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Mark R. Ackermann, DVM, PhD, DACVP Professor, Department of Veterinary Pathology 1600 South 16th Street 2738 College of Veterinary Medicine Iowa State University Ames, Iowa 50011-1250

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Dear Prof Ackermann,

Re: Prexcel-Q

I am pleased to be given a chance to offer my views on the need for further development of the real-time PCR (qPCR) programme, Prexcel-Q. I have been following its development since first hearing about it from Dr Gallup. I have also "test-driven" it and hence am familiar with the current version of the programme.

The number of laboratories using qPCR continues to increase exponentially, as is the case with the variety and range of applications for this technology. Together with colleagues I have been concerned for a long time about the quality of data released using qPCR protocols, and we have recently proposed a set of guidelines ("MIQE") for the publication of papers utilising qPCR (Bustin et al (2009) Clin Chem 55: 611-622). One important, and obviously fundamental, source of errors associated with qPCR experiments arises from mistakes made during the set-up of the qPCR assay. Furthermore, pre-analysis considerations such as testing for

PCR inhibition and optimising the assay are important contributors to a successful assay, but are not generally implemented.

Prexcel-Q is the only programme currently available that allows the user to carry out these analyses systematically and comprehensively. Unlike a large number of other programmes available, Prexcel-Q is not a data analysis programme. Instead it is a tool that provides a much-needed, fundamental building block to guide qPCR set-up procedures. Hence widespread use of Prexcel-Q would be a very useful addition to our armoury for qPCR standardisation.

One essential factor for its acceptability is the development of a GUI that improves its user-friendliness, as the current version is complex and not easy to navigate. Such a version of Prexcel-Q would be much more likely to see widespread adoption and become an integral tool for anyone carrying out qPCR experiments, thus helping to improve the accuracy of scientific data based on qPCR analysis.

I look forward to seeing and using such an advanced version of Prexcel-Q.

Sincerely.

Professor of Molecular Science