Recommendations for testing using the N501Y PCR

For the Coordinated Commission for Clinical Microbiology of the Swiss Society of Microbiology (CCCM-SSM)*
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Content and focus of this document

These recommendations have been written by the Coordinated Commission for Clinical Microbiology of the Swiss Society of Microbiology (CCCM-SSM) on 13th January 2021. The recommendations focus on the emerging N501Y SARS-CoV-2 variants and the role out of a N501Y specific PCR for rapid surveillance of this emerging pathogen. This document is complementary to a recently published document by the Federal Office of Public Health by Michael Bel.

Summary. The CCCM-SSM strongly encourages diagnostic laboratories to establish a N501Y variant specific PCR to support surveillance and control of the emerging variants of concern. Detected variants should trigger an intensified backward and forward contact tracing by cantonal authorities and the N501Y positive isolates should be confirmed using sequencing.

Background

Overall, prevalence of SARS-CoV-2 in the population remains high and the situation is worrisome due to ongoing transmission. **Two newly emerging lineages, the B1.1.7** (UK variant, N501Y.V1) **and the B1.351** (ZA variant, N501Y.V2) **rapidly spread in the United Kingdom, Ireland, and South Africa**. Recent modelling data indicates that the N501Y mutations results in a higher transmissibility. In Switzerland, both variants of concern have been detected shortly before Christmas 2020. Meanwhile, **more than 100 isolates of the B1.1.7 lineage were confirmed in different Swiss laboratories**. Community transmission within Switzerland has also been documented using whole genome sequencing in selected cases. The B1.351 lineage remains rare. Of note, we only explored a small and strongly biased sample set during the past three weeks.

Further background information on the European and global situation can be found at the ECDC and WHO websites:

ECDC: https://www.ecdc.europa.eu/en/publications-data/threat-assessment-brief-rapid-increase-sars-cov-2-variant-united-kingdom
WHO: https://www.who.int/csr/don/31-december-2020-sars-cov2-variants/en/

Currently, most commercial based SARS-CoV-2 specific PCR assays cannot identify these variants of concern. Only one exception is noteworthy: the lineage B1.1.7 has a specific deletion at position 21765-770 of the S gene, resulting in a del 69-70 of the spike gylcoprotein. This deletion results in a S-gene drop out of the Thermo Fisher assay. Due to normal signals of other target genes, this effect was used during the last weeks to identify potential cases of the lineage B.1.1.7. Unfortunately, this deletion is not specific for the UK variant. In the Eastern part of Switzerland, where the S-gene drop out continuously increased, this is due to the lineage B1.258, which also shows the deletion and accounts for the majority of cases. Also the S-gene drop out does not include the B1.351 lineage.

For this reason, several diagnostic laboratories and the Federal Office of Public Health developed a screening strategy to rapidly detect the spread of N501Y variants in Switzerland and reduce further community spread with an intensified backward and forward contact tracing through cantonal authorities. The aim is to control for a 2-3 months time period the prevalence of variants of concern using a second PCR. This strategy may slow down an expected increase of case numbers and pressure on the health care system.

Current strategy:
The current strategy is to establish a N501Y specific PCR detecting the genetic mutation A23063T in a substantial number of diagnostic laboratories. After this **first step to rapidly identify the variant of concern**, as a **second step**, the lineage association should be confirmed using either an amplicon based **sequencing** approach on the S gene or a whole genome based sequencing approach. Confirmation by sequencing to determine the specific lineage is highly recommended, but requires, according to the FOPH, an initiation by the cantonal physician in charge. Laboratories providing diagnostic sequencing service were mentioned on the SSM website and in a separate document detailed instruction and recommendation regarding the sequencing process were published.

Details on the reporting process as well as re-imbursement are specified within a FOPH letter from Michael Bel. Of note, it is likely that the re-imbursement will only be applicable for a specific time window – as soon as the variants of concern are too prevalent, the N501Y specific PCR will become obsolete.

Available PCR assays to determine the N501Y variant:
Different commercial and in house developed PCR system are able to specifically detect the N501Y variant.

Currently, the most common used assay is the SARS Spike N501Y (LightMix Kit) from TIB MOLBIOL (reference number 53-0780-96). This assay has been tested with encouraging results by the national reference center (communicated by Prof. Laurent Kaiser, CRIVE).
Website of TIB MOLBIOL: https://www.tib-molbiol.de/

The CRIVE has also published an *in house* protocol with details on primers and the PCR steps. The document can be access via the following link:
https://www.hug.ch/sites/interhug/files/structures/laboratoire_de_virologie/protocol_amplification_voc_20201201_uk_geneva.pdf

The N501Y specific PCR is already established or will shortly be established at the 5 university centers (Basel, Bern, Geneva, Lausanne, Zurich), Risch AG, Cantonal Hospital Winterthur, and EOC in Bellinzona.

Recommendation key points:
The CCCM-SSM strongly recommends that:

(i) Establishment of a N501Y specific PCR by different diagnostic laboratories.
(ii) Reporting of N501Y confirmed cases and also negative cases (denominator)
(iii) Confirmation using sequencing

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