Clinical Chemistry 63:9
1537–1538 (2017)

Citation Classic

MIQE: A Step Toward More Robust and Reproducible Quantitative PCR

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Featured Article: Bustin SA, Benes V, Garson JA, Hellemans J, Huggett J, Kubista M, et al. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clin Chem 2009;55:611–22.[3]

The concept of using in vitro enzymatic synthesis to amplify DNA was first mooted in 1971 *(1)* and demonstrated in 1985 as the "polymerase chain reaction" *(2)*. PCR enables the detection of a unique DNA sequence amongst a vast background of other, similar DNA molecules. Its remarkable combination of conceptual simplicity and practical accessibility, together with the addition of reverse transcription for detection of RNA, and continuous improvements to reagents, protocols, and instruments has secured PCR's status as today's most versatile and ubiquitous molecular laboratory technique.

In its original guise as an end-point assay, PCR required gel electrophoretic analysis. This method was time-consuming, limited in analytical sensitivity, dynamic range, and resolution, and introduced the potential for contamination. Importantly, end-point PCR was nonquantitative. The introduction of real-time fluorescence-based quantitative PCR (qPCR) changed this by detecting PCR amplicons during the exponential phase using fluorescent reporters *(3)*. With this feature, coupled with no processing after PCR, less contamination, and the potential to carry out statistical analyses, qPCR became the method of choice for quantitative applications.

However, such popularity created a myriad of different protocols, reagents, and analysis methods, which, when combined with different nucleic acid extraction and quality assessment methods, resulted in the publication of implausible and contradictory results. Importantly, the omission of detailed technical information made it challenging to gauge the soundness of qPCR-based results. Specifically, nucleic acid integrity and purity assessments were rarely reported, variability introduced by the reverse transcription step was disregarded, PCR efficiencies were not specified, and normalization procedures were not justified. Publication of erroneous conclusions in the scientific literature became commonplace.

One egregious example of qPCR misuse ostensibly supported a link between measles virus and gut pathology in autistic children. A detailed examination of the qPCR evidence revealed poor assay design, widespread disregard of control results suggesting contamination issues, inadequate nontransparent reporting, and questionable data analysis *(4)*. This provided the final impetus for a group of international scientists to introduce recommendations for qPCR assay design and data reporting. A best practice, commonsense approach of minimum guidelines in categories critical for obtaining reliable results was published in the article discussed here under the acronym MIQE, which stands for "minimum information for the publication of qPCR experiments." This publication is now the second most cited paper in *Clinical Chemistry*.

The MIQE guidelines are now the accepted standard for both optimal qPCR assay design and transparent reporting, actively championed by PCR reagent and instrument manufacturers and many journals. They have also been adapted for diagnostic and clinical applications *(5)*. Furthermore, the increasing popularity of digital PCR has resulted in the publication of MIQE guidelines for digital PCR *(6)* and the challenges of RNA sequencing have resulted in MIQE-inspired guidelines *(7)*.

However, the majority of qPCR publications still do not provide sufficient technical detail *(8)* and, where information is provided, it often invalidates the authors' conclusions *(9)*. Many high–impact factor journals appear to have improved technical reporting standards, but there is actually less transparency today *(10)*. Clearly, technical guidelines are an important step, but unless the scientific community as a whole takes more responsibility, it will continue to be plagued with immaterial, misleading, time- and money-wasting conclusions. The question remains: if a technique as "simple" as qPCR is handicapped by inappropriate use and inadequate scrutiny, what hope is there for the more complex technologies in use today?

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[3] This article has been cited more than 5100 times since publication.
Received January 23, 2017; accepted January 27, 2017.
Previously published online at DOI: 10.1373/clinchem.2016.268953

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 require-*

ments: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

Authors' Disclosures or Potential Conflicts of Interest: *Upon manuscript submission, all authors completed the author disclosure form. Disclosures and/or potential conflicts of interest:*

Employment or Leadership: C.T. Wittwer, *Clinical Chemistry*, AACC.
Consultant or Advisory Role: None declared.
Stock Ownership: None declared.
Honoraria: None declared.
Research Funding: None declared.
Expert Testimony: None declared.
Patents: None declared.

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