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Research article

# SARS-CoV-2 genomic surveillance and reliability of PCR single point mutation assay (*SNPsig® SARS-CoV-2 EscapePLEX CE*) for the rapid detection of variants of concern in Cameroon

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ABSTRACT

*Background:* Surveillance of SARS-CoV-2 variants of concern (VOCs) 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 SNPsig® SARS-CoV-2 EscapePLEX *CE* in detecting VOCs in Cameroon.

*Material and methods:* A laboratory-based study was conducted on SARS-CoV-2 positive nasopharyngeal specimens cycle threshold (Ct)≤30 at the Chantal BIYA International Reference

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Centre in Yaoundé-Cameroon, between April-2020 to August-2022. Samples were analyzed in parallel with Sanger sequencing and (SNPsig® SARS-CoV-2 EscapePLEX CE), and performance characteristics were evaluated by Cohen's coefficient and McNemar test.
*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 %). Globally, the two genotyping methods accurately identified the SARS-CoV-2 VOCs (P = 0.17, McNemar test; Ka = 0.67).
*Conclusion:* Genomic surveillance reveals a rapid dynamic in SARS-CoV-2 strains between epidemiological waves in Cameroon. For wide-spread variant surveillance in resource-limited settings, SNPsig® SARS-CoV-2 EscapePLEX CEkit represents a suitable tool, pending upgrading for distinguishing Omicron sub-lineages.

## 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 [1]. In Africa, 53 countries have been affected as of 2022 with 12,059,691 confirmed cases, with 255,698 deaths and 11,433,772 recoveries [2]. In Cameroon, the first case of SARS-CoV-2 was reported on 6 March 2020. It then spread rapidly nationwide, and by the end of December 2022, there were 123,480 confirmed cases, including1 957 deaths, 120,773 recoveries (recovery rate: 97.8 %), and 1,561,462 people had received at least one dose of vaccine (Ministry of Public Health, 2022) [3]. Alongside these figures, with anecdotal clinical implications, SARS-CoV-2 variants of concern (VOC) have emerged and circulated around the world [4,5].

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) [6].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 [7]. 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 [6]. During the SARS-CoV-2 pandemic, global genomic epidemiological surveillance became crucial to monitoring the emergence of new variants. Current methods for the detection and characterisation of SARS-CoV-2 variants include either sequencing or typing by Single nucleotide polymorphism (SNP) assays. Of note, sequencing is the gold standard for the identification of SARS-CoV-2 variants while SNP is used as a near point-of-care approach for typing of variants in settings with limited laboratory infrastructure. Sequencing is time consuming, cumbersome and is not financially affordable for resource-limited settings (RLS) [8,9]. In this frame, polymerase chain reaction (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. As a conventional sequencing method, Sanger-based assay is advantageous in terms of analysis time and number of samples processed and cost per sample [10]. Nonetheless, partial genome sequencing, on targeted genes of interest, does not allow the detection of recombination and new/additional mutations in non-sequenced areas of the genome.

For rapid interventions in response to the evolution of SARS-CoV-2 variants, Cameroon has acquired a simplified genotyping kit, SNPsig® SARS-CoV-2 EscapePLEX CE 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 resource-limited settings (RLS) as an alternative to sequencing method. The SNPsig® SARS-CoV-2 EscapePLEX CE assay is a new product designed for *in vitro* molecular diagnostics for allelic discrimination of SARS-CoV-2 VOCs (specifically alpha, beta, gamma, delta and omicron variants; see Table 1). 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, K417 N, K417T et P681R [11]. However, it is important to note that existing SNP assays may fail to detect or identify newly emerging variants that carry mutations due to amino

**Table 1**
Table showing the differential mutation profiles exhibited by Alpha Beta, Gamma, Delta, and Omicron SARS-CoV-2 variants with SNPsig® SARS-CoV-2 EscapePLEX CE.

| FAM (E484K) | HEX (K417 N) | ROX (K417T) | Cy5 (P681R) | Result | Indicative of: |
|---|---|---|---|---|---|
| - | + | - | - | Positive for K417 N | Omicron |
| - | - | - | + | Positive for P681R | Delta |
| - | + | - | + | Positive for | Delta with K417 N |
| + | + | - | - | Positive for E484K and K417 N | Beta |
| + | - | + | - | Positive for E484K and K417T | Gamma |
| + | - | - | - | Positive for E484K | Alpha with E484 |
| - | - | - | - | Negative* | Wild type, or other variant |

acid substitutions at sites affecting the primer/probe binding. Given the low-level of 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.

Our study objectives were: (1) to describe the dynamics of viral lineages/variants according waves, and (2) to evaluate the performance of SNPsig® SARS-CoV-2 EscapePLEX CE kit (rapid point mutation assay) for SARS-CoV-2 clade detection.

## 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 (symptomatic or asymptomatic) for COVID-19 by real-time PCR at the Virology Laboratory of the "Chantal BIYA" International Reference Centre (CIRCB) in, Cameroon. CIRCB is a national reference laboratory for SARS-CoV-2 molecular testing and a reference centre for genomic surveillance at country-level.

**Clinical specimens:** The main criteria for sample selection were the cycle threshold values (Ct) $\leq$30 after real-time PCR by DaAn Gene RT-PCR assay that targets the SARS-CoV-2 open reading frame (ORF) and nucleocapsid (N) genes.

### 2.2. Detection of SARS-CoV-2 VOC with Spike gene sanger-based sequencing (gold standard)

Following experience in viral Sanger sequencing protocols [12–14], the protocol for sequencing of SARS-CoV-2 was designed using new primers targeting around 1200bp of the Spike protein within SARS-CoV-2 genome, including the receptor binding domain (RBD). Of note, Spike RBD is relevant to SARS-CoV-2 infectivity, transmission, and antibody-mediated neutralization [15,16]. This region is used to identify SARSCoV-2-VOCs [17,18].

#### 2.2.1. RNA extraction

Viral RNA was manually extracted from 200 μL nasopharyngeal clinical swabs samples using the DaAnGene 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 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 each sample 25 μl reaction mix, 8 μl MgSO 4 (5 μM), 3 μl DNAse- and RNAse-free water, 0.75 μl sense primer (10 μM stock), 0.75 μl antisense primer (10 μM 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 Analyzer) with four different overlapping sequence-specific primers: **38F**, **514F(**TCTCAGCCTTTTCT-TATGGACCT**), 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, 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,USA) in order to eliminate excess primers, unincorporated dideoxynucleotides (ddNTPs), and salts. Capillary electrophoresis was performed using an Applied Biosystems 3500 genetic analyzer (Applied Biosystems, 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 [19]. 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 5 μL of RNA and 25 ml of reaction mixture for each sample (10 μL Master Mix OneStep, 1 μL primers/probes, 4 μL RNase/DNase free water, un volume final de 15 μL). After preparation of the reaction mixture for genotyping, the real-time PCR reaction was performed on a thermocycler (QuantStudio 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 manufacturers' guidelines (Table 1).

### 2.4. 3.4 Data analysis

Descriptive statistics were performed for socio-demographic data and clinical parameters wherever appropriate; median and interquartile range (IQR) were reported for continuous variables. Univariate analyses were performed to assess the association between SARS-CoV-2 VOCs and patients' symptoms, with odds ratio (OR) at 95 % confidence interval. The McNemar test was used to evaluate the reliability of SNPsig® SARS-CoV-2 EscapePLEX CE compared to the sanger sequencing method (gold standard) in the rapid identification of SARS-CoV-2 VOCs. All P values were 2-tailed with 0.05 as the criterion for statistical significance. Cohen's kappa was used to estimate inter-assay concordance and results were interpreted according to the criteria proposed by Landis & Koch: ka = 0.01–0.20 (poor concordance), ka = 0.21–0.40 (fair concordance), ka = 0.41–0.60 (moderate concordance), ka = 0.61–0.80 (strong concordance), and ka = 0.81–1.00 (excellent concordance) [20].

## 3. Results

### 3.1. Characteristics of the study population

A total of 163 participants were enrolled in the study, consisting of 42.94 % (70/163) males and 52.15 % (85/163) females; the median [IQR] age was 37 [28–49] years (min = 2; max = 82); and 30.06 % (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); 95%CI = 72.76–85.64.

### 3.3. SARS-CoV-2 genetic diversity and lineage dynamics overtime

According to the local SARS-CoV-2 molecular epidemiology, variants were found in 91 (70 %) of 130 sequences. The dynamic of SARS-CoV-2 (Fig. 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

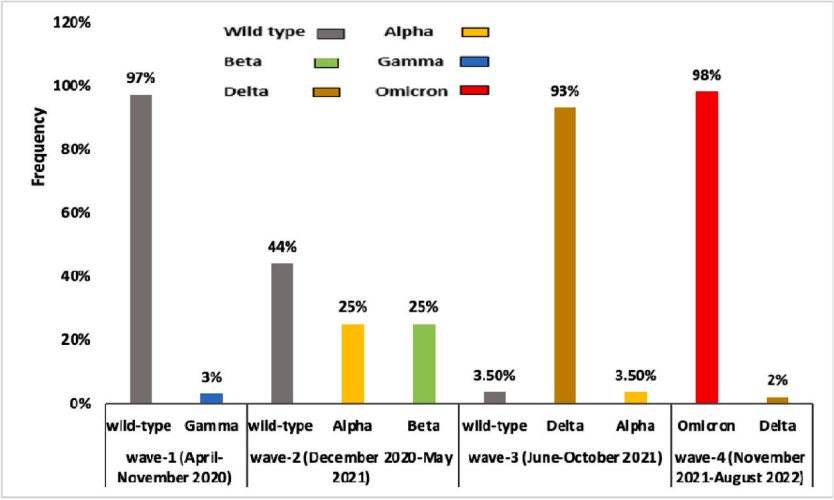


**Fig. 1.** Variants of SARS-CoV-2 strains across different waves, Cameroon, April 2000–August 2022 N = 130.

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 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 2).

### 3.4. Performance characteristics of SNPsig® SARS-CoV-2 EscapePLEX CE assay

Looking at the McNemar test, SARS-CoV-2 VOCs and lineage of origin distribution was not statistically or significantly (P = 0.17) different between the two methods used –Sequencing versus SNP genotyping with strong agreement (Ka [95 % CI] = 0.66 [0.53–0.80]) between the two genotyping methods (Table 3). For the identification of each VOCs, statistical analysis (McNemar test and cohen's Kappa) showed, there was no significant difference with good agreement between the two genotyping methods for the identification of the following SARS-CoV-2 VOCs: Alpha (P = 0.34; Ka = 0.25); Beta-variants (p = 1; Ka = 0.66), Omicron-variant (p = 0.50; ka = 0.96 (Table 4). However, these analyses showed, there was a significant difference between the two tests for the identification of the Delta variant (P = 0.02), despite the excellent agreement observed between the two tests (Ka = 0.82) for the detection of this VOC.

## 4. Discussion

The rapid emergence of SARS-CoV-2 variants required 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 SARS-CoV-2 VOCs is almost exclusively done by next-generation sequencing (NGS) [21,22]. This can be a huge constraint for global surveillance of SARS-CoV-2 mutants, as the equipment and trained personnel to perform NGS are not widely available, particularly in resource-limited settings. Although it has not yet been licensed for identifying new variants, the SNPsig® SARS-CoV-2 EscapePLEX CE is a rapid and cost-effective means to discriminate between one of the previously characterised SARS-CoV-2 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 SNPsig® SARS-CoV-2 EscapePLEX CE kit for rapid VOC typing 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 [23]. Other reports of studies conducted in different settings confirmed that the virus can infect without causing clinical manifestations [24,25].

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 and Omicron. Regarding the dynamics of VOC occurrence (Fig. 1), the first epidemiological wave is characterised by a massive circulation of the lineage of origin (wild type). 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 [26] [–] [28]. The SARS-CoV-2 Omicron variant of concern (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 [29]. 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 [30].

Overall, Spike targeted sequencing through the Sanger approach 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), suggesting that

**Table 2**
Clinical characteristics and SARSCoV-2 variants of concern.

| Variant | Clinical status | | OR (95 % CI) | *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 3**
Global performance of SNPsig SARS-CoV-2 EscapePLEX CE.

| Sequencing results | | | | |
|---|---|---|---|---|
| | | Lineage of origin | Sars-cov-2 Variant | Total |
| SNP-EscapePLEX | Lineage of origin | 33 | 13 | 46 |
| | Sars-cov-2 Variant | 6 | 78 | 84 |
| Total | | 39 | 91 | 130 |
| **$\Delta_{Pb-Pa}$:** 0.054; p = 0.17; Ka (95 % CI) = 0.67 (0.53–0.80) | | | | |

Legend: $\Delta_{Pb-Pa}$: represents the difference between the two proportions; p (p-value): represents the level of significance of the difference; Ka(kappa value): represents the level of agreement between the two tests.

**Table 4**
Performance characteristics of SNPsig SARS-CoV-2 EscapePLEX (capacity to discriminated each VOCs).

| Spike-genome sequencing SNP-EscapePLEX | | | |
|---|---|---|---|
| ***(A) Alpha-variant vs Non Alpha*** | | | |
| | *Alpha* | *Non Alpha* | *Total* |
| *Alpha* | *2* | *7* | *9* |
| *Non Alpha-* | *3* | *118* | *121* |
| *Total* | *5* | *125* | *130* |
| **$\Delta_{Pb-Pa}$:** 0.031; p = 0.340; Ka (95 % CI) = 0.25 (0–0.69) | | | |
| ***(B) Beta-variant vs Non Beta*** | | | |
| | *Beta* | *Non Beta* | *Total* |
| *Beta* | *2* | *2* | *4* |
| *Non Beta* | *1* | *125* | *126* |
| *Total* | *3* | *127* | *130* |
| **$\Delta_{Pb-Pa}$:** 0.008; p = 1.000; Ka (95 % CI) = 0.66 (0.18–1.00) | | | |
| ***(C) Delta-variant vs Non Delta*** | | | |
| | *Delta* | *Non Delta* | *Total* |
| *Delta* | *21* | *0* | *21* |
| *Non Delta* | *7* | *102* | *109* |
| *Total* | *28* | *102* | *130* |
| **$\Delta_{Pb-Pa}$:** 0.054; p = 0.016; Ka (95 % CI) = 0.82 (0.70–0.94) | | | |
| ***(D) Omicron-variant vs Non Omicron*** | | | |
| | *Omicron* | *Non Omicron* | *Total* |
| *Omicron* | *51* | *0* | *51* |
| *Non Omicron* | *2* | *77* | *79* |
| *Total* | *53* | *77* | *130* |
| **$\Delta_{Pb-Pa}$:** 0.015; p = 0.500; Ka (95 % CI) = 0.96 (0.92–1.00) | | | |

Legend: $\Delta_{Pb-Pa}$: represents the difference between the two proportions; p (p-value): represents the level of significance of the difference; Ka (kappa value): represents the level of agreement between the two tes.

low viremia is likely to be associated with sequencing failure [31].Additionally, primers designed for specific regions located inside recombination breakpoints cannot detect recombinant strains. These findings therefore call for the implemention of more sensitive sequencing methods be it for targeted sequencing or for a whole genome sequencing analysis.

As an alternative to sequencing, the SNPsig® SARS-CoV-2 EscapePLEX *CE* Kit has received much attention as a genotyping test for the rapid detection and identification of SARS-CoV-2 VOCs [32,33]. Our analyses showed an overall good concordance with Sanger sequencing in discriminating between variants and wild-type strains (Table 3), thus suggesting its suitability for preliminary identification of VOCs in Cameroon. As concerns its ability to discriminate each VOCs, the kit demonstrated excellent concordance with Sanger sequencing regarding the identification of omicron and delta variants; in line with the manufacturer's finding of 100 % sensitivity for the identification of the omicron-specific K417 N mutation [19]. Interestingly, the statistical significance reported in the SNP performance for the identification of Delta, in the frame of an acceptable concordance between the study assays, underscores possible pre-existing mutants in the sequences not yet integrated in the point mutation device. This therefore calls for a regular updating of such SNP assays in the context of a dynamic outbreak, epidemic or pandemic. These lessons appear very relevant in the preparation and response to future pandemic. However, in comparison to sequencing, our analysis reveals a moderate and a fair concordance respectively for beta and alpha variants. Even though we had a small sample size for these specific VOCs, these findings suggest the need 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.

As study limitations, we did not include negative samples, which would have helped assess both intrinsic and extrinsic features (sensitivity, specificity and predictive values) of SNPsig® SARS-CoV-2 EscapePLEX CE. Also, the small sample size especially for some VOCs such as Gamma did not let us evaluate the capacity of this kit to discriminate this specific variant of concern.

## 5. Conclusion

There was a rapid change in the molecular epidemiology of SARS-CoV-2 in Cameroon, moving from wild-type lineages to Omicron variants and sub-variants. These results underscore the need for genomic surveillance to support the pandemic control strategy. For the rapid detection of viral clades, SNPsig® SARS-CoV-2EscapePLEX CE kit is suitable in identifying SARS-CoV-2 VOCs circulating in RLS within simple PCR facilities available in molecular biology laboratories in RLS. With the emergence of Omicron sub-variants, rapid variant detection tools may need to 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 direct benefits on their clinical conditions with regards to COVID-19 infection.

## Data availability statement

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

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## References


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

[2] P.H.E.O.C. Pheocc, Cameroon COVID-19 situation report, CCOUSP. https://www.ccousp.cm/documentations/rapports-de-situation-covid-19/, 2022. (Accessed 20 December 2022).

[3] Ministry of Public Health, COVID-19 situation report, CCOUSP (2022). Pages 1-5. https://www.ccousp.cm/documentations/rapports-de-situation-covid-19/. (Accessed 26 October 2022).

[4] G. Gabutti, E. d'Anchera, F. Sandri, M. Savio, A. Stefanati, Coronavirus: update related to the current outbreak of COVID-19, Infect. Dis. Ther. 9 (2020) 241–253, https://doi.org/10.1007/s40121-020-00295-5.

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

[6] W.H.O. Who, Tracking SARS-CoV-2 variants. https://www.who.int/activities/tracking-SARS-CoV-2-variants, 2021. (Accessed 4 October 2022).

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

[8] N.A. Pennell, A. Mutebi, Z.-Y. Zhou, M.L. Ricculli, W. Tang, H. Wang, A. Guerin, T. Arnhart, A. Dalal, M. Sasane, K.Y. Wu, K.W. Culver, G.A. Otterson, 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. (2019) 1–9, https://doi.org/10.1200/PO.18.00356.

[9] R. Pereira, J. Oliveira, M. Sousa, Bioinformatics and computational tools for next-generation sequencing analysis in clinical genetics, J. Clin. Med. 9 (2020) 132, https://doi.org/10.3390/jcm9010132.

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

[11] G. Novacyt, Variant testing update: SNPsig® SARS-CoV-2 EscapePLEXT, Novacyt (2021). C:/Users/pc/Downloads/imprim%C3%A9e_1_escapeplex_for_the_detection_of_omicron_technical_bulletin_2.pdf (accessed October 4, 2022).

[12] J. Fokam, E. Ngoufack Jagni Semengue, D. Armenia, D. Takou, B. Dambaya, G. Teto, C.A. Chenwi, A.D. Nka, G.A. Beloumou, S.C.D. Ndjeyep, M.C.T. Tchouaket, N. Fainguem, S.M. Sosso, V. Colizzi, C.-F. Perno, A. Ndjolo, F. Ceccherini-Silberstein, M.M. Santoro, 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 (2022) 115574, https://doi.org/10.1016/j.diagmicrobio.2021.115574.

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

[14] D. Takou, J. Fokam, G. Teto, M.-M. Santoro, F. Ceccherini-Silberstein, A.J. Nanfack, S.M. Sosso, B. Dambaya, R. Salpini, S.C. Billong, C. Gori, C.N. Fokunang, G. Cappelli, V. Colizzi, C.-F. Perno, A. Ndjolo, 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 (2019) 246, https://doi.org/10.1186/s12879-019-3871-0.

[15] C. Pastorio, F. Zech, S. Noettger, C. Jung, T. Jacob, T. Sanderson, K.M.J. Sparrer, F. Kirchhoff, Determinants of Spike infectivity, processing, and neutralization in SARS-CoV-2 Omicron subvariants BA.1 and BA.2, Cell Host Microbe 30 (2022) 1255–1268.e5, https://doi.org/10.1016/j.chom.2022.07.006.

[16] W.T. Harvey, A.M. Carabelli, B. Jackson, R.K. Gupta, E.C. Thomson, E.M. Harrison, C. Ludden, R. Reeve, A. Rambaut, S.J. Peacock, D.L. Robertson, SARS-CoV-2 variants, spike mutations and immune escape, Nat. Rev. Microbiol. 19 (2021) 409–424, https://doi.org/10.1038/s41579-021-00573-0.

[17] M. Matsubara, Y. Imaizumi, T. Fujikawa, T. Ishige, M. Nishimura, A. Miyabe, S. Murata, K. Kawasaki, T. Taniguchi, H. Igari, K. Matsushita, Tracking SARS-CoV-2 variants by entire S-gene analysis using long-range RT-PCR and Sanger sequencing, Clin. Chim. Acta 530 (2022) 94–98, https://doi.org/10.1016/j.cca.2022.03.014.

[18] Public Health England, Investigation of Novel SARS-COV-2 Variant, 2020. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/959438/Technical_Briefing_VOC_SH_NJL2_SH2.pdf.

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

[20] L. Jr, K. Gg, The measurement of observer agreement for categorical data, Biometrics 33 (1977). https://pubmed.ncbi.nlm.nih.gov/843571/. (Accessed 19 October 2022).

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

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

[23] N.N. Fainguem, J. Fokam, E. Ngoufack Jagni Semengue, A. Durand Nka, D. Takou, J. Ageboh Nkembi-leke, C. Alteri, L. Colagrossi, R.B. Yagai, C. Ambe Chenwi, M.C. Tchouaket Tommo, G. Angong Beloumou, A.C. Ka'e, S.C. Ndjeyep Djupsa, A. Abba, L.G. Heunko Yatchou, K. Nnomo Zam, R. Kamgaing, S.M. Sosso, L. Mama, N. Ndembi, V. Colizzi, C.-F. Perno, G. Cappelli, A. Ndjolo, 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 (2022) 2163, https://doi.org/10.4081/jphia.2022.2163.

[24] S.A. Meo, A.S. Meo, F.F. Al-Jassir, D.C. Klonoff, Omicron SARS-CoV-2 New Variant: Global Prevalence and Biological and Clinical Characteristics, 2021.

[25] D.P. Oran, E.J. Topol, Prevalence of asymptomatic SARS-CoV-2 infection, Ann. Intern. Med. 173 (2020) 362–367, https://doi.org/10.7326/M20-3012.

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

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

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

[29] E.C. for D.P, C. Ecdc, Epidemiological update: SARS-CoV-2 Omicron sub-lineages BA.4 and BA.5, Eur. Cent. Dis. Prev. Control (2022). https://www.ecdc.europa.eu/en/news-events/epidemiological-update-sars-cov-2-omicron-sub-lineages-ba4-and-ba5. (Accessed 12 October 2022).

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

[31] T.S. Jørgensen, K. Blin, F. Kuntke, H.K. Salling, T.Y. Michaelsen, M. Albertsen, H. Larsen, 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, 2021, 03.27.21252266, 2021. https://doi.org/10.1101/2021.03.27.21252266.
[32] S.C. Chaintoutis, T. Chassalevris, S. Balaska, E. Mouchtaropoulou, G. Tsiolas, I. Vlatakis, A. Tychala, D. Koutsioulis, A. Argiriou, L. Skoura, C.I. Dovas, 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 (2021) 1015, https://doi.org/10.3390/life11101015.
[33] M. Umair, A. Ikram, Z. Rehman, S.A. Haider, M. Ammar, N. Badar, Q. Ali, M.S. Rana, M. Salman, Genomic diversity of SARS-CoV-2 in Pakistan during the fourth wave of pandemic, J. Med. Virol. 94 (2022) 4869–4877, https://doi.org/10.1002/jmv.27957.