Letter to the Editor

A critical step for relative quantification of mRNAs is selecting the correct internal controls

Dear Editor,

We read with great excitement the manuscript entitled “Measurement of *Pseudomonas aeruginosa* multidrug efflux pumps by quantitative real-time polymerase chain reaction” by Yoneda et al. [1]. The authors described a relative quantitation assay using a real time PCR (RT-PCR) method for multi-drug efflux proteins, MexB and MexY, in several clinical strains of *P. aeruginosa* relative to a reference strain PAO1. Some isogenic mutants of PAO1 were also compared. The relative expression of mRNAs were further validated by comparison to corresponding protein levels. The *rpsL* gene provided the internal control.

The authors stated that the mRNA levels of the isogenic mutants agreed well with those of strain PAO1 and there were only slight discrepancies between mRNA and protein levels of clinical strains. As absolute values for the clinical strains were not given, one can evaluate the results only from Fig. 3. From our assessment of the data in Fig. 3, we believe that there are major differences between mRNA and protein levels for MexY of strains T002, T003 and T004, and even of strain T006 and also for MexB of strain T005. Because of the extremely short half-life of mRNA, only relative levels can be compared, and the results are expressed as “up or down regulated”. From this point of view, for example, the protein levels for MexY of T002, T003 and T004 seem to be normal while corresponding mRNAs are upregulated. It would be very informative to know, on the basis of a statistical analysis, whether these apparent discrepancies are significant.

Whatever the results of such an analysis, there are some discrepancies between mRNA and protein levels. The authors stated that the correlation between protein and mRNA levels among PAO1 and its isogenic mutants is due to their similar genetic backbone, but the discrepancies among clinical strains are because of the genetic diversity. We disagree with such an explanation.

The diversity in genetic background and behavior of prokaryotes in different conditions should have been expected. Moreover, housekeeping genes that are expected to be expressed at a constant level in conditions similar to those used in the assay should be used as a reference. These housekeeping genes are included to normalize the diversity in the background and in the behavior of strains. We think that it is apparent that the internal control used in this study, *rpsL*, failed to normalize the expected differences between clinical strains.

The accuracy of a relative comparison largely depends on the stability of the internal controls. However, the mRNA metabolism of prokaryotes is extremely unstable and, consequently, finding internal control genes that are expressed relatively stably in the assay conditions is critical and must be validated independently. Examples of the evaluation of housekeeping genes in *P. aeruginosa* can be found in two previous studies not mentioned in the manuscript [2,3]. In one of these papers [3], the suitability of six housekeeping genes for use as controls was assessed by real-time quantitative RT-PCR using 23 strains under conditions similar to those used in resistance gene studies. It was concluded that two, rather than one reference gene should be used for normalization. The second study further validated the use of these two genes for pump and porin activities of *P. aeruginosa* [2]. In contrast, two factors leave the conclusions of the study by Yoneda et al. [1] open to question: first, only one gene has been included as a reference; second, this gene, *rpsL*, has not previously been shown by an independent study to be stably expressed among various clinical strains. Surely it would have been better to have selected internal controls similar to those that have been shown to be relatively stable in similar conditions in previous studies?

References


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