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SHORT COMMUNICATION

Repurposing Positive SARS-CoV-2 Antigen Test Devices for Variant Tracking

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Abstract

From the very beginning of the SARS-CoV-2 pandemic, one of the very few common opinions was that to control the expansion of the virus as many as the possible test had to be done. Antigen tests, being affordable and easy and fast to use, represented a great opportunity to expand the testing capacities of many healthcare systems. However, in 2021 with the appearance of the new SARS-CoV-2 variants, variant tracking strategies had to be implemented, which often included needing a second test to determine the variant of the patients diagnosed with antigen tests or not taking these samples into consideration at all. Therefore, we proposed recovering the positive antigen test devices to include them in our routine variant tracking strategy. The recovered positive antigen test devices obtained from 1st April 2021 to 15the January 2022 were analysed following the variant tracking protocol in force. The results obtained were compared to the positive samples detected by RT-PCR which were processed for variant tracking during the same period. 21,304 samples were processed, 6297 from the recovered positive antigen devices and 15,007 from the standard nasopharyngeal swabs. Only 773 (3.63%) samples were no conclusive, 104 (1.65%) from the recovered antigen devices and 669 (4.46%) from the RT-PCR positive group. This difference was statistically significant ($p < 0.01$). Taking this into account the proposed method is suitable and very recommendable, as it is an important measure to have a better and immediate picture of the circulating variants in every community.

Introduction

From the early stages of what later was going to be known as the SARS-CoV-2 pandemic at the beginning of the year 2020, it was determined that with this virus asymptomatic patients could not only test positive [1] but that their samples had comparable viral loads to those of symptomatic patients [2]. Therefore, these people may have an important role in the early spread of the infection. To control this, it was necessary to implement contact tracing and asymptomatic patient testing strategies and consequently to try to implement the highest number of available and accurate tests [3]. This was early on claimed by the Director General of the World Health Organization (WHO) with the now famous statement: "Our key message is: test, test, test".

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In order to achieve the highest amount of performed tests with the shortest turnabout time antigen detection techniques prove to be an important asset [4] [5] and they were included in many SARS-CoV-2 testing policies [6] [7] [8], especially at non-hospital settings, where they have been extensively used.

During the year 2021 with the emergence of the new SARS-CoV-2 variants [9], which in some cases had mutations conferring immune escape [10] or higher infectivity [11], variant tracking strategies had to be implemented. Initially, many clinical microbiology laboratories focus these efforts on the samples that were already at those facilities, just those samples that tested positive by RT-PCR. Nevertheless, these samples may have not represented an accurate picture of what variant was already circulating at the same time, as according to some testing policies the chosen test for ambulatory patients was precisely an antigen test. Thus, increasing the risk of not detecting newly introduced variants at the time for controlling them.

Whilst in some cases some positive patients were appointed to a second test to determine their SARS-CoV-2 variant that was supposed waste of time and funds.

Therefore, we proposed to use the same nasopharyngeal sample used for the antigen test to determine the variant of the positive samples.

The objective of this study is to analyse the performance of recovering the positive nasopharyngeal swabs used for antigen tests to perform a variant tracking test.

Materials and Methods

Sample Selection

Starting on 1st April 2021 the primary care units of integrated health organization, which gives service to around 350,000 inhabitants, submitted the positive Panbio™ COVID-19 Ag Rapid Test Device (Abbot, Spain) they obtained to our clinical microbiology laboratory. For this study, we selected the samples obtained from 1st April 2021 to 15th January 2022. However, during the last week of 2021 and the first 2 weeks of 2022, only a representative fraction of the positive samples could be processed due to the increased number of tests conducted during this period. Those samples were compared to the positive samples detected by RT-PCR with Ct < 35, which were processed for variant tracking during the same period.

Nucleic Acid Extraction

Upon arrival, 1 ml of Universal transport medium was added to the Extraction tube and vortexed vigorously for 30 s. Later the samples were processed on the STARlet System with the STARmag Viral DNA/RNA 200C kit (Seegene, Korea).

Variant Tracking

Both RT-PCR-positive eluates and those eluates from positive antigen tests were processed by RT-PCR identically. At the beginning of the study from the 1st of April to the 3rd of May (Phase I: weeks 15–19), lineage-specific analysis was conducted with the Allplex™ SARS-CoV-2 Variants I Assay (Seegene, Korea) that detects ΔH69/V70-, E484K and N501Y mutations. Samples presenting ΔH69/V70- and N501Y mutations were assigned to the B.1.1.7 lineage. Those specimens with the E484K and N501Y mutations were further examined for K417N and V1176F with the VirSNIPs K417N and V1176F (TIBMolBiol, Germany): those presenting compatible melting temperatures to the K417T and V1176F were assigned to Gamma and those with the K417N and V1176 to Beta. Those samples without any positive target at the Variants I Assay were assigned as no clinically relevant variants. During a second phase from the 3rd of May to the 15th of July (Phase II: weeks 20–29), two additional mutations were searched with VirSNIP assays L452R and P681R. Those samples positive to both assays were assigned as Delta and those assays with only L452R were assigned as Epsilon. During the last stage of the study, from the 15th of July 2021 (Phase III: weeks 30[2021] to 3[2022]) all samples were studied with both Allplex™ SARS-CoV-2 Variants I and Allplex™ SARS-CoV-2 Variants II assays simultaneously and interpreted according to Table 1. A negative control with nuclease-free water and the provided positive control were included for every reaction.

As the Seegene Variants I Assay includes the RNA-dependent RNA polymerase (RdRp) gene as a target, the Cycle threshold (Ct) values of this gene were retrieved as an estimation of the viral load of each sample.

Table 1 Proposed interpretation of Seegene Variants I and II assays and need of sequencing for assigning the samples to corresponding variant

Del 69–70	N501Y	E484K	W152C	K417T	K417N	L452R	Result	Sequencing
+	+	−	−	−	−	−	Alpha	No
+	+	+	−	−	−	−	Alpha with E484K	Yes
−	+	+	−	−	+	−	Beta	No
−	+	+	−	+	−	−	Gamma	No
−	−	−	−	−	−	+	Delta	No
−	−	−	+	−	−	+	Epsilon	Yes
+	−	+	−	−	−	−	Eta	Yes
−	−	+	−	−	−	+	Kappa	Yes
−	−	−	−	−	−	−	Lambda	Yes
+	+	−	−	−	+	−	Omicron	No
−	+	−	−	−	+	−	Possible BA.2 (Omicron)	Yes
−	+	+	−	−	−	−	Possible Mu or C.1.2	Yes
−	−	+	−	+	−	−	Possible Theta or Zeta	Yes
−	−	+	−	−	−	−	Possible B.1.575.2	Yes

Sequencing and Bioinformatic Analysis

The less frequent variants at our study (Epsilon, Eta, Lambda and Mu) or samples with other mutation profiles were sequenced to confirm the findings at the RT-PCR, using a targeted approach with the Ion AmpliSeq SARS-CoV-2 Research Panel (Thermo Fisher Scientific) [12]. The libraries were prepared following the manufacturer's instructions and loaded on a 540 chip and run on the Ion GeneStudio™ S5 System (Thermo Fisher Scientific). Genome assembly was obtained with the IRMAreport plugin [13] and the consistency of the nucleotide calls was checked with Integrative Genomics Viewer (IGV) [14]. Nextclade [15] and Pangolin [16] webApp were used to assign the corresponding clades or lineages.

All sequences included in this study are publicly available at the GISAID repository under the strain name hCoV-19/Spain/PV-HUB-XXXXXXXX/Year collected between 1st April 2021 and 15th January 2022.

Statistical Analysis

Data were expressed as mean and standard deviation. Differences were tested by two-tailed *t* test and proportions by a *Z* test. The values $p < 0.05$ were considered statistically significant.

Results

During the study, 21,304 samples were processed, 6297 from the recovered positive antigen devices (Ag +) and 15,007 from the standard nasopharyngeal swabs (RT-PCR +). For 773 (3.63%) samples no conclusive result could be obtained, 104(1.65%) from the Ag + and 669 (4.46%) from the RT-PCR +. This difference was statistically significant ($p < 0.01$).

The mean RdRp Ct was 18.9 (Sd 4.86) for the Ag + and 21.2 (Sd 6.89) for the RT-PCR +, and this difference was also statistically significant ($p < 0.01$). However, the mean RdRp Ct of the non-conclusive RT-PCR + samples was also significantly higher (33.12) than the rest of the RT-PCR + group (20.1).

Delta was the most frequently found variant in both Ag + with 3666 (58.22%) and RT-PCR + with 7807 (52.02%) groups, followed by Alpha with 2065 (32.79%) in the Ag + group and 3994 (26.61%). The third most frequent variant in our study was Omicron with 381 (6.05%) in the Ag + and 2372 (15.81%) in the RT-PCR + group. (Fig. 1).

However, the proportion of each of them varies greatly during the study (Fig. 2). Considering their weekly distribution, we can observe four different phases than mostly overlap with the three variant tracking protocols described previously. During phase I, Alpha is the predominant variant, representing more than 90% of the tracked variants. During phase II Delta variant erupts and finally during Phase III Delta becomes the predominant variant with almost 100% of the determinations. Phase IV starting at week 50 of 2021 constitutes the replacement of Delta by Omicron.

Epsilon and Eta variants were only found in the RT-PCR + group, whilst Alpha, Beta, Gamma, Delta, Lambda and Mu were found in both groups. For Alpha and Delta, the first detection in both subgroups is coeval. For the Gamma variant the detection in the RT-PCR + precedes the detection in Ag +; however, for Lambda and Mu, the situation is exactly the opposite (Fig. 3).

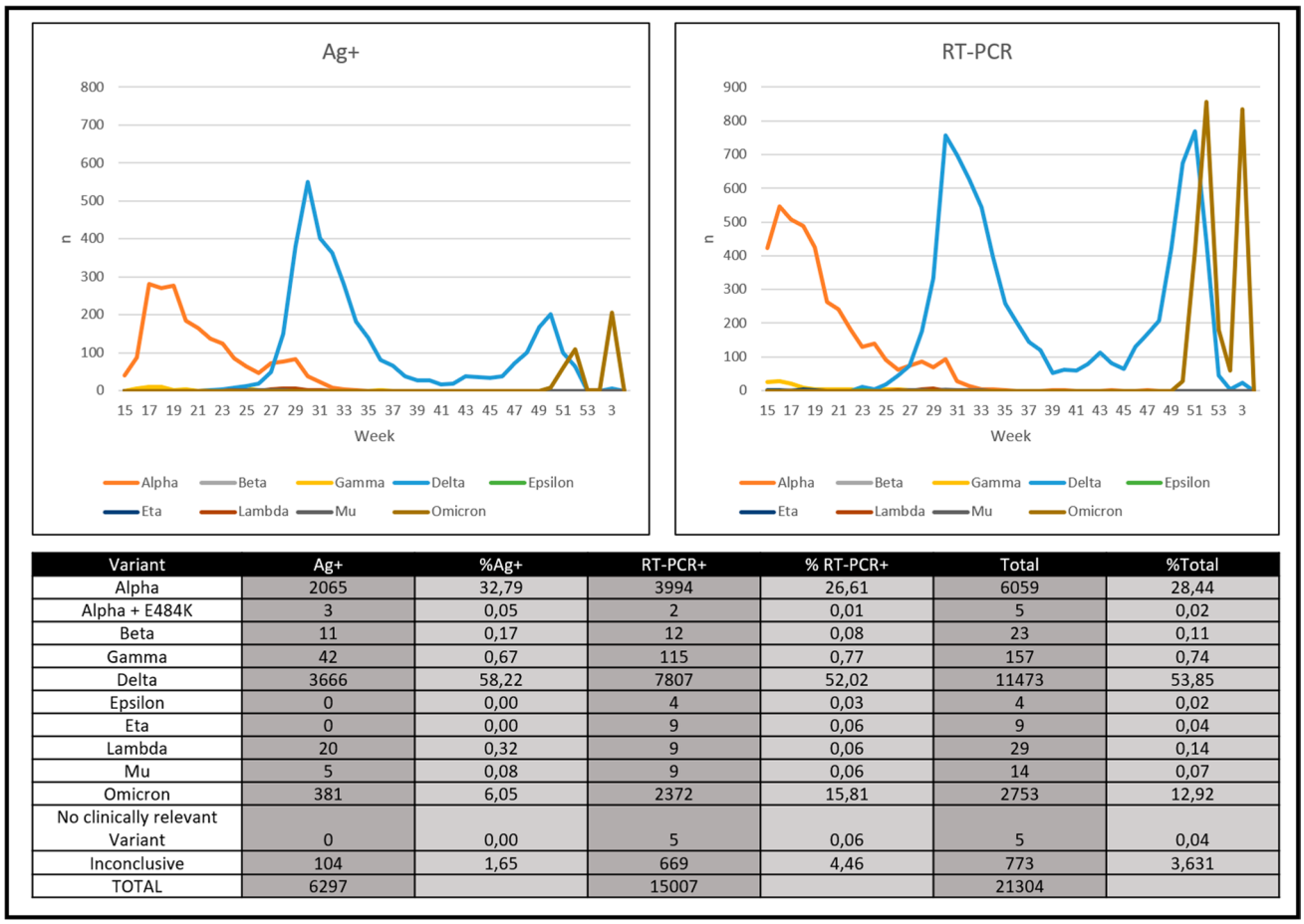
The less frequently found variants such as Epsilon, Eta, Lambda and Mu were correctly assigned by RT-PCR as they were later all confirmed by whole-genome sequencing data.

Conclusion

The results of including the recovered positive antigen devices in the routine variant screening algorithm are quite promising, as the performance of these samples at our study are comparable to the standard nasopharyngeal swabs, even obtaining a lower ratio of non-conclusive results (1.56% vs 4.12%) and lower RdRp Cts than the RT-PCR + group. The expected degradation of the sample without the adequate viral transport medium [17] is probably compensated by the higher viral loads of the samples of the Ag + group. On the one hand, antigen tests are often employed especially at the early stages of the infection [5] when the viral load is presumably higher and on the other hand, the sensibility of these tests rapidly fades with the increasing Cts of the sample [18–20].

Additionally, reusing these samples for variant tracking has several key advantages towards other approaches, especially towards not requiring a second nasopharyngeal swab specifically for determining the SARS-CoV-2 variant. Since a second appointment generates unnecessary duplicity that is time consuming, increases personal and material costs and generates an unnecessary hazard of having a positive patient again at an extraction centre increasing the transmission risk. All of this can be achieved with a little effort between clinical microbiology laboratories and the primary care centres and is a great example of the importance of the coordination between primary care and clinical microbiology laboratories.

Interestingly, the introduction of the Alpha variant coincides with the so-called fourth wave (March–June 2021) at our media, the fifth (July–September 2021) with the emergence of the Delta variant at the third phase of the study [21] and the sixth (December 2021–January 2022) with the



Variant	Ag+	%Ag+	RT-PCR+	% RT-PCR+	Total	%Total
Alpha	2065	32,79	3994	26,61	6059	28,44
Alpha + E484K	3	0,05	2	0,01	5	0,02
Beta	11	0,17	12	0,08	23	0,11
Gamma	42	0,67	115	0,77	157	0,74
Delta	3666	58,22	7807	52,02	11473	53,85
Epsilon	0	0,00	4	0,03	4	0,02
Eta	0	0,00	9	0,06	9	0,04
Lambda	20	0,32	9	0,06	29	0,14
Mu	5	0,08	9	0,06	14	0,07
Omicron	381	6,05	2372	15,81	2753	12,92
No clinically relevant Variant	0	0,00	5	0,06	5	0,04
Inconclusive	104	1,65	669	4,46	773	3,631
TOTAL	6297		15007		21304	

Fig. 1 Weekly and total distribution of variants found in both the recovered positive antigen devices group (Ag +) and the standard nasopharyngeal swabs group (RT-PCR +)

introduction of Omicron. This highlights the necessity of establishing fast and reliable methods for variant tracking.

Epsilon and Eta had a relatively limited spread in this study and where only recovered at the RT-PCR + group, a possible explanation for this could be that in our media most cases of these both variants were imported, and according to the ongoing protocol for variant tracking the diagnostic test chosen for returning travellers should be the RT-PCR. During the first weeks of our study, a local Gamma variant outbreak was under investigation, being RT-PCR the chosen technique for contact tracing. This could explain the preponderance of Gamma in the RT-PCR + group over the Ag + group during Phase I.

Remarkably Lambda and Mu variants were firstly detected in the Ag + group in cases were the suspicion of an imported Variant of Concern or Interest (different to Alpha or Delta) was not high, highlighting the importance of characterizing as many as possible community samples, including the positive antigen tests.

All in all, we think that including the recovered positive antigen devices in the routine variant screening algorithm is suitable, at both logistical and laboratory level, and very recommendable, as it is an important measure to have a better and more immediate picture of the circulating variants and allowing a swift response to the apparition of imported or new variants of clinical relevance in the community.

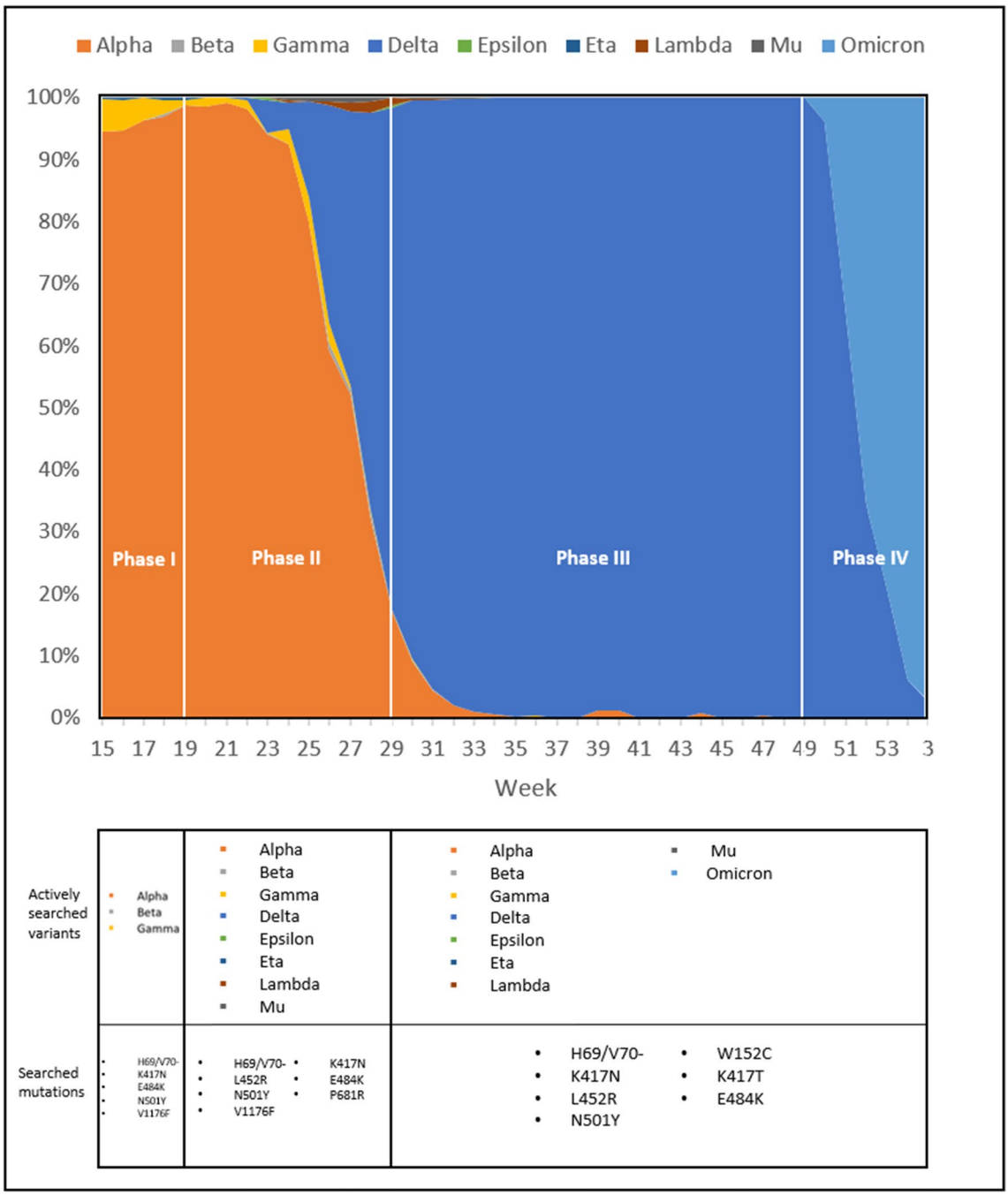


Fig. 2 Representation of the most frequent variant in each Phase of this Study: Phase I (weeks 15–19), Phase II (weeks 20–29), Phase III (weeks 30–49) and Phase IV (50–3) [during last week of 2021 and the first 2 weeks of 2022 only a representative fraction of the positive samples]

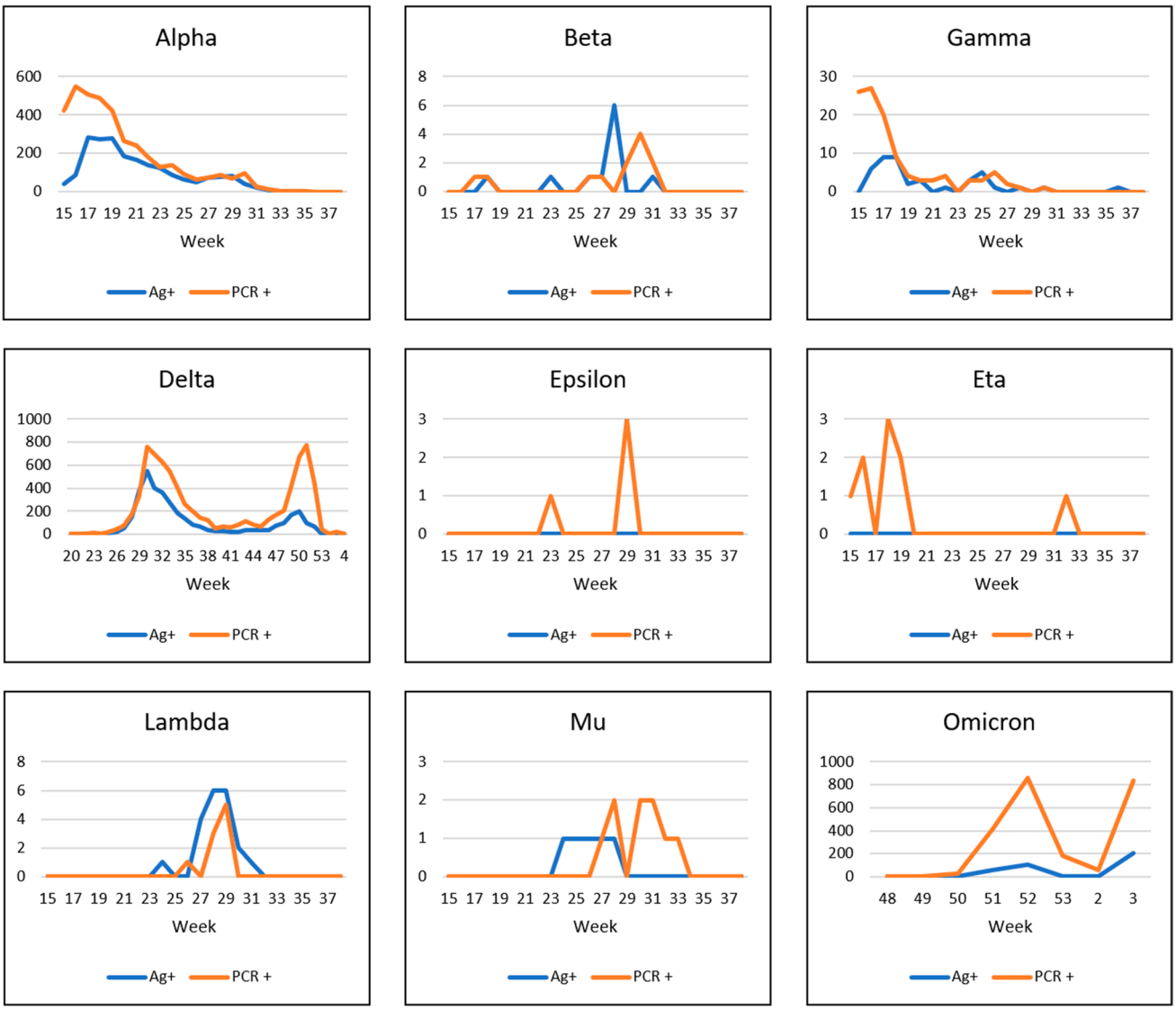


Fig. 3 Weekly distribution of Alpha, Beta, Gamma, Delta, Epsilon, Eta, Lambda, Mu and Omicron variants

Author Contributions MUG participated in the conceptualization, data curation and analysis as well as in the writing of the original draft. All other authors contributed to the writing of the Manuscript. All authors work in the Clinical Microbiology Laboratory led by JLDdTdA.

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Data Availability The analysed data are available under request.

Code Availability Not applicable.

Declarations

Conflict of interest The authors declare no conflict of interest or competing interests.

Ethical Approval Ethical approval for this study was obtained from the Investigation Ethics Committee of the Basurto University Hospital.

Consent to Participate Not applicable.

Consent for Publication Not applicable.

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