Detection of a characteristic melting profile of a SARS-CoV-2 Kappa variant in Italy using the SARS-CoV-2 Variants ELITe MGB® Kit

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ABSTRACT

Background: Although more than a year has passed since the start of the pandemic, SARS-CoV-2 infection still represents a major challenge for public health all over the world due to viral genome capability of gaining rapid mutations. Whole-genome sequencing (WGS) is the gold standard for variant identification, but it is time consuming and relatively expensive. For this reason, assays targeting multiple regions of the SARS-CoV-2 genome may be useful for a rapid traceability of either known or new variants, anyway, not all the manufacturers are able to sustain the rapid development of variants.
Objective: We tested forty nasopharyngeal swabs, resulted positive for the presence of SARS-CoV-2 RNA at low cycle threshold (CT < 25), with SARS-CoV-2 Variants ELITe MGB® Kit, which was designed to identify Nigerian variant, possible UK variant and South African or Brazilian variant.
Results: During the analysis, we noted an atypical melting curve, different from the other variants recognizable by the kit. The subsequent WGS reported this variant as Kappa, so we assess the possibility of "suspecting" the presence of a Kappa variant using SARS-CoV-2 Variants ELITe MGB® Kit.
Conclusions: Rapid variant screening followed by WGS offers the opportunity to study mutation dynamics and quickly identify possible variants of interest (VOI) and/or variants of concern (VOC), which is crucial in virus spreading control. Furthermore, an accurate analysis of the melting peak could be useful to suspect the presence of new variants.

From March 2020 COVID-19 pandemic has affected about 270 million people worldwide with more than five million reported deaths (WHO Coronavirus (COVID-19) Dashboard, 2021) enforcing an effective strategy for the rapid identification and isolation of SARS-CoV-2 infected patients.

As well as for all viruses, also the SARS-CoV-2 genome has the capability of gaining rapid mutations representing an ongoing challenge for Public Health and leading to the characterization, during late 2020, of specific Variant of Interest (VOIs) and Variant of Concern (VOCs), to prioritise global monitoring and research (WHO, 2021).

ECDC (European Centre for Disease Prevention and Control) regularly assesses new evidence on variants detected by epidemiological monitoring based on genomic variant screening and other scientific sources, weekly updating reports available on ECDC's website (ECDC, 2021a, 2021b).

Whilst VOC represents a variant that has been demonstrated to be associated to an increase in transmissibility, more severe disease (e.g., increased hospitalizations or deaths), significant reduction in neutralization by antibodies generated during previous infection or vaccination, reduced effectiveness of treatments or vaccines, or diagnostic detection failures, VOI means a variant presenting known genomic properties and epidemiological or in-vitro evidence that it could imply a significant impact on transmissibility, severity and/or immunity, with a realistic epidemiological impact. However, the evidence is still preliminary or is associated with major uncertainty (CDC, 2021).

Whole-genome sequencing (WGS) data offers a wide range of opportunities to study mutation dynamics, anyway WGS analysis it is time consuming and not all routine laboratories have the instruments and the qualified personnel to sequencing all nasopharyngeal and oropharyngeal samples that are analysed for SARS-CoV-2 RNA detection by

Table 1
Targets and relative melting temperature ranges detectable by SARS-CoV-2 Variants ELITe MGB® Kit.

| Target	Tm (°C)
E484 K WT	63.0–69.0
N501Y WT	51.0–57.0
E484 K MUT	56.0–62.0
N501Y MUT	59.0–66.0

molecular method. (Falzone et al., 2021; Rachiglio et al., 2021; Ntoumi et al., 2021).

For this reason, in accordance with the epidemiological data, the ministry of health requires to regularly send a specific percentage of positive samples to reference laboratories for WGS; anyway, a rapid

identification of VOIs/VOCs could be crucial to help the control of virus spreading.

Up to date, several SARS-CoV-2 variants identification kits have been introduced in the marketplace, useful for the quick identification of the main variants on the samples previously tested positive at PCR-Real time with high CT values, but not all the manufacturers are able to sustain the rapid development of virus variants, so most of those kits are not yet able to detect all emerging variants.

At present, several diagnostic methods for detecting SARS-CoV-2 and SARS-CoV-2 variants have been approved by regulatory agencies worldwide, including immunoassays, PCR-based methods, NGS-based methods and commercialized tools based on different technologies (e.g., LAMP and microarray). This implies that more than 350 different devices have been commercialized, with different performance, such as limit-of-detection (LOD), clinical sensitivity, clinical specificity, and running time. Nevertheless, the RT-PCR based methods are still the most
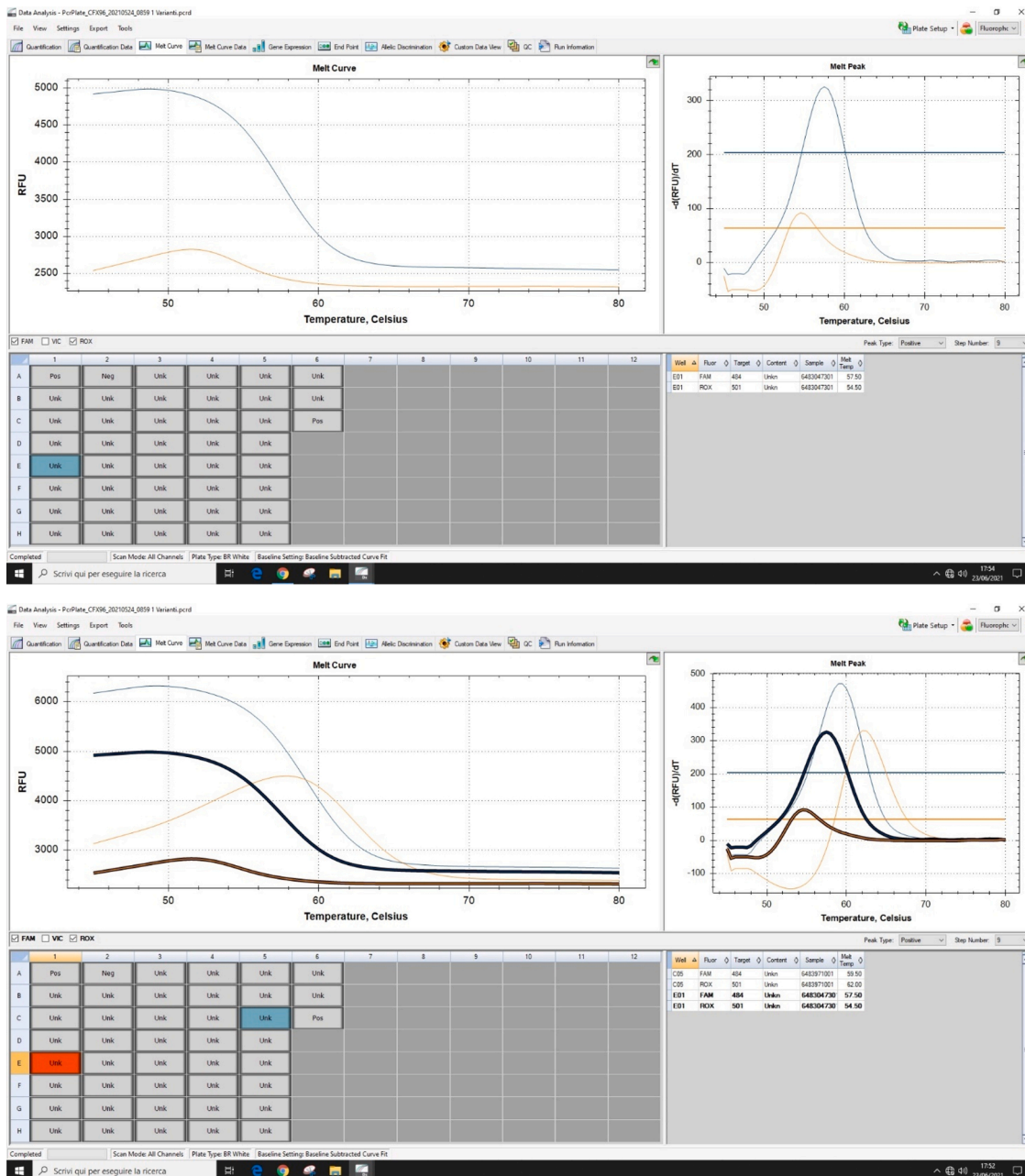
Fig. 1. (a) Kappa variant melting profile (E01). (b) Kappa variant melting profile (E01, in bold) compared to South African/ Brazilian melting profile (C05). (c) Kappa variant melting profile (E01, in bold) compared to UK variant melting profile (from B02 to C04, from E04 to H04).
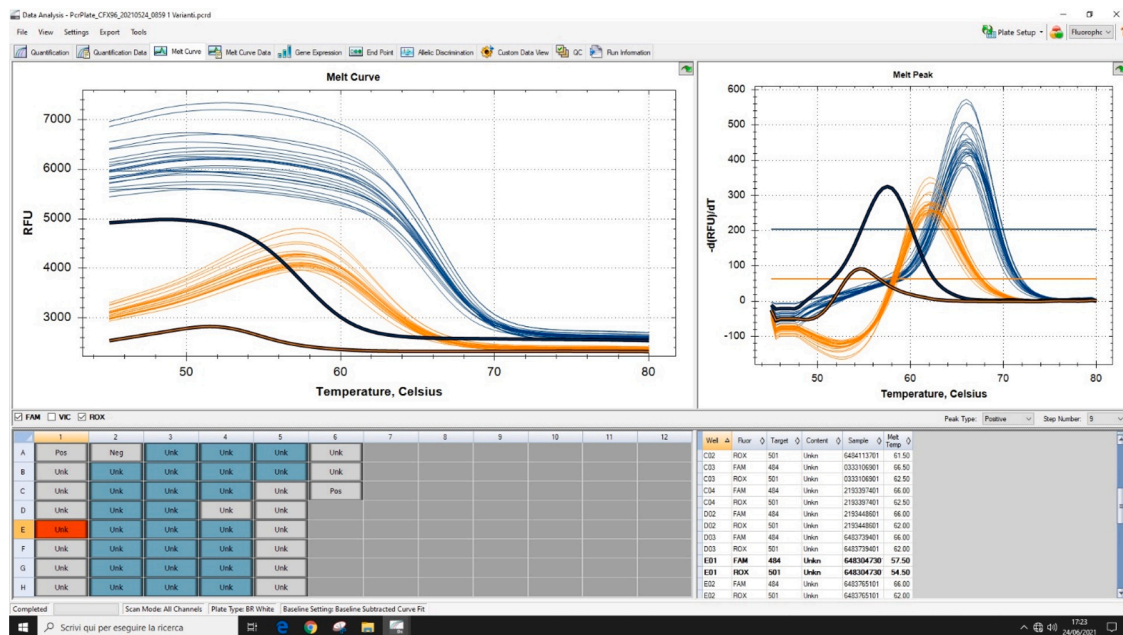
Fig. 1. (*continued*).

reliable and are considered the gold standard for diagnosis of COVID-19 (detection of SARS-CoV-2).On the other hand, NGS whole-genome sequencing is the most powerful method for the molecular character-ization of SARS-CoV-2, for the identification of novel variants during genomic surveillance screening and for the development of genome-based therapeutic approaches. (Falzone et al., 2021; Mukhopadhyay et al., 2021; Hosseini et al., 2020).

In this short communication we want to focus on the possibility of "suspecting" the presence of a Kappa variant through the melting peak analysis using a variant identification kit primarily not designed for this variant.

Among the SARS-CoV-2 variants the B.1.617.1 lineage (Kappa variant, WHO) has been designated as VOI by the World Health Orga-nization from 4th April 2021 (earliest documented positive sample detected in India, October 2020).

The SARS-CoV-2 Variants ELITe MGB® Kit can be used for the qualitative detection and discrimination of the mutations E484 K and N501Y of the S gene of SARS-CoV-2 through Reverse Transcription Real-Time Polymerase Chain Reaction and melting curve analysis with the following interpretation of results:

- absence of both E484 K and N501Y indicates wild type
- presence of E484 K means Nigerian variant
- presence of N501Y indicates possible UK variant
- presence of both E484 K and N501Y denotes South African or Bra-zilian variant.

The specific range of melting temperatures of the targets are repre-sented in Table 1

We tested forty nasopharyngeal swabs, resulted positive for the presence of SARS-CoV-2 RNA at low cycle threshold (CT < 25), with SARS-CoV-2 Variants ELITe MGB® Kit, which was designed to identify Nigerian variant, possible UK variant and South African or Brazilian variant. During the analysis, we noted an atypical melting curve, different from the other variants recognizable by the kit. The subsequent WGS performed on the samples previously analyzed using the SARS-CoV-2 Variants ELITe MGB® Kit revealed that the samplewith an "atypical" melting profile, not corresponding to any variant detectable by the kit, was a Kappa variant (position E01in Fig. 1a). Specifically, during analytical evaluation of the results, we found that this sample

presented a Tm of 57.50 for E484 K, that is within range of Nigerian and South African or Brazilian variant and a Tm of 54.50 for N501Y target, that is within range of wild type (WT). Moreover, comparing the melting curve and melting peaks of this sample to those of the others recogniz-able variant we noted a characteristic profile (Fig. 1b and c).

Although more investigations are needed, this commentary focuses on the possibility of a rapid detection of a suspected Kappa variant using the kit SARS-CoV-2 Variants ELITe MGB® Kit, besides to pointing out that, in addition to the variants investigated, an "anomalous behaviour" of the melting curves, may be suggestive for new or different variants of potential clinical and epidemiological interest, allowing to quickly trace possible clusters. Obviously, the subsequent sequencing remains mandatory.

Data availability

No data was used for the research described in the article.

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CRediT authorship contribution statement

Margherita Scapaticci: Conceptualization, Methodology, Data curation, Writing - original draft. **Andrea Bartolini:** Conceptualization, Methodology, Data curation, Writing - original draft. **Francesca Vitone:** Validation. **Vincenzo Cerreta:** Validation. **Monica Vignoli:** Validation. **Elena Gnudi:** Validation. **Alessandra Frazzoni:** Validation. **Barbara Sitta:** Validation. **Silvia Capitani:** Visualization, Investigation. **Anna-maria Lopriore:** Visualization, Investigation. **Mariapina Donadio:** Visualization, Investigation. **Stefania Chiarastella:** Visualization, Investigation. **Marina Bioli:** Supervision. **Rita Mancini:** Supervision.

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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