Abstract

Background: Worldwide demand for SARS-CoV-2 RT-PCR testing is still high as testing remains central to follow the disease spread and vaccine efficiency. Group testing has been proposed as a solution to expand testing capabilities but sensitivity concerns may limit its impact on the management of the pandemic. Digital PCR (RT-dPCR) has been shown to be highly sensitive and could help by providing larger testing capabilities without compromising with sensitivity.

Methods: We implemented RT-dPCR based COVID-19 group testing on commercially available system and assay (naica® system from Stilla Technologies) and investigated the sensitivity of the method in real life conditions of a university hospital in Paris, France, in May 2020. We tested the protocol in a direct comparison with reference RT-PCR testing on 448 samples split into groups of 8, 16 and 32 samples for RT-dPCR analysis.

Results: Individual RT-PCR testing identified 25/448 positive samples. Using 56 groups of 8, RT-dPCR identified 23 groups as positive, corresponding to 26 true positive samples including 2 samples not detected by individual RT-PCR but confirmed positive by further investigation. 15 of 28 groups of 16 tested positive, corresponding to 25 true positive samples. 14 groups of 32 were fully concordant but this should be confirmed on larger datasets.

Conclusions: Our proposed approach of group testing by digital PCR has a similar to better diagnostic sensitivity compared to individual RT-PCR testing for group up to 16 samples. This approach reduces the quantity of reagent needed by up to 80% while reducing costs and increasing capabilities of testing by up to 10-fold.

Introduction

As contact tracing and a close epidemiological follow-up of COVID-19 remains the cornerstones of the disease control measures in most countries of the Northern hemisphere, many of them have implemented extensive monitoring policies to prevent and control the apparition of new clusters. These policies, requiring important testing capabilities, were exemplified in Wuhan where all 11 Million citizens were tested in 10 days during May 2020. The start of the vaccine campaigns will also need a close follow-up to check vaccination efficiency. Thus, scaling up and maintaining large testing capacities worldwide remains a challenge, with high cost, limited reagents and scarcity of testing equipment or laboratory staff likely to remain limitations.

Group testing or pooling, first suggested by Dorfman in 1943, is a protocol through which individual samples are combined together before running the test (1). The advantage of the method is an overall saving in the number of tests required to screen a given population (2), and thereby an increase in testing capabilities for fixed reagent and instrumentation availability. Savings depend on key parameters such as the disease prevalence and the group size. Group testing protocols using real-time reverse-transcriptase PCR (RT-PCR) have been evaluated and implemented for Covid-19 screening around the world in several experiments using RT-PCR detection techniques, notably in Israel, Germany, California, Nebraska, NY State, and Italy (3–9).

Although these studies show that positive individuals can be detected in pooled samples, it is often with a decreased sensitivity due to dilution and perhaps inhibition effects (3, 5, 7, 9). This can prevent weakly positive specimen from being detected in group samples (3, 8). Concerns about the sensitivity of group testing have been raised by French medical authorities, leading to a negative recommendation on their use in France (10). On the other hand, on the 18th of July 2020, the US FDA (Federal Drug Administration) authorized a first diagnostic test for use with pooled samples containing up to 4 individual swab specimens (11).

Digital PCR (or RT-dPCR) is known for its higher sensitivity over classical RT-PCR (12, 13), including for SARS-CoV-2 detection (15–17), and resistance to PCR inhibitors (14).

In this study we propose a novel group testing protocol using a commercially available SARS-CoV-2 RT-dPCR assay and compare empirically the positive and negative percentage agreement of individual RT-PCR with group testing by RT-dPCR for three group sizes of 8, 16 and 32 samples. We find that, in our condition, group testing by RT-dPCR has a better or similar sensitivity than the reference individual RT-PCR testing for groups of 8 and 16.

Material and Methods

Summary of the method of the comparative study

Overall, 448 patient samples were tested for SARS-CoV-2 by i) individual RT-PCR (gold standard method), ii) RT-dPCR in 56 groups of 8 samples, iii) RT-dPCR in 28 groups of 16 samples and iv) RT-dPCR in 14 groups of 32 samples. In case of discordance between the results of individual RT-PCR testing and group testing in RT-dPCR, samples were re-analyzed individually by RT-dPCR, the gold-standard RT-PCR and a confirmatory RT-PCR assay. The whole protocol is illustrated in Figure 1.

Specimens collection, storage and pooling

Nasopharyngeal swabs of 448 symptomatic patients screened for COVID-19 as hospitalized or seeking the Emergency Department of the Bichat university hospital (Paris, France) between May 6th and May 26th, 2020 were included. All samples were collected in universal transport medium (UTM) (Virocult®, Sigma-Aldrich, Saint-Louis, USA, or eSwab™, Copan, Brescia, Italy) and tested, within 15 hours maximum upon collection, for SARS-CoV-2 detection by RT-PCR (Cobas SARS-CoV-2 test, Roche, Risch-Rotkreuz, Switzerland). Remaining volumes were kept at +5°C and, if above 600 µL, systematically included in the group testing analysis in the same 24 hours. Thus, 125 µL of each included specimen

were randomly mixed with seven others to generate 56 groups of 8 specimens with a final volume of 1 mL per group. The remaining volume of transport medium was stored at +5°C. According to the current French ethical laws, samples used in the current study were only included after the completion of all analysis required for the patient's care.

Detection of SARS-CoV-2 by routine individual RT-PCR testing

All 448 specimens were analyzed individually on a Cobas® 6800 system (Roche, Switzerland) for Covid-19 screening using the Cobas® SARS-CoV-2 Test kit following manufacturer's instruction. Within 11 days maximum (and 20 days for "Sample_25659") upon storage at +5°C, some samples which had different results for RT-PCR and RT-dPCR were reassessed on the Cobas® 6800 system. In case of low remaining amounts of transport medium, the nasal swabs were vortexed once more into the remaining transport medium diluted 1 to 10 with new transport medium.

Extraction of total nucleic acids on grouped samples

All nucleic acids extractions for RT-dPCR assays were performed on a MagNA Pure LC 2.0 (Roche) using the MagNA Pure LC Total Nucleic Acid Isolation Kit (Roche) following manufacturer's instructions. For all sample groups, the total volume of 1 mL was used. For individual samples, 200 µL was diluted with 800 µL of buffer before extraction. Nucleic acids were eluted from 1mL to 50 µL of the elution buffer provided with the kit and stored at +5°C for a maximum of 12 hours before analysis.

Preparation of groups of 16 and 32 individuals

After extraction of the 56 groups of 8 specimen (P8 groups) and prior to viral testing by RT-dPCR, 28 groups of 16 individual samples (P16 groups) were obtained by mixing 15 µL of 2 P8 groups and 14 groups of 32 (P32 groups) were obtained by mixing 10 µL of 2 P16 groups.

Detection of SARS-CoV-2 by grouped RT-dPCR testing using the naica® system

SARS-CoV-2 titration of the grouped samples by RT-dPCR was performed on the naica® system (Stilla Technologies, France) within the next three hours after extraction, using the COVID-19 Multiplex Digital PCR Detection Kit (Stilla Technologies, France/Apexbio, China), allowing detection of the *N* gene, the *ORF1ab* gene and an internal control, as recommended by the manufacturer and described in S1-Supplementary Materials. The naica® system performs digital PCR by partitioning the samples into arrays of up to 30 000 micro-droplets called droplet crystals using a microfluidic Sapphire Chip and two dedicated instruments (Geode and Prism3). The readout has 3 fluorescence channels. The naica® system is for Research-Use Only.

Individual confirmatory testing for SARS-CoV-2 by RT-PCR and RT-dPCR

In case of discrepancies between individual RT-qPCR and grouped RT-dPCR, RT-dPCR results were confirmed by extracting and retesting individually each sample of the group by RT-dPCR and RT-qPCR as previously described and with a third method, the RealStar® SARS CoV-2 RT-PCR Kit (Altona Diagnostics, Germany) (18).

Limit of blank and limit of detection of RT-dPCR

The Limit of Blank for SARS-CoV-2 detection using the group testing approach and the Limit of Detection were evaluated. The methods and results are disclosed in the S2- and S3-Supplementary Materials.

Results

Cohort description from routine RT-PCR testing

Using routine RT-PCR testing, 25 samples were identified as positive out of the 448 samples tested, corresponding to an average test positivity rate of 5.5%. The average Ct value was of 30.0 and 27.3 for the *E* gene and *ORF* gene respectively, with minimum values of 16.5 and 16.3 and maximum values of 38.7 and >40 (not detected) (Figure 2).

Results from grouped RT-dPCR testing

All results for the detection of SARS-CoV-2 by RT-dPCR for grouped testing are presented in Table 1. Because testing was performed systematically as samples came in the laboratory, the groups contain variable numbers of RT-PCR positive samples ("RT-PCR+" samples): 35 with 0, 18 with 1 and 3 with more than 1 RT-PCR+ samples. For the largest group size of 32 samples, only 2 P32 groups had no RT-PCR+ samples.

Detailed results for RT-dPCR in groups of 8

The results, detailed in Tables 1 and 2, are in concordance with the reference individual RT-PCR testing for 52 groups (corresponding for 416 samples), including 32 RT-PCR negative groups and 20 containing at least one RT-PCR+ sample. For the remaining 4 groups, three RT-PCR negative groups tested positive by RT-dPCR ("PCR-/dPCR+" discordances – group IDs: P8_20, P8_28 and P8_39, cf Table 3 and supplementary material 4) and one RT-PCR+ positive group was found negative by RT-dPCR (PCR+/dPCR- discordance – group ID: P8_02). The Ct values for the sample associated with the PCR+/dPCR- discordance (Sample 25659) were 34 and 32.3 for the *E* gene and *ORF1ab* with the Cobas® SARS-CoV-2 assay, respectively. Of note, out of the 8 individual samples with Ct > 35 for the *E* gene, 6

ended up to be the only positive sample in a P8 group and all were detected positive by RT-dPCR. The highest detected Ct values for the *E* gene and *ORF1ab* were 38.7 and >40 (not detected), respectively.

Detailed results for RT-dPCR in groups of 16

The results, detailed in Tables 1 and 2, are in concordance with individual RT-PCR testing for 25 groups (corresponding for 400 samples), including 11 RT-PCR- and 14 RT-PCR+ groups. Among the three groups with discordant results, one presented a PCR-/dPCR+ discordance and 2 PCR+/dPCR- discordances. Of note, out of the 8 individual samples with Ct > 35 for the *E* gene, 5 ended up to be the only positive sample in a P16 group. Two of these groups are responsible for the 2 PCR+/dPCR- discordances. The *E* gene and *OFR1ab* Ct values for these 2 samples were of [36.7; >40 (not detected)] and [36.3; 34.2], while the highest Ct values for a detected single positive sample were [38.3; >40].

Detailed results for RT-dPCR in groups of 32

The results are in concordance with individual RT-PCR testing for all 14 groups (corresponding for 448 samples) and are depicted in Tables 1 and 2. Out of the 8 individual samples with Ct > 35 for the *E* gene, 3 ended up to be the only positive sample in a P32 group. All such 3 P32 groups tested positive by RT-dPCR. The highest corresponding detected Ct values for the *E* gene and *ORF1ab* is of [36.7; >40].

Investigation of the discordances and correlation between RT-dPCR measurements and Ct values

Investigations of the discordances are depicted in S4-Supplementary Materials. The correlation between RT-dPCR measurement and Ct values is presented in S5-Supplementary Materials.

Discussion

In this work, we assessed the positive and negative agreement with individual RT-PCR of group testing combined with digital PCR for SARS-CoV-2 detection. Three different group sizes were investigated using a commercially available digital PCR assay, the COVID-19 Multiplex Digital PCR Detection Kit (Stilla Technologies, France/Apexbio, China). This assay demonstrated a low LoB (at 2 and 0 positive droplets per PCR for *N* and *ORF1ab* genes, respectively) and LoD (at 77 copies/mL, corresponding to 8 copies/reaction, versus 170 copies/mL, corresponding to 34 copies/reaction, for the Altona RT-PCR assay used for direct comparison). This LoD is lower than most estimation for WHO and other reference RT-PCR assays typically ranging between 5 to 500 copies/PCR (19, 20) and between 625 to 1000 copies/mL in the same extraction conditions (18, 19).

For our analysis, we proposed a protocol of group screening performed by RT-dPCR with secondary individual re-testing of positive groups as illustrated in S6-Supplementary Materials. We assessed this protocol by testing in real-life condition 448 consecutive samples grouped by 8, 16 and 32 samples. We observed a better sensitivity than individual RT-PCR testing for groups of 8 samples, with 23 groups of 8 samples tested positive, and including 26 true positive samples, when only 25 samples were identified through individual RT-PCR testing, including one non-conclusive sample (not detected by the confirmatory RT-PCR assay) associated to a PCR+/dPCR- discrepancy. This corresponds to a +8% improvement in sensitivity, excluding the non-conclusive sample. Two among the three samples associated with PCR-/dPCR+ discordances were confirmed as true positive by the confirmatory RT-PCR assay (Altona). To note that, in case of insufficient remaining sample volume for discrepancy analysis, it had to be diluted 1 to 10 with new transport media. This could have an impact on sensitivity of our discrepancy analysis.

Grouped testing by RT-dPCR has a high positive agreement to individual RT-PCR testing for a group size of 16 samples. 15 groups of 16 samples tested positive by RT-dPCR and included a total of 24 true positive samples (22 RT-PCR+ and 2 PCR-/dPCR+ samples). Excluding the non-conclusive sample from

the 25 RT-PCR positive samples, this leads to an improvement of 4% in sensitivity by dPCR in group of 16 compared to individual RT-PCR. However, 2 RT-PCR+ groups tested negative with RT-dPCR, likely explained by high Ct values of the single positive sample included in each of these groups.

Testing in the 14 groups of 32 samples by RT-dPCR has 100% concordance with the reference RT-PCR testing. ~~However, we are careful in drawing conclusions for groups of 32 given the limited data points in this study (14 groups, including only 2 RT-PCR negative groups).~~ As only 14 groups, including only 2 RT-PCR negative groups, we are careful in drawing conclusion for groups of 32. These are still promising results, including 448 individuals, although additional testing would be desirable.

An alternative and even more cost-effective group testing protocol could be to perform the re-testing steps using RT-PCR with Cobas or Altona assays. In these protocols, the sensitivity becomes dependent on the RT-PCR kit used, leading to potential discrepancies with RT-dPCR as observed in our results for groups of 8 samples.

Overall, our data indicates that COVID-19 group testing combined with digital PCR for large group sizes of 8 and 16 samples has better or similar sensitivity than individual RT-PCR testing. The gain in sensitivity of the proposed method is likely due to a combination of *i)* a concentration effect due to performing the pooling prior extraction and performing the extraction step from a large volume of 1 mL of pooled transport medium and *ii)* the intrinsic superior sensitivity of digital PCR compared to RT-PCR, as demonstrated previously for SARS-CoV-2 (15–17) and other viruses (13, 21) detection.

Below standard sensitivity is one of the main reasons why group testing has not been widely adopted for COVID-19 testing, whilst research groups have advocated for its implementation as a solution to the world-wide demand for tests and reagent shortage (2-9). The current study suggests that high sensitivity can be achieved in group testing using digital PCR instead of RT-PCR in the first group screening step. Group testing by RT-PCR is known to enable large-scale, low cost patient screening with minimum reagent consumption (1-3). Digital PCR has higher costs (typical range of 30 € to 50 € per test, varies between test settings) than standard RT-PCR (typical range of 10 € to 20 € per test, varies

between test settings), but significant reagent and cost savings can also be achieved with group testing by digital PCR. Savings will depend on the positivity rate, the group size and the cost of dPCR testing. For test positivity rates below 1% and assuming digital PCR as 2 to 4 times more expensive than individual RT-PCR test, cost reductions of at least 40% and reagents savings of at least 70% are achievable. In similar conditions, but for a test positivity rate of 5%, cost savings will be between 16% and 40% depending on the actual cost of testing by digital PCR. A detailed analysis of reagent and cost savings is given in the S7-Supplementary Materials.

Consequently, group testing by digital PCR can indeed provide large-scale, low cost patient screening with minimum reagent consumption without sacrificing sensitivity. Limitations are 1) that test positivity rates are low, ideally below 1% for the large group sizes contemplated here, and 2) that it requires laboratories trained in molecular assays to implement the manual pooling protocols.

Group testing can be used in various context where testing is not widely available due to testing capacity, economics, or reagent access constraints and where SARS-CoV-2 prevalence is low. In countries where the pandemic is not yet under control or could re-emerge, enhancing testing capacity is essential to control COVID-19 expansion. Increasing the range of people tested amongst contacts with positive cases, but also periodic testing of population in frequent contact with others (e.g. nurses, transportation workers, clerks, etc…) as well as in fragile populations such as nursing homes, or vaccination follow-up, can be part of future strategies against COVID-19 while allowing a relaxation of social distancing measures at the same time. Group testing can help in all of these situations.

Legends of Tables and Figures

Figure 1 : *Schematic of the structure of the comparative study.*

Figure 2: *Distribution of Ct values for the E gene and ORF gene, as measured using individual reference RT-PCR with Cobas® 6800 SARS-CoV-2 assay, for the 25 positive samples.*

Table 1: *Distribution of the samples identified as positive by the routine RT-PCR method (Cobas® SARS-CoV-2 assay) in the groups of 8, 16 and 32 and corresponding RT-dPCR detection of SARS-CoV-2 in the groups.*

Table 2: *Confusion matrix for the groups of 8, 16 and 32 groups of samples.*

Table 3: *Detailed results of confirmatory testing for COBAS-/dPCR+ discordances by individual reassessment using both RT-dPCR and RT-PCR (Altona and Cobas®). NT = "Not tested". ND= "Not detected".*

References

1. Dorfman R. 1943. The Detection of Defective Members of Large Populations. The Annals of Mathematical Statistics 14:436–440.

2. Gollier C, Gossner O. 2020. Group Testing against COVID-19. 2020–04Working Papers. Center for Research in Economics and Statistics.

3. Yelin I, Aharony N, Shaer Tamar E, Argoetti A, Messer E, Berenbaum D, Shafran E, Kuzli A, Gandali N, Shkedi O, Hashimshony T, Mandel-Gutfreund Y, Halberthal M, Geffen Y, Szwarcwort-Cohen M, Kishony R. 2020. Evaluation of COVID-19 RT-qPCR test in multi-sample pools. Clinical Infectious Diseases ciaa531.

4. Schmidt M, Hoehl S, Berger A, Zeichhardt H, Hourfar K, Ciesek S, Seifried E. 2020. FACT- Frankfurt adjusted COVID-19 testing- a novel method enables high-throughput SARS-CoV-2 screening without loss of sensitivity. medRxiv 2020.04.28.20074187.

5. Anderson C, Castillo F, Koenig M, Managbanag J. 2020. Pooling nasopharyngeal swab specimens to increase testing capacity for SARS-CoV-2. bioRxiv 2020.05.22.110932.

6. Hogan CA, Sahoo MK, Pinsky BA. 2020. Sample Pooling as a Strategy to Detect Community Transmission of SARS-CoV-2. JAMA 323:1967.

7. Abdalhamid B, Bilder CR, McCutchen EL, Hinrichs SH, Koepsell SA, Iwen PC. 2020. Assessment of Specimen Pooling to Conserve SARS CoV-2 Testing Resources. American Journal of Clinical Pathology 153:715–718.

8. Griesemer SB, Van Slyke G, St. George K. 2020. Assessment of sample pooling for clinical SARS-CoV-2 testing. preprint, Microbiology.

9. Lohse S, Pfuhl T, Berkó-Göttel B, Rissland J, Geißler T, Gärtner B, Becker SL, Schneitler S, Smola S. 2020. Pooling of samples for testing for SARS-CoV-2 in asymptomatic people. The Lancet Infectious Diseases S1473309920303625.

10. HCSP. 2020. Coronavirus SARS-CoV-2 : poolage des tests RT-PCRRapport de l'HCSP. Haut Conseil de la Santé Publique, Paris.

11. 2020. Coronavirus (COVID-19) Update: FDA Issues First Emergency Authorization for Sample Pooling in Diagnostic Testing. FDA. FDA.

12. Strain MC, Lada SM, Luong T, Rought SE, Gianella S, Terry VH, Spina CA, Woelk CH, Richman DD. 2013. Highly Precise Measurement of HIV DNA by Droplet Digital PCR. PLoS ONE 8:e55943.

13. Huang J-T, Liu Y-J, Wang J, Xu Z-G, Yang Y, Shen F, Liu X, Zhou X, Liu S-M. 2015. Next Generation Digital PCR Measurement of Hepatitis B Virus Copy Number in Formalin-Fixed Paraffin-Embedded Hepatocellular Carcinoma Tissue. Clinical Chemistry 61:290–296.

14. Dingle TC, Sedlak RH, Cook L, Jerome KR. 2013. Tolerance of Droplet-Digital PCR vs Real-Time Quantitative PCR to Inhibitory Substances. Clinical Chemistry 59:1670–1672.

15. Yu F, Yan L, Wang N, Yang S, Wang L, Tang Y, Gao G, Wang S, Ma C, Xie R, Wang F, Tan C, Zhu L, Guo Y, Zhang F. 2020. Quantitative Detection and Viral Load Analysis of SARS-CoV-2 in Infected Patients. Clinical Infectious Diseases ciaa345.

16. Dong L, Zhou J, Niu C, Wang Q, Pan Y, Sheng S, Wang X, Zhang Y, Yang J, Liu M, Zhao Y, Zhang X, Zhu T, Peng T, Xie J, Gao Y, Wang D, Zhao Y, Dai X, Fang X. 2020. Highly accurate and sensitive diagnostic detection of SARS-CoV-2 by digital PCR. preprint, Public and Global Health.

17. Suo T, Liu X, Feng J, Guo M, Hu W, Guo D, Ullah H, Yang Y, Zhang Q, Wang X, Sajid M, Huang Z, Deng L, Chen T, Liu F, Xu K, Liu Y, Zhang Q, Liu Y, Xiong Y, Chen G, Lan K, Chen Y. 2020. ddPCR: a more accurate tool for SARS-CoV-2 detection in low viral load specimens. Emerging Microbes & Infections 1–30.

18. Visseaux B, Le Hingrat Q, Collin G, Ferré V, Storto A, Ichou H, Bouzid D, Poey N, de Montmollin E, Descamps D, Houhou-Fidouh N. 2020. Evaluation of the RealStar® SARS-CoV-2 RT-PCR kit RUO performances and limit of detection. J Clin Virol 129:104520.

19. Visseaux B, Le Hingrat Q, Collin G, Bouzid D, Lebourgeois S, Le Pluart D, Deconinck L, Lescure F-X, Lucet J-C, Bouadma L, Timsit J-F, Descamps D, Yazdanpanah Y, Casalino E, Houhou-Fidouh N, Emergency Department Influenza Study Group. 2020. Evaluation of the QIAstat-Dx Respiratory SARS-CoV-2 Panel, the First Rapid Multiplex PCR Commercial Assay for SARS-CoV-2 Detection. J Clin Microbiol 58.

20. Vogels CBF, Brito AF, Wyllie AL, Fauver JR, Ott IM, Kalinich CC, Petrone ME, Casanovas-Massana A, Muenker MC, Moore AJ, Klein J, Lu P, Lu-Culligan A, Jiang X, Kim DJ, Kudo E, Mao T, Moriyama M, Oh JE, Park A, Silva J, Song E, Takehashi T, Taura M, Tokuyama M, Venkataraman A, Weizman

O-E, Wong P, Yang Y, Cheemarla NR, White E, Lapidus S, Earnest R, Geng B, Vijayakumar P, Odio C, Fournier J, Bermejo S, Farhadian S, Dela Cruz C, Iwasaki A, Ko AI, Landry M-L, Foxman EF, Grubaugh ND. 2020. Analytical sensitivity and efficiency comparisons of SARS-COV-2 qRT-PCR assays. preprint, Infectious Diseases (except HIV/AIDS).

21. Gupta RK, Abdul-Jawad S, McCoy LE, Mok HP, Peppa D, Salgado M, Martinez-Picado J, Nijhuis M, Wensing AMJ, Lee H, Grant P, Nastouli E, Lambert J, Pace M, Salasc F, Monit C, Innes AJ, Muir L, Waters L, Frater J, Lever AML, Edwards SG, Gabriel IH, Olavarria E. 2019. HIV-1 remission following CCR5Δ32/Δ32 haematopoietic stem-cell transplantation. Nature 568:244–248.

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