties based on validation and interlaboratory study data
- Increasing interest in reporting uncertainties in proficiency tests
- ML and Bayesian treatments rare and will take time to understand
  - Intuition is a poor guide to sound priors
- Uncorrected bias is a bigger problem than new uncertainty methods
- Small degrees of freedom (≤2) will remain common …

Acknowledgements

- Simon Cowen*
  - Volume variation in dPCR*
- Philip Wilson
  - Bayesian modelling for dPCR*

- Funding
  - UK Chemical and Biological Measurement programme
  - *EMRP NEW04 - Novel mathematical and statistical approaches to uncertainty evaluation

Conclusion

From Reproducibility to Bayes
(via GUM 1995)
is
not an evolutionary course

it is a
vital range of options