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Dear Editor

Re: MIQE: Information for Publication of Quantitative Real-Time PCR Experiments

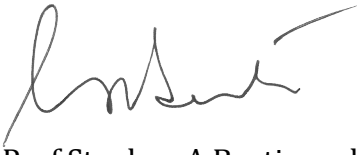
Many research publications employ quantitative real time PCR (qPCR) for nucleic acid analysis. However, most provide inadequate experimental detail, many apply unsuitable data analysis with the result that conclusions are often inappropriate and inconsistent between laboratories. As a result, the quality of qPCR data is variable, manuscripts can be difficult to evaluate by reviewers, and the studies may be impossible to repeat. Consequently, there is a clear need for a common publication standard that comprehensively describes qPCR experiments.

The recently published "MIQE" guidelines provide a uniform standard for reporting qPCR data and delineate the level of detail needed to enable appropriate interpretation of qPCR experiments. These guidelines are modeled after similar recommendations for microarrays, proteomics, and others under the umbrella of MIBBI (Minimum Information for Biological and Biomedical Investigations, http://www.mibbi.org). MIQE was developed by an international

consortium of academic scientists with a conscious focus to avoid any commercial bias.

For manuscripts that use qPCR, please consider adopting these MIQE standards. We suggest that authors include the accompanying checklist upon initial submission, enabling even non-expert reviewers to assess the validity of the qPCR data and any conclusion drawn. Attached please find an expanded justification for the need of these guidelines, the published article and the qPCR checklist that can also be found at http://www.RDML.org/MIQE.

Yours sincerely,



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MIQE Justification

Real-time quantitative PCR (qPCR) has become a ubiquitous tool in molecular biology. The data obtained from this technique have served to underpin the conclusions promulgated in a great number of research and diagnostic publications. qPCR technology is supported by a large number of suppliers of assorted reagents, kits, plasticware and instruments; hence experiments are performed using countless variations of already disparate protocols. There are varying standards of template and assay quality control and data analysis can be highly subjective, even when interpreted by an ever growing range of software tools. Unsurprisingly, the absence of agreement on how best to perform qPCR assays and analyse the resulting data translates into inconsistency and contradiction when reporting experimental results. This interferes with the readers' ability to make sense of qPCR-based results and restricts their capacity to reproduce published data.

In practice, this has adverse consequences and results in the corruption of our scientific literature with a multitude of publications reporting insignificant, conflicting and plainly wrong results. A high profile example is provided by the publication [1] and subsequent retraction [2] of a Science "breakthrough of the year" in 2005. Of more direct consequence, perhaps, is the shocking misuse of qPCR technology to support the speculation surrounding a link between the MMR vaccine and autism. When analysed correctly, the results proved to be entirely due to poorly implemented experimental technique [3], a conclusion upheld by recent verdicts at the USA vaccine court trials.

We have spent a considerable amount of time highlighting and addressing this problem and, as a group, have become only too aware of the poor standards

characteristic of qPCR assay design, execution and interpretation. In our opinion, the major problem with the use and interpretation of qPCR data is the lack of information that characterises most papers utilising this technology. The majority of publications do not provide sufficient experimental detail to permit the reader to evaluate critically the quality of the results presented or to repeat the experiments. Specifically, detailed information about sample acquisition and handling, RNA quality and integrity, primer and probe characteristics, reverse transcription details, PCR efficiencies and data analysis parameters are frequently omitted, and sample normalisation is habitually performed against single reference genes without adequate justification.

MIQE [4] is a set of guidelines that describe the minimum information necessary for evaluation of qPCR experiments. Included is a checklist to accompany the initial submission of a manuscript to the publisher. By providing all relevant experimental conditions and assay characteristics, reviewers can assess the validity of the protocols used. Full disclosure of all reagents, sequences, and analysis methods is necessary to enable other investigators to reproduce results. MIQE details should be published either in abbreviated form or as an online supplement. Following these guidelines will encourage better experimental practice and transparency, allowing more reliable and unequivocal interpretation of quantitative PCR results. In addition the RDML consortium has developed a universal format for the exchange of qPCR data that is independent of the qPCR instrument being used. This allows for easy reanalysis of submitted data.

Editors of scientific journals should be aware of the MIQE guidelines and the RDML data format [5]. Please consider them for inclusion in your

instructions for authors. Additional information on these guidelines and data standards can be found at http://www.RDML.org, http://www.RDML.org/MIQE and in their respective publications (PMID: 19246619 & 19223324).

[1]T. Huang, H. Bohlenius, S. Eriksson, F. Parcy, and O. Nilsson, The mRNA of the Arabidopsis gene FT moves from leaf to shoot apex and induces flowering. Science 309 (2005) 1694-6.
[2]H. Bohlenius, S. Eriksson, F. Parcy, and O. Nilsson, Retraction. Science 316 (2007) 367.
[3]S.A. Bustin, RT-qPCR and molecular diagnostics: no evidence for measles virus in the GI tract of autistic children Eur Pharm Rev Dig 1 (2008) 11-16.
[4]S.A. Bustin, V. Benes, J.A. Garson, J. Hellemans, J. Huggett, M. Kubista, R. Mueller, T. Nolan, M.W. Pfaffl, G.F. Shipley, J. Vandesompele, and C.T. Wittwer, The MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments. Clinical Chemistry 55 (2009) 609-620.
[5]S. Lefever, J. Hellemans, F. Pattyn, D.R. Przybylski, C. Taylor, R. Geurts, A. Untergasser, and J. Vandesompele, RDML: structured language and reporting guidelines for real-time quantitative PCR data. Nucleic Acids Res (2009).