

Abstract

Lyophilized Matrix Containing Ready-to-Use Primers and Probe Solutions for Standardization of Real-Time PCR and RT-qPCR Diagnostics [†]

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Abstract: Real-time molecular techniques have become the reference methods for the direct diagnosis of pathogens. The reduction of steps is a key factor in order to decrease the risk of human errors resulting in invalid series and delayed results. We describe here a process involving the preparation of oligonucleotide primers and a hydrolysis probe in a single tube at predefined optimized concentrations that are stabilized via lyophilization (Lyoph-P&P). Lyoph-P&P was compared to the classic protocol using extemporaneously prepared liquid reagents, assaying (i) sensitivity, (ii) long-term stability at 4 °C, and (iii) long-term stability at 37 °C, mimicking transportation without a cold chain. Two previously published molecular assays were selected for this study. They target two emerging viruses that are listed on the blueprint of the WHO to be considered for preparedness and response actions: *chikungunya virus* (CHIKV) and *Rift Valley fever phlebovirus* (RVFV). The results of our study demonstrate that (i) Lyoph-P&P is stable for at least four days at 37 °C, supporting shipping without the need of a cold chain, (ii) Lyoph-P&P rehydrated solution is stable at 4 °C for at least two weeks, (iii) the sensitivity observed with Lyoph-P&P is at least equal to, and often better than, that observed with liquid formulation, and (iv) the validation of results observed with low-copy specimens is rendered easier by higher fluorescence levels. In conclusion, Lyoph-P&P holds several advantages over extemporaneously prepared liquid formulations and merits consideration as a novel real-time molecular assay for implementation into a laboratory with routine diagnostic activity. Since the meeting, this concept has been applied to the COVID-19 situation: two diagnostic assays (E gene and RdRp) have been developed and can be ordered on the European Virus Archive catalog (<https://www.european-virus-archive.com/detection-kit/lyophilized-primers-and-probe-rt-pcr-2019-ncov-e-gene>; <https://www.european-virus-archive.com/detection-kit/lyophilized-primers-and-probe-rt-pcr-sars-cov-2-rdrp-gene>).

Keywords: freeze-drying; lyophilization; PCR; diagnosis; virus; pathogen; TaqMan; emerging; epidemic



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