Optimization of RT-PCR chemistry for a new infectious disease testing system

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Background

Infectious diseases are among the most prevalent diseases globally and cause serious health and economic burdens.[1] Timely and accurate detection of pathogens is needed for treatment decisions and to stop the transmission chain.[2]

The role of molecular diagnostics in clinical microbiology has steadily grown over the past decades due to the high sensitivity, speed, and possibility of automation of the methods.[3] One of the most established molecular methods is quantitative reverse transcription PCR (RT-PCR).[4]

In this work, RT-PCR conditions were optimized for a new molecular testing system for rapid infectious disease diagnostics, which is under development in the healthtech company Uniogen. The aim of the work was to find glycerol-free reverse transcriptase (RT) and DNA polymerase enzymes and optimal reaction compositions suitable for the dry chemistry to be used with the new system.

Methods

In total 6 DNA polymerases and 8 RT enzymes were compared in quantitative RT-PCR using PCR controls (Figure 1) . The enzyme pairs with the highest performance were selected for RT concentration and PCR buffer optimization. Ten pairs of enzymes were dried on PCR test chips for stability monitoring.

Figure 1. Two-enzyme one-tube RT-PCR setup using hydrolysis probes and Uniogen's PCR test chips.

Results

The enzyme comparison showed variations in S/B and Ct results across different enzyme pairs.

Amplification was efficient over a wide range of RT concentrations (Figure 2).

Use of detergents and higher KCl concentration (50 vs. 30 mM) resulted in higher specific fluorescent signal (Figure 3).

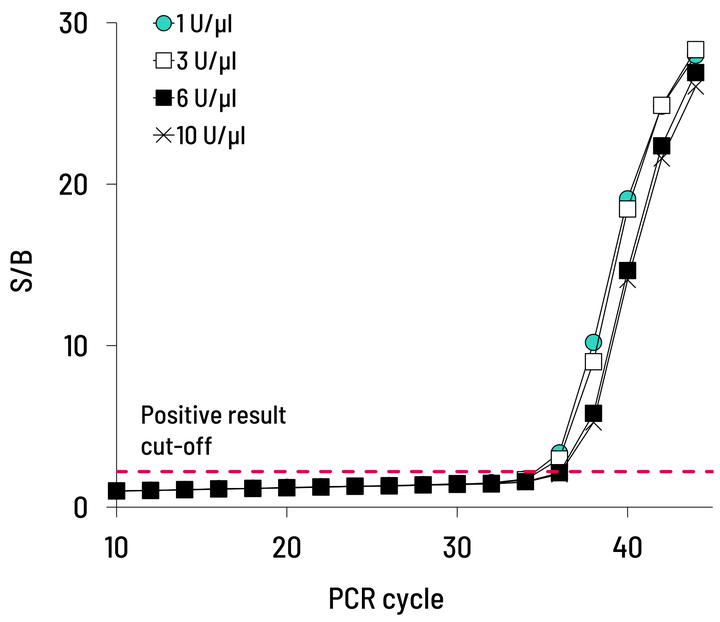

Figure 2. Effect of RT concentration on specific fluorescence signal. Mean of S/B plotted against PCR cycle for 4 replicates.

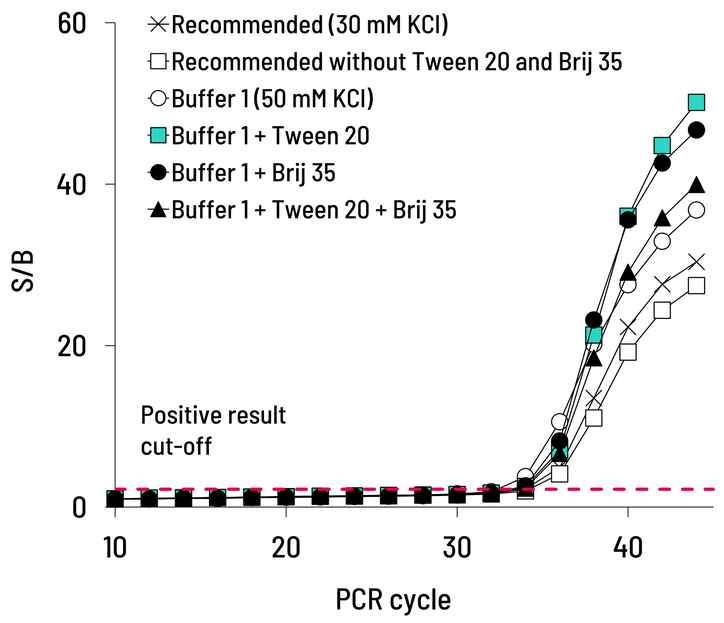

Figure 3. Effect of buffer composition on specific fluorescence signal. Mean of S/B plotted against PCR cycle for 3 replicates.

Enzymes exhibited stability after dried on the PCR test chip. Based on the S/B and Ct results, there was no significant loss of enzyme activity after 8 weeks of storage at +28 °C (Figure 4).

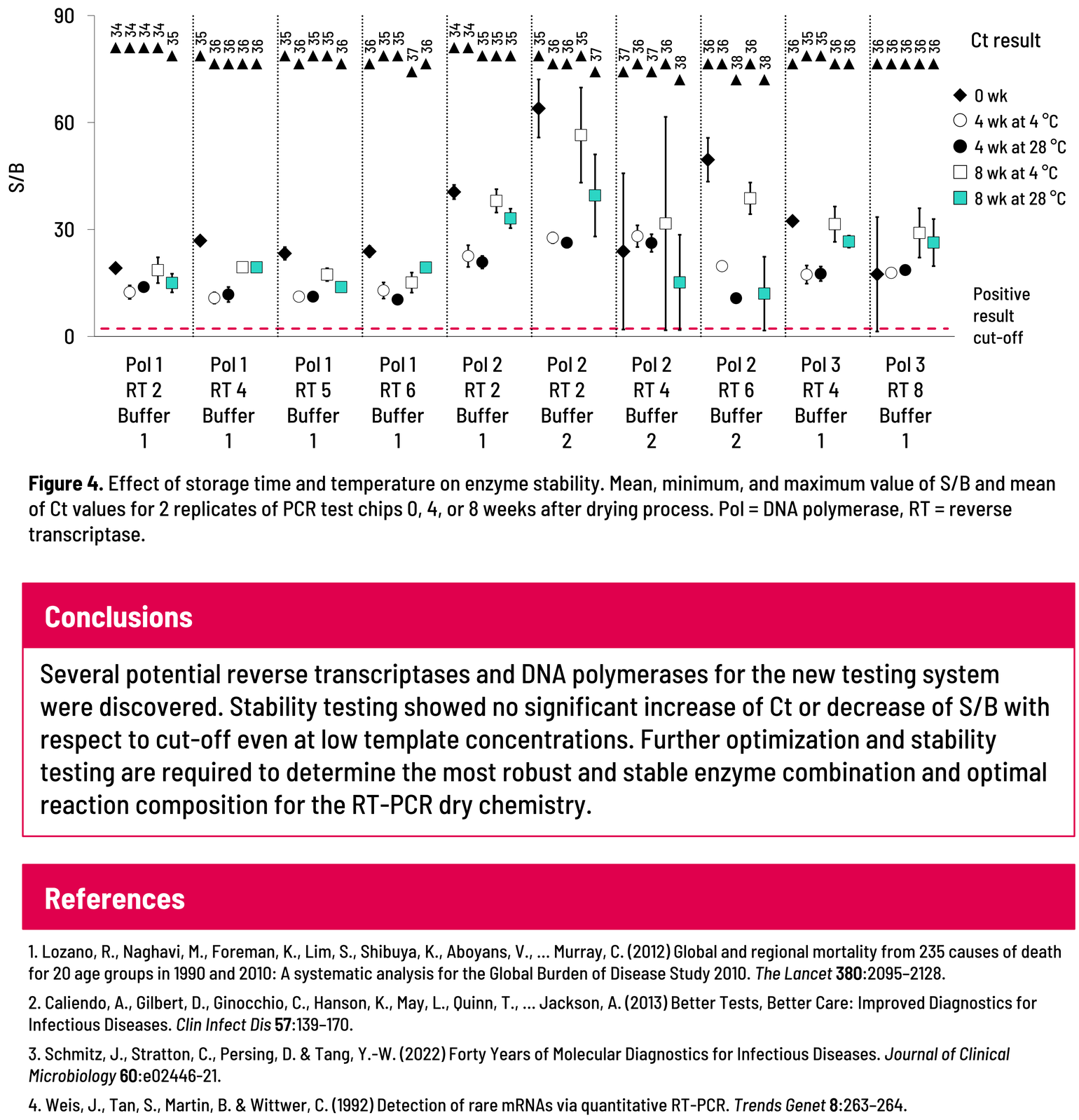

Figure 4. Effect of storage time and temperature on enzyme stability. Mean, minimum, and maximum value of S/B and mean of Ct values for 2 replicates of PCR test chips 0, 4, or 8 weeks after drying process. Pol = DNA polymerase, RT = reverse transcriptase.

Conclusions

Several potential reverse transcriptases and DNA polymerases for the new testing system were discovered. Stability testing showed no significant increase of Ct or decrease of S/B with respect to cut-off even at low template concentrations. Further optimization and stability testing are required to determine the most robust and stable enzyme combination and optimal reaction composition for the RT-PCR dry chemistry.

References

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