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Covid-19: which samples for which tests?

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The government's policy for fighting the Covid-19 epidemic is based on the triptych "Test, Alert, Protect". This strategy is based on different laboratory techniques, each type of test being dependent on a sampling method. Among the available tests aimed at detecting the presence of SARS-CoV-2 in a sick or asymptomatic person, the choice must take into account anatomical, virological and epidemiological data on the one hand, and the diagnostic or epidemiological objective on the other hand. During the infectious cycle, viremia is inconstant, low and short term, there is no viruria and fecal elimination is variable. Since the highest amounts of virus are found in the upper and lower airways, the methods used to collect viral material favor in practice the nasopharyngeal swab, the reference technique, and the salivary swab [1].

I. Nasopharyngeal swabs

While it is easy to collect a saliva sample, obtaining a sample of nasopharyngeal secretion is more difficult because of the first approach: it is indeed a question of guiding a flexible swab through the nasal cavity to reach the nasopharynx 5 to 6 cm behind the nasal orifice. This sampling technique, which is not without risks, must be based on a good knowledge of anatomical landmarks.

1 - Anatomical reminder of the nasal cavities and the nasopharynx

The depth of the nasopharyngeal swab should take into account the complexity of the nasal anatomy and its congenital variations or related to an underlying nasal-sinus pathology.

The nasal cavity is a narrow space connecting the nasal vestibule forward to the nasopharynx behind. The two nasal cavities, right and left, are separated by a septum which is frequently deviated; as a result, the two nasal cavities are rarely symmetrical. When a swab is introduced into the nasal cavity, it is necessary to have a spatial representation of the organ to be crossed, each nasal cavity having the shape of a parallelepiped:

-below, the lower wall separates the nasal cavity from the mouth; it is the nasal floor that the swab should follow from front to back, thus conditioning the position of the head of the sampled person.

-at the top, the upper wall separates the nasal cavity from the endocranium, containing the brain. This roof of the nasal cavity also supports the organ of smell. This is a danger zone that must be avoided by placing the head correctly when taking the sample.

-in front, the anterior zone is the nasal vestibule across which the swab will be introduced.

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-in the back, the posterior side communicates with the nasopharynx, the sampling site.

-inside, the septum separates the nasal cavity from the opposite nasal cavity. This septum may be subject to anatomical abnormalities which can complicate the sampling.

- outside, the nasal cavity is mainly related to the sinuses of the face and the orbit. In its lower part, it supports the inferior nasal turbinate that the swab must follow, in parallel to the nasal floor (diagram). It is from this side wall that elements can interfere with the swab, such as polyps from the sinus cavities.

Once the end of the swab has passed through the nasal cavity and entered the nasopharynx, it is in a cube shaped cavity, which is the site of the collection on the posterior side.

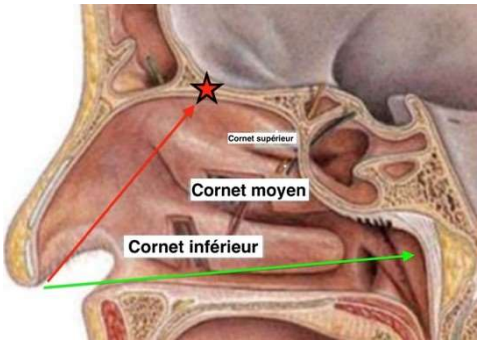

Figure: The near horizontal arrow indicates the route to be taken by the swab.

The difficulties that can be encountered when crossing the nasal cavity are mainly due to anatomical variations, more rarely pathological ones These anomalies are frequent. They are essentially:

- a deviation of the septum: these anomalies are common. They can be simple, watch glass shape, obstructing one nasal cavity but leaving the opposite one wide open. But they can be complex, zig-zagging, leading to an obstruction of both nasal cavities. As they are of cartilaginous or bony origin, it is not possible to force the passage without the risk of causing mucous wounds and bleeding, as the mucous lining of the septum is fragile and highly vascularized.
- hypertrophy of the lower nasal cone: this anomaly is also frequent since it is a marker of chronic rhinitis, its prevalence is estimated at more than 10% of the population. The mucous membrane of the lower nasal cone is soft and can be pushed back with caution when the swab is introduced into the nasal cavity.
- polyps: often present in any sinus disease of the face, their size varies considerably from one patient to another. The largest are observed in patients with naso-sinus polyposis, whose semiological marker is a former loss of smell which can be found in questioning. If they are not too large, the polyps can be pushed back by the swab when it passes through the nasal cavity.
- a nasal or sinus surgery: these are routine ENT operations (surgery of the septum, lower nasal cone, and especially the sinuses of the face) to be sought by questioning the patient on his surgical history before any sampling. Indeed, major sinus surgery, such as ethmoidectomy, can weaken the roof of the nasal cavity which is no longer protected by the various endo-cavitary structures. It is therefore important to be careful when

taking the sample and to carefully follow the floor of the nasal cavity, without branching up.

2 - The nasopharyngeal sampling technique

As soon as contamination occurs, SARS-CoV-2 replicates in the cells of the nasal mucosa, spreads along the upper airways and then into the respiratory tree. The ideal sampling site for testing for the presence of viral genetic material is the nasopharynx, the first targets of the virus being the hair cells of the upper airways.

The sampler must be protected (FFP2 mask, goggles, overshirt, gloves).

The sampled person must be reassured. The nature of the actions that will be performed must be explained to him. Before the sample is taken, the patient must be questioned on:

-the existence of frequent nasal symptoms such as nasal obstruction, specifying the obstructed side, which will guide the sampling on the opposite side;

-the presence of a major loss of sense of smell, associated with nasal obstruction, which may lead to the discovery of polyps in the nasal cavities;

-surgical history: operations on the sinuses of the face (especially ethmoidectomy) requiring the procedure to be performed with caution, away from the upper part of the nasal cavity.

Technically, the most important point is to "visualize" the path of the swab, which should be located in the lower third of the nasal cavity, fleeing the upper two thirds. To do this, the swab must follow the lower nasal cone whose direction is strictly parallel to the floor of the nasal cavity. This lower nasal cone is about one centimeter above the floor of the nasal cavity.

To do this, the patient must have the head straight with the chin parallel to the floor. The head should never be stretched out, let alone hyperextended, as this will naturally direct the swab towards the areas of the nasal cavity to be avoided, especially the roof. In addition, the sampling site would not be suitable as far from the nasopharynx. The swab therefore follows the floor slowly and steadily reaches the tail of the lower nasal cone or even the posterior wall of the nasopharynx. It is then necessary to rub gently and not "scrub" the mucous membrane. The cells are collected at the end of the swab withdrawn without any brutal gesture. It is therefore a smear.

In case of resistance, one should never try to force the swab into the nasal cavity as this may cause a wound of the nasal septum and bleeding.

The training of the samplers is simple: it consists of a face-to-face teaching of about one hour and can ideally be assisted by an ENT specialist who can illustrate this training with the film of a nasal fibroscopy, which is very didactic to describe the anatomy of a nasal cavity as well as possible.

II. Saliva samples

Saliva, which in the form of sputum or aerosols, serves as a vehicle for person-to-person transmission of SARS-CoV-2, can also be used for its detection. The virus can enter the saliva from the mucous membranes of the lower and upper respiratory tract where it replicates, but also through infection of the salivary glands [2].

In practice, a saliva sample can be collected by spitting into a sterile container (tube or sufficiently flared jar) or by sublingual pipetting. This is a self-collection method and is suitable for mass screening. On the other hand, the heterogeneous composition of the saliva fluid requires a number of precautions. The collection of the sample must comply with some preconditions (not having eaten, drunk or smoked for half an hour). Then, a homogenization and fluidization step is necessary before transferring the sample to a storage tube, these manipulations exposing the sample to a risk of contamination and overloading the pre-analytical step [3]. Saliva sampling is commonly performed in the UK, Germany and the USA. In France, the Haute Autorité de Santé (HAS) has recommended it for people in whom nasopharyngeal sampling is difficult, such as young children, the elderly or the mentally retarded. Unfortunately, its practice is still marginal due to pre-analytical technical constraints and a sensitivity estimated by the HAS to be lower than the nasopharyngeal sampling [4], but considered to be equivalent by other meta-analysis [5].

III. Which tests for which objectives?

Virus testing can be part of a clinical (confirming the diagnosis of SARS-CoV-2 infection) or epidemiological (screening for SARS-CoV-2 carriers) approach.

1 - Diagnostic sampling

These samplings are primarily intended for patients with symptoms suggestive of Covid-19, people living in the same household and their recent contacts. Nasopharyngeal sampling followed by RT-PCR remains the reference method, to be preferred in all these indications and each time the result of another technique leaves a doubt or is in disagreement with the clinic. Moreover, the spread of SARS-CoV-2 new variants, which have appeared in Great Britain, South Africa and Brazil, requires the development of new screening RT-PCR and multiplex PCR kits to detect them before confirmation by sequencing.

Saliva sampling followed by RT-PCR offers a very useful alternative when nasopharyngeal sampling is difficult or even dangerous.

2 - Epidemiological sampling

The first aim is to identify the transmission chains, downstream and upstream of confirmed cases, in order to interrupt them.

More broadly, virological tests contribute to the epidemiological surveillance by providing essential indicators for controlling the epidemic. In order to monitor the circulation of SARS-CoV-2 and its variants, representative samples of regional, departmental and local populations, corresponding to activity groups (hospitals, businesses, schools) or communities (EHPAD, USLDs, medico-social establishments, prisons) should be tested at short intervals.

In this population-based screening framework, which is essential for the early detection and containment of outbreaks of a resurgent epidemic, saliva sampling (followed by RT-PCR), which has the advantage of simplicity, can be taken as a first-line test [6].

However, since RT-PCR analysis always requires a minimum response time of 24 hours, it is desirable to develop new techniques that will enable rapid results to be obtained. For example, the RT-LAMP (Loop-mediated isothermal Amplification) isothermal amplification system, which can be used on an integrated system without RNA extraction, can provide a result in 60 minutes. In the LAMP-seq version, the addition of a barcode system assigned to each sample would make it possible to analyze a mixture of several hundred to several thousand samples in a single amplification step [7]. This fast and inexpensive method would be particularly suitable for mass screening activities, but it has not yet been validated.

The unstable epidemiological situation that has prevailed in France for several weeks now makes it urgent to approve screening methods with acceptable analytical performance and offering the dual advantage of easy sampling and rapid results.

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