INSTITUTE OF MOLECULAR AND TRANSLATIONAL MEDICINE

Identification of SARS-CoV-2 variants through post PCR high resolution melting

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Introduction

- Detection of SARS-CoV-2 RNA in nasopharyngeal swabs is the gold standard for detection of COVID-19 infection.
- Distinguishing different SARS-CoV-2 strains from each other is crucial for surveying the evolution of the virus in a population and may guide clinical and epidemiological efforts in the regional fight against COVID-19.

Aim of study

- To establish a rapid, reliable and inexpensive genotyping method, that distinguishes mutation from AAA to gAA at amino acid position 484 of the spike 1 protein.

Methods

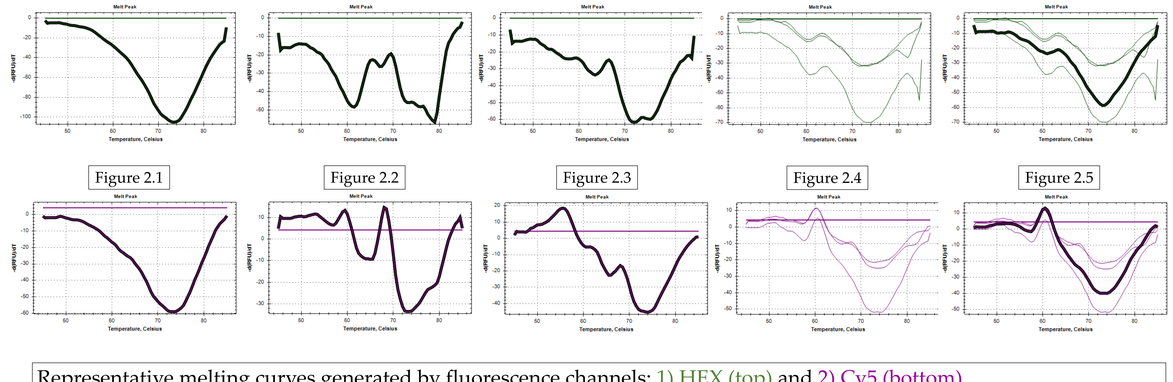
- Sloppy molecular beacon assay SMB-484 from paper of Banada P, *et al.* J Clin Microbiol. 2021;59(10):e0084521.
- Modifications were used to increase the signal from sloppy molecular beacon, and to allow checking for amplification independently from beacon signal.
- Applied to 2 synthetic DNA standards with artificial mutations in S1 gene, 6 samples with gCA (Omicron) variant, 2 samples with gAA variant, and 93 samples without variant information.

Results and conclusion

- FAM signal from double -strand amplicon does not allow to distinguish variants at position 484 (data not shown).
- Both Cy5 signal from probe designed for AAA variant and HEX signal from probe designed for gAA variant allow to distinguish synthetic probes from each other and from the real RNA samples.
- Cy5 signal from probe designed for AAA (vs gAA) variant does not allow to distinguish gAA from gCA variant.
- HEX signal from probe designed for gAA (vs AAA) variant allows distinguishing gAA from gCA variant by a melting curve shift.
- Out of 93 samples without variant information, 14 require further investigation by replicating, sequencing, or re-testing by other variant-specific PCR because their melting curves look different from the rest.

Figure 1.1 | Figure 1.2 | Figure 1.3 | Figure 1.4 | Figure 1.5

Figure 2.1 | Figure 2.2 | Figure 2.3 | Figure 2.4 | Figure 2.5



Representative melting curves generated by fluorescence channels: 1) HEX (top) and 2) Cy5 (bottom).
1) negative control, 2) synthetic standard I, 3) synthetic standard II, 4) three verified Omicron samples (sequenced), 5) **sample of interest*, compared to Omicron samples.
*sample of interest – not a verified variant, but resembles the curve pattern of the three Omicron samples

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References

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