L'UTILISATION DE SALIVE ET DES ÉCOUVILLONS NASAUX POUR LA DÉTECTION DU SARS-COV-2 - MISE À JOUR SEPTEMBRE 2021

RAG sous-groupe Testing – 6 september 2021; validé en RMG 9 septembre 2021

Note : Les recommandations actuelles sont susceptibles d'être modifiées en fonction de nouvelles informations et/ou de l'évolution de l'épidémie.

Recommandations principales :

- Le RAG Testing considère que les recommandations actuelles concernant l'utilisation d'échantillons de salive et d'écouvillons nasaux antérieurs sont toujours valables.
- Leur utilisation est déjà possible dans plusieurs situations et cela devrait être communiqué plus clairement aux prestataires de soins de santé.
- Les échantillons de salive sont actuellement déjà considérés comme une alternative acceptable pour les tests RT-PCR dans :
  - les patients présentant des symptômes <=5 jours ;
  - lorsqu'un prélèvement nasopharyngé ou un prélèvement combiné nez-gorge est très difficile ou impossible, par exemple en cas de malformation de la cloison nasale, chez les patients très jeunes, chez les patients souffrant de troubles psychiatriques ou chez les patients qui ressentent une douleur ou un inconfort excessif lors du prélèvement nasopharyngé ou du prélèvement combiné nez-gorge ;
  - dépistage répété ;
  - le dépistage avant de participer à un événement (seulement sous la supervision d'un professionnel de santé).
- Les échantillons de salive sont obtenus de préférence après un raclage de gorge, comme décrit dans le protocole préparé à cet effet.
- Les écouvillons nasaux antérieurs ou mi-turbiné sont déjà considérés comme une alternative acceptable pour les:
  - tests RT-PCR ou Ag rapides chez les patients présentant des symptômes <=5 jours dans les cas où le patient ressent trop de douleur ou d'inconfort avec un écouvillon nasopharyngé ou combiné nez-gorge ;
  - autotests.

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CONTEXT

In a recent advice on the testing, quarantine and isolation procedures, it was recommended to overall maintain the current testing procedures, as long as the epidemiological situation remains worrisome (alarm level 2). Because of the higher risk of post-vaccination infections by the Delta variant, testing indications in fully vaccinated people were expanded. For some indications it was not recommended to systematically test, but to encourage people to self-test. For this reason, access to testing should be made more easy and the RAG testing was requested to give an advice on alternative sampling and testing methods to be explored, for example a broader use of saliva specimens.

The current recommendations with regards to the use of saliva specimens are summarized in annex.

DISCUSSION

- Since the last advices on the use of saliva samples, there appears to be no new scientific evidence with regards to its performance.
- Recommendations by international agencies have not been altered, and also the neighboring countries continue to provide the same guidance.
- The current Belgian recommendations on the use of saliva samples were already broadened and it is now also accepted for testing (with RT-PCR) symptomatic patients (with symptoms <=5 days) and pre-event screening of asymptomatic people, in addition to the already existing indications in repetitive screenings and when a nasopharyngeal swab or combined nose-throat swab is very difficult or impossible.
- It appears that health care providers are not well aware of when saliva specimens can be used, and there is a need to better communicate the current guidance.
- A systematic Cochrane review on the use of saliva specimens in preparation has concluded that the type of saliva specimen makes an important difference, and that enhanced saliva (after deep throat clearing or gargling) performs better than simply spitting. The current protocol that was developed for the collection and analysis of saliva samples for SARS-CoV-2 already instructs that saliva is preferably obtained by scraping the throat, but also this protocol does not appear to be known by health care providers[1].
- Scientific evidence with regards to the use of saliva for rapid Ag testing continues to be worrisome.
- Also the current recommendations with regards to the use of anterior nasal samples, another more acceptable alternative sampling method, appear to be insufficiently known[2]. The current recommendation is that they can be used, for either RT-PCR or a rapid Ag test, in symptomatic patients during the first 5 days of symptoms and in situations where a patient experiences too

[1] See : 20201130_Advice RAG_Saliva sampling_NL.pdf (sciensano.be) or 20201130_Advice RAG_Saliva sampling_FR_0.pdf (sciensano.be)

[2] See : 20210517_Advice_RAG_Use of nasal swabs_NL.pdf (sciensano.be) or 20210517_Advice_RAG_Use of nasal swabs_FR.pdf (sciensano.be)

much pain or discomfort during the nasopharyngeal swabbing. They are also accepted for self-testing.

RECOMMENDATIONS

- To maintain the current recommendations with regards to the use of saliva and anterior nasal swabs.
- To better communicate to health care providers what specimens are allowed in what circumstances.

SCIENTIFIC LITERATURE

Sensitivity of RT-PCR on saliva vs. on nasopharyngeal swabs in symptomatic individuals

An extensive review of the literature is available in previous advices[3]. Since then, more studies have been published, but not changing substantially the conclusions based on earlier studies.

The large majority of the research is among symptomatic people (hospitalized patients or people attending an OPD or an emergency department). Several systematic reviews and meta-analyses came to same conclusions (1–9):

- RT-PCR on saliva samples in symptomatic people generally has a somewhat lesser sensitivity than on nasopharyngeal swabs (NPS) - a loss of about 2-5% - and detects about 85% of positive cases.
- Viral load in saliva samples is usually higher than in nasopharyngeal samples, indicating that it are mostly cases with a low viral load that are undetected.
- Sensitivity is almost equal to nasopharyngeal swabs in patients with recent/severe symptoms or high viral load.
- Most reviews conclude that saliva is an acceptable alternative specimen collection method in a context of diagnosis in ambulatory care.
- There is some evidence that sensitivity in self-collected samples is lower than in saliva specimens collected under supervision (10).

Performance in asymptomatic individuals

Evidence on the performance of saliva specimens among asymptomatic people, for screening purposes, is still less extensive, but increasing. The most relevant studies are summarized below. Conclusions are hard to make, because of the often large discrepancy between study findings and the often low number of positive samples included. Some authors conclude that saliva is an appropriate sample for screening purposes, while others conclude it is not. All studies that included both symptomatic and asymptomatic people found consistently a lower sensitivity in asymptomatic than in symptomatic persons.

[3] See : 20210614_Advice_RAG_Saliva and self-collected nose-throat swabs_NL.pdf (sciensano.be) or 20210614_Advice_RAG_Saliva and self-collected nose-throat swabs_FR.pdf (sciensano.be)

Herrera et al. assessed concordance between NPS and saliva among 2017 asymptomatic healthcare and office workers in Mexico (11). 178 (8.4%) tested positive with NPS and 152 (7.2%) with saliva. Using positive with either sample as a reference and excluding inconclusive results, the sensitivity was 94.5% for NPS and 81.4% for saliva. However, saliva had a lower number of inconclusive results and showed a significantly higher concentration of both total RNA and viral copies than NPS.

Norizuki et al. assessed, over a 7 days period, the sensitivity of different tests on nasopharyngeal, anterior nasal and saliva samples taken from 20 asymptomatic air travelers who had tested positive with RT-PCR on a NPS and were under quarantine in Japan (12). On a total of 97 samples tested, the sensitivity compared to RT-PCR on NPS was 69% for RT-PCR on a nasal swab and 64% for RT-PCR on saliva, comparable to the sensitivity of a rapid Ag test (Fujirebio) on a NPS (60%). Sensitivity of an automated Ag test (Lumipulse) on saliva was 55%. Among 33 samples with viral load ≥ 104 copies/sample, sensitivity was 100% for both RT-PCR on a nasal swab and RT-PCR on saliva (which was equal to the sensitivity of the rapid Ag test on NPS), and 91% for an automated Ag test on saliva.

Yokota et al. compared the utility of RT-PCR for mass screening, using NPS and saliva samples in 2 cohorts of in total asymptomatic persons in Japan: a contact-tracing cohort (161 people) and an airport quarantine cohort (1763 people) (13). In the contact-tracing cohort, 41 people tested positive on the NPS and 44 on the saliva samples. In the airport quarantine cohort, 5 people tested positive on NPS and 4 on saliva. The sensitivities of NPS and saliva were 88% (46/52) and 92% (48/52), respectively. Viral load was equivalent between saliva and NPS.

Mendoza et al. implemented a pooled surveillance testing program for asymptomatic SARS-CoV-2 infections in K-12 schools and universities in New York (14). Students, faculty and staff were tested 1,2 times per week using saliva specimens (SalivaClear™). They compared the detection of SARS-CoV-2 in saliva and in the reference testing nasopharyngeal swab modality in 20 positive and 100 negative samples, and found 100% agreement. The median Ct value of the nasopharyngeal swab was substantially higher than the median Ct value of the concurrently collected saliva specimens (30.4 and 21.0, respectively).

A study in French Guyana compared saliva and NPS samples taken from 776 people during outreach screening campaigns, of which 39% were asymptomatic (15). Of the 162 people that tested positive on either sample, 152 (94%) tested positive on the NPS and 86 (53%) on saliva. Sensitivity of saliva, using NPS positive samples as the reference, was substantially lower among asymptomatic people (only 24%) than among symptomatic people (77%). Sensitivity was much better in samples with a high viral load (83% when Ct value<30) and all samples that tested positive on saliva and negative on NPS had a high viral load (<25).

A study in France compared NPS and saliva, using two different RT-PCR procedures, among people attending community screening centers (16). 1451 participants were enrolled, of which 571 presented with symptoms and 564 were high-risk contacts. 129 tested positive on the NPS and 167 on saliva. Sensitivity of saliva, using a positive RT-PCR on either sample as reference, was 93% and 87%, depending on the procedure used, and was higher than the sensitivity of the RT-PCR on NPS (65%). Sensitivity was higher in symptomatic than in asymptomatic people (95% vs. 91%, and 92% vs. 78%, for the two procedures respectively). Viral load was overall higher in saliva than in NPS. Interestingly, consumption of alcohol, coffee, and food, smoking, or teeth brushing within 30 min before sampling had no impact on diagnostic accuracy.

A recent research letter in JAMA reported the results of a prospective study among household contacts in the US (17). Paired nasopharyngeal and saliva samples were collected every 3 to 7 days and tested with RT-PCR for up to 4 weeks or until 2 negative nasopharyngeal test results. SARS-CoV-2 was detected in 524 nasopharyngeal and 318 saliva specimens. Saliva sensitivity was highest in samples collected during the first week of infection (71.2%) but decreased each subsequent week. Participants who presented with COVID-19–associated symptoms had significantly higher saliva sensitivity compared with asymptomatic participants (88.2% vs 58.2). The authors conclude that saliva was sensitive for detecting SARS-CoV-2 in symptomatic individuals during initial weeks of infection, but sensitivity in asymptomatic SARS-CoV-2 carriers was less than 60% at all time points. Their results suggest that saliva-based RT-PCR should not be used for asymptomatic COVID-19 screening.

Performance in children

A literature review specifically on the performance of RT-PCR testing on saliva samples in children is available in previous advices. Since the last advice, a few additional studies have been published (18–22), but the problems with most of these studies persist. Many have very small sample sizes and include mostly older children, with very few children less than 6 years old. Almost all studies are in symptomatic children.

For older children (>10 years) performance appears to be similar as in adults (see above), but no definite conclusion can be made for small children (<6 years old).

Sensitivity of rapid Ag tests on saliva vs. on nasopharyngeal swabs

A literature review specifically on the use of saliva for rapid Ag testing is available in the advice on the subject of 17 May 2021[4]. Since then, a few additional studies have been published (see below), but the conclusion remains largely the same. There are too many studies that found an alarmingly low sensitivity, even when Ct values are low, to consider it an appropriate sampling method for rapid Ag testing.

In a letter to the editor, Seitz et al. reported the results of an evaluation of a rapid Ag test (Xiamen Zhongsheng Langjie Biotechnology Co.) on saliva taken from asymptomatic people participating in a voluntary mass screening program in Austria (23). Forty people participated, 18 with positive rapid Ag test results on NPS and 22 not undergoing NP sampling. Of the 18 positive cases, 8 (44%) tested positive on saliva, and of the 5 samples with a Ct value <=30, 3 (60%) tested positive.

Schildgen et al. assessed the performance of three rapid Ag tests on throat washing (TW) samples (24). Sixty TW samples that had tested positive with RT-PCR and 8 TW samples that had tested negative were retested with the rapid Ag tests. Sensitivity in 23 symptomatic patients was 30%, 40% and 100% for the RapiGen, Panbio and SD-Biosensor tests, respectively, and 31%, 39% and 85% in 27 asymptomatic persons. Specificity ranged from 7.7% to 85% in symptomatic and from 14% to 93% in asymptomatic.

Uwamino et al. retested 117 NPS specimens and 73 saliva samples with positive results on RT-PCR and with enough residual volume with a rapid Ag test (Espline SARS-CoV-2 RAD kit -

[4] See: 20210517_Advice_RAG_Use of saliva for rapid Ag testing_NL.pdf (sciensano.be) or 20210517_Advice_RAG_Use of saliva for rapid Ag testing_FR.pdf (sciensano.be)

FUJIREBIO) on the day of sample collection (25). Seventy-two percent of NPS specimens that were collected within 4 days of symptom onset were positive for the rapid Ag test test. However, the positivity rate of NPS specimens collected 5 days after symptom onset was less than 30%, and those of saliva samples were lower than 30% in each time period. On the other hand, none of the saliva samples that tested negative with the rapid Ag test were positive on culture.

INTERNATIONAL RECOMMENDATIONS

ECDC

Since the last RAG advice on the use of saliva specimens, ECDC has not published any new guidance. The recommendations of the last Technical Report (May, 2021) remain therefore valid:

- For symptomatic patients as an alternative to nasopharyngeal swabs for RT-PCR tests within the first five days after symptom onset or when practical considerations make nasopharyngeal swabbing difficult
- Optionally, for screening asymptomatic individuals who are required to self-test frequently for occupational or other reasons. Screening of asymptomatic individuals using saliva for RT-PCR can also be considered as an alternative method if nasopharyngeal swabs cannot be obtained, e.g. in case of shortages of swabs, in very old or disabled individuals, and to increase acceptance for repeated testing.
- When using saliva as a sample material, its limitations need to be considered.
- Nasopharyngeal swabs remain the preferred sample option for persons with high risk of exposure to a positive COVID-19 case.
- Not enough available data to recommend diagnosis based on saliva samples in children.

France

Also France has not issued any new guidance since the last RAG advice. The use of saliva is approved by the 'Haute Autorité de santé (HAS)' in:

- symptomatic people for whom nasopharyngeal swabbing is difficult or impossible (deviation of the nasal septum, very young patients, patients with psychiatric disorders...);
- as an alternative in high-risk contacts for whom a nasopharyngeal swab is not feasible;
- for large-scale targeted screening, especially if it is repeated regularly, for example in schools, universities, health care settings or nursing homes.

The 'Haut Conseil de la Santé Publique' (HCSP) prioritizes the use of saliva specimens for the following populations:

- Repetitive screening and contact testing in health care professionals;
- Repetitive screening and contact testing in hospitalized patients;
- Repetitive screening and contact testing in nursing home residents;
- Screening of nursing home staff and visitors.

It further prioritizes saliva sampling for:

- When nasopharyngeal swabbing is not possible;
- Repetitive screenings, in particular among health staff and nursing home staff.

The Netherlands

RIVM has maintained its recommendations on the use of saliva specimens. It approves it for testing children under 6 years of age (using an Oracol sponge) and in exceptional cases for other patients of all ages in the care of the disabled and in (psycho)geriatrics, in whom it is impossible to take naso- and oropharyngeal swabs

Germany

The Robert Koch Institute does not disapprove the use of saliva specimens for COVID-19 testing, but warns that the sensitivity may be more or less inferior to the reference method. The use of these sample materials should therefore take place taking into account the respective setting and in close consultation with the laboratory.

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ANNEX: CURRENT RECOMMENDATIONS ON THE USE OF SALIVA SPECIMENS FOR SARS-COV-2 TESTING

The current recommendations on the use of saliva for RT-PCR testing are available in the RAG advice 'Saliva and self-collected nose-throat swabs' of 14 June 2021[5] and on the use of saliva for rapid antigen testing in the RAG advice 'Use of saliva for rapid Ag testing' of 17 May 2021[6].

Use of saliva for RT-PCR testing

- A nasopharyngeal swab or a combined nose-throat swab are the preferred samples for SARS-CoV-2 testing.
- Saliva specimens are a valid alternative in the following circumstance:
    - Symptomatic patients with symptoms <=5 days (but rapid Ag test on a nasopharyngeal swab remains the first choice)
    - If a nasopharyngeal swab or combined nose-throat swab is very difficult or impossible. Examples are: deviation of the nasal septum, very young patients,

[5] See : 20210614_Advice_RAG_Saliva and self-collected nose-throat swabs_NL.pdf (sciensano.be) or 20210614_Advice_RAG_Saliva and self-collected nose-throat swabs_FR.pdf (sciensano.be)
[6] See: 20210517_Advice_RAG_Use of saliva for rapid Ag testing_NL.pdf (sciensano.be) or 20210517_Advice_RAG_Use of saliva for rapid Ag testing_FR.pdf (sciensano.be)

patients with psychiatric disorders, or patients experiencing too much pain or discomfort during the nasopharyngeal or combined nose-throat swabbing.
    - Repetitive (weekly) screening of asymptomatic people.
    - Pre-event screening of asymptomatic people, if under close supervision of a health care provider or other trained person.

- The use of saliva is not advised in asymptomatic high-risk contacts and arriving/returning travelers
- The use of saliva is permitted in departing travelers, if it is approved by the country destination, under close supervision of a health care provider or other trained person

Use of saliva for rapid antigen testing

- Until the reasons for the sometimes very low performance of rapid Ag tests on saliva is clarified: maintain the recommendation not to use rapid Ag tests on saliva specimens.