

Resource Summary Report

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SalivaDirect: RNA extraction-free SARS-CoV-2 diagnostics

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Protocol Information

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Group: Coronavirus Method Development Community, XPRIZE Rapid Covid Testing

Summary: SalivaDirect is an RNA-extraction free, dual-plexed RT-qPCR method for SARS-CoV-2 detection. It can be broadly implemented as it (1) does not require saliva collection tubes containing preservatives, (2) does not require specialized equipment for RNA extraction, and (3) is validated for use with products from multiple vendors. Thus, the simplicity and flexibility of SalivaDirect means that it is not as affected by supply chain bottlenecks as some other assays. Our method is RNA-extraction free which enables testing of low volume and minimally processed saliva in dual-plexed RT-qPCR for SARS-CoV-2 detection. Saliva will be treated with proteinase K followed by a heat inactivation step, and is then directly used as input in the dual-plexed RT-qPCR test. Our aim was not to design new primers and probes for RT-qPCR testing, but rather to use validated primer and probe sets (N1 and RP) developed by the US CDC. The human Ribonuclease P (RP) probe was modified with a different fluorophore so that the primer/probe set could be combined in a duplex assay, reducing the number of tests to 1 assay with 2 sets. Version 2 includes: Optimized thermocycler conditions Locally validated alternative options for Proteinase K, RT-qPCR master mix, and thermocyclers Use of 8-strip tubes for sample processing step, due to contamination issues in 96-well plates. Version 3 has been updated to remove steps for sample self-collection. Version 4 has updated Ct thresholds for the ABI 7500 Fast Dx.

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