

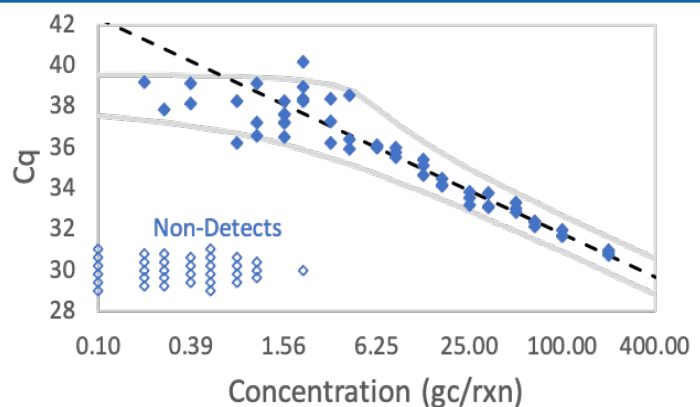
REMEMBERING MICROBIOLOGY BASICS IN qPCR DATA ANALYSIS

Implications for wastewater-based epidemiology

KEY MESSAGES

- qPCR-based approaches reliant on calibration curves cannot explain non-detects or low concentrations relevant in wastewater-based epidemiology surveillance
- Established quantitative microbiology tools were applied to improve evaluation of gene copy concentrations and their uncertainty
- By explaining C_q data at low concentrations, an enhanced standard curve model enables better qPCR-based inference about disease prevalence

Figure 1: C_q values do not follow the log-linear standard curve model at low concentrations, but are explained by an enhanced standard curve model (shown as 95% probability bounds). Non-detects shown at arbitrary C_q values



WHY WAS THIS DONE?

qPCR has been used in Canada during the SARS-CoV-2 pandemic as part of wastewater-based epidemiology surveillance of disease prevalence in populations. Many questions have been raised about interpretation and handling of non-detects and results at low concentrations that approaches grounded in calibration using a log-linear standard curve model cannot explain.

The rich history of mechanistic models for quantitative microbiology data is reflected in digital PCR but not qPCR. This project incorporated those concepts into qPCR data analysis to build a framework for evaluating uncertainty in concentration estimates. Evaluation of uncertainty is needed to distinguish changes that may have public health significance from random noise.

“Current quality regulations dictate that any result from an analytical laboratory should be given with an associated uncertainty estimate...” – **Burns et al., 2005**

APPROACH

Foundational theory for the log-linear standard curve model ignores the discrete nature of microorganisms and their genes. An enhanced standard curve model addressing this flaw provides superior representation of qPCR data at low concentrations (Fig. 1). Consistent with digital PCR theory, non-detects arise by chance in samples with low numbers of target genes and are not missing or censored data. This new model was used to evaluate uncertainty in parameters such as amplification efficiency as well as concentrations of environmental samples. It seamlessly formalizes assimilation of data from technical replicates including non-detects.

FINDINGS

1) The log-linear standard curve model is satisfactory in the linear dynamic range, but the enhanced model improves estimation of low concentrations

2) Non-detects are concentration estimates of zero—not missing or censored data—and should not be omitted or substituted with arbitrary values

3) It is better to show uncertainty in all concentration estimates than to partition adequately reliable results using a poorly defined limit of quantification

“...although qPCR has frequently been proclaimed a touchstone or a gold standard, in practice this “standard” is a variable one, and the reporting of results requires considerable sophistication of analysis and interpretation”

– **Bustin et al., 2009**

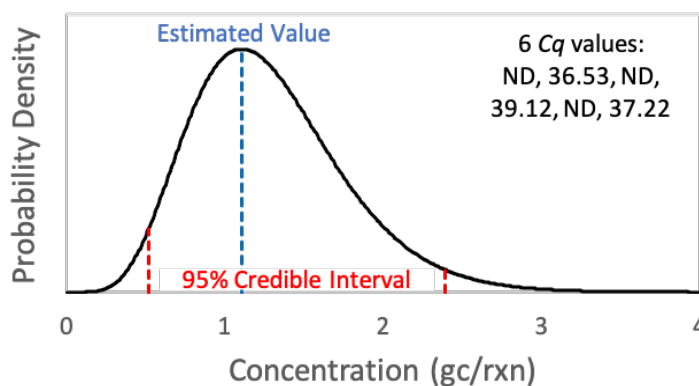


Figure 2: Uncertainty in qPCR-based concentration estimates and 95% credible interval to indicate precision

IMPLICATIONS

The newly developed approach:

- is consistent with over a century of statistical approaches applied to other quantitative microbiology methods and based on modelling of well-understood mechanisms
- corrects erroneous interpretations and handling of non-detects in qPCR
- helps labs to understand and address how uncertain reported values are
- seamlessly reveals the quantitative information available in non-detects and Cq values below the linear dynamic range of the standard curve
- circumvents the need for a poorly defined and quantified limit of quantification (LOQ)
- allows qPCR-based concentration values to be plotted with error bars



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