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Recommendations of the CCCM-SSM SARS-CoV-2 diagnostics working group on the indications and limitations of the SARS-CoV-2 PCR testing from saliva

Content and focus of this document

These recommendations were written based on the request from the FOPH to prepare a short guideline regarding either (i) using rapid antigen tests (on-site) and/or (ii) using pooled saliva molecular biological analysis methods (in art. 16 EpG labs) for people without symptoms in long-term facilities and schools. The final version of these recommendations have been adapted in April 2021 by the CCCM-SSM members in the weeks before and during the committee meeting that took place on 21 April.

The use of antigen tests in an asymptomatic population is generally not recommended by the CCCM-SSM, outside specific situations outlined in the CCCM-SSM recommendations on antigen testing [1–2]. Thus, this document will mainly focus on saliva RT-PCR samples pooling and it complements the recent recommendations of the CCCM-SSM on saliva RT-PCR [3].

Background

Overall, the COVID-19 situation remains worrisome due to the emergence of several variants of concern (VOCs) exhibiting mutations at position 501 of the S protein. This mutation to a tyrosine (Y) in the receptor-binding domain is considered to increase the contagiousness of the SARS-CoV-2 due to increased affinity with the receptor [4]. One of these VOCs, the UK variant, was shown to exhibit 17 mutations scattered on various proteins, an increased mutations rate, and might also exhibit an increased replication in the respiratory epithelium [5]. Moreover, increased contagiousness has been especially well documented in Great Britain with the UK variant with increased effective reproduction number by 0.4 to 0.7 [6]. If this high contagiousness is due to the mutation 501Y (i.e. due to the higher affinity of the Spike protein with the ACE receptor), then three VOCs might share this phenotype: one each from United Kingdom (UK variant = B.1.1.7), from South Africa (SA variant = B.1.351), from Brazil (BZ variant = P.1), and from India (B.1.614.2).

In this setting, efforts have been undertaken to detect this variant by implementing specific search of the VOCs when (i) there is an epidemiological link, either contact to a person infected with one of these VOCs, or a person with a travel history in one of the countries where such VOCs are endemic (especially United Kingdom, South Africa, Brazil, and India), or when (ii) a large cluster is documented. In addition, prospective and retrospective surveillance has been undertaken showing that the UK variant is now present in Switzerland and in the process of replacing the other variants, with a growing proportion of variants rising across Switzerland to >95%. Reference can be added: https://pubmed.ncbi.nlm.nih.gov/33806013/

This increased contagiousness of the VOCs may be thus take place possibly due to either:

a) a better entry of the virus in cells thanks to improved affinity of the S protein (the key needed to enter the cell) with the receptor expressed at the cell surface;
b) a possible prolonged excretion of the virus (more than ten days, which corresponds to the current duration of patients isolation);
c) a higher viral load due to improved replication in lung (and nasopharyngeal) epithelial cells.

If hypothesis a) or b) hold true, then the VOCs might be more contagious even without presenting a higher viral load, i.e. without presenting an increased viral load in the nasopharyngeal cavities. This is important given (i) the documented much lower (1,000 times less) analytical sensitivity of antigen tests performed on nasopharyngeal samples as compared to the RT-PCR performed on nasopharyngeal samples (current gold standard) and (ii) the documented lower viral load (10 to 100 times less) in the saliva samples as compared to nasopharyngeal samples.

Due to the increasing presence of VOCs in Switzerland (especially the UK variant), more strict measures have been implemented from 18 January 2021 at the national level (soft lock-down). However, according to WHO recommendations, schools have not been closed due to the major impact on children and adolescent health such a measure may have.

A possible role of children in transmission is considered by some experts given (i) the observed median viral load similar to that observed in adults and (ii) the lower seroprevalence due to the mild disease, leading likely to weaker and shorter duration antibody response, and hence to apparent low seroprevalence at distance of outbreaks wave and possible reinfections cycles in this population, especially in the less than 12-year-olds, for which mask usage is difficult to implement.

Thus, there is a clear intention from political authorities to now strengthen the screening in schools, in order to specify whether these experts opinions are true and to be able to perform selected quarantines if this holds true. However, addi-

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tional difficulties that may have impaired case detection in this pediatric population is the commonly asymptomatic infection and the poor acceptance of the relatively invasive nasopharyngeal sampling in children. Thus, in some regions such as the Canton Vaud, screening of children has now been switched to usage of saliva RT-PCR, based on the excellent results of a recent study conducted by Dr Asner (pediatrics) and Dr Greub (microbiologist) in symptomatic children.

In this context, large scale screening of children, even when asymptomatic, is now considered by the FOPH, especially given a possible role of children in transmission chains. In addition, since the mortality of COVID-19 has especially been huge in the elderly with large clusters in long-term facilities, such a regular mass screening in asymptomatic individuals is also considered in such a setting. In this context, the FOPH asked the CCCM-SSM to prepare the present recommendation, specifically focusing on the pooling of saliva samples on-site and/or in the lab. Using rapid Ag tests (on-site) is also considered by the FOPH for case finding in long-term care facilities or schools with asymptomatic populations.

Antigen test in an asymptomatic population

Most clinical studies that showed convincing data regarding the use of antigen tests have been performed by including symptomatic subjects (fever, cough, and onset of symptoms within four days). This is among others, what was reported by Schwob et al., where a clinical sensitivity of 87% has been documented in patients with an acute infection [7]. This relatively good clinical sensitivity is mainly due to the very high viral load during the first four days of the disease. Later, in subjects with a mean infection duration of 11 days, we observed about 50% of sensitivity [8]. This sensitivity was even worse among the patients with an antibody response since none of the subjects with IgA in the nasopharyngeal samples were positive by antigen testing (due to competition of IgA in the secretion with the antibodies used in the test to detect the N protein) [8].

For asymptomatic subjects, the use of the antigen test on nasopharyngeal samples has been recommended by the CCCM-SSM for few specific situations when rapid triaging of patients entering the hospital may be of added value to prevent viral transmission at the emergency ward, especially when rate of test positivity is above 20%, i.e. during peaks (waves) of the current epidemic [1–2]. However, at CHUV, the clinical sensitivity of antigen tests on nasopharyngeal samples in such asymptomatic subjects was low, of about 35%, and this sensitivity was even lower – only 28% – in another Swiss hospital, where the antigen test was done directly at the bedside using the Roche antigen test [9]. Even more worrisome, in this setting with a low prevalence, despite the rather good specificity of about 99% of this antigen test, is that the positive predictive value was of only 50% [9].

Here, it is important to underline that the antigen test exhibits an analytical sensitivity of about 100,000 to 1,000,000 copies/ml as compared to the sensitivity of RT-PCR which is of 10 to 100 copies/ml (i.e 1,000 to 10,000 times less). Thus, a fair sensitivity is only obtained with nasopharyngeal antigen tests in subjects with very high viral load (>1 million copies/ml). Such a very high viral load is uncommonly observed in an asymptomatic population (outside recent transmission events) since viral loads in asymptomatic subjects are generally 100 times lower than in symptomatic subjects [10] and since acute infection may not be suspected in asymptomatic populations, outside specific clusters, household contact or any other epidemiological hint for recent exposure.

Thus, the **CCCM-SSM do NOT recommend the use of antigen tests** for routine screening of asymptomatic populations, outside specific epidemiological conditions suggesting active transmission. Due to the lower amount of viruses in saliva, likely interference of saliva with antigen tests and the overall poor sensitivity of antigen tests, the antigen tests may NOT be recommended on saliva, even in symptomatic subjects with less than four days of symptoms.

Thus, as already outlined in the CCCM-SSM recommendation [1–2], the SARS-CoV-2 specific antigen test should in principle follow the published guidelines from the FOPH, i.e. is intended

(i) for patients with symptoms of a respiratory infection with less than four days duration;
(ii) for patients managed in an outpatient setting with general less severe symptoms and in no need for hospitalization or intensive care medicine;
(iii) not for patients working in the health care system;
(iv) not for patient in close contact with vulnerable people, e.g. nursing at home;
(v) not for patients belonging to a specific high-risk population (see FOPH website).

Outside these indications, whenever possible, RT-PCR should be preferred (see below).

It may still be acceptable to use the antigen tests, for instance, when an elderly person in home care is highly suspected to get contaminated, in order to rapidly identify the persons positive with the highest risk of transmission. However, the CCCM-SSM considers that cohorting in such elderly care centers should not be done based on antigen results given the high rate of false negative results estimated to 20% among symptomatic subjects with less than four days of COVID-19 symptoms [7–8]. The sensitivity will drop quite significantly for patients with symptoms for more than four days and among infected asymptomatic individuals [10].

RT-PCR in saliva

The current gold standard for virus diagnostics is the RT-PCR [11]. This paragraph summarize the main aspects already discussed in the CCCM-SSM recommendation on saliva RT-PCR [3]. First, nasopharyngeal swabs are considered to be the best sample to detect SARS-CoV-2 [11]. However, saliva may be used for mass testing using PCR for many reasons. Saliva can easily be collected, and the collection procedure is more comfortable for the patients. Saliva RT-PCR is especially recommended for children or the elderly [3]. The collection of saliva also does not require specifically trained personnel, and everybody is used to "swab" their teeth with a toothbrush three times a day, making buccal swabs easy to propose. Thus, even **self-collection of saliva** by using a mouth swab may be proposed. RT-PCR performed on self-made buccal swabs represents a very good solution to decrease the workload of testing centers and to in-

crease accessibility and acceptance of tests (for instance with packages available in all pharmacies and/or in post offices). Samples can then – once sampling is done at home – be safely transported to a laboratory for RT-PCR testing by surface mail like any other patient sample (UN 3373 standard). Such packages for home testing have been made available in Lausanne just before the Christmas holidays and are especially appreciated by the oncologists for the vulnerable patients that may be repeatedly tested, without need to expose themselves to other potentially contagious persons in testing centers. It was also used for healthcare workers at CHUV.

RT-PCR testing from saliva has been studied in **symptomatic subjects** [7, 10]. When sampling is done properly, this approach may exhibit sensitivities of 95% [4] in patients with acute symptoms of COVID-19 (≤ 4 days). Moreover, RT-PCR testing from saliva exhibits a specificity of more than 99.9%.

RT-PCR from saliva will detect subjects that exhibits more than 10,000 copies/ml in nasopharyngeal swabs and still exhibiting about a 100 times better sensitivity as compared to antigen tests on nasopharyngeal samples [3].

Due to the high sensitivity of RT-PCR, this approach is also suitable to test any subjects with more than seven days of symptoms as well as **asymptomatic individuals.** Recently, we have shown that among patients hospitalized in the internal medicine ward or in the ICU, the PCR on saliva (buccal swabs) exhibited a sensitivity of 69% that contrasted to the sensitivity of 4% to 8% observed using antigen tests on the same samples [12]. Moreover, the saliva PCR was even clearly better in terms of sensitivity than the nasopharyngeal antigen test that was only positive in 35% to 45% of subjects, despite the fact that these exact same samples were PCR positive in as many as 98% of cases [12].

Detailed recommendations on saliva sample collection and PCR systems compatible with such sample is provided in the recommendation of the CCCM-SSM [3].

Saliva pooling outside laboratories

RT-PCR may be subject to contamination due to the amplification process. Moreover, the risk of cross-contamination between samples from different persons is especially high with SARS-CoV-2 since some subjects (so-called superspreaders) may have a very high viral load, even in the saliva. Thus, the CCCM-SSM do NOT recommend the pooling inside laboratories. The laboratories that still intend to propose such in-lab pooling should ensure quality by using the following measures: pooled samples should only be handled in laboratories with experienced technical personnel, appropriate laboratory equipment and infrastructure for diagnostics, e.g. safety bench for potential contagious materials, a quality assurance concept, and also access to samples for verification and validation.

This situation is not the case outside of such laboratory dedicated to diagnostics, and hence pooling directly at schools or in long-term facilities may be problematic, with significant risks of loss of traceability and of cross-contamination of samples. Thus, the CCCM-SSM does only recommend pooling at school in a highly dynamic epidemic situation with high incidence of more than 1,000 persons/100,000 inhabitants per week (see below). Then, pooling of four or five samples only will be recommended since in such high incidence, pooling of a larger number of samples will not make sense since the positivity rate of a pool, that necessitates to retest all patients belonging to that pool, will be counterproductive. Even in such epidemic situations, if there is no reagents shortage and if there is sufficient access to PCR, the CCCM-SSM would recommend to focus on individual PCR testing instead of pooling since pooling impacts the time to results negativley.

Conclusion – recommended test indications for RT-PCR from individual saliva obtained by buccal swabs

Nasopharyngeal RT-PCR remains the gold standard for patients who require hospitalization or are criticaly ill in order to provide a precise diagnosis. Saliva-based PCR assays are predominantly suited for outpatient scenarios or specific symptomatic and asymptomatic cohorts.

- **Any symptomatic outpatient,** especially when symptoms started more than four days ago; even if the symptoms started less than four days ago, a PCR test should be preferred to the antigen test for individuals exposed to vulnerable subjects (persons living in the same household or healthcare workers)
- Diagnosis of symptomatic **pediatric outpatients**
- **Any subject exposed to a confirmed patient (contact),** at day 5 after exposure, in order to triage between isolation and quarantine for ten days
- **Screening tool at borders** for symptomatic and asymptomatic persons entering Switzerland from a country with a higher prevalence (except commuters) to avoid unnecessary quarantine
- **Asymptomatic persons,** for instance in a pharmacy, surgery or dedicated testing center or by self-collection of saliva using pre-prepared kits, available in pharmacies, train stations, ski resorts, and other touristic locations
- **Subjects at risk of infection with a variant of concern (VOC).** The saliva and nasopharyngeal PCR are especially recommended (over antigen testing) for any subject that has travelled to an area endemic for a VOC or exposed to someone infected by a VOC since (i) antigen tests when performed using the "dry swab" approach do not provide access to material for further sequencing, and (ii) antigen tests are more likely than PCR to give false negative results.
- To increase acceptance **to anyone** (fulfilling criteria of an antigen test but) **preferring a saliva PCR test** in order to avoid a nasopharyngeal sample (and to get a better sensitivity)

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Date and versions
First draft prepared from 17 to 19 January 2021 and shared with the Federal Office of Public Health (FOPH) on 20 January 2021.
Second current draft modified in April 2021 and validated on 21 April 2021 by the CCCM-SSM members.

Referenzen

Online unter www.sulm.ch/d/pipette -> Aktuelle Ausgabe (Nr. 3-2021)