Sensitivity testing – QPCR versus LAMP and effect of SDT inactivation buffers

Purpose

This testing was to establish relative sensitivity of LAMP testing to QPCR testing in the presence of SDT and SDT-Complete inactivation buffers relative to a water control.

Results

Serial ten-fold dilutions were made of an extracted Hela RNA stock in water and then assayed using the published CDC RNAse P primer set by QPCR. This provided the calibration curve shown in Fig 1A, with an estimated 10-100 copies per reaction reached at a log -5 dilution. Results at log -6 dilution showed a stochastic positive response as expected at low copy number. The trendline fitted through the data shows an excellent fit with low residuals.

The stock RNA dilutions were then tested using the NEB published Actin LAMP control primer set. Total sample volumes were made from a 3:1 ratio of buffer to RNA stock. This provides the data shown in the three dilution curves in Fig 1B.

It can be seen that the limit of detection (LOD) is around 10 fold lower for the LAMP reactions. The results show good linearity to the -4 log dilution and then variability around the -5 log dilution. There is an offset of around 10 cycles (about 5 minutes) in SDT-complete in comparison to the SDT buffer, although the calibration curves are colinear suggesting inhibition of the reaction is not effecting sensitivity until the background limit is reached. The dotted red line shows the limit when some negative control samples start to show 'false positives' due to background DNA amplification.

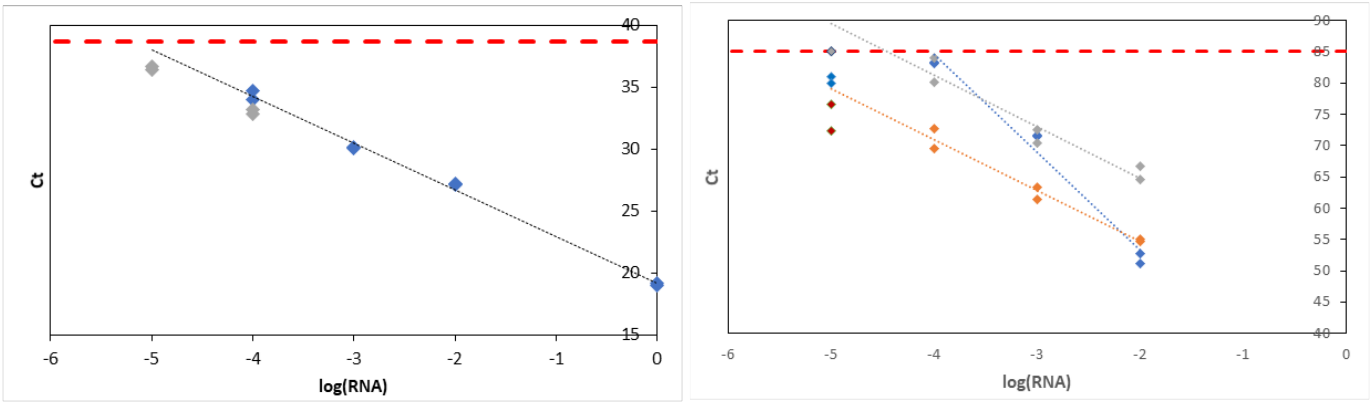

Fig1A (left) Ct values determined by QPCR of diluted stock Hela RNA. Two datasets are shown in blue and grey. The grey dataset included an additional log -5 dilution. There is a slight offset between the two sets, which is expected from run-to-run variation. The limit of sensitivity of the reaction, where occasional 'false positives' may be detected is shown by the dotted red line. Fig 1B (right) Ct values from fluorescent LAMP reactions (NEB master mix plus dsDNA binding dye). The buffers used for dilution are water (blue), SDT (orange) and SDT-complete (grey). Trend lines based on the first three dilutions are shown for each. The dotted red line shows the limit of sensitivity of the LAMP reaction.

Conclusions

The SDT and SDT-complete buffers show good compatibility with LAMP-based detection reactions with around a 10-fold lower LOD than an equivalent QPCR reaction. The true variation in LOD between QPCR and LAMP is not proved by these results as the RNA targets are not identical in the QPCR and LAMP reactions. However, it is shown that the inactivating buffers are compatible with the LAMP reaction. The SDT buffer shows a similar sensitivity to samples diluted in water. The SDT-Complete buffer shows some increase in Ct, which is presumed due to inhibition of the amplification reaction by the RNAse inhibitor present in this buffer. However, this may only result in an increase in detection time rather than a reduction in LOD and reaction sensitivity if compared to a background reaction using the same buffer. Further work is needed to validate this conclusion for 'real world' swab-samples that may contain additional inhibitors.

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