PLOS ONE

RESEARCH ARTICLE

Self-collection and pooling of samples as resources-saving strategies for RT-PCR-based SARS-CoV-2 surveillance, the example of travelers in French Polynesia

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Citation: Aubry M, Teiti I, Teissier A, Richard V, Mariteragi-Helle T, Chung K, et al. (2021) Self-collection and pooling of samples as resources-saving strategies for RT-PCR-based SARS-CoV-2 surveillance, the example of travelers in French Polynesia. PLoS ONE 16(9): e0256877. https://doi.org/10.1371/journal.pone.0256877

Editor: Etsuro Ito, Waseda University: Waseda Daigaku, JAPAN

Received: June 17, 2021

Accepted: August 17, 2021

Published: September 2, 2021

Data Availability Statement: All relevant data are within the manuscript and its Supporting information files.

Funding: The COV-CHECK PORINETIA surveillance strategy has been funded by the Government of French Polynesia. The Laboratory for Urgent Response to Biological Threats at the Institut Pasteur of Paris provided primers for the sequencing of the SARS-CoV-2 genome. The funders had no role in study design, data collection

Abstract

In French Polynesia, the first case of SARS-CoV-2 infection was detected on March 10th, 2020, in a resident returning from France. Between March 28th and July 14th, international air traffic was interrupted and local transmission of SARS-CoV-2 was brought under control, with only 62 cases recorded. The main challenge for reopening the air border without requiring travelers to quarantine on arrival was to limit the risk of re-introducing SARS-CoV-2. Specific measures were implemented, including the obligation for all travelers to have a negative RT-PCR test for SARS-CoV-2 carried out within 3 days before departure, and to perform another RT-PCR testing 4 days after arrival. Because of limitation in available medical staff, travelers were provided a kit allowing self-collection of oral and nasal swabs. In addition to increase our testing capacity, self-collected samples from up to 10 travelers were pooled before RNA extraction and RT-PCR testing. When a pool tested positive, RNA extraction and RT-PCR were performed on each individual sample. We report here the results of COVID-19 surveillance (COV-CHECK PORINETIA) conducted between July 15th, 2020, and February 15th, 2021, in travelers using self-collection and pooling approaches. We tested 5,982 pools comprising 59,490 individual samples, and detected 273 (0.46%) travelers positive for SARS-CoV-2. A mean difference of 1.17 Ct (CI 95% 0.93–1.41) was found between positive individual samples and pools (N = 50), probably related to the volume of samples used for RNA extraction (200 μL versus 50 μL, respectively). Retrospective testing of positive samples self-collected from October 20th, 2020, using variants-specific amplification kit and spike gene sequencing, found at least 6 residents infected by the Alpha variant. Self-collection and pooling approaches allowed large-scale screening for SARS-CoV-2 using less human, material and financial resources. Moreover, this strategy allowed detecting the introduction of SARS-CoV-2 variants of concern in French Polynesia.

and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

Introduction

Cases of respiratory infection (coronavirus disease 2019, COVID-19) caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) were first reported in December 2019 in Wuhan City, Hubei Province, China [1]. Because of the disease global expansion, COVID-19 was declared by the World Health Organization as a public health emergency of international concern on January 30th, 2020 [2], and was then characterized as a pandemic on March 11th [3].

French Polynesia is a French overseas collectivity in the South Pacific with ca 190,000 inhabitants living on the island of Tahiti, and 90,000 inhabitants distributed on 73 other islands scattered on a surface area as wide as Europe [4]. The first case of SARS-CoV-2 infection was detected on March 10th, 2020, in a resident of Tahiti returning from France [5]. To stop viral transmission, the population of French Polynesia was confined and international air traffic was interrupted on March 20th and 28th, respectively. Only residents or foreigners showing a compelling reason were allowed to enter French Polynesia provided they were quarantined on arrival. Confinement was eased from April 20th in most islands as no active circulation of SARS-CoV-2 had been detected, then was fully leveraged on May 21st. Between March 10th and June 25th, among 5,390 patients tested for a suspicion of SARS-CoV-2 infection, a total of 62 were found positive, including 32 imported cases, and no COVID-19 related death was recorded [6].

Tourism is an important financial resource for French Polynesia [7]. Consequently it was urgent to reopen the international air traffic, but without imposing a quarantine on arrival so as not to deter tourists from traveling. At the same time, a strategy needed to be found to protect the population from a new introduction of the virus, especially in remote touristic islands where health facilities are limited. Screening for SARS-CoV-2 all travelers arriving in French Polynesia was impossible because of the lack of medical staff to collect naso-pharyngeal swabs, and of laboratory staff trained in molecular biology to analyze the collected samples. Moreover, the number of available test kits for RNA extraction and RT-PCR assays was limited due to the global shortage.

A prospective study conducted in Australia on 236 ambulatory patients showed that the performance of self-collected throat and nasal swabs was at least equivalent to that of throat and nasal/naso-pharyngeal swabs collected by health workers for the detection of SARS-CoV-2 [8]. Thus, self-collection would offer a reliable alternative to health worker collected samples, and would reduce the need for trained medical staff. Moreover, several studies demonstrated that pooling of samples prior to RNA extraction and RT-PCR testing was reliable compared to the analysis of individual samples for SARS-CoV-2 detection [9–13]. This method proved efficient to increase testing capacity by saving reagents and laboratory-staff worktime, thus allowing large-scale screening of asymptomatic populations.

We describe here the use of self-collected and pooled samples as resources-saving strategies for RT-PCR-based surveillance of SARS-CoV-2 in travelers entering French Polynesia. From July 15th, 2020, to February 9th, 2021, all travelers had to comply with a testing protocol (COV-CHECK PORINETIA) which combined 2 consecutive RT-PCR tests for the detection of SARS-CoV-2 [14]. The first one was performed within 3 days before departure from an individual oro- and/or naso-pharyngeal sample collected by a health worker. A negative RT-PCR result was required to allow departure. The second RT-PCR test was performed 4 days after arrival from a pool including up to 10 oral and nasal samples self-collected by the travelers. In case of positive RT-PCR, all samples included in the pool were re-tested individually. Moreover, from February 2021, due to the worldwide emergence of SARS-CoV-2 variants of public health concern [15], self-collected samples found positive by SARS-CoV-2 RT-PCR

were re-tested using variant-specific amplification kit. Both retrospective and prospective investigations conducted from samples self-collected by travelers over the past four months showed the introduction of the Alpha variant in French Polynesia.

Methodology

Procedure before departure

Within 3 days before departure, each traveler (including minors) had to register online on the « Electronic Travel Information System » platform (ETIS, https://www.etis.pf/), implemented by the government of French Polynesia since July 11th, 2020. The ETIS form contained information about the status of the traveler (resident or non-resident of French Polynesia), and personal information including name, gender, date of birth, passport number, mobile phone number (local or international), e-mail address, geographical address in French Polynesia for residents, or country of residence and contact-person in case of emergency for non-residents. The traveler had to declare to have tested negative for SARS-CoV-2 by RT-PCR within 3 days prior to departure, certify to present no symptoms of COVID-19 at boarding, agree to comply with all sanitary rules required by the government of French Polynesia (including wearing a mask in public areas, avoiding close contact with people as much as possible, reporting any symptom suggestive of COVID-19), certify to have a travel insurance for non-French citizens and agree to assume all health costs incurred in French Polynesia, and accept to perform a self-test for SARS-CoV-2 detection using a kit provided upon arrival. Finally, non-resident travelers had to accurately describe their itinerary by indicating arrival and departure dates and flight numbers, and each visited island with the dates of stay as well as the name and contact of the accommodation(s). Data collected on the ETIS platform were stored in accordance with applicable General Data Protection Regulation (GDPR) laws. They could be consulted only by the French Polynesia health authorities and used for the protection of public health and epidemiological research. Once finalized, the traveler received the receipt of the ETIS form at the email address indicated for the registration. Each traveler was identified by a unique ETIS number and a QR code. On the day of departure, the traveler had to present the ETIS receipt and the proof of negative RT-PCR test at check-in to be allowed to board the flight.

Self-sampling kit distribution on arrival

On arrival at Tahiti-Faa'a international airport (Tahiti), each traveler 6 years of age or greater and staying more than 4 days in French Polynesia received a self-sampling kit consisting of a zipper plastic bag containing 2 swabs (one for the oral sampling and the other for the nasal sampling), a tube with 2–3 ml of viral transport medium (VTM) or universal transport medium (UTM), and a plastic bag approved for the transport of bio-hazardous materials with an absorbent inside. With the kit were also provided in both French and English languages an information notice presenting the protocol implemented for COVID-19 surveillance of travelers, an instruction sheet describing how to proceed to sample self-collection, and the list of the health care centers located on the different islands of French Polynesia where self-collected samples could be dropped off (S1 File).

The date of completion of the self-collection of samples was indicated on the kit and corresponded to the 4th day after arrival in French Polynesia, excluding weekends because of the closure of most health care centers (if the 4th day fell on a Saturday, the test was brought forward to the previous Friday, and if it fell on a Sunday the test was postponed to the following Monday). The choice of the 4th day for sample self-collection was based on the mean incubation period, *ie* the delay between the exposure to the virus and the apparition of symptoms of COVID-19, which had been estimated to 5–6 days in previously published studies [16–18].

Since the first RT-PCR testing had to be performed within 3 days prior to departure, the delay between possible infection and detectable viremia would correspond to 2–6 days (mean 4 days) after arrival in French Polynesia.

Each kit was identified by a unique number represented by a barcode affixed to both the zipper plastic bag and the tube containing the transport medium. Prior to giving the kit to the traveler, the kit number was matched to the traveler's ETIS number using an application installed on mobile digital tools (tablet or smartphone) that successively scanned the barcode on the kit and the QR code on the traveler's ETIS receipt. Those data were sent automatically to the server of the ETIS platform to be associated with the traveler's identity. The data allowing the link between the kit number and the identity of the traveler could only be viewed by the physicians of the Surveillance office from the Ministry of Health of French Polynesia.

Sample self-collection and shipment

Two days after arrival, each traveler received an automatic message from the tourism department of French Polynesia at the e-mail address provided in the ETIS form, as a reminder to perform samples self-collection on the date indicated on the kit. Travelers could refer to the instruction sheet received upon arrival and to the video tutorial posted online by the tourism department (available at: https://www.youtube.com/watch?v=pYcOp6tk9bo&feature=emb_logo). Moreover, a call center open 7 days a week and an e-mail address were available to answer travelers' questions.

In order to increase the probability of collection of viral particles at the respiratory tract, the traveler had to collect both nasal and oral samples. Briefly, the traveler had to insert the swab provided for the nasal sampling into each nostril until some resistance was felt, and rub the walls 4 times (the swab provided for the nasal sampling had a large cotton tip to prevent the traveler from pushing it too deep and getting hurt). In addition, the traveler had to insert the swab provided for the oral sampling into the mouth and rub several times inside of cheeks, the top and the bottom of the tongue, the palate, and the lower and upper gums. Then, the traveler had to insert the 2 swabs into the tube containing the transport medium and close it, turn the tube 3 times to mix the samples with the medium, put the tube into the bio-hazard plastic bag and seal it, and finally put the bio-hazard plastic bag back into the zipper plastic bag.

The traveler had to keep the self-collected samples at a temperature between +4˚C and +8˚C until dropping it off at the Institut Louis Malardé (ILM, Papeete, Tahiti) or one of the health care centers on the list provided with the kit. Travelers staying in a hotel, guesthouse or on a cruise ship participating in the surveillance strategy also had the opportunity to drop off their self-collected samples at the lobby of their accommodation. Then, the accommodation was responsible for forwarding the self-collected samples to the nearest health care center under the temperature conditions recommended during transport. In Tahiti, self-collected samples were collected from health care centers every weekday. In the other islands, self-collected samples dropped off at the health care centers were shipped by boat or plane to Tahiti once to several times a week, depending on the frequency of sea and air rotations (Fig 1). All self-collected samples were ultimately delivered to ILM.

Analysis of self-collected samples

Pool size validation. In order to increase testing capacity for SARS-CoV-2 detection at ILM, we assessed the use of pooled samples from different travelers before viral RNA extraction and RT-PCR steps. A previous study had shown that 8 to 32 individual samples could be pooled without compromising the benefit of the pooling strategy for a SARS-CoV-2 prevalence ranging from 2 to 0.1% [19]. Since travelers entering French Polynesia had a negative

Fig 1. Air and maritime connections for the shipment of travelers' self-collected samples from the islands with health care centers participating in the COV-CHECK PORINETIA surveillance strategy, to Tahiti where the Institut Louis Malardé is located; a surface area as wide as Europe.

https://doi.org/10.1371/journal.pone.0256877.g001

SARS-CoV-2 RT-PCR test dating less than 3 days before departure, the expected prevalence among this population was low.

In order to assess the use of a pool size of 10 individual samples, we performed 10-fold serial dilutions of a nasopharyngeal sample collected from a patient tested RT-PCR positive for SARS-CoV-2 in French Polynesia in March 2020 (Fig 2). Then, we mixed 50 μL of each 10-fold dilution with 50 μL of 9 nasopharyngeal samples collected from patients tested RT-PCR negative for SARS-CoV-2, with a final volume of 500 μL per pool. We subsequently performed viral RNA extraction and RT-PCR.

Viral RNA extraction was performed on the NucliSENS® easyMag® instrument (bioMérieux, France). A volume of 50 μL of each 10-fold dilution or 500 μL of each pool was mixed with 50 μL or 100 μL of magnetic silica, respectively. The final elution volume was set up at 50 μL. To check the validity of the extraction process, 10 μL of internal positive extraction control (LightMix Modular EAV RNA Extraction Control kit, TIB MOLBIOL, Germany) were added to each dilution or pool.

Duplex RT-PCR was performed on the CFX96 Touch Real-Time PCR Detection System (Bio-Rad, USA), using 2 different protocols.

In the first protocol, 7.5 μL of each extracted RNA were added to 12.5 μL of reaction mixture containing 10 μL of 2X Reaction buffer, 0.32 μL of MgSO4, and 0.8 μL of RT/Taq mixture provided with SuperScript™ III Platinum™ One-Step qRT-PCR Kit (Invitrogen, USA), 0.5 μL of primers and probe mixture from LightMix® Modular SARS and Wuhan CoV E gene kit or from LightMix® Modular Wuhan CoV RdRP-gene kit, and 0.5 μL of primers and probe

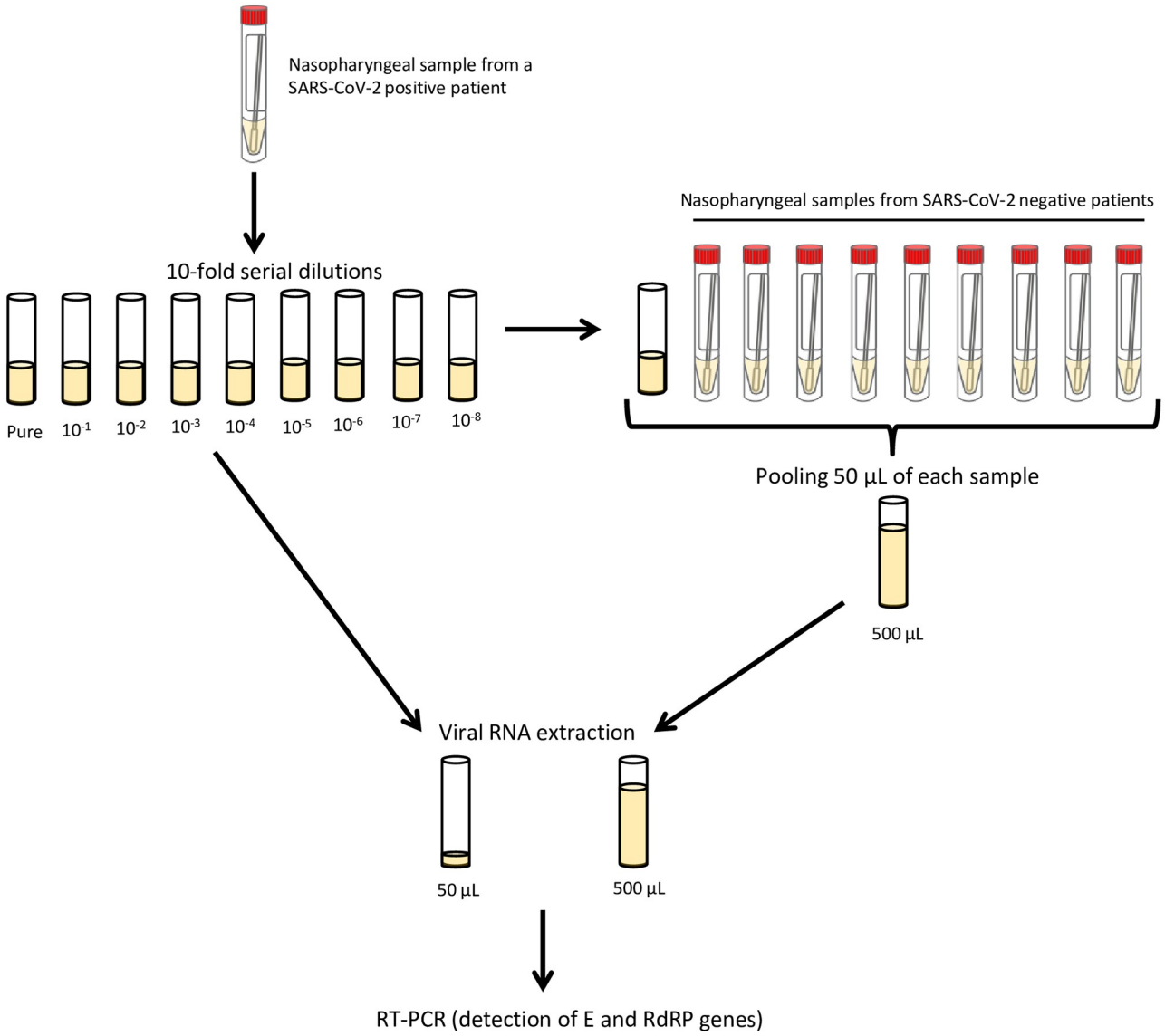

Fig 2. Protocol for the evaluation of the pooling strategy.

https://doi.org/10.1371/journal.pone.0256877.g002

mixture from LightMix® Modular EAV RNA Extraction Control kit (TIB MOLBIOL, Germany) [20]. Reverse transcription was carried out at 55˚C for 5 min, followed by the initial denaturation and polymerase activation steps at 95˚C for 5 min, 45 cycles of amplification at 95˚C for 5 sec, 60˚C for 15 sec and 72˚C for 5 sec, and a final cooling step at 40˚C for 30 sec.

In the second protocol, 5 µL of each extracted RNA were added to 15 µL of reaction mixture containing 10 µL of 2X Reaction buffer and 0.5 µL of Reverse Transcriptase provided with iTaq Universal Probes One-Step kit (Bio-Rad, USA), 0.5 µL of primers and probe mixture from LightMix® Modular SARS and Wuhan CoV E gene kit or from LightMix® Modular Wuhan CoV RdRP-gene kit, and 0.5 µL of primers and probe mixture from LightMix® Modular EAV RNA Extraction Control kit. Reverse transcription was carried out at 50˚C for 10 min, followed by the initial denaturation and polymerase activation steps at 95˚C for 3 min, and then 45 cycles of amplification at 95˚C for 15 sec and 60˚C for 30 sec.

Pooling of samples. Upon receipt at ILM, the barcode of each self-collected sample was scanned, and up to 10 self-collected samples were randomly grouped. A new barcode assigned to the group of self-collected samples was scanned and stuck on a tube containing 2 mL of NucliSENS lysis buffer (BioMérieux, France). Under a class II biological safety cabinet, the tubes containing the transport medium and the 2 swabs used for nasal and oral samples self-collection were removed from the bio-hazard and zipper plastic bags, and vortexed for 30 sec. Then, 50 μL of transport medium were taken from each of the grouped tubes and transferred into the pooling tube containing the lysis buffer. The pooling tube was subsequently vortexed for 10 sec and incubated at room temperature for at least 10 min before the extraction step.

RNA extraction and RT-PCR. Viral RNA extraction and duplex RT-PCR were performed from pooled self-collected samples as described above, using LightMix® Modular SARS and Wuhan CoV E gene kit and LightMix® Modular EAV RNA Extraction Control kit. If a pool tested positive, 200 μL of each self-collected sample were individually subjected to viral RNA extraction, by adding 50 μL of magnetic silica and 10 μL of internal positive extraction control. The presence of SARS-CoV-2 RNA was confirmed by the detection of specific RdRP gene using LightMix® Modular Wuhan CoV RdRP-gene kit, with similar conditions of RT-PCR as described above.

Confirmatory test

Barcode numbers corresponding to individual self-collected samples tested positive were sent to the Surveillance office from the Ministry of Health. Using the data provided on the ETIS platform, travelers were contacted and immediately isolated. A naso-pharyngeal swab was collected from each traveler by a health worker and tested by RT-PCR to confirm SARS-CoV-2 infection.

Detection of variants

Individual self-collected samples found positive for SARS-CoV-2 between October, 2020, and February, 2021, were re-tested using the VirSNiP SARS-CoV-2 Spike N501Y Kit (TIB MOLBIOL, Germany) that specifically detects the mutation N501Y found in the spike (S) protein of the Alpha, Beta and Gamma variants [15], and reagents from SuperScript™ III Platinum™ One-Step qRT-PCR Kit. Briefly, 7.5 μL of extracted RNA were added to 12.5 μL of reaction mixture containing 10 μL of 2X Reaction buffer, 0.30 μL of MgSO4, and 0.8 μL of RT/Taq mixture provided with SuperScript™ III Platinum™ One-Step qRT-PCR Kit, and 0.5 μL of primers and probe mixture from VirSNiP SARS-CoV-2 Spike N501Y Kit. Reverse transcription was carried out at 55˚C for 5 min, followed by the initial denaturation and polymerase activation steps at 95˚C for 5 min, and then 45 cycles of amplification at 95˚C for 5 sec, 60˚C for 15 sec and 72˚C for 5 sec. The presence of the 501Y mutation was revealed by the amplification curve with a melting temperature of 61.2±2˚C.

To identify the variant lineage (Alpha, Beta or Gamma), the complete S gene (nucleotide positions 21,563 to 25,384) was sequenced with the 3500 series genetic analyzer (Applied Biosystems, USA), using primers provided by the Laboratory for Urgent Response to Biological Threats (CIBU) at the Institut Pasteur (Paris, France), and reagents from the Big Dye Terminator V3.1 kit (Applied Biosystems, USA). Partial sequences were cleaned and assembled using the Sequencher 4.10 software (Gene Codes Corporation, USA). The final sequence was uploaded on the GISAID CoVsurver (available at https://www.gisaid.org/epiflu-applications/covsurver-mutations-app/) that automatically determines nucleotide/amino acid mutations and deletions compared to the reference sequence hCoV-19/Wuhan/WIV04/2019.

Statistical analysis

Data were analyzed with GraphPad Prism 7.04. The paired t test was used to compare RT-PCR results between individual and pooled samples. P values < 0.05 were considered to be significant.

Ethics

The COV-CHECK PORINETIA surveillance strategy was implemented by the government of French Polynesia (order No. 525 CM of May 13th, 2020, amended by order No. 961 CM of July 8th, 2020; published in full in the Official Journal 2020 No. 79 NS of July 9th, 2020). The approval for the use of data produced within the framework of the COV-CHECK PORINETIA surveillance strategy was obtained from the ethics committee of French Polynesia (No. 90 CEPF of June 15th, 2021). All biological samples were de-identified before researchers accessed the samples.

Results

Validation of the pooling strategy

To assess the impact of pooling samples on SARS-CoV-2 detection sensitivity, we compared RT-PCR results (Ct values) obtained following viral RNA extraction from 50 μL of 10-fold serial dilutions of a positive nasopharyngeal sample, and from 500 μL of pools containing 50 μL of each diluted sample with 50 μL of 9 negative nasopharyngeal samples. Because of the limited number of positive nasopharyngeal samples available at the time the assessment was performed and the short delay before reopening the air border, only one sample could be tested.

Reagents from 2 different RT-PCR kits (SuperScript™ III Platinum™ One-Step qRT-PCR Kit or iTaq Universal Probes One-Step kit) were used to detect the SARS-CoV-2 E and RdRP genes. Whatever the RT-PCR kit used and the gene detected, Ct values were not significantly different between individual and pooled samples, with p-values ranging from 0.051 to 0.699 (Table 1).

Detection of SARS-CoV-2 infection in travelers

From July 15th, 2020, to February 15th, 2021, we tested 5,982 pools comprising 59,490 individual self-collected samples, and found 238 pools (3.98%) positive for SARS-CoV-2 E gene (S1 Table). RNA extraction and RT-PCR subsequently performed on individual samples confirmed that 273 travelers were positive for SARS-CoV-2 specific RdRP gene detection. Among the pools tested, 211, 23, 4 and 1 contained respectively 1, 2, 3 and 4 positive individual samples (Fig 3a–3c). The prevalence of positive self-collected samples among all travelers tested was 0.46%.

RNA extracted from individual samples of the first 50 positive pools reported in French Polynesia (among those containing only one positive individual sample) were also tested for the detection of the SARS-CoV-2 E gene (Fig 4). A mean difference of 1.17 Ct (CI 95% 0.93–1.41) was found between positive individual and pooled samples, following RNA extraction from 200 μL and 50 μL of sample, respectively.

Detection of variants

Among 143 positive individual samples self-collected by travelers from October 20th, 2020, 8 were also found positive using variants-specific amplification kit. All travelers had arrived from Metropolitan France. Sequencing of the complete S gene (3,822 bp) from 6 samples

Table 1. Comparison of individual test Ct value with pool Ct value using 10-fold serial dilutions of a positive nasopharyngeal sample, and 2 different RT-PCR kits for the detection of SARS-CoV-2 E and RdRP genes.

Dilution	SuperScript™ III Platinum™ One-Step qRT-PCR Kit				iTaq Universal Probes One-Step kit			
	E gene detection		RdRP gene detection		E gene detection		RdRP gene detection	
	Individual	Pool	Individual	Pool	Individual	Pool	Individual	Pool
–	13.67	14	13.42	13.53	13.67	13.92	13.49	13.74
10^{-1}	17.04	17.1	16.61	16.73	16.9	17.12	16.84	16.99
10^{-2}	20.43	20.34	20.5	20.26	20.41	20.29	20.79	20.38
10^{-3}	23.54	23.48	23.37	23.24	23.55	23.72	23.48	23.49
10^{-4}	27.12	27.29	27.04	27.02	27.17	27.33	27.1	27.28
10^{-5}	30.5	31.26	30.35	30.32	30.63	30.78	30.33	30.61
10^{-6}	34.05	34.57	35.71	34.47	35.71	34.2	34.63	35.04
10^{-7}	38.73	39.82	44.11	ND	41.71	ND	43.59	44.6
10^{-8}	ND	ND	ND	ND	ND	ND	ND	ND

ND: SARS-CoV-2 RNA not detected.

https://doi.org/10.1371/journal.pone.0256877.t001

identified nucleotide mutations specific to the Alpha variant (Table 2). The 2 remaining travelers tested positive using variants-specific amplification kit were closely related to the 2 other travelers for whom sequencing of the S gene revealed the presence of mutations specific to the Alpha variant.

Discussion

Since the emergence of SARS-CoV-2 in China in December 2019, the virus has been circulating worldwide [21]. Several countries, including French Polynesia, rapidly closed their borders to prevent the introduction of new COVID-19 cases [5]. In contrast to other countries in the Pacific which have maintained their borders closed, French Polynesia reopened international air traffic from July 15th, 2020, while COVID-free at that time [6, 22], in order to revive tourism-related economic activity.

The requirement to have a negative RT-PCR test for SARS-CoV-2 prior to departure to French Polynesia had been in place since April 2020 for residents returning from abroad. They also had to observe a period of isolation after arrival and perform another RT-PCR test before their discharge. Despite these control measures, 2 travelers tested positive at the end of their period of isolation on June 26th, 2020 [23], suggesting that they had been infected during the last days preceding their departure from Metropolitan France. These observations supported the idea that in addition to the pre-departure RT-PCR test, a second post-arrival test was needed to detect travelers who may have been infected just before departure.

The COV-CHECK PORINETIA surveillance strategy, consisting in testing the samples self-collected by travelers 4 days after their arrival, enabled to detect SARS-CoV-2 infection in 273 travelers among 59,490 travelers tested between July 15th, 2020, and February 15th, 2021. Consistent with the results of a previous study showing that up to 11 individual samples could be pooled for a SARS-CoV-2 prevalence of 1% [19], the prevalence of SARS-CoV-2 infections found at 0.46% in travelers confirms that our pooling strategy including up to 10 different self-collected samples was relevant for screening travelers entering French Polynesia.

Travelers suspected of being infected by SARS-CoV-2 were immediately contacted by the Surveillance office from the Ministry of Health and placed in isolation. A nasopharyngeal swab was collected by a health worker and tested by RT-PCR to confirm the infection. People who

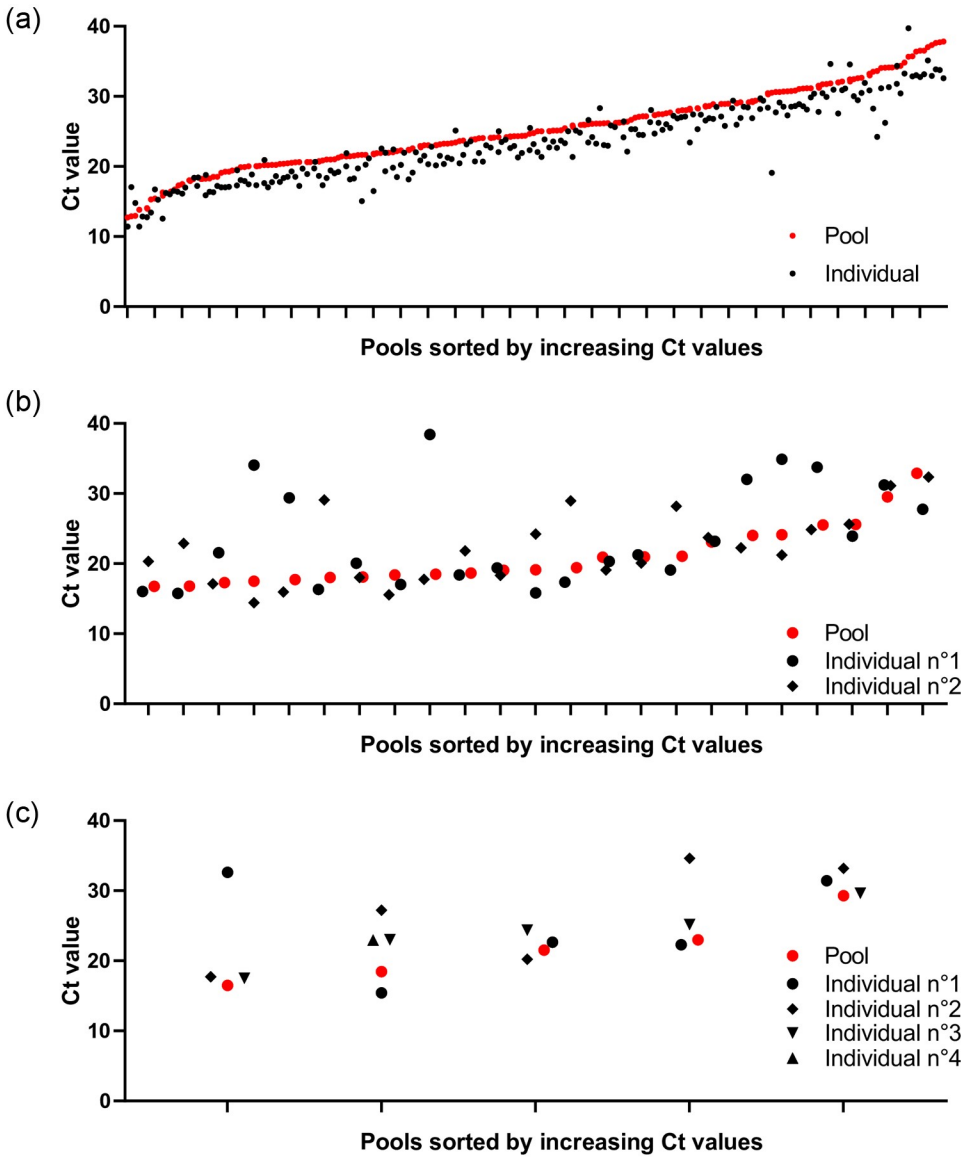

Fig 3. Graph showing Ct values for the detection of SARS-CoV-2 E gene in pools, and of RdRP gene in individual self-collected samples. A) Pools (N = 210) containing 1 positive individual sample; B) Pools (N = 23) containing 2 positive individual samples; C) Pools (N = 5) containing >2 positive individual samples.

https://doi.org/10.1371/journal.pone.0256877.g003

had stayed with the suspected cases were also tested. Positive cases were repatriated to Tahiti as soon as possible in order to benefit from appropriate medical care, especially in the event of the appearance of severe symptoms, and also to protect the population of the islands from SARS-CoV-2 contamination. This strategy enabled the early detection of the first case of COVID-19 since the re-opening of the borders on a cruise ship, and prevented the contamination of other passengers [24].

The COV-CHECK PORINETIA surveillance strategy had some limitations. Indeed, of the 61,397 travelers who were given a self-test kit on arrival, 1,907 (3.11%) did not return their self-collected samples, despite reminders. In addition, since self-collection was not supervised by a health worker, it was impossible to check whether the travelers had correctly collected

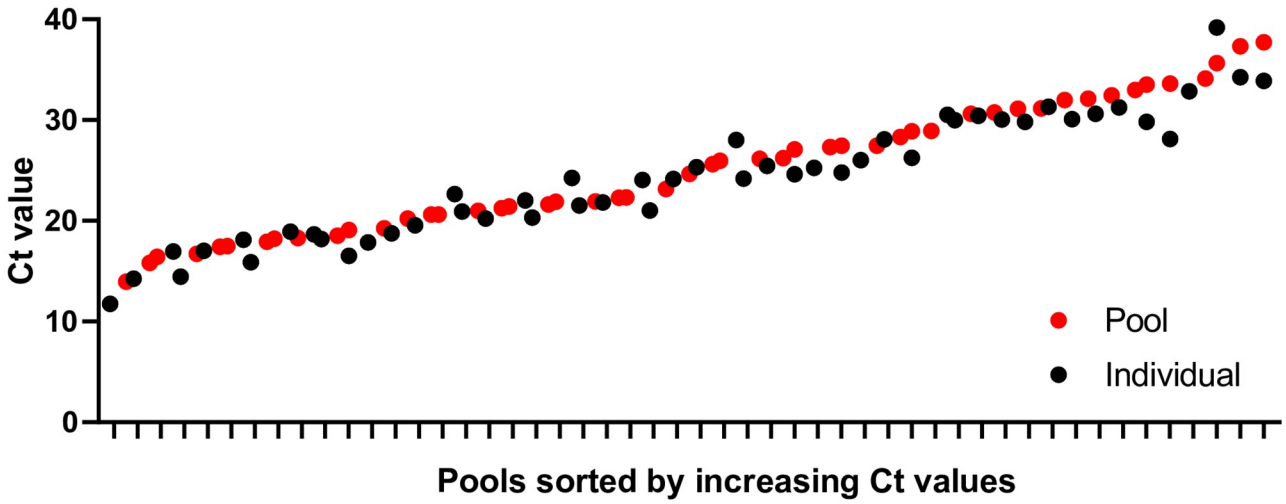

Fig 4. Graph showing Ct values for the detection of SARS-CoV-2 E gene in positive individual and pooled self-collected samples.

https://doi.org/10.1371/journal.pone.0256877.g004

their samples by strictly following the instructions supplied with the kit. Finally, the pooling samples strategy resulted in a slight loss of sensitivity of the RT-PCR assay compared to individual samples testing (on average 1.17 Ct for the detection of the E gene), probably related to the volume of sample extracted (50 μL versus 200 μL, respectively).

Despite these limitations, the surveillance strategy contributed to protect most remote French Polynesia islands with limited health infrastructures from the introduction of SARS--CoV-2 [25]. In addition to the surveillance strategy implemented since July 15th, 2020, travelers were required to apply barrier measures during their stay, thus limiting the risk of contamination of the islands' population, and to report to the health authorities any symptoms suggestive of COVID-19. Despite these obligations, an asymptomatic traveler whose self-collected sample tested positive but who had not complied with the barrier measures during the 4 days preceding the test generated the first cluster of COVID-19 cases in Tahiti [6]. As of February 15th, 2021, 18,293 cases of COVID-19 and 135 deaths related to SARS-CoV-2 infection were recorded [26].

Table 2. Mutations specific to the Alpha variant detected in the S gene of SARS-CoV-2 strains isolated from self-collected samples of 6 travelers.

Date of reception at ILM (month/year)	Traveler 1	Traveler 2	Traveler 3	Traveler 4	Traveler 5	Traveler 6
	12/2020	01/2021	01/2021	02/2021	02/2021	02/2021
Amino acid substitutions	N501Y	N501Y	N501Y	N501Y	N501Y	N501Y
	A570D	A570D	A570D	A570D	A570D	A570D
	D614G	D614G	D614G	D614G	D614G	D614G
	P681H	P681H	P681H	P681H	P681H	P681H
	T716I	T716I	T716I	T716I	T716I	T716I
	S982A	S982A	S982A	S982A	S982A	S982A
	D1118H	D1118H	D1118H	D1118H	D1118H	D1118H
Amino acid deletions	H69	H69	H69	H69	H69	H69
	V70	V70	V70	V70	V70	V70
	H144	H144	H144	H144	H144	H144

https://doi.org/10.1371/journal.pone.0256877.t002

Given the worldwide emergence of several variants of SARS-CoV-2 with an estimated increased transmissibility of up to 70% [27], self-collected samples of travelers that had been found positive from October 20th, 2020, using SARS-CoV-2 specific RT-PCR, were retrospectively tested with a variant-specific kit. Subsequent sequencing of the S gene revealed that at least 6 travelers had been infected with the Alpha variant before entering French Polynesia. Rapid isolation of these travelers prevented the wide spread of the variant, with only 4 secondary cases reported as of February 23rd, 2021 [25].

Conclusion

Depending on the evolution of the epidemiological context of COVID-19 in French Polynesia, the strategy consisting in pooling samples self-collected by travelers was initially used to detect the introduction of SARS-CoV-2 in a COVID-free area with limited medical staff and material resources, then more recently to prevent the emergence of variants of concern. Our results showed that pooling samples had a minor impact on the sensitivity of the RT-PCR test. In order to facilitate SARS-CoV-2 screening in the population, more and more countries have validated the use of self-collected samples [28–30]. Therefore, self-collection and pooling approaches should be considered to prevent, or at least limit, the introduction of COVID-19 cases when tourist trade resumes in countries whose borders are currently closed, and for mass population screening in countries with active SARS-CoV-2 circulation.

Supporting information

S1 File. Documents provided with the self-sampling kit.
(PDF)

S1 Table. RT-PCR results (Ct values) for the detection of SARS-CoV-2 E and RdRP genes in positive individual and pooled samples self-collected by travelers from July 15th, 2020, to February 15th, 2021.
(PDF)

Acknowledgments

We acknowledge the staff of the Ministry of Health, the Ministry of Tourism, Tahiti-Faa'a airport, and the Institut Louis Malardé for their involvement in the COV-CHECK PORINETIA surveillance system. We also thank the staff of hotels and guest houses for participating in receiving samples and sending them to health care centers. Finally, we especially thank Marie Solignac (Institut Louis Malardé) for designing the map of French Polynesia.

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