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SHORT COMMUNICATION

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Positive SARS-CoV-2 RT-qPCR of a nasal swab spot after 30 days of conservation on filter paper at room temperature

Guillaume André Durand[1,2] | Abdennour Amroun[2] | Gilda Grard[1,2] | Cyril Badaut[1,2]

[1]French Armed Forces Biomedical Research Institute, National Reference Laboratory for Arboviruses, Marseille, France

[2]Unité des Virus Émergents (UVE: Aix-Marseille Univ-IRD 190-Inserm 1207), Marseille, France

Correspondence
Guillaume André Durand, CNR Arbovirus, IRBA, Laboratoire 114, Institut Hospitalo-Universitaire Méditerranée Infection, 19-21 boulevard Jean Moulin, 13005 Marseille, France.
Email: gdurand@protonmail.com

Abstract

We tested the use of nasal swabs spotted onto filter paper (Whatman 3M) for the molecular diagnosis of SARS-CoV-2 infection. Spots of a positive nasal swab in conservation medium (B.1.177 strain, 21Ct) were still positive (duo E-gene/IP4) after 10, 20, and 30 days of conservation at room temperature, with Ct values of 28, 27, and 26, respectively. Direct spotting of the swab at bedside (omicron strain) still gave a positive result after 10 days in two RT-qPCR systems: 33.7 Ct using duo E-gene/IP4, and 34.8 using a specific Omicron system. Spotting of a dilution range of media spiked with the Delta (strain 2021/FR/0610, lineage B 1.617.2) and Omicron strains (strain UVE/SARS-CoV-2/2021/FR/1514) showed a threshold of 0.04 $TCID_{50}$ after 10 days of conservation. We show, for the first time, that this simple and low-cost conservation method can be used to store samples for RT-qPCR against SARS-CoV-2 for up to at least 1 month.

KEYWORDS

cellular effect, disease control, research and analysis methods, RNA extraction, RNA stability, SARS coronavirus, virus classification

1 | INTRODUCTION

As COVID-19 has spread around the world, concerns have arisen about its diagnosis in countries with weak healthcare networks.[1] Diagnosis currently relies on RT-qPCR amplification from nasal swabs. According to the World Health Organization (WHO), swabs should be quickly delivered to the laboratory and shipping and storage should be performed at 2°C–8°C or the sample frozen to −20°C or −70°C in case of a delay.[2] These recommendations require cost-effective and temperature-controlled transport, which is particularly difficult to implement in developing countries, which contributes to under-diagnosis of the true incidence of the disease.[3] The WHO has estimated that six of seven COVID-19 infections go undetected in Africa.[4] In our laboratory, we routinely use blood spots dried onto filter paper for enzyme-linked immunoassay and RT-qPCR of arboviruses. This low-cost approach allows for rapid sampling, easy transport (room temperature, letter format), and easy laboratory processing[5] and is convenient for diagnosis and epidemiological research in southern countries. As arboviruses are also RNA viruses, we postulated that it would be possible to amplify the SARS-CoV-2 genome from dried spots of nasal samples on filter paper. Here, we tested, for the first time, RT-qPCR directly from dried nasal swabs for the diagnosis of SARS-CoV-2 infection.

2 | MATERIALS AND METHODS

Three experiments were conducted. First, we tested an already known positive sample, stored for 11 months at −80°C in homemade sample buffer (0.2% bovine serum albumin, 30 mM HEPES, 100 µg/100 µl Pen/Strep, phosphate-buffered saline, pH 7.2). This nasal sample comes from Marseille, France, and was collected in December 2020. All diagnostic investigations were already performed, and written consent of the patient was obtained. The strain was B.1.177 (ON203054). Forty microlitres of the sample was diluted 1:10 and three 100-µl spots applied to filter paper (Whatman 3M; Scheilcher & Schuell). The filter paper was kept for 10 min at room temperature (20°C) inside a laminar flow hood to avoid contamination and then

packaged in a plastic bag and stored for 1 month at room temperature (20°C). After 10, 20, and 30 days, RNA was manually extracted using a QIAamp Viral RNA Minikit, according to the manufacturer's instructions (QIAGEN AG). The envelop and IP4 genes of SARS-CoV-2 were amplified on a CFX thermocycler (Bio-Rad), as previously described.[6]

The second experiment consisted of an analytical test. Two strains were used: Delta (strain 2021/FR/0610, lineage B 1.617.2) and Omicron (strain UVE/SARS-CoV-2/2021/FR/1514). Both came from the European Viral Archive (https://www.european-virus-archive.com). Each strain was spiked into Dulbecco's modified Eagle medium without fetal bovine serum and a 1:10 serial dilutions performed to obtain a final amount of virus near the LOD of our RT-qPCR. Then, 100µl of each dilution was spotted onto filter paper and allowed to dry at room temperature for 10 min. A T0 was performed using 160 µl of each dilution. After storage for 10 or 30 days at room temperature inside a plastic bag, RNA was extracted from the paper as described above. The entire experiment was performed in a NSB3 biosafety laboratory.

Finally, the third experiment consisted of direct spotting of a nasal swab at the bedside from a positive volunteer. This patient was previously diagnosed with Omicron SARS-CoV-2 infection and had only few symptoms. Written consent for this experiment was obtained. The sample was collected 5 days after symptoms onset. The nasal swab was directly spotted onto the filter paper, then homogenized into homemade buffer and spotted once again. The rest of the conservation buffer (160 µl) was used for RNA extraction as the day 0 control (T0). Filter papers were maintained for 10 days at room temperature before RNA was extracted for RT-PCR, as previously described. In addition to the duo RT-qPCR system E-gene/IP4, we tested a specific Omicron RT-qPCR system.[7]

3 | RESULTS

The first experiment consisted of RT-qPCR on samples from paper filters from a positive nasal sample after 10, 20, or 30 days of conservation at room temperature, compared to the extemporaneous positive test (T0), with a Ct value of 21. All spots were positive, with Ct values of 28, 27, and 26 from days 10, 20, and 30, respectively (Figure S1). The second experiment consisted of an analytical evaluation using the Omicron and Delta strains. The results are summarized in Table 1. RNA was detected for both strains after 10 and 30 days. The Omicron strain was still detected for an inoculum of 0.04 $TCID_{50}$, whereas the Delta strain was detected until 0.4 $TCID_{50}$. We found a median Ct gap of 3.7 for the Delta strain and 3.5 for the Omicron strain relative to the T0. Between T10 and T30, the Ct gap was 2.5 and 1.3, respectively.

Finally, the last experiment consisted of the direct spotting of a positive swab onto the filter, at the bedside, before conservation at room temperature. The extemporaneous sample was positive with both RT-qPCR systems: 33.3 Ct (E-gene/IP4) and 35.3 Ct (Omicron). After 10 days, the filter paper was RT-PCR positive with 33.7 Ct (E-gene/IP4) and 34.8 Ct (Omicron).

4 | DISCUSSION

Here, we demonstrate that it is possible to amplify the SARS-CoV-2 genome from a dried spot of a nasal swab on filter paper, even after 30 days of conservation at room temperature. The direct spotting of a nasal swab at the bedside was as sensitive as direct RT-qPCR on diluted buffer media, despite 10 days of conservation. The dried spot method allow amplification of 0.04 $TCID_{50}$ viral particles after 30 days of conservation. The dried spot appears to not be infectious, given the failure of isolation of the virus after 20 days in cell culture (Vero cells ATCC CCL81). We did not evaluate the effect of various temperatures, notably high temperatures, such as those observed in Africa. These issues all need to be properly evaluated in a further study to determine the limits of this technique in detail. However, we have performed the proof of concept and felt it important to report these results to the community. This technique could be helpful for diagnosis and in the epidemiological and research fields. For example, this approach could be useful for the sampling and transport for COVID-19 diagnosis in rural countries, providing better epidemiological follow-up of the epidemic. This could help governments in the management of the crisis. The dried spot technique could also theoretically be used for environmental or wild-animal sampling for SARS-CoV-2 reservoir research, given the ability to sequence from a dried spot. In conclusion, we believe that dried nasal swab samples could help in the management of the pandemic at many levels, notably in southern countries, given the low cost and ease of use of dried spots on filter paper.

TABLE 1 RT-qPCR amplification of SARS-CoV-2 genomes from spots dried onto filter paper after 10 and 30 days of conservation at room temperature

	Delta strain					Omicron strain				
$TCID_{50}$	4	0.4	0.04	0.004	0.0004	4	0.4	0.04	0.004	0.0004
Dilution	−1	−2	−3	−4	−5	−1	−2	−3	−4	−5
T+0	27.5	32	40	38	–	25	28.5	31	35	39
T+10	32	35	–	–	–	28	31	36	–	–
T+30	35	37	–	–	–	30	33	36	–	–

Note: Negative results (Ct > 40) are represented by –.

AUTHOR CONTRIBUTIONS

Guillaume André Durand: coordinated the project and performed the experiments. **Abdennour Amroun**: involved in the preparation and titration of virus strains. **Gilda Grard and Cyril Badaut**: participated in the writing of the article.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Not applicable.

ETHICS STATEMENT

Not applicable.

ORCID

Guillaume André Durand http://orcid.org/0000-0003-3348-9618

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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