Data Handling Pipeline Description: Since numerous miRNAs have been shown to be present in the circulation, these so-called circulating miRNAs have emerged as potential biomarkers for disease. However, results of qPCR studies on circulating miRNA biomarkers vary greatly and many experiments cannot be reproduced. Missing data in qPCR experiments often occur due to off-target amplification, non-analyzable qPCR curves and discordance between replicates. The low concentration of most miRNAs leads to most, but not all missing data. Therefore, failure to distinguish between missing data due to a low concentration and missing data due to randomly occurring technical errors partly explains the variation within and between otherwise similar studies. Based on qPCR kinetics, an analysis pipeline was developed to distinguish missing data due to technical errors from missing data due to a low concentration of the miRNA-equivalent cDNA in the PCR reaction. Furthermore, this pipeline incorporates a method to statistically decide if concentrations from replicates are sufficiently concordant, which improves stability of results and avoids unnecessary data loss. By going through the pipeline's steps, the result of each measurement is categorized as either valid, invalid or undetectable. Together with a set of imputation rules, the pipeline leads to more robust and reproducible data as was confirmed experimentally. Using two validation approaches, in two cohorts totalling 2214 heart failure patients, we showed that this pipeline increases both accuracy and precision of qPCR measurements. In conclusion, this statistical data handling pipeline improves the performance of qPCR studies on low expressed targets such as circulating miRNAs.

The files provided with this document are example databases with the data format and the syntax (in both R and SPSS) to simplify the use of the above described pipeline. The steps in the pipeline differ between measurements performed in duplicate and in triplicate. Syntaxes and example databases are therefore provided for both types of data. The example databases are provided in both excel and SPSS format. If you have any questions or suggestions, please contact the authors of the paper.

For duplicate measurements

Example database duplicates.xls

Example database duplicates. sav

Syntax duplicates.sps

Syntax duplicates.R

For triplicate measurements

Example database triplicates.xls

Example database triplicates. sav

Syntax triplicates.sps

Syntax triplicates.R

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FAQs

- **Q.**
  A.

References

- [de Ronde et al.](#) Practical data handling pipeline improves performance of qPCR-based circulating miRNA measurements. RNA, Epub ahead of print. 2017

Version History

Data Handling Pipeline version 2017.0 released Febuary 2017

First release with publication of paper.