Early detection of SARS-CoV-2 variants using traveler-based genomic surveillance at four

US airports, September 2021- January 2022

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1   **Abstract**

2   We enrolled arriving international air travelers in SARS-CoV-2 genomic surveillance, using

3   molecular testing of pooled nasal swabs, and sequencing positive samples for viral sublineage.

4   Traveler-based genomic surveillance provided early warning variant detection; we reported the

5   first U.S. Omicron BA.2 and first BA.3 in North America, weeks before next reported detection.

6   **Key Words**: SARS-CoV-2; genomic surveillance; international travelers

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Background

Despite layered mitigation measures, international travel during the COVID-19 pandemic continues to facilitate global spread of SARS-CoV-2, including novel variants of concern (VOCs). On November 26, 2021, B.1.1.529 (Omicron) was designated a VOC by the World Health Organization [1]. On December 6, 2021, as part of measures to reduce Omicron introduction and spread, the requirement for a negative SARS-CoV-2 test taken before air travel to the United States was shortened from three days to one day [1]. Although SARS-CoV-2 genomic sequencing has increased significantly during the pandemic [2], gaps remain in early detection of emerging variants among arriving travelers.

In September 2021, the Centers for Disease Control and Prevention (CDC), in collaboration with private partners, implemented a voluntary SARS-CoV-2 genomic surveillance pilot program.We initially enrolled travelers on certain flights from India during the Delta surge. On November 28, we expanded the program to include travelers arriving from countries with high travel volumes, including those where Omicron was first detected.

Methods

Design, Setting, and Participants

During September 29–November 27, 2021, the surveillance program included travelers arriving on seven direct flights from India at three international airports: John F. Kennedy, New York (September 29), Newark Liberty, New Jersey (October 4), and San Francisco, California (October 12); Hartsfield-Jackson Atlanta International Airport, Georgia was added on November 28, 2021. During November 28-January 23, 2022, travelers on flights from India, South Africa, Nigeria, the United Kingdom, France, Germany, and Brazil on approximately 50 flights per day

3

1   were enrolled. (Figure 1a). Participants were 18 years or older, provided informed consent, and

2   completed demographic, clinical, and travel history questions.

3   *Sample Collection*

4   Participants could opt-in for, in-airport pooled nasal swab self-collection at-home saliva sample

5   collection 3-5 days after arrival, or both (Supplementary Figure 1). For in-airport pooled

6   sampling, travelers self-collected a dry lower nasal swab sample. Samples were placed in

7   collection tubes with 5–25 other samples and shipped to the Concentric Laboratory Network..

8   During September 29–November 27, samples were pooled based on the flight number. During

9   November 28-January 23, samples were pooled based by country of flight origination. For at-

10   home kits, travelers were asked to collect a saliva sample on day 3–5 after arrival and send it to

11   the laboratory.

12   *Laboratory Testing*

13   All samples underwent SARS-CoV-2 reverse transcription polymerase chain reaction (RT-PCR).

14   After November 27, samples were tested for S-gene target failure (SGTF) using TaqPath

15   COVID-19 assay [3]. All positives underwent whole genome sequencing and variant sublineage

16   determination. Reverse transcribed RNA was amplified using the ARTICv3 protocol [4].

17   Amplicons were pooled and prepared using standard protocols. For Illumina sequencing,

18   samples underwent tagmentation and were sequenced on NovaSeq 6000 (2x50 bp; Illumina). For

19   rapid sublineage identification, a ligation-based library was prepared and sequenced on GridION

20   (Oxford Nanopore) as described in supplemental methods.

21   *Reporting*

1　All travelers participating in pooled testing were advised to submit their at-home kit for

2　individual testing. Individual results were reported to participants via a secure digital portal and

3　to public health authorities per CDC reporting guidelines; pooled results were not reported to

4　participants [5]. Sequence data from positive samples were uploaded to GISAID, and select

5　samples were provided to CDC for viral culture and further characterization.

6　*Statistical Analysis*

7　For this analysis we focused on pooled testing for variant detection and thus included pooled

8　results only. Using Chi-square tests conducted in R 4.0.3, we assessed differences in pooled

9　positivity rates by flight country of origin. This activity was reviewed by CDC and conducted

10　consistent with applicable federal law and CDC policy.[1]

11　**Results**

12　During September 29, 2021–January 23, 2022, we enrolled 16,149 (~10%) of an estimated

13　161,000 eligible travelers, yielding 1,454 sample pools. Overall, 221 (16%) of 1,367 pooled

14　samples (average pool size 11 swabs) tested were SARS-CoV-2-positive. The median turnaround

15　time from sample collection to sequencing was 11 business days (range, 5 - 20). For select

16　samples, we performed expedited sequencing within 48 hours to confirm validity of SGTF as an

17　early indicator for Omicron. Positivity among pooled samples was 1.8% (6/338) during

18　September 29–November 27. After November 27, 2021, it was 20.9% (215/1029) and it varied

19　by country of flight origin; 43.5% (40/92) in South Africa, 32.6% in Brazil (15/46), 25% in

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[1] See e.g., 45 C.F.R. § 46.102(l)(2); 21 C.F.R. part 56; 42 U.S.C. §241(d); 5 U.S.C. §552a; 44 U.S.C. §3501 et seq

1 France (30/120), 18.4% in the UK (30/163), 17.8% in Germany (38/123), and 15.7% (62/395) in

2 India (p < 0.001) (Supplementary Table 1).

3 Before November 28, all sublineages were Delta (B.1.617-like), apart from one undetermined

4 sublineage. During November 28–January 23, 67% (145/215) of positive pooled samples

5 collected were Omicron, (B.1.1.529-like), 5% (11/215) were Delta (B.1.617-like), and the

6 remaining 27% (59/215) of sublineages could not be determined due to low sample sequencing

7 coverage (Figure 1b and Supplementary Table 2). Of 145 Omicron sequences, 112 exhibited

8 complete or partial SGTF sublineage. Omicron sublineages included BA.1 (100), BA1.1 (12),

9 BA.2 (26), and BA.3 (1), BA.2 + Orf1a:M85 (1), and BA.2 + S:R346K (1). Four samples were

10 identified as Omicron, but sublineage could not be determined due to low sequencing coverage.

11 A sample collected on December 14 was the first reported BA.2 in the United States, 7 days

12 earlier than any other U.S. report (Figure 1c). Similarly, a sample collected on December 3 was

13 the first reported BA.3 in North America, 43 days before the next report [6].

14 **Discussion**

15 The traveler-based SARS-CoV-2 genomic surveillance program was able to identify early

16 importation of variants, including Omicron sublineages BA.2 and BA.3before they were reported

17 elsewhere in the United States and North America, respectively. Overall, 16% of pooled tests

18 were positive, with 21% positivity following Omicron emergence. We detected a large

19 proportion of positive post-arrival pooled samples even though passengers were required to have

20 a negative sample collected within one day pre-departure

21 Possible reasons for high pooled test-positivity on arrival despite negative pre-departure testing

22 include timing of infection and testing (i.e., before infection was detectable), use of testing

23 modalities with lower sensitivity [7], or infection soon after pre-departure testing [8, 9]. If

passengers had infections that were undetected in pre-departure testing, longer flight times may have allowed for passengers in their incubation period to convert to a positive result after arrival. [9]. Finally, it is possible that fraudulent test results were used to meet pre-departure testing requirements [10].

Pooled testing in this program is advantageous as it enables efficient, large volume sampling and increases testing throughput while conserving resources. This can be valuable for continued detection when prevalence of SARS-CoV-2 infection is low. The pooled testing design minimizes dilution and reduces loss of sensitivity by pooling during collection. Each Concentric network laboratory is validated to ensure molecular assay sensitivity of 1,500 viral copies/ml. The disadvantage of pooled testing is an inability to directly link test results with individual-level data. Follow-up individual testing, such as the at-home test kits collected in our program (data not presented), provide an additional opportunity to capture linkable meta-data.

With ~ 10% participation rate, we detected sublineageBA.2 and BA.3 weeks before they were reported by other US and North American sequencing efforts. The country-level proportions of variants that we identified were consistent with those reported by national and global sequencing programs [2]. Our study suggests that when COVID-19 rates are high, as during the Omicron surge, a 10% participation rate would be sufficient to detect relatively rare susublineages. Sample size calculations for variant detection require a more complicated approach that will include models and simulations to maximize variant detection at different global prevalence rates while also reducing resource allocation. As the pandemic evolves, the program may include additional modalities, such as wastewater sample collection or air sampling from aircrafts, that enable SARS-CoV-2 monitoring in low prevalence settings and are not dependent on individual passenger participation.

Detection of imported emerging infectious diseases has traditionally focused on travelers presenting to health clinics after symptom onset [12]. COVID-19 presents unique challenges since transmission often occurs before symptom onset or in asymptomatic persons [7]. By the time of variant detection, there is often widespread community transmission. Many countries have required testing for arriving travelers to limit introduction and spread of SARS-CoV-2 [11] yet few utilize traveler-based viral genomic surveillance to detect novel variants and provide detailed epidemiological data. Earlier detection of novel SARS-CoV-2 variants allows researchers and public health officials the needed time to gather information about transmissibility, virulence, and vaccine effectiveness, enabling adjustments to treatment and prevention strategies [2].

This traveler-based genomic surveillance program underscores the importance of public-private partnerships in achieving public health priorities in an ever-changing pandemic, and the utility of surveillance tools beyond traditional individual testing. The program's scalability and adaptability, including the ability to rapidly add locations and expedite sequencing, were key factors for success. Traveler-based SARS-CoV-2 genomic surveillance provides a model of pathogen detection that can be used as an early warning, sentinel system for future outbreaks.

NOTES

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Disclaimer

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1
2   FIGURE LEGEND:
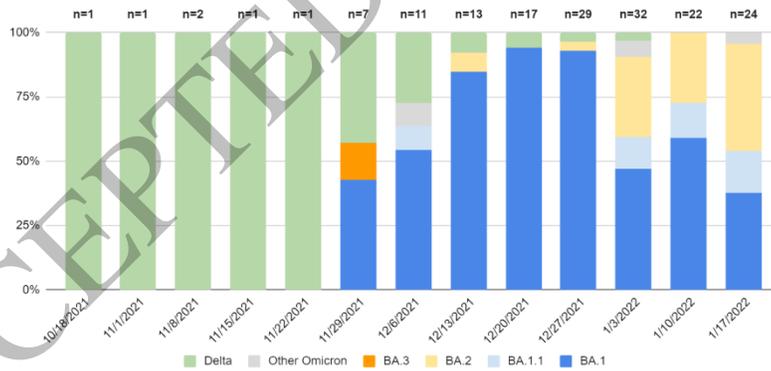3   A.   Traveler-based SARS-CoV-2 Genomic Surveillance, program score during
4   September 29, 2021 – January 23, 2022
5   B. Proportions of variants detected, by collection week, pooled testing

Figure 1.

A. Traveler-based SARS-CoV-2 Genomic Surveillance, program scope during September

29, 2021 – January 23, 2022

	Surveillance Period	
	September 29, 2021, to November 27, 2021	**November 28, 2021, to January 23, 2022**
Countries in Scope	India	India South Africa Nigeria Brazil France United Kingdom Germany
Airports in Scope	EWR JFK SFO	ATL EWR JFK SFO

B. Proportions of variants detected, by collection week, pooled testing