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Rapid detection of SARS-CoV-2 by pulse-controlled amplification (PCA)

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1. Introduction

The gold standard for diagnosis of acute COVID-19 is currently reverse transcription real-time PCR (RT-qPCR). The method is highly sensitive and can detect fewer than 10 copies of the virus genome (Corman et al., 2020). The workflow from sample to result takes about 4–6 hours and includes extraction of the viral RNA before setting up and running the RT-qPCR reaction. Including the time for sample transportation, in-house laboratory logistics, analysis and communication of test results, sample turnaround times easily add up to 24–48 hours and more. Thus, there is an urgent need to cut down the time of SARS-CoV-2 diagnostics.

PCA (pulse-controlled amplification) is a qPCR-related nucleic acid amplification technology that allows detection of a nucleic acid target within a few minutes (Müller et al., 2020). It differs from conventional qPCR in that the reaction is bound to a solid phase and is driven by the novel concept of local heating, meaning that only the immediate area around the solid phase is heated, as opposed to a global heating of the entire reaction volume. Thus, it resembles a microfluidic device, but without the requirement for microfluidic channels and mechanisms. PCA does not require RNA extraction from the sample material and the test is performed using a lightweight (< 1 kg) device (Pharos Micro, GNA Biosolutions, Martinsried, Germany) that can run off battery power if necessary. PCA is therefore ideally suited for a point of care setting or for use in a drive-through testing station, thereby obviating sample transport. The technology has already been used successfully for DNA targets. In a proof-of-concept study assays for the detection of MRSA and Yersinia pestis were developed (Müller et al., 2020). Both assays allowed detection of the target DNA in under 10 min. Furthermore, for Yersinia pestis a detection was demonstrated for crude sample material without nucleic acid extraction in a simulated bioterror scenario in the field, with the operators wearing full PPE and the Pharos Micro device running off battery power. With this in mind, we adapted PCA for RNA targets and developed a RT-PCA assay to detect SARS-CoV-2 directly from swab samples without prior RNA extraction. The aim was to establish a detection system for SARS-CoV-2 that is a) faster than RT-qPCR and b) does not rely on a laboratory environment and can therefore be used, e.g., at drive-through or mobile testing stations to provide fast test results and allow immediate action on positive test results.

2. Material and methods

2.1. Sample material

SARS-CoV-2 virus culture, strain IMB-1, was grown on Vero E6 cells. Cell culture supernatant containing viral particles was treated either with 4 volumes of AVL buffer (Qiagen, Hilden, Germany) and 1 vol of ethanol or heated to 80 °C for 10 min to inactivate the virus.

Clinical samples consisted of residual diagnostic sample material, i.e. swab samples in universal transport medium. The use of residual clinical specimens in this study was in accordance with the statement of the Central Ethics Committee of the German Medical Association on the further use of human body materials for the purposes of medical

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2.2. Pulse controlled amplification (PCA) - technology

PCA technology is based on thermocycling using local heating for the denaturation step. The amplification takes place on an array of 75 gold-coated tungsten wires with a diameter of 15 μm, which are immersed in the reaction solution and carry one of the primers. Short electric pulses of a few hundred microseconds heat the wires to roughly 95 °C and thereby denature double stranded DNA near the wires. During the brief heating period, the temperature field diffuses only a few microns away from the surface of the wires, thus effectively heating only a minute fraction (<1%) of the reaction volume. After the pulse, the heat dissipates nearly instantaneously into the bulk of the solution. This results in very short amplification cycles of 1.5–5 seconds, depending on the assay and target sequence. In the Pharos Micro prototype, wires are embedded in a disposable chip that has eight wells, i.e. eight reactions can be run simultaneously. Amplification of the target sequence is detected in real time via hydrolysis probes, similar to qPCR.

2.3. PCA assay for the detection of SARS-CoV-2

Primers and probe sequences used in this study are listed in Table 1. Pharoas reaction chips were functionalized with the thiolated reverse primer as described by Müller et al. (Müller et al., 2020). Sample material (i.e. swab samples in virus transport medium) was treated either with 4 volumes of AVL buffer (Qiagen, Germany) and 4 volumes of ethanol or simply heated to 80 °C in a small heating block for 10 min without adding any lysis buffer. 45 μL of treated sample material and 15 μL of hybridization buffer (GNA Biosolutions) were added to a well in the chip and incubated for 5 min at ambient temperature to allow hybridization of viral RNA to the reverse primer immobilized on the wires. The liquid was then removed and the well rinsed with 50 μL wash buffer (GNA Biosolutions). Optionally, this initial binding step can be repeated to improve sensitivity of the test. 50 μL of the RT-PCA mastermix (containing 7 mM MgCl₂, 100 nM E-Sarbeco-P1-Rc, 450 nM E-Sarbeco-F, 1x PCA mastermix/ 0.09 x RT mix/ 1x booster mix [GNA Biosolutions]) were added to each well. PCA settings for the Pharos Micro were 5 min reverse transcription at 55 °C, with a lid temperature of 63 °C and base temperature of 55 °C, followed by a 60 s thermalizing step (to equilibrate the reaction solution to the combined annealing and elongation temperature of 67 °C base and 74 °C lid). For denaturation, 250 μsec pulses were applied to the array of wires suspended in the solution, with the pulses being repeated every 3 s for 800 cycles. With these settings, the entire run is completed within 40 min. The majority of positive samples are detected within 15 min. We never observed any positive signals after 25 min. Therefore, while we used 800 cycles during development of the assay, in practice this can be shortened to 500 cycles, which would cut the runtime down to about 28 min.

### Table 1

| Sequence 5’-3’ | Reference |
|---------------|-----------|
| E_Sarbeco-F   | ACAGTTACGTAAATATGTAATAGGTT | [1] |
| E_Sarbeco-R   | Thiol - AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAATACGAGGAAAACACCA | [1] with thiol anchor and spacer |
| E_Sarbeco-P1-Rc | /iSp9/ATATTGGGAAATGTGGCATGAGTGTG | [1] |
| E_Sarbeco-P1-Rc | FAM-CCAGGCGACGTAGATGCTAGTTGAT | Reverse complement of [1] |

2.4. RT-qPCR

Viral RNA was extracted for RT-qPCR using the QiaAmp Viral RNA extraction kit (Qiagen, Hilden, Germany) following the manufacturer’s protocol.

Primers and probes as well as PCR parameters were used as described by Corman et al. (Corman et al., 2020) The reactions were carried out on a MIC (Magnetic Induction Cycler, BMS, Australia) using Qiagen One-Step RT-PCR reagents.

3. Results

We developed a PCA assay for SARS-CoV-2 targeting the E gene. The primer and probe sequences were modified from the assay published by Corman et al. (Corman et al., 2020) to adapt it to the Pharos system, adding a thiolated anchor sequence for the reverse primer to enable it to bind to the gold surface of the wires and using the reverse complement of the published probe sequence (Table 1). Using the normal probe sequence or attaching the anchor to the forward primer was not successful for the PCA assay.

The aim was to arrive at a workflow that allows fast, sensitive and specific detection of SARS-CoV-2 without the need for prior RNA extraction. This is especially important with regard to the global supply shortages in RNA extraction kits seen over the last few months. We used AVL buffer (Qiagen, Hilden Germany) to lyse the viral particles, but did not perform a subsequent complete column-based RNA purification. AVL contains guanidinium thiocyanate, which is a common component of lysis buffers in commercial RNA extraction kits and is known to denature proteins and lyse cells (Mason et al., 2003). However, it is also known to inhibit conventional PCR (Thompson et al., 2014). This problem can largely be overcome by the bind/wash preconditioning step used in PCA (see methods section). Guanidinium thiocyanate is classified as a hazardous substance with corresponding transport restrictions, which may prevent its use in a non-laboratory setting. Therefore, as an alternative, and to reduce the necessary pipetting steps and simplify the workflow, we also tested inactivation and lysis of the viral particles in the sample by heat treatment (80 °C for 10 min). The efficacy of both treatments to inactivate SARS-CoV-2 was confirmed by plaque assay. There was no significant difference in the results of the PCA reactions with either treatment (data no shown).

Using a dilution series of SARS-CoV-2 viral particles grown on Vero E6 cells and inactivated with AVL buffer and ethanol we successfully detected 10e⁶ copies/μl of viral target RNA in 5 min 32 s and 10e⁴ copies/μl in 17 min 39 s (Fig. 1A). Probit analysis showed the limit of detection (LOD) (Forootan et al., 2017) to be at 4.9 copies/μl (replicates of eight; 95 % confidence interval 3.8–8.2 copies/μl) (Fig. 2). A positive detection is defined as the measured fluorescence crossing the threshold fluorescence. The time it takes to reach this threshold (time to threshold - ttt value) is inversely proportional to the target copy number in the sample. Thus, the ttt value in PCA is analogous to the ct (threshold cycle) value given in qPCR. However, in contrast to qPCR, where a 10-fold dilution of target copy number is associated with an increase in ct value of 3.3, the ttt value in PCA does not follow such a strictly linear correlation with target copy number, particularly at low target concentrations (Fig. 1A). Therefore, PCA has to be regarded as a semi-quantitative, rather than quantitative technique.

We then tested 83 clinical samples that had been confirmed to be positive for SARS-CoV-2 using RT-qPCR targeting the E gene. A representative graph of the PCA results with these samples is shown in Fig. 1B. The concentration of target RNA in the 83 clinical samples ranged from 2.6 × 10⁷ to 7.4 × 10⁸ copies/μl (quantified according to (Wölfe et al., 2020)). This corresponds to RT-qPCR ct values between 17.95 and 32.53. Out of the 83 samples 74 (89.2 %) tested positive in PCA, with ttt values ranging from 5 min 28 s to 24 min 26 s (Fig. 3). 100 % (n = 32) of the samples with a RT-qPCR ct value < 25 (corresponding to 1.6 × 10³ copies/μl) tested positive with PCA.
To test diagnostic specificity, we used 40 clinical samples that had been confirmed to be negative for SARS-CoV-2 by RT-qPCR. All reactions were negative.

To test analytical specificity, we used a panel of 31 viral and bacterial pathogens that are relevant for differential diagnosis of respiratory infections (listed in Table 2). All reactions were negative.

4. Discussion & conclusion

Fast diagnostics of COVID-19 is crucial for management of the pandemic. Currently, RT-qPCR, which takes several hours to complete, is still the gold standard. In recent months, however, a number of faster alternatives based on different platforms and technologies have come onto the market that deliver results within an hour or less. On the one hand there are isothermal NAAT (nucleic acid amplification technology) methods like LAMP (loop-mediated amplification) (Dao Thi et al., 2020; Park et al., 2020; Yang et al., 2020; Zhang et al., 2020) or RPA (recombinase polymerase amplification) (Behrmann et al., 2020), some of them in combination with a CRISPR-cas-based detection (Broughton et al., 2020; Ding et al., 2020; Joung et al., 2020; Lucia et al., 2020). On the other hand, there are fully integrated cartridge systems like Cepheid’s GeneXpert (Cepheid, Sunnyvale, USA) and Abbott’s IDnow (Abbott, North Chicago, USA). Further to these nucleic acid based technologies, the first antigen tests in lateral flow format are beginning to emerge, e.g. Covid-19 Ag Respi Strip (Coris Bioconcept, Gembloux, Belgium) or SOFIA2 SARS Antigen FIA (Quidel, San Diego, USA).

Apart from speed, spreading SARS-CoV-2 diagnostics over several different technologies can also be advantageous for a very practical reason. During the peak of COVID-19 infections in March-May 2020, where diagnostics was based almost exclusively on RT-qPCR, there were global supply shortages in PCR reagents, RNA extraction kits and platform-specific plasticware. For management of the ongoing pandemic and potential future surges of infections, having alternatives to RT-qPCR can alleviate the supply problems.

The published isothermal NAAT assays for SARS-CoV-2 slightly lag behind the sensitivity of RT-qPCR, with LODs of 5–40 copies/μl (Behrmann et al., 2020; Broughton et al., 2020; Dao Thi et al., 2020; Ding et al., 2020; Joung et al., 2020; Lucia et al., 2020).
The figure includes 74 out of 83 tested samples. The remaining 9 samples were positive with RT-qPCR but negative with PCA.

Fig. 3. Comparison of the performance of PCA and standard RT-qPCR using clinical sample material (swab samples in universal transport medium). The figure includes 74 out of 83 tested samples. The remaining 9 samples were positive with RT-qPCR but negative with PCA.

Table 2

| Acinetobacter baumannii | Bacillus anthracis | Burkholderia cepacia | Burkholderia mallei | Burkholderia pseudomallei | Burkholderia thailandensis | Candida albicans | Clostridium freundii | Enterobacter aerogenes | Enterococcus faecalis | Escherichia coli | Francisella tularensis tularensis | Haemophilus influenzae | Klebsiella pneumoniae | Legionella pneumophila | Moraxella catarrhalis | Neisseria meningitidis | Propionibacterium acnes | Proteus mirabilis | Pseudomonas aeruginosa | Serratia marcescens | Staphylococcus aureus | Staphylococcus epidermidis | Stenotrophomonas maltophilia | Streptococcus pneumoniae | Streptococcus pyogenes | Strep. Influenza A virus | Strep. Influenza B virus |
|-------------------------|-----------------|---------------------|----------------------|--------------------------|--------------------------|-----------------|-------------------|---------------------|---------------------|-----------------|-------------------------|----------------------|----------------------|------------------|------------------------|----------------------|-------------------|---------------------|---------------------|---------------------|----------------------|------------------------|---------------------|---------------------|
| 1                      | 2               | 3                   | 4                    | 5                        | 6                         | 7                | 8                 | 9                    | 10                  | 11              | 12                         | 13                    | 14                    | 15                | 16                      | 17                    | 18                | 19                  | 20                  | 21                  | 22                    | 23                    | 24                | 25                  | 26                  | 27                  | 28                  | 29                  | 30                  | 31                  |

et al., 2020; Joung et al., 2020; Lucia et al., 2020; Park et al., 2020; Yang et al., 2020; Zhang et al., 2020) in contrast to 1–5 copies/reaction seen with some RT-qPCR assays (Corman et al., 2020). A detection directly from swab samples without prior RNA extraction has been described for some isothermal PCR assays (Dao Thi et al., 2020) although this leads to some loss of sensitivity. The PCA assay described in this study is similar to isothermal NAAT assays in terms of sensitivity, but is superior in terms of time to result (within 15–20 min as opposed to 30–60 min for isothermal NAAT).

Cartridge-based systems have the obvious appeal of ease-of-use, since a sample can be added directly to the cartridge without any prior treatment and then inserted into the analyzer. Abbott’s IDnow delivers a positive result in 5 min, a negative one in 13 min and claims a LOD of 125 copies/mL sample (although this sensitivity has recently been questioned (Basu et al., 2020; Smithgall et al., 2020)), while Cepheid’s Gene Xpert can detect 10 copies/mL in 45 min, which rivals the sensitivity of RT-qPCR on any open system. The disadvantage of cartridge systems is sample throughput. While the IDnow analyzer is certainly small enough for field or point-of-care use, it can only analyze one sample at a time. The Gene Xpert system is available with 1 up to 80 modules, but the multi-module devices are too bulky for field or POCT use. In comparison, the Pharos Micro PCA device can analyze up to eight samples simultaneously in a small, lightweight device. A further disadvantage of cartridge-based tests is that they are closed systems, i.e. the target, primer and probe sequences they use are not publicly available.

Lateral flow assays (LFAs) are a classic example of POCT tests. They deliver a result in 15–20 min and usually do not require any sample processing or instrumentation (although reader devices are available for some LFAs to provide semi-quantitative results) and require only minimal training to perform. As such, they are ideally suited for use in the field or at POCT. However, LFAs also have a notoriously bad analytical sensitivity and usually fall several orders of magnitude short of the LOD of PCR (Zasada et al., 2015). The COVID-19 Ag Respi-Strip (Coris Bioconcept, Gembloux, Belgium), a lateral flow assay based on detection with gold particles, has a diagnostic sensitivity of 60 % (76.7–85.7 % for samples with a RT-qPCR ct value <25). The Sofia2 SARS Antigen FIA (Quidel, San Diego, USA), a fluorescence immuno assay has a diagnostic sensitivity of 80 %, with a LOD of 8.5 × 10^2 viral particles/mL. In contrast to the Coris Bioconcept test, the Quidel test requires an analyzer to detect the fluorescence signal.

The herein described PCA test is similar to LFAs in terms of time to result and fieldability, but provides higher sensitivity than LFAs. Its sensitivity is comparable to that of isothermal NAAT techniques, but does not quite achieve RT-qPCR levels. It is, however, faster than isothermal NAAT and considerably faster than RT-qPCR. With regard to sensitivity, the LOD of 4.9 copies/μl (or 4.9 × 10^2 copies/mL) determined for PCA is well below the mean viral load/mL measured in 3303 patients across all age groups of 5.18–6.39 (platform LC480, Roche) and 4.90–6.12 (platform Cobas, Roche) (Jones et al., 2020). Viral load in swab samples in the first 5 days after onset of symptoms was found to range between 10^3 – 10^6 /swab (Wölfel et al., 2020). Based on these figures, the SARS-CoV-2 PCA assay is likely to be able to detect the vast majority of COVID-19 infections in clinical samples. In any case, it will be able to diagnose COVID-19 in the clinical most relevant highly contagious patients with high viral load.

While RT-qPCR and associated RNA extraction require a lab
environment, PCA can be operated outside stationary laboratory facilities, even in the field (Müller et al., 2020). We envisage PCA as a potential point-of-care test for healthcare professionals, presumably in combination with more complex and time-consuming confirmatory tests. SARS-CoV-2 PCA tests can be used for a fast analysis of individual samples at or near point-of-care facilities to allow identification and immediate action on smaller clusters of infection or, with multiple PCA devices running in parallel, at drive-through testing stations with an on-site capacity of up to 2000 tests per day per 10 devices. With this approach most contagious patients, which show highest viral loads before the onset of symptoms (He et al., 2020; Rothe et al., 2020) and in the early phase of the disease (Wölfel et al., 2020), as well as potential "silent carriers" could be rapidly identified and quarantined.

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Author contributions

KZ planned the study, analyzed the data and wrote the manuscript, MW carried out the experimental work, RE cultured SARS-CoV-2 and tested inactivation of RE-attenuated SARS-CoV-2. All authors read and approved the final manuscript.

Declaration of Competing Interest

The authors declare no conflict of interest.

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References

Basu, A., Zinger, T., Inglima, K., Woo, K., Atie, O., Yusaitis, L., See, B., Aggero-Rosenfeld, M.E., 2020. Performance of Abbott ID NOW COVID-19 rapid nucleic acid amplification test in nasopharyngeal swabs transported in viral media and dry nasal swabs, in a New York City academic institution (preprint). Microbiology. https://doi.org/10.1101/2020.05.11.089896.

Behrmann, O., Bachmann, I., Spiegel, M., Schramm, M., El Wahed, A.A., Dobler, G., Dame, G., Hufert, F.T., 2020. Rapid detection of SARS-CoV-2 by low volume single tube reverse transcription recombinase polymerase amplification using plexor®-based quantitative PCR (qPCR). Biomol. Detect. Quantif. 12, 1–6. https://doi.org/10.1016/j.bdq.2017.04.001.

He, X., Lau, E.H.Y., Wu, P., Deng, X., Wang, J., Hao, X., Lau, Y.C., Wong, J.Y., Guan, Y., Tan, X., Mo, X., Chen, Y., Lao, B., Chen, W., Hu, F., Zhang, Q., Zhong, M., Wu, Y., Zhao, L., Zhang, F., Cowling, B.J., Li, F., Leung, G.M., 2020. Temporal dynamics in viral shedding and transmissibility of COVID-19. Nat. Med. 26, 672–675. https://doi.org/10.1038/s41588-020-0869-9.

Jones, T.C., Mühlemann, B., Veith, T., Biele, G., Zuchowski, M., Hoffmann, J., Stein, A., Edelmann, A., Corman, V.M., Drosten, C., 2020. An analysis of SARS-CoV-2 viral load by patient age (preprint). Infectious Diseases (except HIV/AIDS). https://doi.org/10.1016/j.ijid.2020.08.12548.

Joung, J., Ladha, A., Saito, M., Segel, M., Bruneau, R., Huang, M.W., Kim, N.-G., Yu, X., Li, J., Walker, B.D., Greninger, A.L., Jerome, K.R., Gootenbeek, J.S., Abudayleh, O. O., Zhang, F., 2020. Point-of-care testing for COVID-19 using SHERLOCK diagnostics (preprint). Infectious Diseases (except HIV/AIDS). https://doi.org/10.1016/j.ijid.2020.04.009121.

Lucia, C., Federico, P.-B., Alegranda, G.C., 2020. An ultrasensitive, rapid, and portable coronavirus SARS-CoV-2 sequence detection method based on CRISPR-Cas12 (preprint). Mol. Biol. https://doi.org/10.1007/s00294-020-91127-X.

Mason, P.E., Neilson, G.W., Dempsey, C.E., Barnes, A.C., Craciunshank, J.M., 2003. The hydration structure of guanidinium and thiocyanate ionic implications for protein stability in aqueous solution. Proc. Natl. Acad. Sci. 100, 4557–4561. https://doi.org/10.1073/pnas.0735920100.

Müller, K., Dalen, S., Holowachuk, S.A., Stehr, J., Zwinglmairer, K., BaersSENS, F., Ullerich, L., Stoeker, K., 2020. Pulse Controlled Amplification - a new powerful tool for front-line diagnostics. medRxiv. https://doi.org/10.1101/2020.07.01.20144097.

Park, G.-S., Ku, K., Baek, S.-H., Kim, S.-J., Kim, S.I., Kim, B.-T., Maeng, J.-S., 2020. Development of reverse transcription loop-mediated isothermal amplification assays targeting severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). J. Mol. Diagn. 22, 729–735. https://doi.org/10.1016/j.jmoldx.2020.03.006.

Rothe, C., Schunk, M., Sohann, P., Bretzel, G., Frosch, G., Wallrauch, C., Zimmer, T., Thiel, V., Janke, C., Guggemos, W., Seifalmia, M., Drosten, C., Vollmar, P., Zwinglmairer, K., Zange, S., Wölfel, R., Hoelscher, M., 2020. Transmission of 2019-nCoV infection from an asymptomatic contact in Germany. N. Engl. J. Med. 382, 970–971. https://doi.org/10.1056/NEJMoa2001468.

Smithgall, M.C., Scherberkova, I., Whitter, S., Green, D.A., 2020. Comparison of Cepheid Xpert Xpress and Abbott ID Now to Roche cobas for the Rapid Detection of SARS-CoV-2. J. Clin. Virol. 128, 104428 https://doi.org/10.1016/j.jcv.2020.104428.

Thompson, R.E., Duncan, G., Mc Cord, B.R., 2014. An investigation of PCR inhibition using plexor®-based quantitative PCR and short tandem repeat amplification. J. Forensic Sci. 59, 1517–1529. https://doi.org/10.1111/1556-4609.12556.

Wölfel, R., Corman, V.M., Guggemos, W., Seifalmia, M., Zange, S., Müller, M.A., Niemeyer, D., Jones, T.C., Vollmar, P., Rothe, C., Hoelscher, M., Bleicker, T., Brünink, S., Schneider, J., Ehmanna, R., Zwinglmairer, K., Drosten, C., Wendtner, C., 2020. Virological assessment of hospitalized patients with COVID-19. Nature 581, 465–469. https://doi.org/10.1038/s41586-020-2196-x.

Yang, W., Wang, D., Wang, Q., Xu, M., Zhao, Q., Zhou, Y., Zhao, H., Wang, L., Xu, Y., Wang, J., Han, S., Wang, P., Pei, F., Wang, Y., 2020. Rapid detection of SARS-CoV-2 using reverse transcription RT-LAMP method (preprint). Infectious Diseases (except HIV/AIDS). https://doi.org/10.1101/2020.03.19.998724.

Zhang, Y., Oidaura, N., Xiong, J., Sun, I., Nyanabah, R.O., Wei, H., Tanner, N.A., 2020. Rapid molecular detection of SARS-CoV-2 (COVID-19) virus RNA using colorimetric LAMP (preprint). Infectious Diseases (except HIV/AIDS). https://doi.org/10.1101/2020.02.26.2002837.