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Passengers screening for SARS-CoV-2 infection at Rome Fiumicino Airport: a strategy aimed at limiting the spreading of the infection through COVID-free air flights revealed the airport role as a formidable sentinel center for monitoring pandemic trends and viral variants circulation

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Summary

Background and aims: At the Fiumicino Rome Airport, passengers can undergo the antigen or molecular test for SARS-CoV-2 at the COVID-19 Test Area (CTA), in compliance with the boarding regulations on air flights and those for entering the destination countries. The aim of this study was to describe the adaptability and usefulness of using rapid molecular tests designed for point of care (POC) in a context such as the CTA of Rome Fiumicino airport, by describing the volume and activities performed in the last period of the state of emergency in Italy, July 10, 2021- March 31, 2022.

Materials and methods: Rapid molecular tests for SARS-CoV-2 were carried out with ID NOW™ COVID-19 assay on the ID NOW™ platform using dry swabs collected from passengers (or airport staff) at the CTA. Swabs were stored at room temperature and analyzed within 1-2 hours of collection. Sequencing for SARS-CoV-2 variants of concern (VOC) identification was carried out on swabs dipped in universal transport medium (UTM), after new swab gathering from consenting passengers resulted positive with the rapid molecular assay. A proficiency panel prepared with the viral isolate 2019-nCoV/Italy INMI1 was used to evaluate analytical sensitivity; clinical sensitivity was tested by performing real-time polymerase chain reaction (RT-PCR) tests on 50 swabs obtained from passengers who previously resulted positive to ID NOW™ COVID-19 assay.

Results: In total, 14632 rapid molecular tests for SARS-CoV-2 were performed at CTA and 5.6% resulted positive. Sequence analysis of samples with Ct value <25 (61,2 %) with real-time RT-PCR test allowed detection of all the main VOCs: B.1.617.2 (Delta) in 18% of samples, BA.1 (Omicron-1) in 34% of positive swabs and BA.2 (Omicron-2) lineage in 9% of samples. The BA.2 variant was detected in the Lazio region for the first time at CTA, in one sample obtained from an Italian passenger com-

ing from Doha, Qatar. A proficiency panel for SARS-CoV-2 detection was used to establish the limit of detection of ID NOW™ COVID-19 assay. For clinical sensitivity evaluation, 50 subjects with positive results by ID NOW™ COVID-19 assay were tested with routine real-time RT-PCR method in the laboratory and confirmed for SARS-CoV-2 detection.

Conclusions: The great success among passengers, demonstrated by the large number of rapid molecular tests performed, confirmed the appreciation of this service by the users. Our experience at CTA showed that airports, railways and maritime stations should represent strategic surveillance centers for monitoring of circulation of respiratory pathogens, and may have a direct impact on the implementation of prevention and control strategies for public health against the spreading of infectious diseases. Indeed, SARS-CoV-2 data obtained at the CTA faithfully reflected the Italian epidemic curve trend of SARS-CoV-2 and its variants. The rapid molecular ID NOW™ COVID-19 assay used at CTA has proven to be suitable for use in a special context, such as the Rome Fiumicino Airport, though it was designed for use in POC. Due to the simplicity of use, robustness of method and technology that allows the return of results in a few minutes, this system revealed itself to be an optimal tool at the CTA, ensuring passengers boarding on COVID-free flights in a short time and security.

Introduction

During the COVID-19 pandemic, in response to the health emergency connected to the spreading of the new coronavirus SARS-CoV-2 and the associated COVID-19 pathology, restrictive measures were adopted around the world for the movement of people, locally, nationally and internationally according to the epidemic curves trend. In many cases, restrictive measures were limited to specific foreign states and territories; other times, they were limited to short periods; or else they were reserved only for certain destinations. Before the introduction of certifications issued following the completion of the vaccination cycle or recovery from COVID-19 (Green Pass), negative antigenic and molecular tests were used as a certificate of entry in almost all countries, accompanying personal documents. Today, a negative test is still required to enter some countries and it is one of the conditions to obtain EU digital COVID certificate Green Pass.

Rome Fiumicino Airport was the first in Europe to set up a COVID-19 Test Area (CTA) for the exclusive use of passengers with a boarding pass. The CTA, born from the collaboration between Aeroporti di Roma (ADR) and the National Institute for Infectious Diseases (INMI) Lazzaro Spallanzani, is a service available for all departing and arriving passengers (and airport staff) who need to perform a quick swab for SARS-CoV-2. The purpose of the CTA is to provide passengers with reports certifying negativity to SARS-CoV-2 infection in real time, to facilitate access to COVID-free flights and to avoid fiduciary isolation after arriving in the countries of destination.

The activity at the CTA, which began on April 6, 2021, initially with antigen tests and then with the addition of molecular tests, immediately proved to be of great convenience and interest, as confirmed by the high number of tests, especially during Christmas 2021 and New Year holidays. The CTA is still active today. The Special Regional Continuity Care Units for COVID-19 (USCAR) staff, consisting of 20 health workers, executes swabs and antigen tests, giving results in a few minutes (max 20 minutes). Molecular tests are carried out by biologists of the Laboratory of Virology INMI through rapid procedure (by isothermal amplification of nucleic acids technology) or classical method (based on real-time polymerase chain reaction (RT-PCR) or transcription-mediated amplification (TMA)), based on response times required by passengers. The rapid molecular test allows to obtain results in about 30 minutes, as compared to the classic molecular procedure, giving results within 24 hours. In both cases, the result of the molecular test reaches the user directly by e-mail or text message, depending on the choice made when booking the exam. Classical molecular tests based on RT-PCR are carried out at the Virology Laboratory of INMI, while rapid molecular tests are carried out directly in the CTA headquarters, where a small laboratory has been set up, complete with all equipment.

Rapid molecular tests are performed using the ID NOW™ diagnostic system (Abbott Rapid Diagnostics Inc., Charlottesville, USA) with the ID NOW COVID-19 assay. ID NOW™ is a newly introduced diagnostic platform for the rapid qualitative detection of infectious diseases, designed for use in high-activity, POC hospital environments. Thanks to LAMP (loop-mediated isothermal amplification) technology, one of the most valid procedures for rapid diagnosis of infectious diseases [3,7,6,5], results are available in a few minutes with important advantages in clinical management: *i.e.* immediate arrangement for patient's isolation in the emergency room or in community, quick transfer to another location dedicated to positive patients, *etc*. Furthermore, LAMP technology offers numerous advantages over traditional RT-PCR: it does not require sophisticated and expensive instruments such as thermal cyclers; unskilled personnel can perform it; it is a highly sensitive and specific technique; it provides results with accuracy comparable to those obtained with traditional PCR. These characteristics make this technology ideal for contexts far from diagnostic laboratories, such as POC, with the advantage of avoiding sample shipment, which in some contexts can be problematic, complex and expensive. The aim of this study was to describe the adaptability and usefulness of a rapid molecular method designed for POC in a completely different context, such as the CTA of Rome Fiumicino Airport. The volume and type of activities performed in the last period of the state of emergency in Italy, from July 10, 2021 to March 31, 2022, at the CTA and overall results were reported and discussed.

Materials and methods

Samples analyzed

Rapid molecular tests are performed on direct (dry) swabs carried out on passengers (or airport staff) at the CTA, stored at room temperature and analyzed within 1-2 hours of collection. For the sequencing test, swabs dipped in universal transport medium (UTM) were used, stored at 4°C and sent to the laboratory at the end of the day.

ID NOW™ COVID-19 assay

ID NOW™ COVID-19 assay (Abbott Rapid Diagnostics, Inc.) is carried out according to the manufacturer's directions on the ID NOW™ platform. The procedure detects a highly conserved region of the viral genome, the RNA-dependent RNA polymerase (RdRp) [1]. As indicated by the manufacturer, this assay is reserved for symptomatic people, who are within 7 days of the onset of symptoms suggestive of SARS-Cov-2 infection. Preferred samples are direct swabs (nasal swab, nasopharyngeal swab, throat swab), which must be processed as soon as possible

after collection, within 1h (if stored at 15-30°C) or within 24h (if stored at 2-8°C). The results are qualitative and available in 13 minutes; in the case of SARS-CoV-2 detection, the result is available on average within 6 minutes from the start of the test. The CTA laboratory is equipped with four ID NOW™ instruments. The ID NOW™ COVID-19 rapid molecular assay was approved by the Food and Drug Administration (FDA) in March 2020 and received the CE mark in the same year, while it was introduced in Italy in March 2021.

Preparation of a proficiency panel for the evaluation of the analytical sensitivity of the ID NOW™ COVID-19 test

Serial dilutions of SARS-CoV-2 isolate (named 2019-nCoV/Italy-INMI1; full genome sequence GISAID accession numbers: EPI_ISL_410545) [2] ranged from 2x10^6 to 2x10^2 viral RNA copies/mL (corresponding to 1000 and 0.1 TCID50/mL) were extracted using QIAamp Viral RNA mini kit (Qiagen, Hilden, Germany) and amplified by qRT-PCR using RealStar® SARS-CoV-2 RT-PCR Kit 1.0 (Altona Diagnostic GmbH, Hamburg, Germany) on Rotor-GeneQ Real-Time 5-plex cycler (Qiagen, Hilden, Germany). RNA copies/mL were based on a standard curve prepared through serial dilutions of the EURM-019 single stranded RNA (ssRNA) fragments of SARS-CoV-2 including different target genes [4]. A negative sample containing cell culture medium only was included in the panel.

Sequencing analysis

Sequence analysis was performed on swabs of passengers who tested positive for SARS-CoV-2 rapid molecular test. The passengers who gave their consent (40.3%) were subjected to a new swab in UTM stored at 4°C until shipment to the laboratory carried out the same day or the day after. Nucleic acid extraction was performed using the automated Qiasymphony system (Qiagen, Hilden, Germany) and subsequent amplification by classical PCR. A region of the S gene (aa 390-623) comprising the SARS CoV-2 receptor binding domain was amplified. The individual sequences obtained were compared, by alignment, with the original Wuhan virus sequence (NC_045512.2). The specific pattern of mutations observed allowed identifying the variant of the virus.

Results

Evaluation of analytical sensitivity of the ID NOW™ COVID-19 assay

Analytical sensitivity of the ID NOW™ COVID-19 test was assessed with a proficiency panel containing SARS-CoV-2 total RNA ranging from 2x10^6 to 2x10^2 copies/mL. The analysis was performed by adding 100µL of each dilution in the sample receiver and proceeding according to the manufacturer's test protocol. The assay was able to detect the lowest SARS-CoV-2 RNA dilution (200 copies/mL), confirming the declared analytical sensitivity (Table 1).

Clinical sensitivity was evaluated by testing 50 positive swabs obtained from subjects that resulted positive with ID NOW™ COVID-19 assay. Swabs in UTM were analyzed with the PCR-based Simplexa™ COVID-19 direct assay. All (100%) results were confirmed positive, despite different extraction methods and different amplification technology, based on real-time RT-PCR.

Descriptive analysis of the molecular tests performed at the Covid-19 Test Area

At the beginning of the diagnostic activity with the molecular assay for SARS-CoV-2, rapid molecular tests were carried out only as confirmation of positive antigen tests, according to the regulations in force at that time and the number of tests performed was limited. Then, following the introduction of stricter regulations, the trend of requests for molecular tests greatly increased (Figure 1). Overall, over the period analyzed, from July 10, 2021, to March 31, 2022, 14,632 molecular tests for SARS-CoV-2 were performed with ID NOW™ COVID-19 assay. Swabs resulted positive were n=828 (5.6%). Apart from a service interruption from October 6 to 22, 2021, the activity was carried out at sustained levels, at an average rate of 62.7 tests per day, with over 150 tests per day on average during end-year holidays. (Figure 1). The maximum number of tests carried out in one day was 199 molecular swabs.

The number of positive and negative swabs obtained monthly with the percentage of positive samples was represented in Figure 2A. In December 2021, the positive swabs were 240, in January 2022 they were 254, in February 2022 were 82 and in March 2022 were 65. The trend of positivity of tests observed among passengers in transit at the CTA perfectly overlapped with the Italian epidemiological curve (https://www.epicentro.iss.it/coronavirus/sars-cov-2-sorveglianza-dati) for the same period (Figure 2B).

A brief description of demographic characteristics of passengers who underwent the rapid molecular test with ID NOW™ COVID-19 assay was shown in Table 2 and the distribution of age was shown in Figure 3.

Throughout the analyzed period, episodes of environmental or instrumental contamination never occurred, thanks to single cartridges containing all the amplification components enclosed in internal reaction chambers not accessible during the process. In total, test repetitions were slightly less than 0.5%, due to unsuitable swabs: in this case, the assay was repeated on a new sample.

Sequencing of SARS-CoV-2 positive swabs

In the period analyzed (November 27, 2021 - March 30, 2022), n=307 swabs were sent to the main laboratory for sequenc-

Table 1. Proficiency panel.

Sample ID	SARS-CoV-2	Proficiency Panel (2019-nCoV/Italy INMI 1 isolate) Viral RNA Copies/mL	$TCID_{50}$/mL COVID-19 results	ID NOW™
1	+	$2x10^6$	1000	Detected
2	+	$2X10^4$	10	Detected
3	+	$2X10^3$	1	Detected
4	+	$2X10^2$	0.1	Detected
5	-	0	0	Not Detected

ing. After evaluation of relative viral load (Ct) by classical PCR, samples with Ct value <25 were sequenced (n=188; 61.2%). The B.1.617.2 (Delta) variant was detected until January 11, 2022, in n=57 (18%) samples. The presence of BA.1 (Omicron-1) lineage has been observed starting from December 4, 2021 (n=104; 34%) and BA.2 (Omicron-2) lineage was detected in n=27 (9%) samples. The BA.2 (Omicron-2) lineage was detected for the first time in the Lazio region in one sample obtained from an Italian passenger coming from Doha, Qatar.

Table 2. Demographic characteristics of passengers subjected to rapid molecular test at CTA with ID-NOWTM COVID-19 assay.

Passengers' demographic characteristics	N (%)
Total	14632
Diagnosis results, N (%)	
Positive	828 (5,6)
Negative	13804 (94,3)
Gender, N (%)	
Male	7877 (53,8)
Female	4825 (33)
Unknown	1930 (13,2)
Age	
Media	41,4
≤18	767 (5,24)
>18	12545 (85,74)
Unknown	1320 (9,02)

Conclusions

The establishment of the CTA at Rome Fiumicino Airport was necessary to ensure Covid-free flights during the most critical phase of the pandemic, in order to keep air transport of people and goods active. Currently, the CTA is still active and requests for antigen and molecular tests remain necessary to satisfy different boarding rules of the international airlines (antigen tests or molecular tests necessary for boarding), to comply with the rules of entry into countries (often different between countries, and modified several times over time) and to avoid fiduciary isolation (quarantine) upon arrival in some countries of destination.

From the first day, the CTA immediately proved to be useful for passengers, due to the fact that it offers the possibility to perform molecular tests in a short time and directly at boarding, especially when unaware of having to present negative tests for SARS-CoV-2 (with antigen or molecular test) at check-in.

However, it is interesting to note that, over time, the service carried out by CTA has proved to be a formidable sentinel centre for monitoring the progression of the pandemic and the circulation of SARS- CoV-2 variants. In fact, even considering that molecular tests were not performed on all passengers in transit at the airport, the number of positive cases found in the period analyzed showed a trend to parallel that observed on a national scale. In addition, the CTA service allowed to collect numerous swabs positive for SARS-CoV-2 that underwent sequencing analysis during the period of national and regional surveys of viral variants organized by the Italian Ministry of Health. The sequence analysis of swabs collected among passengers at the CTA demonstrated the presence of all the main viral strains prevalent, such as the delta and omicron variants of SARS-CoV-2.

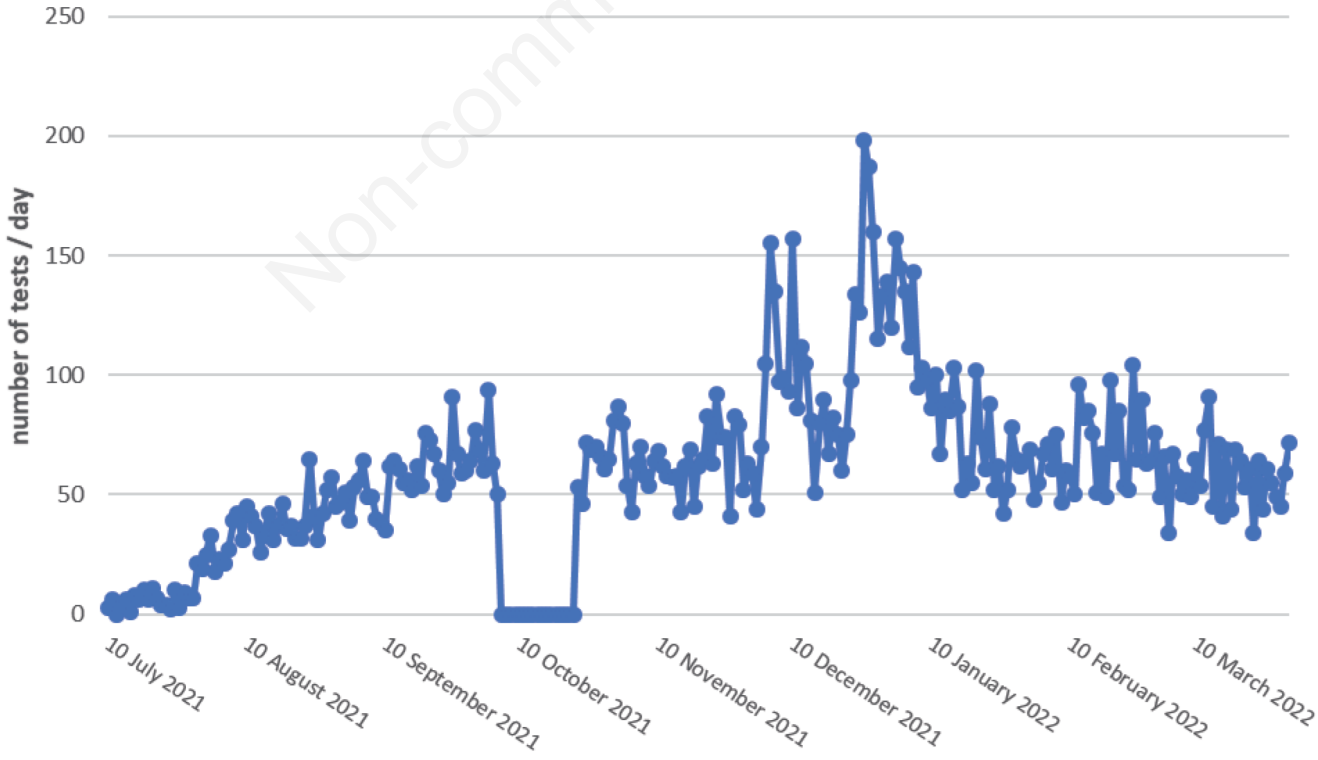

Figure 1. Graphical representation of the number of tests performed daily on swabs with ID NOW™ COVID-19 assay at Fiumicino CTA from 10 July 2021 to 31 March 2022. The activities have been temporarily interrupted for a short time since from 6 to 22 October 2021.

Although designed for use in high activity and POC hospital environments, ID NOW™ diagnostic platform for the rapid qualitative detection of SARS-CoV-2 infection has proven to be extremely versatile and suitable for use in a different context than that for which it was designed, such as the Rome Fiumicino Airport. Due to the simplicity of use, the robustness of the method and the technology utilized that allows the return of results in a few minutes, this system revealed to be an optimal tool at the CTA, ensuring passengers boarding on COVID-free flights in a short time.

The evaluation of analytical and clinical sensitivity of the ID NOW™ COVID-19 assay with the proficiency panel prepared with the 2019-nCoV/Italy INMI 1 viral isolate and with a small number of positive swabs repeated with molecular test based on RT-PCR, confirmed high quality of results in terms of sensitivity and reliability of the results, even with different viral variants. Furthermore, since the assay is based on recognition of RdRp gene of SARS-CoV-2, a highly conserved gene, the diagnostic system has been demonstrated to be able to detect all the variants of SARS-CoV-2 in circulation, to date, with no impact on assay performance.

The introduction of the service for screening of passengers with antigenic and rapid molecular tests at the Rome Fiumicino airport, where tens of thousands of people, mainly asymptomatic, pass through each day, has proved to be a valid strategic initiative for

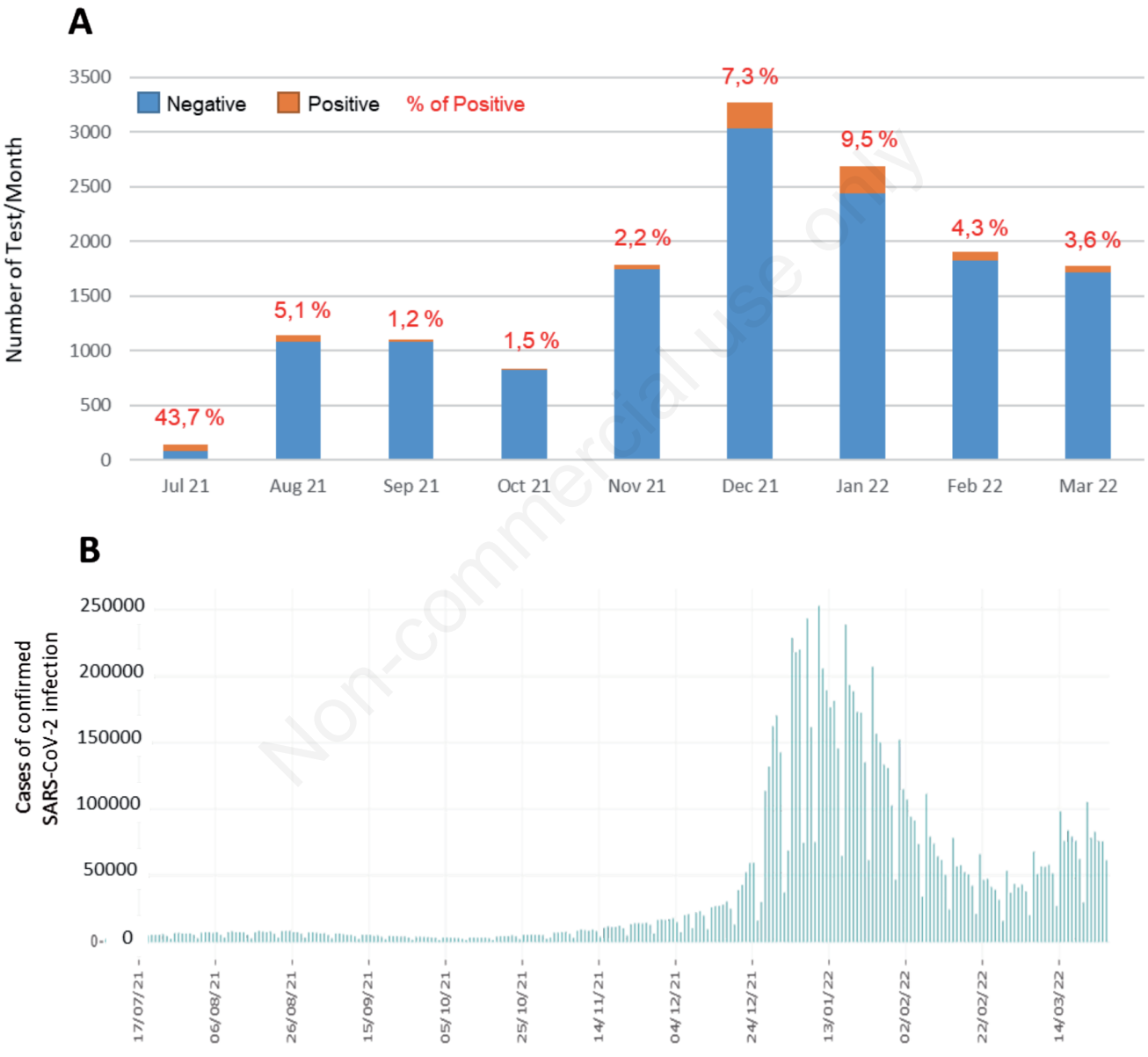

Figure 2. (A) Graphical representation of the number of tests performed monthly at Fiumicino CTA. In blue, the number of negative SARS-CoV-2 tests; in orange, the number of positive tests. The percentages shown in red above each column represent the positive tests observed each month. (B) Cases of confirmed SARS-CoV-2 infection reported in Italy, by date of sample/diagnosis from 10 July 2021 to 31 March 2022. In this period, trend of positive cases overlapped perfectly with that observed at Fiumicino CTA. The data were collected through the integrated surveillance system of COVID-19 in Italy and were elaborated by the ISS integrating epidemiological and microbiological data provided by the regions/autonomous provinces and by the SARS-CoV-2 national reference laboratory at the ISS (produced by the COVID-19 Task force of the Department of Infectious Diseases and the IT Service Istituto Superiore di Sanità). Free data available at: https://www.epicentro.iss.it/en/coronavirus/sars-cov-2-dashboard.

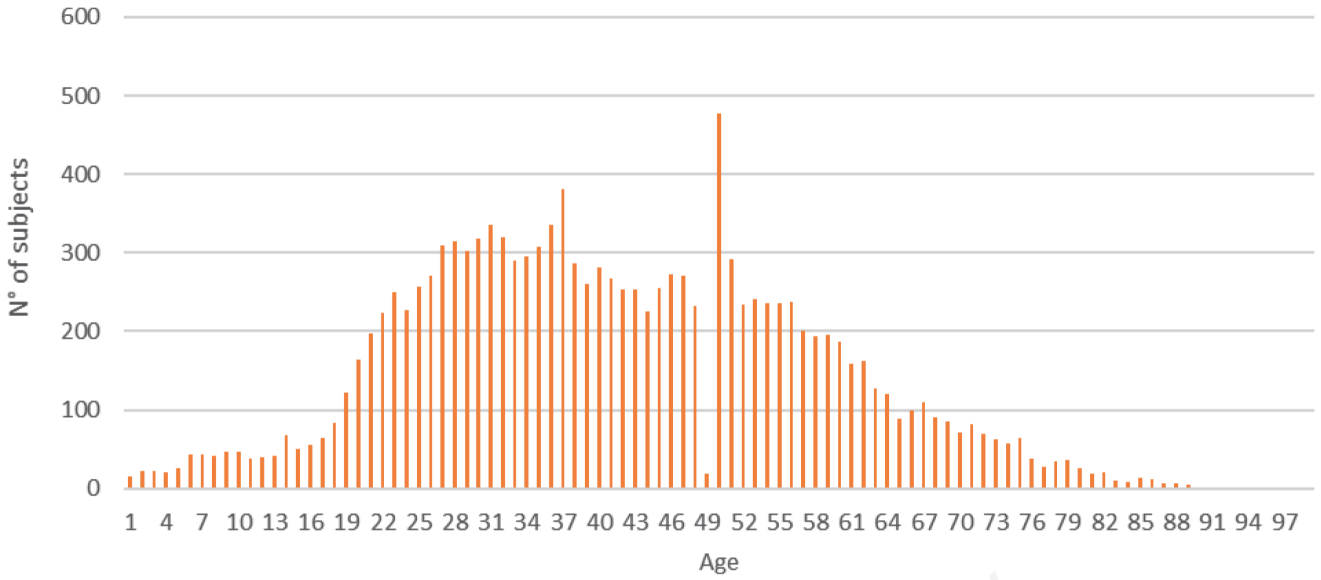

Figure 3. Age distribution of passengers who underwent rapid molecular test with ID NOW™ COVID-19 assay at Fiumicino Covid-19 Test Area.

monitoring of the spread of SARS-CoV-2 infection, after the decision to close the airports and the interruption of flights. After Fiumicino, indeed, the establishment of the COVID-19 test area has been taken as an example in numerous other national and international airports. The ID NOW™ COVID-19 system has been or is currently used at the airports of numerous countries, such as Bulgaria, Hungary, Poland, Dubai, Canada, French Guyana, Jamaica, El Salvador, Paraguay, France, Bahrain.

The CTA experience gained at Fiumicino airport indicates that airports, railway and maritime stations may represent strategic places for monitoring of respiratory infections circulation and in the future, they could become important surveillance centres for controlling of various respiratory pathogens circulation, with direct impact on implementation of prevention and control strategies for public health protection towards the spread of infectious diseases.

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