Letter to the Editor

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Pooling for SARS-CoV-2-testing: comparison of three commercially available RT-qPCR kits in an experimental approach

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To the Editor,

The solution to the resource problem in series testing for SARS-CoV-2 could be pool testing. Pool testing is not a new invention, it has been known since the 1940s [1] and has become indispensable in some areas: Pool tests for blood-borne infectious agents (For example HIV and HCV) have been a recognized standard in the screening of blood donors for many years [2]. Pool testing is also common practice among epidemiologists and tropical medicine specialists to get an overview of the spread of vector-transmitted virus infections (e.g. dengue, yellow fever); for this purpose, mosquitoes are regularly collected and pooled for testing in the supposed distribution areas. However, due to the dilution effect, pooled biomarker tests may have a lower sensitivity than single-sample tests. This means that the diagnostic target (in this case the viral RNA of SARS-CoV-2) from an infected sample is diluted by target-free samples in the pool and may approach the detection limit for the test.

For the present study the effectivity of pool testing for SARS-CoV-2 in three commercially available PCR kits was investigated. We selected three oropharyngeal gurgel

specimens (which were tested positive earlier for SARS-CoV-2) for spiking experiments to determine the detection limits of the methods. The viral loads of each specimen as a gold standard were obtained from dilutions (undiluted/1:2/10/100/100/10.000) according to a modified published protocol [3]. Briefly, RNA was isolated on an EZ1 Advanced XL workstation using the Virus Mini Kit (Qiagen, Hilden, Germany). SARS-CoV-2 RNA was quantified by reverse-transcription quantitative real-time PCR (RT-qPCR) in the *E* gene and results were reported as genome copies per milliliter (c/mL). The lower limit of detection (LoD95) was 300 copies (c)/mL.

Specimen pools were prepared as follows: a pool always contained one PCR-positive sample (oropharyngeal gurgel specimen, 150 µL) with the defined dilution and the remaining volume of PCR-negative samples (tested negative by the Roche system) defined by the pool size (e.g. for a pool of 5, one positive sample [150 µL] was pooled with four negative, each of 150 µL). We isolated RNA from pooled specimens using extraction kits as recommended by the manufacturers (Roche: NucleoSpin RNA Virus (Macherey-Nagel, Dueren, Germany) or for Viasure the BEXS Ready Viral DNA/RNA kits (Inno-train Diagnostik, Kronberg, Germany)). Then we conducted one-step RT-qPCR with the LightCycler® Multiplex RNA Virus Master (target *E* gene) and with the Viasure SARS-CoV-2 Real time PCR Detection Kit (*N* gene/*ORF* gene) on Light Cycler 480 II Instrument (Roche Diagnostics, Mannheim, Germany; bestbion, Köln, Germany). The same pools were analyzed with a second qualitative test system: the Xpert Xpress™ SARS-CoV-2 assay (cartridge system including extraction step and amplification targeting the *E* and *N2* genes) on a GeneXpert instrument (Cepheid, Sunnyvale, CA, USA).

The protocol described above was reproduced with three different patient samples with high viral loads: the viral loads of the SARS-CoV-2 positive samples were between 4.4×10^7 c/mL (undiluted) and 4.4×10^3 (dilution 1:10.000). An effect of pooling was obvious: in one patient sample the lowest obtained viral load was 3.0×10^2 c/mL when the original sample with a virus load of 4.4×10^3 c/mL

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Figure 1: Virus loads (A) and corresponding CT values of single (pool size 0; red circle) vs. pooled samples (pool size 2 (open square)/5 (green triangle)/10 (open triangle) and 30 (black square)) of three commercially available tests for the *E* gene assay (B), *E* and *N2* gene assay (C) and *N* and *ORF* gene assay (D) (CT = cycle threshold) given from one patient sample.

In two further pooling approaches with different patient samples (data not shown) only one test kit (Roche) was able to detect specific RNA in all dilutions/pool sizes. Therefore, an area of “uncertain sensitivity” for low virus loads was indicated (blue square) for the test kit of Cepheid/Viasure.

was pooled with 29 negative samples (Figure 1A). Only one commercially available test system (Figure 1B) was able to detect specific RNA in all dilutions of all of the three patient samples in the pooling approach. With the other two test kits, reliable and reproducible detection was achieved when the initial sample concentration was at least 3×10^4 c/mL, indicating a higher limit of sensitivity; detection of RNA in all samples of the dilution approach was possible in only 1/3 of the samples with the test kit from Cepheid/Viasure (therefore an area of “uncertain sensitivity” was indicated in Figure 1C, D). The Δ CT value in SARS-CoV-2 RNA positive samples (CT difference between un-pooled and pooled sample) irrespective of the amplified gene region was up to of 2.60 in pools of 2 and 6.50 in pools of 30 across all different test kits.

There are publications on the effectiveness of pooling in SARS-CoV-2 diagnostics, comprising data from in-house and commercially available assays [4–7]. In the present study three common commercially available test systems were compared with regard to pooling sensitivity. The experimental approach (which comprises three different samples) shows that it is possible to identify specific SARS-CoV-2 RNA also with a low concentration (3×10^4 c/mL) after pooling with 29 negative samples in all test kits. However, samples with lower viral loads ($\leq 10^3$ c/mL or with a CT value > 35) could be reproducibly detected only with the test kit of Roche suggesting a lower sensitivity limit. This should be kept in mind particularly since pooling influences the test sensitivity: the present data clearly demonstrated higher Δ CT values for pools of 30 compared to pools of 2. In this view the present data are in line with others who presented data on pooling experiments where the CT value differs from pooled samples and original sample up to five [5], and pooling up to 50 samples was possible [8], albeit with a reduction in analytical sensitivity [9].

There are increasing problems with the reagent supply with regard to the PCR diagnostics of COVID-19. Pool testing could therefore be an option to analyze a larger number of samples in a reagent-saving manner: in this view pool testing is effective when the prevalence is below 15%: in a principle to proof publication, a saving of up to 80% of test kits is expected with a pool size of 11 and a prevalence rate of 1% [5, 10]. If the prevalence is too high, too many pools have to be retested and pool testing becomes ineffective.

The present concept has yet to prove itself in routine practice and for each PCR test, but we are confident that it can make an essential contribution to the increasing demand for high frequency testing in and outside of hospitals.

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