Test Center Denmark (TCDK) at Statens Serum Institute daily screens a very large number of samples by an initial PCR and then performs a follow up variant PCR on all positive samples utilizing a panel of variant specific PCR assays. One of the PCR assays used is an allelic discrimination assay for mutation L452R. This was implemented in July 2021 and was based on available sequence information for detection of the Delta-variant. SSI has chosen to use the wild type (WT) codon L452 as a marker for the Omicron variant, in order to be able to discriminate Omicron from the, until recently, completely dominating Delta -variant. So far, all WGS confirmed omicron cases have been positive for this marker (= Wildtype in codon 452). Although several other variants also have wild types in this codon (e.g. the Alpha variant), essentially only the Delta variant has been observed during the last 4 months in the Danish testing program. Therefore, it is reasonable to make the assumption that, at the present date in Denmark, all or nearly all WT L452 are Omicron positive. The advantage of using the WT L452 marker, is that it is independent of any heterogeneity around deletion 69/70, as this assay is not dependent of the so-called S gene drop-out, but rather specific for the 452 marker.

In order to investigate the specificity of the assay early on; we initially tested this close to the introduction into the Test Center in July 2021. This has been described in the attached preprint from MedRxiv; Rapid surveillance platforms for key SARS-CoV-2 mutations in Denmark https://doi.org/10.1101/2021.10.25.21265484 . This analysis indicates a very high sensitivity (above 99.9%) and specificity of this assay, including a false positive rate of around 1 per 1700-1900 initially positive samples, thus equal to around 3 false positives identified per 5000 positive samples tested.

Recently, data from Variant PCR from October 2021 was utilized to determine the specificity of WT 452 as a marker for the Omicron Variant. As mentioned above, we have assumed that nearly all positive samples would be positive for Delta and not Omicron during this period. For this data set, the initial variant PCR test included 54150 positive samples, of which all but 4 samples were correctly identified as Delta and confirmed by WGS. This entails a specificity of 99.993% % (95% confidence interval using Clopper-Pearson is 99.981-99.998%), equal to a false positive rate of 0.1-1 false positive per 5 000 COVID positive samples. This very low rate of false positives, lower than the initial validation mentioned in the preprint, is partly due to the very high accuracy of sample handling in the automated Danish test setup. Subsequently, from the 22nd November to 8th December 2021, 489 Omicron positive samples have been detected by variant PCR in Denmark using this method. At this point in time we have subjected 83 of these to WGS, and all but two of them confirmed as Omicron. One of these was due to a mislabeling caused by the manual system used for extra fast sequencing of this sample, while the other one, was due to a virus variant also having the wildtype sequence at 452, namely the AZ.5 variant which is nearly absent in Denmark, this only being the third sample detected with this variant and therefore not of major consequence for our method.

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In addition, please find attached the SOP at the manuscript describing our Variant-PCRs. In connection with the methods described in the preprint and the SOP, we want to note that:

Based on the shortage of production capacity of BHQplus probes due to high demands during the pandemic, alternative probes with the same sequence for the L452R PCR but an alternative modification (LNA) can be ordered instead. The probes have been validated and can be used in small and large scale screening using an allelic discrimination analysis. The PCR protocol as well as the primer remain the same for the L452R PCR as described in the preprint.

Alternative LNA probes (if the BHQplus probes are not available):

452R_mutation_LNA_delta_variant: HEX-TAT AAT TAC +C+GG TA+T AGA TTG TTT AG-BHQ1

L452_WT_LNA_omicron_variant: CF Red 610-TAT AAT TAC +C+TG TA+T A+GA TTG TTT AG-BHQ2

Kind Regards,

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