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Recommendations of CCCM-SSM SARS-CoV-2 diagnostics working group on quality controls for SARS-CoV-2 PCR and antigen testing

SARS-CoV-2 specific diagnostics currently used in routine settings and with sufficient evidence on test performance includes SARS-CoV-2 RT-PCR from nasopharyngeal swabs and saliva and SARS-CoV-2 rapid antigen tests from nasopharyngeal swabs.

Background

These test methods are currently recommended for use in patients with an acute onset of a respiratory infectious disease (see websites of FOPH and SSM for further details).

Some of the SARS-CoV-2 rapid antigen tests are validated in detail by the CCCM-SSM and the assays fulfilling the validation criteria will be published on the FOPH website (white list). As the performance of SARS-CoV-2 rapid antigen tests is largely unknown and the assays will be used by less experienced personnel and in specific non- or less health care associated settings, a detailed validation prior to market release was conducted under the mandate of the FOPH.

For various reasons it is important to continuously assess the quality of SARS-CoV-2 specific diagnostic assays in use: (i) Assays may show production lot specific differences in performance; (ii) SARS-CoV-2 continues to evolve and may change its diagnostic targets due to a diagnostic selection pressure – a phenomenon which has been observed in the past for many pathogens; (iii) companies may update or improve testing systems affecting the test performance; and (iv) new population and scenarios may be included (e.g. lower viral loads, asymptomatic people, different sample material).

Due to the high dynamic of the ongoing SARS-CoV-2 pandemic and the newly available diagnostic tests on the market (with often unknown test performance) it is very important to regularly check reliable online resources for news on SARS-CoV-2 related diagnostics.

The following list is not complete but provides some core documents:

- The European Centre for Disease Prevention and Control (ECDC): <https://www.ecdc.europa.eu/en/novel-coronavirus/laboratory-support>
- World Health Organization (WHO): <https://www.who.int/publications/i/item/diagnostic-testing-for-sars-cov-2>
- Centers for Disease Control (CDC), USA: <https://www.cdc.gov/coronavirus/2019-ncov/lab/index.html>
- Centre national de référence pour les infections virales émergentes (CRIVE), Switzerland: <https://www.hug.ch/laboratoire-virologie/centre-national-reference-pour-infections-virales>.
- Swiss Society of Microbiology (SSM): www.swissmicrobiology.ch

Internal quality assessments

Recently a new SARS-CoV-2 run control has become available and is recommended by the European Society of Clinical Virology (https://www.nibsc.org/about_us/latest_news/covid-19_run_control.aspx) – apparently these controls are compatible with large fully automated systems for SARS-CoV-2 PCRs. We also strongly recommend the regular usage of an internal PCR quality control in automated systems.

External quality assessments

External quality assessment is a cornerstone in introducing new test methods and ensuring a baseline quality level. This is even more important in a pandemic situation, when a novel, previously unknown pathogen is circulating, and microbiological expertise and diagnostic knowledge are limited. Diagnostic trust in testing systems and strategies can be ensured and improved with quality assessments. External quality assessments also allow, if sufficiently scaled, to compare the test performance of in house developed and commercial assays.

To date not many external quality assessment results have been published. The RECOVER network with a European network recently published their experience from the first wave of spring 2020 and noted an overall high performance of RT-PCR based systems. Whereas all core samples were detected in 315 of 365 (86.3%) of the participating laboratories, the ring trial also found that some commercial assays showed significant lower test performance.¹ Similar results were reported by colleagues from South Korea, noting that a total of 110 (93%) laboratories reported correct results for all qualitative tests. However, 29 (25%) laboratories had one or more outliers according to cycle threshold values.² More worrisome results were reported from Austria, where only 40 of 67 (60%) laboratories detected all positive samples and 37% of the laboratories did not detect the weakest positive samples, resulting in the recommendation to improve the test sensitivity by focusing on RNA extraction and/or PCR based detection.³

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Similar, another European ring trial reported a variable sensitivity in molecular detection of SARS-CoV-2 in expert laboratories. Only 27 of the 68 participating laboratories tested all core samples correctly. The risk of false-negative tests increased significantly with lower SARS-CoV-2 concentrations.⁴

Obviously, an external quality assessment and the discussed publications only reflect a cross sectional aspect of a given time frame. However, these results show that the testing performance is variable and there is clearly room for improvement. A continuous quality assessment program can in depth assess the diagnostic performance over a longer time period. We can assume that assays which continuously fail will be improved or removed from the market.

Various well recognized quality control institutions have started an external quality assessment program for SARS-CoV-2 related molecular diagnostics, most of them are currently at a pilot stage.

Further information can be found on the following websites of

- UK NEQAS: <https://ukneqas.org.uk/news/new-covid-19-uk-neqas-pilot-scheme-developments/>
- INSTAND e.V.: https://www.instand-ev.de/en/news/detail/news/ringversuch-416-virusimmunologie-sars-cov-2-ak-september-2020-teilnahmedokumente-sind-online/?tx_news_pi1%5Bcontroller%5D=News&tx_news_pi1%5Baction%5D=detail&cHash=0892c6d6af7a42917ad595163cd55778
- QCMD: <https://www.qcmd.org/index.php?pageId=49&pageVersion=EN>

SARS-CoV-2 RT-PCR from nasopharyngeal swabs and saliva

The current gold standard for SARS-CoV-2 diagnostics is the RT-PCR. In house developed RT-PCR tests were rapidly introduced at all Swiss University centers, based on early released genomic sequences and initial cross-validation with the national reference laboratory, and were subsequently utilized to validate and implement new upcoming commercial RT-PCR tests at various sites. Meanwhile, most laboratories use a combination of slower high-

throughput robotic based and rapid single cartridge based RT-PCR systems.

The overall diagnostic quality of molecular diagnostics in Switzerland is high, as diagnostics of pathogens is only performed in laboratories with experienced and specifically trained technical personnel, respective laboratory equipment and infrastructure for diagnostics, e.g. safety bench for potential contagious materials, a quality control concept (including internal and external quality controls), and also access to samples for verification and validation. Outside of a laboratory environment many of the laboratory benefits are missing. Conducting molecular diagnostics outside of a laboratory environment (under responsibility of an accredited medical laboratory) is more challenging and requires special attention. Whereas some testing systems have an internal technical quality control, this control only assesses the analytical quality and not the overall diagnostic process. A quality control should test all aspects of the diagnostic process (pre- to post-analytics).

The CCCM-SSM therefore recommends that:

(i) Internal and external quality controls for SARS-CoV-2 molecular diagnostics have to be organized. This may include European EQA programs such as UK NEQAS, QCMD or INSTAND e.V., once they become broadly available. For the moment, it is strongly recommended that laboratories participate in EQA programs in Switzerland.

(ii) A more structured and nationwide EQA program should be initiated. Establishment of EQA expectations and guidelines, involving CCCM-SSM, CRIVE, FOPH and Swissmedic, is currently prioritized. CCCM-SSM also actively supports and promotes the implementation of quality controls for SARS-CoV-2 diagnostics. Providing sets of standardized samples with a broad range of viral loads would help to gain critical experience in test performances.

(iii) Every test site conducting molecular diagnostics should perform an internal control on a regular basis. Namely, every 100 sample should be in parallel assessed by the at-

tached laboratory using an established RT-PCR system.

(iv) Especially, in the case of molecular diagnostics outside of a laboratory environment, an EQA can provide critical insights. Test sites and capacities have been reported in the past to C. Metzger (COVID-19 Laboratory Coordination Switzerland). We recommend that any test site under responsibility of an accredited medical laboratory, reports the site, the testing device in use, and the associated test capacity in a similar fashion. This would allow to get an overview of molecular diagnostic devices outside of a laboratory environment. These sites should participate in EQAs via the responsible attached laboratories, reflecting the specific testing system in use and sample materials (nasopharyngeal swab, saliva) commonly tested.

Based on the previously reported variable test performance, it is clearly needed that testing sites performing molecular diagnostics participate and conduct an internal and external quality assessment on a regular basis.

SARS-CoV-2 specific antigen tests

Of note, for SARS-CoV-2 antigen testing so far no external quality assessment has been conducted (Pubmed Search with terms EQA, SARS-CoV-2, and antigen; date 27 January 2021). To date no external quality assessment has been conducted for rapid antigen tests. In order to allow a continuous assessment after the market release for the implementation and use of these tests according to the testing guidelines defined by FOPH, the FOPH should be allowed to conduct and/or request, in collaboration with a CCCM-SSM expert board, a post-market release validation. This should be based on conflicting reports regarding the

rapid antigen test performance, such as laboratory reports regarding discrepant results. Thereby, even a test on the FOPH white list may again be removed if a doubtful quality could be confirmed by a CCCM-SSM laboratory.

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References

1. Matheussen V, Corman VM, Donoso Mantke O, et al. International external quality assessment for SARS-CoV-2 molecular detection and survey on clinical laboratory preparedness during the COVID-19 pandemic, April/May 2020. Euro Surveill 2020; 25(27).
2. Sung H, Han MG, Yoo CK, et al. Nationwide External Quality Assessment of SARS-CoV-2 Molecular Testing, South Korea. Emerg Infect Dis 2020; 26(10): 2353–60.
3. Gorzer I, Buchta C, Chiba P, et al. First results of a national external quality assessment scheme for the detection of SARS-CoV-2 genome sequences. J Clin Virol 2020; 129: 104537.
4. Fischer C, Mogling R, Melidou A, et al. Variable sensitivity in molecular detection of SARS-CoV-2 in European Expert Laboratories: External Quality Assessment, 2020. J Clin Microbiol 2020.

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