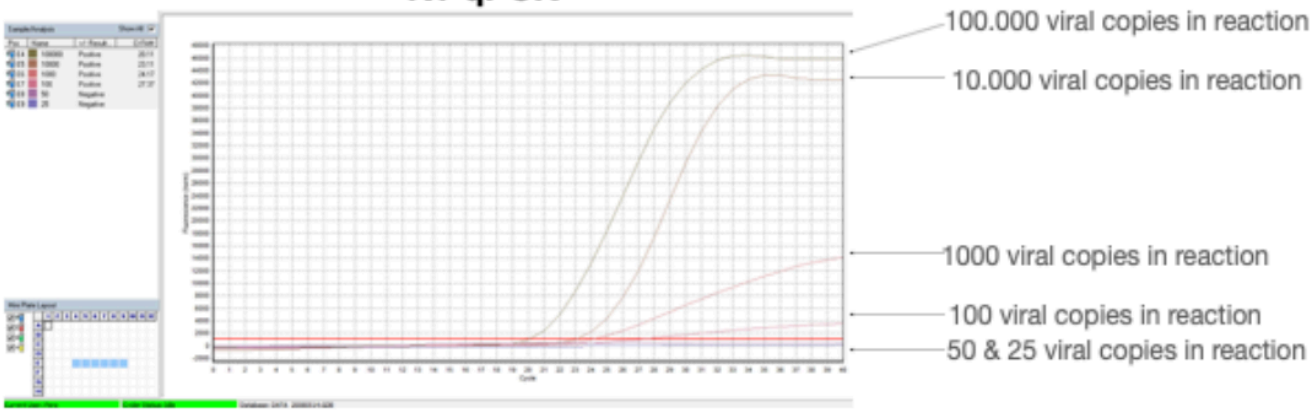
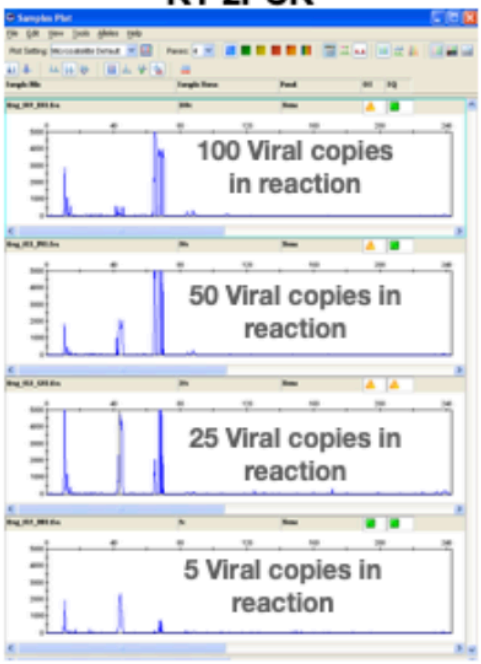
Detection of SARS-Cov-2 RNA: RT-zPCR vs. RT-qPCR Sensitivity Comparison

The RT-zPCR could reliably detect **<5** viral copies while the RT-qPCR, the current standard method for detection of SARS-CoV-2, needs >1000 viral copies for reliable detection.

RT-qPCR



RT-zPCR



Methods: The PCR primer sequences and probes were according to the CDC-recommended diagnostic assay for amplification of the N1 protein-encoding region of the SARS-CoV-2 virus. Both methods, the RT-zPCR and the RT-qPCR used the same reagents, the same PCR mix, and the same cycling protocol. They differed only in the number of cycles, 30 for the RT-zPCR and 40 for the real-time RT-qPCR, respectively. The control SARS-CoV-2 genomic RNA (IDT) allocated in the quantities of 200, 100, 50, 25, and 5 copies in the PCR reactions were transcribed and amplified by one-step RT-zPCR method, followed by the load-preparation step on a separate well-plate medium per RT-zPCR workflow, that included denaturation and separation with HiDi Formamide of the amplified fluorescent targets and mixing with the LIZ500 Size Standard (ThermoFisher) on a shaker at a medium speed (1500rpm) for 15 minutes. An AB 9700 PCR cycler (ThermoFisher) was used for the RT-zPCR amplification, while AB 3730xl Genotyper (Thermo Fisher) was used for detection. In addition, 100000, 10000, 1000, 100, 50, and 25 viral copies were amplified and detected by the RT-qPCR method per CDC protocol on the Eppendorf RT-PCR MasterCycler.

100x sensitivity of RT-zPCR over RT-qPCR method leads to early detection of viral infectious agents such as SARS-CoV-2