Pool testing from throat wash and nasopharyngeal swabs for SARS-CoV-2 RNA with high sensitivity

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BACKGROUND

High cost of nucleic acid testing (NAT), finite NAT testing capacities, finite capacities for taking swabs and insufficient sensitivity of point of care antigen testing generates a need for new approaches. Many existing protocols for pool testing reduce sensitivity and do not allow resolving of pools. We therefore developed a pool test with high sensitivity and still allows resolving of pools from different materials.

METHODS

Optimal pool size was calculated as 10 samples/pool. 200µl of each sample are pooled with addition of a synthetic RNA as processing control. High volume nucleic acid extraction and concentration is performed on a 24-well Kingfisher flex system. 25µl of eluate are pipetted to 25µl of PCR master mix containing primers and probes for E-Gene/RdRP-gene and internal control. A total of 920 samples plus controls can be tested in one PCR run. Resolving positive pools was performed with the residual original sample individually with the Abbott Alinity m SARS-CoV-2 assay.

RESULTS

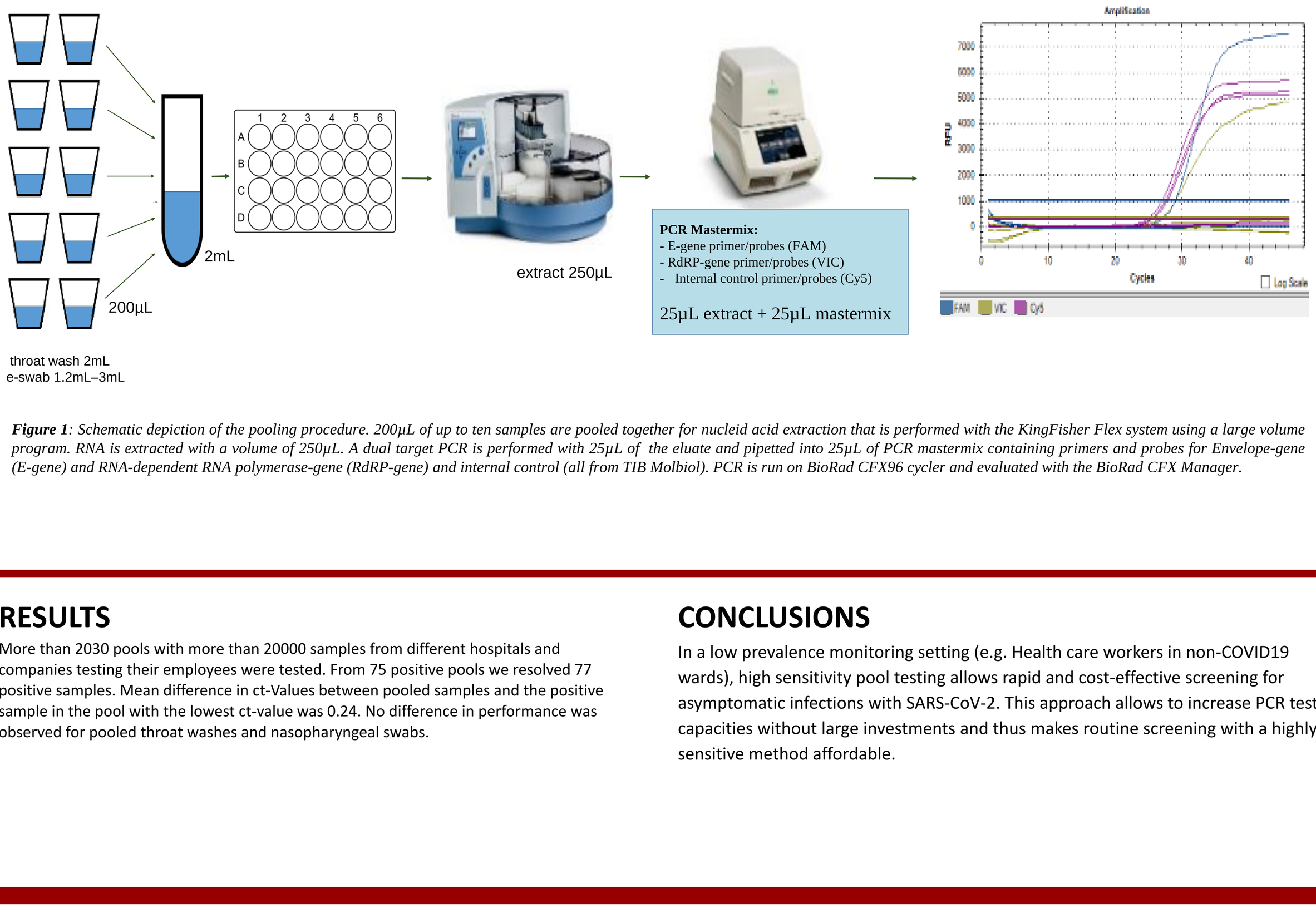

***Figure 1**: Schematic depiction of the pooling procedure. 200µL of up to ten samples are pooled together for nucleid acid extraction that is performed with the KingFisher Flex system using a large volume program. RNA is extracted with a volume of 250µL. A dual target PCR is performed with 25µL of the eluate and pipetted into 25µL of PCR mastermix containing primers and probes for Envelope-gene (E-gene) and RNA-dependent RNA polymerase-gene (RdRP-gene) and internal control (all from TIB Molbiol). PCR is run on BioRad CFX96 cycler and evaluated with the BioRad CFX Manager.*

RESULTS

More than 2030 pools with more than 20000 samples from different hospitals and companies testing their employees were tested. From 75 positive pools we resolved 77 positive samples. Mean difference in ct-Values between pooled samples and the positive sample in the pool with the lowest ct-value was 0.24. No difference in performance was observed for pooled throat washes and nasopharyngeal swabs.

CONCLUSIONS

In a low prevalence monitoring setting (e.g. Health care workers in non-COVID19 wards), high sensitivity pool testing allows rapid and cost-effective screening for asymptomatic infections with SARS-CoV-2. This approach allows to increase PCR testing capacities without large investments and thus makes routine screening with a highly sensitive method affordable.