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SARS-CoV-2 Genomic Surveillance and Reliability of PCR Single Point Mutation Assay (SNPsig® EscapePLEX) for the Rapid Detection of Variants of Concern in Cameroon

--Manuscript Draft--

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Abstract:	Background: Surveillance of SARS-CoV-2 variants of concern (VOC) and lineages is crucial for decision-making.Our objective was tostudy the SARS-CoV-2 clade dynamics across epidemiological waves and evaluate the reliability of SNP EscapePLEX-kit in detecting VOC in Cameroon. Material and Methods: A laboratory-based study was conducted on SARS-CoV-2 positive nasopharyngeal specimens (Ct-value<30) at the Chantal BIYA International Reference Centre in Yaoundé-Cameroon, between April-2020 to August-2022. For each sample, Sanger-sequencing and SNP-EscapePLEX-kit were performed, using sequencing as gold standard to evaluate the performance of SNP-EscapePLEX. Results: Of the 130 sequences generated, SARS-CoV-2 clades during wave-1 (April-November 2020) showed 97%(30/31) wild-type lineages and 3%(1/31) Gamma-variant; wave-2 (December-2020 to May-2021), 25%(4/16) Alpha-variant, 25%(4/16) Beta-variant, 44%(7/16) wild-type and 6%(1/16) mu; wave-3 (June-October 2021), 94%(27/29) Delta-variant, 3%(1/29) Alpha-variant, 3%(1/29) wild-type; wave-4 (November-2021 to August-2022), 98%(53/54) Omicron-variant and 2%(1/54) Delta-variant. Omicron sub-variants were BA.1(47%), BA.5(34%), BA.2(13%) and BA.4(6%). Overall sensitivity and specificity of SNP-Escaplex was 84%[78-87] and 89%[76-95] respectively, with 75%[63-76] and 100%[96-100] respectively for Delta-variant; and 96%[90-96] and 100%[93-100] for Omicron-variants. Conclusion: Genomic surveillance reveals a rapid dynamic in SARS-CoV-2 strains between epidemiological waves in Cameroon. For wide variant surveillance in resource-limited settings, EscapePLEX-kit represents a suitable tool, pending upgrading for distinguishing Omicron sub-lineages. Keywords: SARS-CoV-2, variants of concern, SNPsig®EscapePLEX , Cameroon Background: Surveillance of SARS-CoV-2 variants of concern (VOC) and lineages is crucial for decision-making.Our objective was to study the SARS-CoV-2 clade dynamics across epidemiological waves and evaluate the reliability of SNP EscapePLEX-kit in detecting VOC in Cameroon. Material and Methods: A laboratory-based study was conducted on SARS-CoV-2 positive nasopharyngeal specimens (Ct-value<30) at the Chantal BIYA International Reference Centre in Yaoundé-Cameroon, between April-2020 to August-2022. For each sample, Sanger-sequencing and SNP-EscapePLEX-kit were performed, using sequencing as gold standard to evaluate the performance of SNP-EscapePLEX. Results: Of the 130 sequences generated, SARS-CoV-2 clades during wave-1 (April-November 2020) showed 97%(30/31) wild-type lineages and 3%(1/31) Gamma-variant; wave-2 (December-2020 to May-2021), 25%(4/16) Alpha-variant, 25%(4/16) Beta-variant, 44%(7/16) wild-type and 6%(1/16) mu; wave-3 (June-October 2021), 94%(27/29) Delta-variant, 3%(1/29) Alpha-variant, 3%(1/29) wild-type; wave-4 (November-2021 to August-2022), 98%(53/54) Omicron-variant and 2%(1/54) Delta-variant. Omicron sub-variants were BA.1(47%), BA.5(34%), BA.2(13%) and BA.4(6%). Overall sensitivity and specificity of SNP-Escaplex was 84%[78-87] and 89%[76-95] respectively, with 75%[63-76] and 100%[96-100] respectively for Delta-variant; and 96%[90-96] and 100%[93-100] for Omicron-variants. Conclusion: Genomic surveillance reveals a rapid dynamic in SARS-CoV-2 strains between epidemiological waves in Cameroon. For wide variant surveillance in resource-limited settings, EscapePLEX-kit represents a suitable tool, pending upgrading for distinguishing Omicron sub-lineages.
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To the Editor-In-Chief,
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SUBMISSION OF MANUSCRIPT FOR PUBLICATION

We herein submit our manuscript entitled "**SARS-CoV-2 Genomic Surveillance and Reliability of PCR Single Point Mutation Assay (*SNPsig®EscapePLEX*) for the Rapid Detection of Variants of Concern in Cameroon**", for publication in **Journal of Virological Methods**.

With the rapid spread and evolution of SARS-CoV2 preoccupying variants of concern (Alpha, Beta, Gamma, Delta, Omicron), countries all over the globe have set-up various ranges of tools and public approaches to adequately respond to the pandemic. Our manuscript hereby present results from a simplified genotyping kit based on qPCR-multiplex approach, implemented in Cameroon as an alternative to high throughput sequencing, and developed to increase the feasibility and efficiency of genomic surveillance of SARS-CoV-2 variants in low and middle income countries. In the frame of the genomic surveillance of SARS-CoV-2 in Cameroon, our objective was to describe the SARS-CoV-2 dynamics across epidemiological waves and evaluate the reliability of SNP EscapePLEX-kit in detecting all variants of concerns. Sharing this evidence with readers of the **Diagnostic Microbiology& Infectious Disease** (clinicians, virologists and policies-makers) will contribute substantially in limiting the spread and adequately framing the response against SARS-CoV2 not just across other resource-limited settings, but even beyond.

The manuscript has not been submitted and is not under consideration elsewhere. All authors approved this final version.

Thank you in advance for your time and attention, and we hope that our manuscript will be deemed acceptable for publication in the *Heliyon* .

Cordially yours,

Dr Joseph Fokam
The Corresponding author, on behalf of the co-author

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SARS-CoV-2 Genomic Surveillance and Reliability of PCR Single Point Mutation Assay (*SNPsig®EscapePLEX*) for the Rapid Detection of Variants of Concern in Cameroon

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Abstract

Background: Surveillance of SARS-CoV-2 variants of concern (VOC) and lineages is crucial for decision-making.Our objective was to study the SARS-CoV-2 clade dynamics across epidemiological waves and evaluate the reliability of SNP EscapePLEX-kit in detecting VOC in Cameroon.

Material and Methods: A laboratory-based study was conducted on SARS-CoV-2 positive nasopharyngeal specimens (Ct-value<30) at the Chantal BIYA International Reference Centre in Yaoundé-Cameroon, between April-2020 to August-2022. For each sample, Sanger-sequencing and SNP-EscapePLEX-kit were performed, using sequencing as gold standard to evaluate the performance of SNP-EscapePLEX.

Results: Of the 130 sequences generated, SARS-CoV-2 clades during wave-1 (April-November 2020) showed 97%(30/31) wild-type lineages and 3%(1/31) Gamma-variant; wave-2 (December-2020 to May-2021), 25%(4/16) Alpha-variant, 25%(4/16) Beta-variant, 44%(7/16) wild-type and 6%(1/16) mu; wave-3 (June-October 2021), 94%(27/29) Delta-variant, 3%(1/29) Alpha-variant, 3%(1/29) wild-type; wave-4 (November-2021 to August-2022), 98%(53/54) Omicron-variant and 2%(1/54) Delta-variant. Omicron sub-variants were BA.1(47%), BA.5(34%), BA.2(13%) and BA.4(6%). Overall sensitivity and specificity of *SNP-Escaplex* was 84%[78-87] and 89%[76-95] respectively, with 75%[63-76] and 100%[96-100] respectively for Delta-variant; and 96%[90-96] and 100%[93-100] for Omicron-variants.

Conclusion: Genomic surveillance reveals a rapid dynamic in SARS-CoV-2 strains between epidemiological waves in Cameroon. For wide variant surveillance in resource-limited settings, EscapePLEX-kit represents a suitable tool, pending upgrading for distinguishing Omicron sub-lineages.

Keywords: SARS-CoV-2, variants of concern, SNPsig®EscapePLEX , Cameroon

1 Introduction

Coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), has spread worldwide with several implications (Chen et al., 2020). In Africa, 53 countries have been affected so far, with 12,059,691 confirmed cases (with 255,698 deaths and 11,433,772 recoveries) (PHEOCC, 2022). In Cameroon, the first case of SARS-CoV-2 detection was reported on 6 March 2020. The infection then spread rapidly and nationwide, with up to 123 480 confirmed cases, 1 957 deaths, 120 773 recoveries (recovery rate: 97.8%), and 1 561 462 people received at least one dose of vaccine (Ministry of Public Health, 2022). Alongside these figures, with anecdotal clinical implications, SARS-CoV-2 variants of concern (VOC) have emerged and circulated around the world (Gabutti et al., 2020; Sanyaolu et al., 2021).

On 11 May 2021, the World Health Organisation (WHO) designated four different VOCs, including the B.1.1.7 (Alpha lineage ; UK in September 2020), B.1.351 (Beta lineage ; South Africa in May 2020), P.1 (Gamma lineage; Brazil in November 2020) et B.1.617.2 (Delta lineage ; India in October 2020) (WHO, 2021).WHO designated the Pango B.1.1.529 line as Omicron (first case reported from South Africa, in November 2021), a VOC which has spread rapidly around the world (Weil et al., 2022). Each of these VOCs is characterized by a combination of mutations, some of which may also increase the virulence of SARS-CoV-2 and its ability to evade vaccines or other social and public health measures (WHO, 2021).During the SARS-CoV-2 pandemic, genomic epidemiological surveillance around the world became crucial to monitoring the emergence of new variants. Current methods for the detection and characterisation of SARS-CoV-2 variants including: Next generation sequencing (NGS), Spike gene Sanger-based sequencing and screening SNP (Single nucleotide polymorphism) assays. The NGS essay is the gold standard for the identification of SARS-CoV-2 variants. However, this technique requires quite a lot of time to implement and is not

financially accessible for resource-limited settings (RLS) (Pennell et al., 2019; Pereira et al., 2020). Beside a well implemented Sanger sequencing approach (notably through Homemade protocols), PCR Point Mutation Assay (SNPsig®EscapePLEX) can be an alternative for the identification of VOCs, as it can provide very fast results with less consumables and reagents. Of note, scientific evidence shows that the Sanger method is very advantageous in terms of analysis time (15 hours for Sanger sequencing versus 51 hours for NGS) and cost per sample (20 times lower for Sanger sequencing) (Dorlass et al., 2021).

For rapid interventions in response to the evolution of SARS-CoV-2 variants, Cameroon has acquired a simplified genotyping kit, SNPsig®EscapePLEXsig of the "NOVACYT GROUP" company (www.novacyt.com). This kit was developed to increase the feasibility and efficiency of genomic surveillance of SARS-CoV-2 variants in low and middle income countries (LMIC) as an alternative to sequencing method. The SNPsig®-SARS-CoV-2-EscapePLEX assay is a new product designed for in vitro molecular diagnostics for allelic discrimination of SARS-CoV-2 VOCs. The procedure follows the standard real-time PCR method. This multiplex qPCR typing method allows discriminatory and simultaneous identification of four clinically significant mutations in the SARSCoV-2 Spike genome: E484K, K417N, K417T et P681R (NOVACYT, 2021). However, it is important to note that existing SNP assays may fail to detect or identify newly emerging variants that do carry a specific due to amino acid substitutions at sites affecting the primer/probe binding. Given the low-level of evidence on genomic surveillance of SARS-CoV-2 variants in RLS, and the need for SARS-CoV-2 rapid variant detection, it would be of paramount importance to understand the changes in circulating viral clades and their potential impacts on the performance of rapid variant detection assays available locally.

Within the frame of genomic surveillance, we therefore sought to study the dynamics of viral lineages/variants and to evaluate the performance of SNPsig®EscapePLEXsig kit as a rapid point mutation assay for SARS-CoV-2 clade surveillance in RLS like Cameroon.

2 Materials and methods

2.1 Study design

Study type and population: A laboratory-based study was conducted from April 2020 throughout August 2022 on nasopharyngeal specimens of individuals confirmed positive to COVID-19 by real-time PCR at the Virology Laboratory of the "Chantal BIYA" International Reference Centre in Yaoundé, Cameroon. Briefly, CIRCB a national reference laboratory for SARS-CoV-2 molecular testing and a reference centre genomic surveillance at country-level.

Clinical specimens: The main criteria for sample selection were the mean of cycle threshold (Ct) value under 35 (ORF1ab, N genes; for *DaAn*Gene RT-PCR assay).

2.2 Detection of SARS-CoV-2 VOC with Sanger Spike-sequencing

Following experience in Sanger sequencing protocol used as standard for viral genotyping (Fokam et al., 2022, 2011; Takou et al., 2019), the protocol for sequencing of SARS-CoV-2 was designed using new primers to amplify a fragment of the SARS-CoV-2 genome encoding part of the Spike protein.

2.2.1 RNA extraction:

Viral RNA was manually extracted from 200 µL nasopharyngeal clinical swabs samples using the *DaAn*Gene viral RNA Mini kit according to the manufacturer's protocol (www.daangene.com). SARS-CoV-2 -RNA was then processed directly for conventional reverse transcription and amplification.

2.2.2 Reverse transcription and PCR amplification:

Viral RNA was retro-transcribed and amplified using the kit One-Step Invitrogen (SuperScript® One-Step for long templates RT/ PCR; Foster City, CA) and 2 different sequence-specific primers (5'-3'): **38F (-**GTCAGTGTGTTAATCTTACAACCAG-**)** as the as the forward, and **1191R (-**TGCATAGACATTAGTAAAGCAGAGA-**)** as the reverse, (the given position refers to the Wuhan strain of SARS-CoV-2). The RT-PCR reaction contained for for each sample 25 µl reaction mix, 8 µl MgSO 4 (5 mM), 3 µl DNAse- and RNAse-free water, 0.75 µl sense primer (10 mM stock), 0.75 µl antisense primer (10 mM stock), 1 µl RNAseOUT (5 U/ µl Invitrogen), 1.5 µl RT-Taq (Superscript III RT/Platinum high fidelity) and 10 µl of extracted RNA. The RT-PCR conditions consisted of an initial step of 1 cycle at 50°C for 30 min; 1 cycle of 94°C for 2 min; 40 cycles (95°C, 30 s; 52°C, 30 s; 72°C, 90 s); a final elongation step of 1 cycle at 72°C for 10 min. The expected cDNA is about 1200 base pairs (bp)in length (position 38[orf]-1191 [orf]). For each PCR reaction, positive and negative controls were used to ensure the effectiveness of the reaction and the absence of cross-contamination, respectively. Amplification results were revealed after agarose-gel electrophoresis and positive results were kept for the sequencing process. Then PCR products were purified through the ExoSAP-IT™ kit (Applied Biosystems™, Lithuania).

2.2.3 Sequencing reaction (Sanger method):

The amplified products from the ORF region were completely sequenced in the sense and antisense orientations using an automated sequencer (ABI 3500 Genetic Ana- lyzer) with four different overlapping sequence-specific primers: **38F**,**514F(**TCTCAGCCTTTTCTTATGGACCT**),** **655R (**CCTGAGGGAGATCACGCACTA**)** and **1191F**. The reaction mixture for the sequencing reaction contained 1.5 µl ABI PRISM Big Dye Terminator V3.1, 6.5 µl big dye diluent (from the kit), 4.8 µl DNAse/ RNAse-free water water, 3.2 µl primer (1 µM stock) and 2 µl of purified cDNA .The sequencing conditions were as follows: 35 cycles (96°C, 10 s; 55°C, 10 s; 60°C, 4 min); 1 cycle of 4°C for 30 min. The sequencing product was purified by gel filtration

chromatography using Sephadex G-50 resin (Sigma-Aldrich) in order to eliminate excess primers, unincorporated dideoxynucleotides (ddNTPs), and salts. Capillary electrophoresis was performed using an Applied Biosystems 3500 genetic analyzer (Applied Biosystems, Tokyo, Japan).

2.2.4 SARS-CoV-2 sequence analysis

The sequences were aligned assembled and edited by the reference sequence using SeqScape Version 2.7. Spike Sequence were interpreted using the COV19 database Stanford algorithm (https://covdb.stanford.edu) and NCBI (National Center for biotechnology information). SARS-CoV-2 Spike gene nucleotide sequences were submitted to GenBank using Bankit (https://www.ncbi.nlm.nih.gov/WebSub/).

2.3 Detection of SARS-CoV-2 VOC with SNP genotyping

SNP genotyping reaction was performed using the commercial SNPsig® SARS-CoV-2 (EscapePLEX CE) kit (PrimerDesign, UK); according to the manufacturer's protocol (PRIMER DESIGN, 2022). This kit can be used on any thermocycler able to detect fluorescence in **the FAM, HEX/VIC, ROX and Cy5** emission channels. The kit also includes primers for confirmation of a positive SARS-CoV-2 result. The kit contains all the necessary items to perform the test. The RT-qPCR reaction contained 5µL of RNA and 25ml of reaction mixture for each sample (10µL Master Mix OneStep, 1µL primers/probes, 4µL RNase/DNase free watter, un volume final de 15µL). After preparation of the reaction mixture for genotyping, the real-time PCR reaction was performed on a thermocycler (QuantStidio 7 Flex, Applied Biosystems, by thermos Fisher) according to the following program: reverse transcription for 10min at 55°C, enzyme activation for 2min at 95°C, 45 cycle of (denaturation for 10s at 95°C, hybridization and elongation for 60s at 60°C). Mutations and variants were interpreted according to the kit manufacturers' guidelines.

2.4 Data Analysis

Descriptive statistics were performed for socio-demographic data and clinical parameters wherever available. Median and interquartile range (IQR) were reported for continuous variables. Chi-square (χ^2) and Fisher's exact test were used for comparison, and the significance level was set at $P \leq 0.05$. The SNP-ExcaPLEX kit sensitivity and specificity, were computed with their 95% confidence interval (CI). Cohen's kappa was used to estimate inter-assay concordance and results were interpreted according to the criteria proposed by Landis & Koch: k=0.01-0.20 (poor concordance), k=0.21-0.40 (fair concordance), k=0.41-0.60 (moderate concordance), k=0.61-0.80 (strong concordance), and k=0.81-1.00 (almost perfect concordance)(Jr and Gg, 1977). The status of the sample was defined as "true positive"/"true negative" when sequencing data agreed with the results obtained with SNPsig SARS-CoV-2EscapePLEX and considered "false positive"/"false negative" when in disagreement.

3 Results

3.1 Characteristics of the study population

A total of 163 participants were enrolled in the study, consisting of 45.16% (70/163) males and 54.84% (85/163) females; the median [IQR] age was 37 [28-49] years (min=2; max=82); and 30% (49/163) of the study population reported a COVID-19 related symptom. Half of the study population had a PCR Ct-value below 19 [16-23] cycles (min=9; max=33.5).

3.2 Sanger sequencing assay performance

Out of the 163 positive nasopharyngeal specimens processed, 88.96 % (145/163) were successfully amplified after RT-PCR. Regarding sequencing (by use of the Sanger method), 130 Spike-sequences were successfully generated out of the 145 processed (89.65 %), giving an overall sequencing performance of 79.75% (130/163) [72.76-85.64] (95%CI).

3.3 SARS-CoV-2 Genetic diversity and lineage dynamics

According to the local SARS-CoV-2 molecular epidemiology, variants were found in 91(70 %) of 130 sequences. The dynamic of SARS-CoV-2 (Figure 1) during wave-1 (April-November 2020) showed 97% (30/31) wild-type lineages and 3% (1/31) Gamma-variant; wave-2 (December 2020-May 2021) showed 25% (4/16) Alpha-variant, 25% (4/16) Beta-variant, 44% (7/16) wild-type lineages and 6% (1/16) mu; wave-3 (June-October 2021) showed 93% (27/29) Delta-variant, 3.5% (1/29) Alpha-variant, 3.5% (1/29) wild-type lineages; wave-4 (November 2021-August 2022) showed 98% (53/54) Omicron-variant and 2% (1/54) Delta-variant. Omicron sub-variants were 47% (25/53) BA.1, 34% (18/53) BA.5, 13% (7/53) BA.2 and 6% (3/53) BA.4.

Univariate analysis showed that only the Omicron variant was significantly related to the clinical status of patients (OR=0.10[0.03-0.37]; P=0.001). The prevalence of this variant was significantly lower in symptomatic patients (p=0.01) (Table 1).

3.4 Clinical performance of SNP-EscapePLEX assay

The profile of each reported viral strain was not statistically significantly different between the two methods used – Sequencing vs. EscapePLEX, indicating possible interchangeability of these variant screening assays (Table 2).

Overall sensitivity and specificity of SNP-ESCAPLEX was 84%[78-87] and 89%[76-95] respectively, which fall within the range of an acceptable performance in accuracy for the detection of COVID-19 variants in circulation (Table 3).

According to viral strains, the sensitivity and specificity of SNP-Escaplex on Delta-variant was 75% [63-76] and 100% [96-100] respectively; the sensitivity and specificity of SNP-Escaplex on Omicron-variants was 96% [90-96] and 100% [93-100] respectively respectively, indicating a very high accuracy in detecting Omicron from other pre-existing SARS-CoV-2 variants (Table 4).

4 Discussion

The rapid emergence of SARS-CoV-2 variants requires immediate deployment of surveillance tools. Rapid detection of these variants is essential as their spread may have an impact on transmission rates, diagnostic procedures, disease severity, or vaccine response. To date, the monitoring of the occurrence and circulation of VOCs is almost exclusively done by NGS (Bhoyar et al., 2021; Chiara et al., 2021). This can be a huge constraint for global surveillance of SARS-CoV2 mutants, as the equipment and trained personnel to perform NGS are not widely available, particularly in resource-limited settings (RLS). Although it has not yet been licensed for identifying new variants, the SNPsig® SARS-CoV-2 kit (EscapePlex) is a rapid and cost-effective mean to discriminate between one of the previously characterised SARS-CoV2 variants. This study allowed us to assess the molecular epidemiology of VOCs, and then, in comparison to the Sanger sequencing result, to validate the performance of the SNPescaPLEX kit for rapid VOC screening nationwide.

The majority of our study population were asymptomatic (70%; 114/163), even in advent of omicron variant, which was found in this study to be very infectious but inversely proportional with symptomatology and thus disease severity. A previous study conducted in Cameroon observed a similar clinical profile, reporting specifically 4% of symptomatic patients (Fainguem et al., 2022). Other reports of studies conducted in different settings confirmed that the virus can infect without causing clinical manifestations (Oran and Topol, 2020;Meo et al., 2021).

In the analysis of the molecular epidemiology of SARS-CoV-2, Sanger genotyping allowed us to detect all VOCs in Cameroon, including Alpha, Beta, Gamma, Delta. Regarding the dynamics of VOC occurrence (Figure 1), the first epidemiological wave is characterised by a massive circulation of the lineage of origin. Gamma-VOC was identified in only one strain during this

period, suggesting that community transmission had not occurred in the country with this particular VOC, or the sample size was not representative. In the second wave we observe the appearance of the Alpha and Beta variants in co-circulation with the wild lineage. In the third wave we observe strong circulation of the Delta variant, with a significant decrease in the prevalence of the Alpha variant (3.5%). In the fourth wave, corresponding to the current wave, Omicron variant predominates at about 100%. This epidemiological profile observed in Cameroon since the beginning of the pandemic corresponds to that observed in several other countries of the world(Afrin et al., 2022; Eales et al., 2022; Fujino et al., 2021). The SARS-CoV-2 Omicron variant of concern (l B.1.1.529), which became dominant in many countries in early 2022, comprises several sub-variants. In our context, in the first quarter (January-March 2022) the BA.1 sub-variant was the only one in circulation (100%). This was quickly replaced by the sub-variants BA.2 at 75% (3/4) and BA.4 at 25% (1/4) in the second quarter (April-June 2022). In the third quarter, we observe the appearance of the BA.5 sub-variant (75%) which co-circulates mostly with BA.2 and BA.4. These results are still in line with the European epidemiological profile, which is driven by the BA.4 and BA.5 sub-variants (ECDC, 2022). This profile also reveals the absence of other omicron sub-variants such as BA.3 and recombinant forms in our context, probably suggesting a low efficacy of the primers used for these sub-lineages and sub-variants (Tegally et al., 2022).

Overall, Sanger sequencing demonstrated a success rate of approximately 79.75% (130/163). A similar result was obtained previously, reporting an estimated overall sequencing success rate of 85.1% (166/195) and suggested that low viremia is likely to be associated with sequence failure using the Sanger approach (Jørgensen et al., 2021); calling thus for these patients, to implement more sensitive sequencing methods such as the NGS – with the advantage of enabling whole genome sequencing for better appreciation of viral diversity.

When comparing the two methods (EscaPLEX versus Sanger), the prevalence rates of each variant were not significant (P>0.05) (Table3). However, as opposed to Sanger

sequencing, EscaPLEX could not identify the Gamma, implying potential low effectiveness of this new kit in detecting certain VOC as observed in a study in Burkina Faso, where the frequency of the Beta variant (56.6%) analysed with the SNPsig® SARS-CoV-2 kit (EscapePlex) was lower than with the SNPsig® VariPLEX™ kit (75.6%)(Soubeiga et al., 2022). As an alternative to sequencing, the SNP_SARSCoV-2-EscapePLEX Kit has received much attention as a genotyping test for the rapid detection and identification of SARS-CoV-2 VOCs (Chaintoutis et al., 2021; Umair et al., 2022). Overall, for its ability to discriminate between variants and wild-type strains, our analyses show that the SNP_SARSCoV-2-EscapePLEX kit has high sensitivity (84% [78-87]) and specificity (89% [76-95]), and would be suitable for preliminary identification of VOC in Cameroon. In terms of its ability to discriminate each SARSCoV-2 variant, this kit demonstrated good concordance with sequencing (Ka= 0.97 [0.93-0.98]) and a better ability to discriminate the Omicron variant with a sensitivity of 96% [93-98]. This result is in line with the manufacturer's finding of 100% sensitivity for the identification of the omicron-specific K417N mutation according to the interpretation algorithm (PRIMER DESIGN, 2022). However, in comparison to sequencing, our analysis reveals a zero sensitivity (0%) for the detection of the Gamma variant. This result is also in agreement with the manufacturer who found a null sensitivity for the identification of K417N (which, in association with E484K mutation confirms infection by a Gamma variant; see table2). This finding therefore advocates for an improvement of the clinical performance of the kit in order to fit to the molecular epidemiology within the country; enabling quick detection, and subsequent adequate response to circulating VOCs.

5 Conclusion

There is a rapid change in the molecular epidemiology of SARS-CoV-2 in Cameroon, moving from wild-type lineages to Omicron variants and sub-variants. This evidence underscores the need for genomic surveillance to support the pandemic control strategy. For the rapid detection of viral clades, SNPsig SARSCoV-2 EscapePLEX kit is suitable in identifying VOC circulating in RLS within simple PCR facilities available in sevral molecular

biology laboratories in RLS. With the emergence of Omicron sub-variants, rapid variant detection tools should be upgraded to distinguish between sub-variants.

Ethics considerations

Ethical approval was obtained from the Cameroon National Ethics Committee for research on human health (2022/01/1430/CE/CNERSH/SP), within the frame of the EDCTP (European and Developing 358 Countries Clinical Trials Partnership) PERFECT-Study (RIA2020-EF3000). A study information sheet was provided to each individual, and a written informed consent was obtained from all study participants. Confidentiality was ensured by the use of unique identifiers and data were kept in a password encrypted computer with limited access. Results were returned free of charge to each participant for a direct benefits on their clinical conditions with regards to COVID-19 infection.

Credit authorship contribution statement

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Data availability:

SARS-CoV-2 sequences generated in this study are available in Genbank under the following accession numbers: OQ248255 – OQ248384.

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Declaration of Competing Interest

The authors have declared no competing interest.

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References:

Afrin, S.Z., Islam, M.T., Paul, S.K., Kobayashi, N., Parvin, R., 2022. Dynamics of SARS-CoV-2 variants of concern (VOC) in Bangladesh during the first half of 2021. Virology 565, 29–37. https://doi.org/10.1016/j.virol.2021.10.005

Bhoyar, R.C., Jain, A., Sehgal, P., Divakar, M.K., Sharma, D., Imran, M., Jolly, B., Ranjan, G., Rophina, M., Sharma, S., Siwach, S., Pandhare, K., Sahoo, S., Sahoo, M., Nayak, A., Mohanty, J.N., Das, J., Bhandari, S., Mathur, S.K., Kumar, A., Sahlot, R., Rojarani, P., Lakshmi, J.V., Surekha, A., Sekhar, P.C., Mahajan, S., Masih, S., Singh, P., Kumar, V., Jose, B., Mahajan, V., Gupta, V., Gupta, R., Arumugam, P., Singh, A., Nandy, A., V, R.P., Jha, R.M., Kumari, A., Gandotra, S., Rao, V., Faruq, M., Kumar, S., G, B.R., G, N.V., Roy, S.S., Sengupta, A., Chattopadhyay, S., Singhal, K., Pradhan, S., Jha, D., Naushin, S., Wadhwa, S., Tyagi, N., Poojary, M., Scaria, V., Sivasubbu, S., 2021. High throughput detection and genetic epidemiology of SARS-CoV-2 using COVIDSeq next-generation sequencing. PLOS ONE 16, e0247115. https://doi.org/10.1371/journal.pone.0247115

Chaintoutis, S.C., Chassalevris, T., Balaska, S., Mouchtaropoulou, E., Tsiolas, G., Vlatakis, I., Tychala, A., Koutsioulis, D., Argiriou, A., Skoura, L., Dovas, C.I., 2021. A Novel Real-Time RT-PCR-Based Methodology for the Preliminary Typing of SARS-CoV-2 Variants, Employing Non-Extendable LNA Oligonucleotides and Three Signature Mutations at the Spike Protein Receptor-Binding Domain. Life 11, 1015. https://doi.org/10.3390/life11101015

Chen, N., Zhou, M., Dong, X., Qu, J., Gong, F., Han, Y., Qiu, Y., Wang, J., Liu, Y., Wei, Y., Xia, J., Yu, T., Zhang, X., Zhang, L., 2020. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. The Lancet 395, 507–513. https://doi.org/10.1016/S0140-6736(20)30211-7

Chiara, M., D'Erchia, A.M., Gissi, C., Manzari, C., Parisi, A., Resta, N., Zambelli, F., Picardi, E., Pavesi, G., Horner, D.S., Pesole, G., 2021. Next generation sequencing of SARS-CoV-2 genomes: challenges, applications and opportunities. Brief. Bioinform. 22, 616–630. https://doi.org/10.1093/bib/bbaa297

Dorlass, E.G., Lourenço, K.L., Magalhães, R.D.M., Sato, H., Fiorini, A., Peixoto, R., Coelho, H.P., Telezynski, B.L., Scagion, G.P., Ometto, T., Thomazelli, L.M., Oliveira, D.B.L., Fernandes, A.P., Durigon, E.L., Fonseca, F.G., Teixeira, S.M.R., 2021. Survey of SARS-CoV-2 genetic diversity in two major Brazilian cities using a fast and affordable Sanger sequencing strategy. Genomics 113, 4109–4115. https://doi.org/10.1016/j.ygeno.2021.10.015

Eales, O., de Oliveira Martins, L., Page, A.J., Wang, H., Bodinier, B., Tang, D., Haw, D., Jonnerby, J., Atchison, C., Ashby, D., Barclay, W., Taylor, G., Cooke, G., Ward, H., Darzi, A., Riley, S., Elliott, P., Donnelly, C.A., Chadeau-Hyam, M., 2022. Dynamics of competing SARS-CoV-2 variants during the Omicron epidemic in England. Nat. Commun. 13, 4375. https://doi.org/10.1038/s41467-022-32096-4

ECDC, E.C. for D.P. and C., 2022. Epidemiological update: SARS-CoV-2 Omicron sub-lineages BA.4 and BA.5 [WWW Document]. Eur. Cent. Dis. Prev. Control. URL https://www.ecdc.europa.eu/en/news-events/epidemiological-update-sars-cov-2-omicron-sub-lineages-ba4-and-ba5 (accessed 10.12.22).

Fainguem, N.N., Fokam, J., Ngoufack Jagni Semengue, E., Durand Nka, A., Takou, D., Ageboh Nkembi-leke, J., Alteri, C., Colagrossi, L., Yagai, R.B., Ambe Chenwi, C., Tchouaket Tommo, M.C., Angong Beloumou, G., Ka'e, A.C., Ndjeyep Djupsa, S.C., Abba, A., Heunko Yatchou, L.G., Nnomo Zam, K., Kamgaing, R., Sosso, S.M., Mama, L., Ndembi, N., Colizzi, V., Perno, C.-F., Cappelli, G., Ndjolo, A., 2022. High concordance in SARSCoV-2 detection between automated (Abbott m2000) and manual (DaAn gene) RT-PCR systems: The EDCTP PERFECT-Study in Cameroon. J. Public Health Afr. 13, 2163. https://doi.org/10.4081/jphia.2022.2163

Fokam, J., Ngoufack Jagni Semengue, E., Armenia, D., Takou, D., Dambaya, B., Teto, G., Chenwi, C.A., Nka, A.D., Beloumou, G.A., Ndjeyep, S.C.D., Tchouaket, M.C.T., Fainguem, N., Sosso, S.M., Colizzi, V., Perno, C.-F., Ndjolo, A., Ceccherini-Silberstein, F., Santoro, M.M., 2022. High performance of integrase genotyping on diverse HIV-1 clades circulating in Cameroon: toward a successful transition to dolutegravir-based regimens in low and middle-income countries. Diagn. Microbiol. Infect. Dis. 102, 115574. https://doi.org/10.1016/j.diagmicrobio.2021.115574

Fokam, J., Salpini, R., Santoro, M.M., Cento, V., D'Arrigo, R., Gori, C., Perno, C.F., Colizzi, V., Nanfack, A., Gwom, L.C., Cappelli, G., Takou, D., 2011. Performance evaluation of an in-house human immunodeficiency virus type-1 protease-reverse transcriptase genotyping assay in Cameroon. Arch. Virol. 156, 1235–1243. https://doi.org/10.1007/s00705-011-0982-3

Fujino, T., Nomoto, H., Kutsuna, S., Ujiie, M., Suzuki, T., Sato, R., Fujimoto, T., Kuroda, M., Wakita, T., Ohmagari, N., 2021. Novel SARS-CoV-2 Variant in Travelers from Brazil to Japan. Emerg. Infect. Dis. 27, 1243–1245. https://doi.org/10.3201/eid2704.210138

Gabutti, G., d'Anchera, E., Sandri, F., Savio, M., Stefanati, A., 2020. Coronavirus: Update Related to the Current Outbreak of COVID-19. Infect. Dis. Ther. 9, 241–253. https://doi.org/10.1007/s40121-020-00295-5

Jørgensen, T.S., Blin, K., Kuntke, F., Salling, H.K., Michaelsen, T.Y., Albertsen, M., Larsen, H., 2021. A rapid, cost efficient and simple method to identify current SARS-CoV-2 variants of concern by Sanger sequencing part of the spike protein gene. https://doi.org/10.1101/2021.03.27.21252266

Jr, L., Gg, K., 1977. The measurement of observer agreement for categorical data. Biometrics 33.

Meo, S.A., Meo, A.S., Al-Jassir, F.F., Klonoff, D.C., 2021. Omicron SARS-CoV-2 new variant: global prevalence and biological and clinical characteristics.

Ministry of Public Health;, 2022. COVID-19 Situation Report. CCOUSP. URL https://www.ccousp.cm/documentations/rapports-de-situation-covid-19/ (accessed 10.26.22).

NOVACYT, G., 2021. Variant Testing Update: SNPsig® SARS-CoV-2 EscapePLEXT. Novacyt. URL file:///C:/Users/pc/Downloads/imprim%C3%A9e_1_escapeplex_for_the_detection_of_omicron_technical_bulletin_2.pdf (accessed 10.4.22).

Oran, D.P., Topol, E.J., 2020. Prevalence of Asymptomatic SARS-CoV-2 Infection. Ann. Intern. Med. 173, 362–367. https://doi.org/10.7326/M20-3012

Pennell, N.A., Mutebi, A., Zhou, Z.-Y., Ricculli, M.L., Tang, W., Wang, H., Guerin, A., Arnhart, T., Dalal, A., Sasane, M., Wu, K.Y., Culver, K.W., Otterson, G.A., 2019. Economic Impact of Next-Generation Sequencing Versus Single-Gene Testing to Detect Genomic Alterations in Metastatic Non–Small-Cell Lung Cancer Using a Decision Analytic Model. JCO Precis. Oncol. 1–9. https://doi.org/10.1200/PO.18.00356

Pereira, R., Oliveira, J., Sousa, M., 2020. Bioinformatics and Computational Tools for Next-Generation Sequencing Analysis in Clinical Genetics. J. Clin. Med. 9, 132. https://doi.org/10.3390/jcm9010132

PHEOCC, P.H.E.O.C., 2022. Cameroon COVID-19 Situation Report. CCOUSP. URL https://www.ccousp.cm/documentations/rapports-de-situation-covid-19/ (accessed 12.20.22).

PRIMER DESIGN, 2022. Instruction for Use SNPsig® SARS-CoV-2 EscapePLEX™ CE 96 Tests 4–12.

Sanyaolu, A., Okorie, C., Marinkovic, A., Haider, N., Abbasi, A.F., Jaferi, U., Prakash, S., Balendra, V., 2021. The emerging SARS-CoV-2 variants of concern. Ther. Adv. Infect. Dis. 8, 20499361211024372. https://doi.org/10.1177/20499361211024372

Soubeiga, S.T., Charlotte, K., Zoure, A.A., Compaoré, T.R., Zida, S., Kagembega, A., Sagna, T., Dabiré, C., Ouedraogo, O., Lasisi, N., Kabore, A., Zida, F.M., Fofana, B., Somé, S., Nikiema, A., Kambire, D., Zongo, D., Soulama, I., Sawadogo, C., Gampini, S., Ouedraogo, H.G., 2022. SARS-CoV-2 Variants Screening in Burkina Faso. J. Med. Microbiol. Infect. Dis. 10, 135–140. https://doi.org/10.52547/JoMMID.10.3.135

Takou, D., Fokam, J., Teto, G., Santoro, M.-M., Ceccherini-Silberstein, F., Nanfack, A.J., Sosso, S.M., Dambaya, B., Salpini, R., Billong, S.C., Gori, C., Fokunang, C.N., Cappelli, G., Colizzi, V., Perno, C.-F., Ndjolo, A., 2019. HIV-1 drug resistance testing is essential for heavily-treated patients switching from first- to second-line regimens in resource-limited settings: evidence from routine clinical practice in Cameroon. BMC Infect. Dis. 19, 246. https://doi.org/10.1186/s12879-019-3871-0

Tegally, H., Moir, M., Everatt, J., Giovanetti, M., Scheepers, C., Wilkinson, E., Subramoney, K., Moyo, S., Amoako, D.G., Baxter, C., Althaus, C.L., Anyaneji, U.J., Kekana, D., Viana, R., Giandhari, J., Lessells, R.J., Maponga, T., Maruapula, D., Choga, W., Matshaba, M., Mayaphi, S., Mbhele, N., Mbulawa, M.B., Msomi, N., Consortium, N.-S., Naidoo, Y., Pillay, S., Sanko, T.J., San, J.E., Scott, L., Singh, L., Magini, N.A., Smith-Lawrence, P., Stevens, W., Dor, G., Tshiabuila, D., Wolter, N., Preiser, W., Treurnicht, F.K., Venter, M., Davids, M., Chiloane, G., Mendes, A., McIntyre, C., O'Toole, A., Ruis, C., Peacock, T.P., Roemer, C., Williamson, C., Pybus, O.G., Bhiman, J., Glass, A., Martin, D.P., Rambaut, A., Gaseitsiwe, S., Gottberg, A. von, Oliveira, T. de, 2022. Continued Emergence and Evolution of Omicron in South Africa: New BA.4 and BA.5 lineages. https://doi.org/10.1101/2022.05.01.22274406

Umair, M., Ikram, A., Rehman, Z., Haider, S.A., Ammar, M., Badar, N., Ali, Q., Rana, M.S., Salman, M., 2022. Genomic diversity of SARS-CoV-2 in Pakistan during the fourth wave of pandemic. J. Med. Virol. 94, 4869–4877. https://doi.org/10.1002/jmv.27957

Weil, A.A., Luiten, K.G., Casto, A.M., Bennett, J.C., O'Hanlon, J., Han, P.D., Gamboa, L.S., McDermot, E., Truong, M., Gottlieb, G.S., Acker, Z., Wolf, C.R., Magedson, A., Chow, E.J., Lo, N.K., Pothan, L.C., McDonald, D., Wright, T.C., McCaffrey, K.M., Figgins, M.D., Englund, J.A., Boeckh, M., Lockwood, C.M., Nickerson, D.A., Shendure, J., Bedford, T., Hughes, J.P., Starita, L.M., Chu, H.Y., 2022. Genomic surveillance of SARS-CoV-2 Omicron variants on a university campus. Nat. Commun. 13, 5240. https://doi.org/10.1038/s41467-022-32786-z

WHO, W.H.O., 2021. Tracking SARS-CoV-2 variants [WWW Document]. URL https://www.who.int/activities/tracking-SARS-CoV-2-variants (accessed 10.4.22).

Figure and Tables

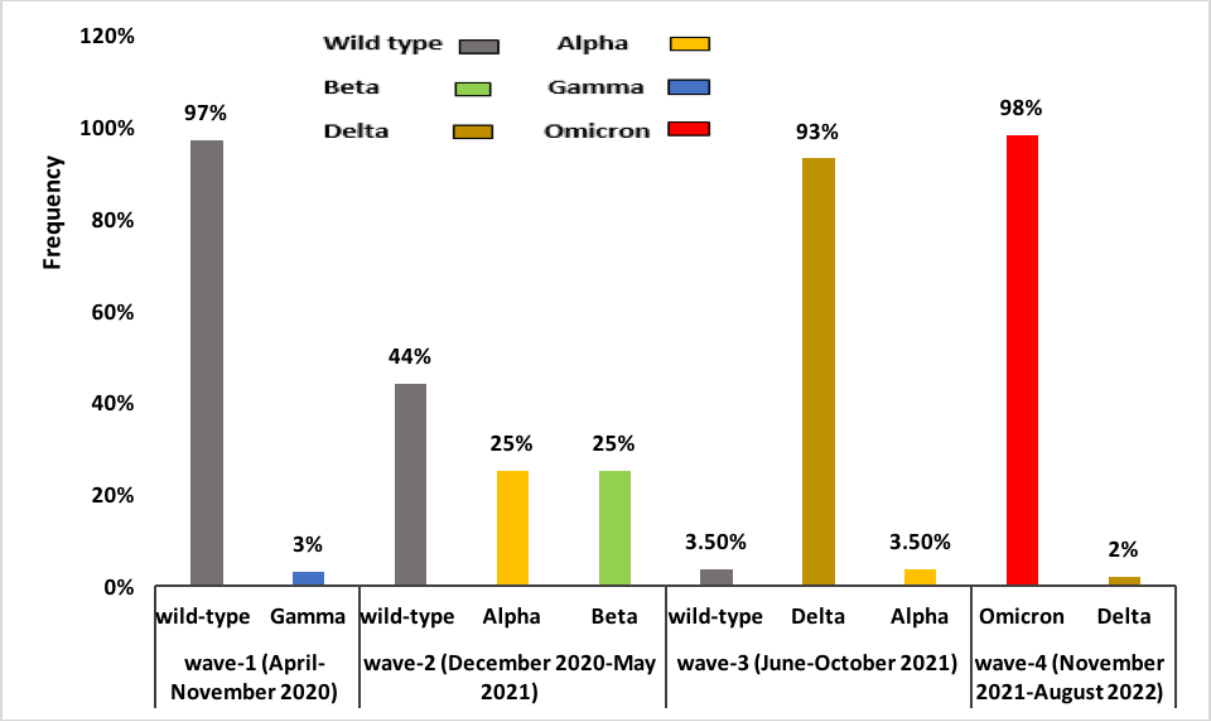

Figure 1: Dynamics of SARS-CoV-2 strains across different waves

Table 1: Univariate analysis of biological and clinical characteristics and SARSCoV-2 variants Of Concern.

Variant	Clinical status		OR (IC95%)	P-Value
	Asymptomatic	Symptomatic		
Lineage of origin	24	15	1	
Alpha	3(60%)	2(40%)	1.11[0.16-7.40]	0.91
Beta	1(33.33%)	2(66.66%)	3.33[0.28-39.01]	0.34
Gamma	0	1(100%)	--	--
Delta	17(60.71%)	11(39.28%)	1.08[0.40-8-2.90]	0.87
Omicron	50(94.33%)	3(05.67%)	0.10[0.03-0.37]	0.001

Table 2: SARS-CoV-2 Variant and Lineage of origin detected using Sanger sequencing and SNP- ExcaPLEX kits.

VOCs	Sanger sequencing	SNP- ExcaPLEX	P-Value
Wild-type	30.00 (39/130)	35.38 (46/130)	0.35
Alpha	3.85(5/130)	6.92 (9/130)	0.24
Beta	2.31(3/130)	2.31 (3/130)	1.00
Gamma	0.77(1/130)	0.00 (0/130)	0.32
Mu	0.77(1/130)	0.00 (0/130)	0.32
Delta	21.54(28/130)	16.15 (21/130)	0.26
Omicron	40.77(53/130)	39.23(51/130)	0.80

Table 3: Clinical Performance of SNPsig SARS-CoV-2 EscapePLEX

		Sequencing result		
		Sars-cov-2 Variant	Ligneage of origine	Total
SNP-EscapePLEX)	Sars-cov-2 Variant	78	4	82
	Ligneage of origine	15	33	48
Total		93	37	130
Sensitivity (Se)=84%[78-87]; Speficity(Sp)=89%[76-95]; Ka(Kappa)= 0.67 [0.51-076]				

Table4: Intrinsic features of RT-PCR genotyping Kit

Spike-genome sequencing		
SNP-EscapePLEX		
(A) Alpha-variant vs Non Alpha		
	Alpha	*Non Alpha*
Alpha	*78*	*4*
Non Alpha-	*15*	*33*
Se=84% [78-86]; Sp=89%[76-96]; PPV=95%[89-98] ; NVP=69%[59-74] Ka= 0.67 [0.51-0.76]		
(B) Beta-variant vs Non Beta		
	Beta	*Non Beta*
Beta	*2*	*2*
Non Beta	*1*	*125*
Se=67% [14-97]; Sp=98%[97-99]; PPV=50%[10-76] ; NVP=99%[97-1000] Ka= 0.56 [0.09-0.83]		
(C) Gamma-variant vs Non Gamma		
	Gamma	*Non Gamma*
Gamma	*0*	*0*
Non Gamma	*1*	*129*
Se=0% ; Sp=100%		
(D) Delta-variant vs Non Delta		
	Delta	*Non Delta*
Delta	*21*	*0*
Non Delta	*7*	*102*
Se=75% [63-75] ; Sp=100% [96-100]; PPV=100%[84-100];NVP=94%[74-94] Ka= 0.82 [0.65-0.83]		
(E) Omicron-variant vs Non Omicron		
	Omicron	*Non Omicron*
Omicron	*51*	*0*
Non Omicron	*2*	*77*
Se=96% [93-98]; Sp=100% [95-100]; PPV=100%[94-100] ; NVP=97%[95-100] Ka= 0.97 [0.93-0.98]		
Se=Sensitivity; Sp= Specificity; PPV= Positive Predictive Value; NPV= Negative Predictive Value		

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: