Detecting delivery of a microRNA-based drug

P. Winata[1], E. McGowan[1] and N. Nassif[1], G. Reid[2]

[1] University of Technology, Sydney, [2] Asbestos Disease Research institute

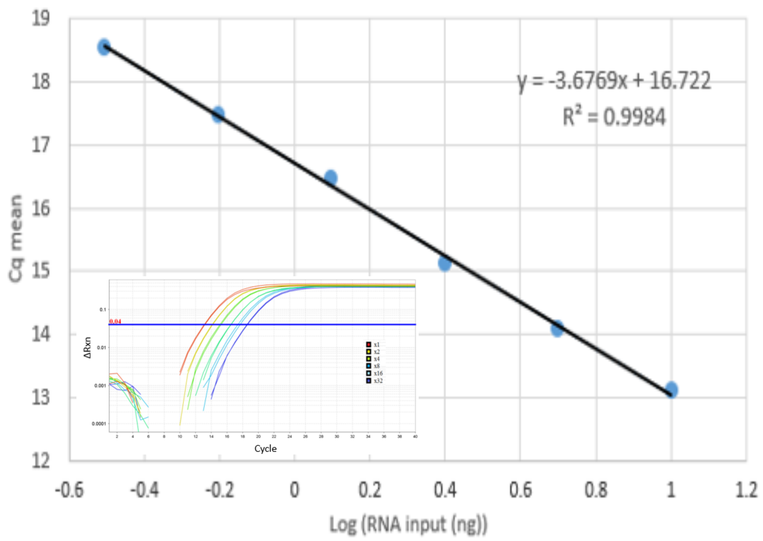
Introduction

Malignant pleural mesothelioma (MPM) is a malignancy arising from the cells lining the pleural cavity. Similar to other cancers, expression of the tumour suppressive microRNA miR-16 have been reported to be downregulated. Re-expression of miR-16 in cell culture and animal models decreased tumour growth. Recently, the use of a novel microRNA mimics based on the miR-16 sequence have been developed, and this concept has entered clinical trial. As the mimics used have a novel sequence, it should be possible to detect their delivery using RT-qPCR. Here we report the efficiency and specificity of three RT-qPCR platforms in detecting these novel microRNA mimics.

Results

A. RT-qPCR assay efficiency

We assessed the efficiency of three microRNA detection platforms using synthetic RNAs corresponding to these endogenous and novel microRNA sequences. To assess this, two-fold dilution series of each synthetic RNA were utilised to produce an amplification plot (inset) which was used to generate the standard curve (shown here for miR-16??).



From the standard curve, the slope was used to calculate the percentage efficiencies of each RT-qPCR assay design:

Life Technologies

microRNA	Slope value	Efficiency factor	Efficiency (%)
Hsa-miR-16	-3.6696	1.87	87.29 %
conmiR-15/107.2	-3.8764	1.81	81.12 %
conmiR-15/107.4	-4.1018	1.75	75.31 %

miRXes

microRNA	Slope value	Efficiency factor	Efficiency (%)
Hsa-miR-15a	-3.3669	1.98	98.16 %
conmiR-15/107.2	-3.9772	1.78	78.42 %
conmiR-15/107.4	-4.5389	1.66	66.08 %

Exiqon

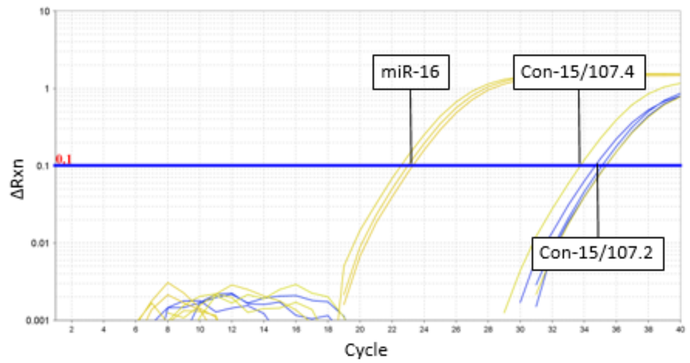
microRNA	Slope value	Efficiency factor	Efficiency (%)
Hsa-miR-16	-4.2067	1.73	72.87 %
conmiR-15/107.2	-3.7472	1.85	84.87 %
conmiR-15/107.4	-3.6769	1.87	87.05 %

These results indicate a greater efficiency of Life Technologies and miRXes platforms in detecting endogenous microRNAs than novel microRNAs, with the opposite true for the Exiqon kit.

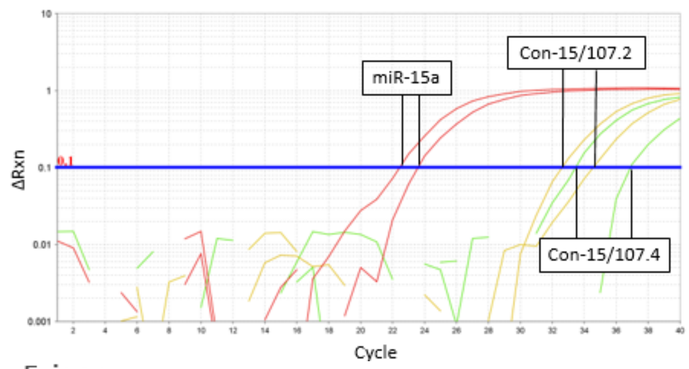
B. RT-qPCR assay specificity

We tested specificity of each RT-qPCR platform by assaying endogenous and novel microRNA mimics in untreated MPM cell lines. As only endogenous microRNA should be detected, this gives a good indication of the specificity of the assays.

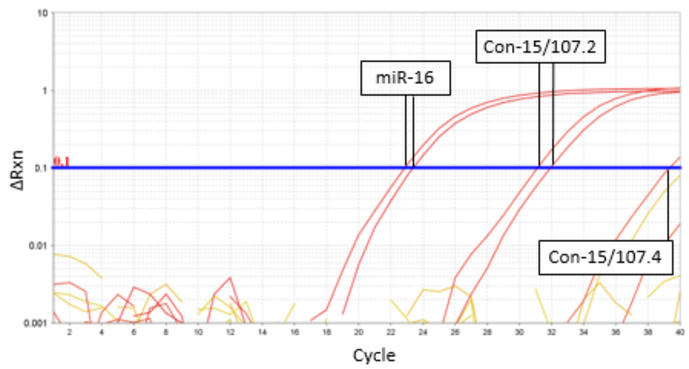
Life Technologies



miRXes



Exiqon



As only miR-15a and mIR-16 are present in these templates, only those assays should produce a signal. These results indicate an inability of RT-qPCR platforms to specifically detect microRNAs of related sequence.

Conclusion

None of the RT-qPCR platforms tetsed are sufficiently specific in detecting the novel microRNA sequences. Another method, for example miR-Seq, will be required to detect mimic delivery to tumour cells in patients.