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Development of an Indexed Score to Identify the Most Suitable Biological Material to Assess SARS-CoV-2

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Abstract: Introduction: The rapidly contagious process of respiratory viruses such as SARS-CoV-2 makes it urgent to multiply testing for diagnostics to identify the active viral shedding cases (current infection, carriage state or, residual viral RNA) and decrease the risk of transmission to other patients and healthcare professionals. Although nasopharyngeal swabs (NPSs) are the most common specimen type used for COVID-19 diagnosis, they require supervision by a professional, and concerns have been raised regarding healthcare personnel exposure, difficulty in collection, and patient discomfort. Viral RNA can also be detected in specimens such as saliva, blood, bronchoalveolar lavage fluid, sputum, faeces, and urine. This study aimed to provide updated information about the most suitable biological material to diagnose SARS-CoV-2, considering the risk assessment, specialization needed, test cost, complexity of the collection, and sample treatment associated with the different types of specimens. Methods: An extensive search of scientific review articles was made to collect information about the biological specimens to identify SARS-CoV-2 in the urine, sputum, nasopharyngeal, oropharyngeal, bronchoalveolar (BAL), saliva, faeces, and blood. For this purpose, an index score was developed based on seven categories: Materials and Equipment; Infection Risk for the Health Professional; Infection Risk for the Patient; Collection; Cost; Specialized HR; and RNA Extraction Type. Results and Discussion: Each criterion from the index score was quoted from 1.0 to 5.0, and a sum was made to classify which specimen is the best choice to diagnose SARS-CoV-2, according to the chosen parameters. Data indicated that urine specimens are the most elementary biological sample to access. Regarding RNA extraction, NPSs, OPS, and BAL presented the maximum score. However, BAL has the lowest score regarding associated costs. Concerning sputum and saliva, all the aspects were evaluated with a score of 5.0 except for the RNA Extraction Type in sputum. Regarding the total scores of the multiple specimens, the lowest corresponds to BAL with a score of 1.7, followed by blood with 3.1 and NPSs and OPS with 3.6 and 3.7, respectively. Urine and faeces have the same value, 4.4, sputum has 4.9, and the highest and maximum possible value corresponds to saliva with 5.0, making this last specimen the most suitable for all considered parameters. Conclusion: Although OPS and NPSs are the most used specimens, there are better alternatives. Among all the specimens of the respiratory system, saliva is the most cost-effective specimen for performing SARS-CoV-2 diagnosis. Even though these infections are usually diagnosed clinically based on symptoms and local epidemiology, the identification of the specific pathogen may affect clinical management and be crucial for containing potential outbreaks.

Keywords: SARS-CoV-2; COVID-19; biological material; score; risk

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1. Introduction

Coronavirus Disease 2019 (COVID-19) caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2, formerly known as 2019-nCoV) was first reported in China

and has been spread worldwide. On 11 March 2020, the World Health Organization (WHO) announced COVID-19 as a public health emergency of international interest. According to the last report of January 2024, over 700 million confirmed cases and almost 7 million deaths have been recorded worldwide [1]. The rapidly contagious nature of SARS-CoV-2 makes it urgent to multiply testing for COVID-19 diagnostics, to identify the active viral shedding cases (current infection, carriage state or residual viral RNA) and decrease the risk of transmission to other patients and healthcare professionals, including transmission from asymptomatic people [2,3].

Currently, the gold standard method for the identification of SARS-CoV-2 is quantitative reverse transcription polymerase chain reaction (RT-qPCR) of upper respiratory tract samples collected with naso- and/or oropharyngeal swabs (NPSs and OPSs) [4–6].

Although the NPS is the most common specimen type used for COVID-19 diagnosis, it requires the skills of a trained health professional to perform sample collection [7]. Furthermore, there is concern regarding the exposure of healthcare staff, procedure complexity, and patient discomfort during collection [8].

Viral transmission can occur through dispersal in the air of droplets from the respiratory tract generated when the patient talks, coughs, or sneezes, even in asymptomatic individuals. As saliva is one of the secreted fluids [9] that can be self-collected without supervision by the drooling technique, the use of this specimen as an alternative to NPSs and OPS has been suggested [7,10].

Viral RNA can also be found in blood [11], detecting SARS-CoV-2 RNA in plasma or serum could be an alternative diagnostic approach [12]. Additionally, this type of sample is convenient in some occasions, such as when the patient is in a coma or unresponsive [12].

Bronchoalveolar lavage fluid (BLA) samples can also be an option because it is a very good specimen with a high viral detection rate. However, the collection procedure is extremely invasive and requires the use of specific medical material, and it is typically collected from patients with severe illness or those undergoing mechanical ventilation [13].

Moreover, although previous studies have reported that most symptomatic COVID-19 patients have low sputum production levels [14], which makes specimen collection difficult in many clinical settings [8], this biological fluid can also be considered a viable option since it is a non-invasive method [14].

With the available information regarding this disease, it is clear that the most common symptoms are related to the respiratory tract system; however, gastrointestinal symptoms such as nausea and diarrhoea have also been observed [15]. Therefore, transmission through stool shedding or the oral–faecal route is possible, making faeces specimens another target for SARS-CoV-2 identification methods [16]. In addition to gastrointestinal symptoms, several cases of acute kidney injury have also been noticed and, for that reason, urine specimens are being considered as another option for COVID-19 diagnosis and an alternative specimen for the use of swabs [17].

This comprehensive scientific analysis aims to provide updated information about the most suitable, cost-efficient and safe biological sample to identify SARS-CoV-2 using the qRT-PCR methodology, considering the risk assessment, specialization required for sampling, test cost and complexity of the collection and sample treatment associated with the different types of specimens: urine, sputum, nasopharyngeal, oropharyngeal, bronchoalveolar, saliva and faeces. For this purpose, an indexed score was developed based on seven categories, namely: Materials and Equipment; Infection Risk for the Health Professional; Infection Risk for the Patient; Collection; Cost; Specialized Human Resources; RNA Extraction Type.

It is important to note that this comprehensive scientific analysis is the baseline used for a research project with the application of a saliva self-collection kit for the diagnosis of SARS-CoV-2 developed by H&TRC—Health and Technology Research Center in ESTeSL—Escola Superior de Tecnologia da Saúde, Instituto Politécnico de Lisboa.

2. Methodology

A comprehensive scientific analysis was carried out to determine the types of samples used to diagnose SARS-CoV-2. Based on scientific research, it was assumed that the sample for diagnostic examination for SARS-CoV-2 can be extracted from various physiological systems within the human body, including: (1) Urinary—urine; (2) Respiratory—sputum, nasopharyngeal (NPSs), oropharyngeal (OPS), bronchoalveolar (BAL); (3) Digestive—saliva and faeces; and (4) Circulatory—blood (Figure 1). Data comparing the accuracy of RT-PCR testing suggest that the test sensitivity may vary by the type of specimen.

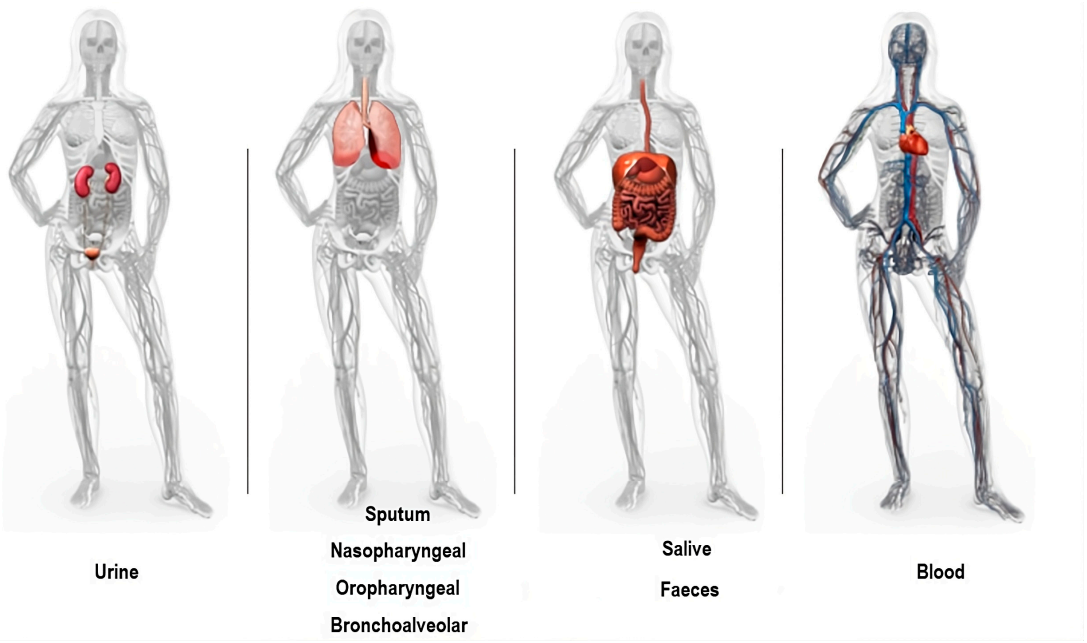

Figure 1. Graphical representation of the biological materials in the study.

Outbreaks caused by infectious microorganisms such as SARS-CoV-2 enclose significant morbidity and mortality rates and endorse significant health threats for the professionals involved in specimen collection for diagnosis purposes as well as for the patients. Thus, the collection of information on suspect cases as well as the collection of clinical specimens for laboratory diagnosis is crucial. The World Health Organization described guidelines for the collection of clinical specimens focused on the safety of field and laboratory investigators, as well as patients [1]. These guidelines focused on materials and techniques employed for the gathering, manipulation, and transportation of specimens, and were duly considered in the formulation of this research. The purpose of this assessment was to create a score for seven (7) different parameters regarding biological specimen collection in suspected human cases for SARS-CoV-2 laboratory diagnosis (urine, sputum, nasopharynx swab, oropharynx swab, bronchoalveolar specimen, saliva, faeces, and blood) [1], which will provide up-to-date information to identify the most suitable method based on the WHO guidelines of clinical specimen collection and for laboratory testing for coronavirus disease (COVID-19). For this, we created a score of 1 to 5 (see Supplementary Materials). (1) Materials and equipment we included were all necessary materials for specimen collection including specimen holders, swabs, medical equipment and individual protection equipment, storage temperature until testing in-country laboratory and recommended temperature for shipment; (2) potential risks regarding the health professional were took in consideration of the type of specimen collection, namely if the specimen can be self-collected or if it requires a health professional for the collection, the direct contact between the health professional and the patient, the proximity and the time necessary for the collection of the specimen as well as the individual protection equipment needed by the professional during the procedures; (3) the patient risk, including the use of medical equipment and the need

for transport to specific medical facilities for the sample collection; (4) we also considered the invasive nature of the sampling protocol; (5) the economic cost both for patients and for the health system, including all materials, equipment and individual protection devises; (6) the need for highly specialized and trained health professionals; and (7) the complexity for RNA extraction protocols considering each specimen.

3. Results and Discussion

The comprehensive scientific analysis presented in this study takes into consideration the index developed by the authors, based on a literature review and personal and professional knowledge. Figure 2 represents the sampling methods considered in this assessment as well as the respective biological specimens.

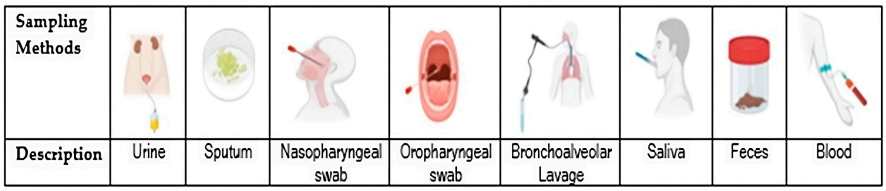

Figure 2. Biological specimen collection in suspected human cases of SARS-CoV-2.

For each type of sampling method, various factors were taken into consideration, such as the necessary materials and equipment for specimen collection, potential risks for health professionals, risks for patients, the invasive nature of the sampling protocol, economic costs, and the need for specialized health professionals, as explained in Table 1.

Table 1. Index of Scoring Criteria in detail.

Sampling Methods	Criteria Application
Urine	The urine specimen is vulnerable to tampering through dilution or adulteration. Urine samples pose a minimal risk of infection, although there is a possibility of sample spillage resulting in insufficient volume. It is advised to wear gloves during the handling of urine samples. They have the potential to be self-collected, which presents an advantage as it eliminates the need for a trained health professional to carry out the sampling. Low cost. Medium accessibility.
Sputum	Clinical diagnostic sputum tests are designed to identify the underlying causes of lower respiratory tract infections and various other diseases. Moreover, these tests serve as an effective means of assessing the efficacy of clinical treatment. When a patient presents with pneumonia, performing a sputum culture is imperative. However, one major drawback of relying solely on sputum smears for diagnosis is that a significant number of pulmonary cases may go undetected. The effectiveness of sputum smears in detecting disease is more pronounced in cases of cavitary pulmonary disease among patients with a forceful cough. It is worth noting that the cough and spit method may lead to slight discomfort due to repeated coughing. Once more, sputum samples have the potential to be self-collected, which eliminates the need for a trained health professional for sampling. Low cost. Medium accessibility.
Nasopharyngeal swab	The nasopharyngeal swab is commonly employed for the identification of several viruses and bacterial infections. It is generally regarded as a safe and well-tolerated technique, although a multitude of complications have been frequently documented, including but not limited to retained swabs, epistaxis, and cerebrospinal fluid leakage. These complications are often linked with high-risk factors such as severe septal deviations, pre-existing defects in the skull base, and previous sinus or transsphenoidal pituitary surgery. Professionals must possess adequate anatomical knowledge and employ appropriate techniques to carry out nasopharyngeal COVID-19 testing. Healthcare professionals must conduct these tests in a dedicated and sterilized room to prevent the transmission of the virus. Healthcare providers conducting the nasopharyngeal swab test must adhere to the prescribed personal protective measures, encompassing an N95 mask, disposable cap, goggles, gown, latex gloves, and shoe covers. Despite potential discomfort and extended result waiting periods, this method remains paramount for its heightened sensitivity and accuracy owing to the elevated virus concentration in the nasopharyngeal region. Medium cost. Medium accessibility.

Table 1. *Cont.*

Sampling Methods	Criteria Application
Oropharyngeal swab	Oropharyngeal (OPS) sampling is considered a more practical method to use. Before being extracted, the swab is gently twisted a few times and directed towards the posterior wall of the oropharynx. A broader variety of swab items can be used to get OPS samples, and while self-collection may be possible, it is advised that a healthcare professional collect OP swabs. Performing throat cultures on individuals who have an irritated epiglottis is not recommended. Acute respiratory obstruction and bacterial infection can both be exacerbated by swabbing the epiglottis. Healthcare professionals must conduct these tests in a dedicated and sterilized room to prevent the transmission of the virus. All healthcare professionals administering the oropharyngeal swab test are required to don the recommended personal protective equipment (PPE), which should include an N95 mask, disposable cap, goggles, gown, latex gloves, and shoe covers. Medium cost. Medium accessibility.
Bronchoalveolar Lavage	Bronchoalveolar lavage fluid (BAL) samples may be considered as a viable option due to their characteristics as a highly effective specimen with a notable rate of detection. However, in the collection process, a bronchoscope is passed through the mouth or nose into an appropriate airway in the lungs, with a measured amount of fluid introduced and then collected for examination. This sample is typically obtained from patients who are severely ill or undergoing mechanical ventilation. Bronchoalveolar lavage serves as a valuable and secure procedure for sampling the cellular components of the lung. BAL, as a diagnostic tool, can be employed to accurately diagnose various infections and acquire material for culture and sensitivity analysis. The most prevalent risks associated with this procedure are akin to those observed in flexible bronchoscopy, including transient hypoxemia, post-BAL fever (which can be seen in up to 30% of patients), bronchospasm, and, very rarely, pneumothorax. This procedure can only be performed by a trained physician, and it is required to wear the recommended personal protective equipment (PPE), which should include an N95 mask, disposable cap, goggles, gown, latex gloves, and shoe cover. Very high cost. Low accessibility.
Saliva	Saliva testing and collection may be a simple, low-cost sample technique that causes the user as little discomfort as possible. It is also a reliable, user-friendly, portable, and scalable platform for the diagnosis of diseases. The type of saliva sampled—whole saliva or saliva generated by certain glands—as well as whether the sample was taken following stimulation will determine the outcome. The amount of spit and the device's capacity to retrieve the biomarkers determine which one is best. Moreover, the salivary composition can be influenced by several factors, including the flow rate, pH, temperature, food, and age (with infants, children, and the elderly exhibiting the most fluctuation). However, since new, extremely sensitive technologies have been developed, saliva has a lower level of analytes. Additionally, the potential to be self-collected eliminates the need for a trained health professional for sampling. Low cost. High accessibility.
Faeces	SARS-CoV-2 is frequently detected in faecal specimens even after negative results from throat swabs during the post-symptomatic phase. The presence of the virus in the stool seems to be comparable among patients with and without gastrointestinal symptoms. It is crucial to acknowledge that the stool sample might contain infectious pathogens, which can be transmitted to others. Consequently, it is mandatory for all healthcare professionals handling the samples to wear the prescribed personal protective equipment (PPE), including an N95 mask, disposable cap, goggles, gown, latex gloves, and shoe covers. The performance of a faecal culture testing does not entail any associated risks and can be self-collected. Low cost. Medium accessibility.
Blood	In certain scenarios, such as when dealing with patients in a comatose state, the act of sampling through the oropharyngeal or nasopharyngeal route is rarely achievable, thus making blood sampling a viable alternative. Furthermore, the utilization of blood tests may pose fewer risks for the medical personnel carrying out the sampling compared to the use of respiratory swabs. It is important to acknowledge that the identification of viral RNA-aemia might not indicate an active infection, but rather signify a post-viral infection period. As this approach involves an invasive methodology, there exists the potential for adverse effects such as pain, swelling, bruising, discoloration of tissue, or scarring around the vein from which the blood sample is drawn. It is plausible that some of these tissue alterations and scarring could persist permanently. Additionally, these collection procedures must be conducted exclusively by licensed technicians with the proper collection material including collection tubes, needles, blood collection chairs and sterilization materials. Every healthcare professional must use the specified personal protective equipment (PPE), comprising an N95 mask, disposable cap, goggles, gown, latex gloves, and shoe covers. High cost. High accessibility.

A score was given to each sample regarding the parameters discussed in Table 1 along with the complexity of RNA extraction (which will be addressed separately in the subsequent sections). Our score, presented in Table 2, demonstrated that the higher score corresponds to saliva (5.0) and the bronchoalveolar specimen had the lowest score (1.7). Detailed description of each criterion and the corresponding score is included in Table S1.

Table 2. Index of Scoring Criteria: 1–5 attributed scores considering (1) materials and equipment; (2) potential risks regarding the health professional; (3) the patient risk; (4) the invasive nature of the sampling protocol; (5) the economic cost both for patients and for the health system; (6) the need for highly specialized and trained health professionals; and (7) the complexity of RNA extraction protocols. The scores were attributed according to the data presented in Table 1 (except for RNA extraction type, which is detailed in the subsequent sections).

	Human Body Systems							
	Urinary		**Respiratory**				**Digestive**	**Circulatory**
	Urine	**Sputum**	**NPS**	**OPSs**	**BAL**	**Saliva**	**Faeces**	**Blood**
Materials and Equipment	5	5	4	4	1	5	5	3
Infection Risk for the Health Professional	5	5	3	3	2	5	5	4
Infection Risk for the Patient	5	5	3	3	1	5	5	4
Collection	5	5	3	4	1	5	5	2
Cost	5	5	4	4	1	5	5	3
Specialized HR	5	5	3	3	1	5	5	3
RNA Extraction Type $\overline{x}$	1	4	5	5	5	5	1	3
Score $[\overline{x}]$	4.4	4.9	3.6	3.7	1.7	5.0	4.4	3.1

Figure 3A presents the average scores of the seven selected criteria for all studied specimens. Based on this figure, it is possible to observe that the most suitable specimen is "saliva", with an average score of 5.0. Figure 3B–E show the average scores defined for the urinary, respiratory, digestive and circulatory systems, respectively.

(**A**)

Figure 3. *Cont.*

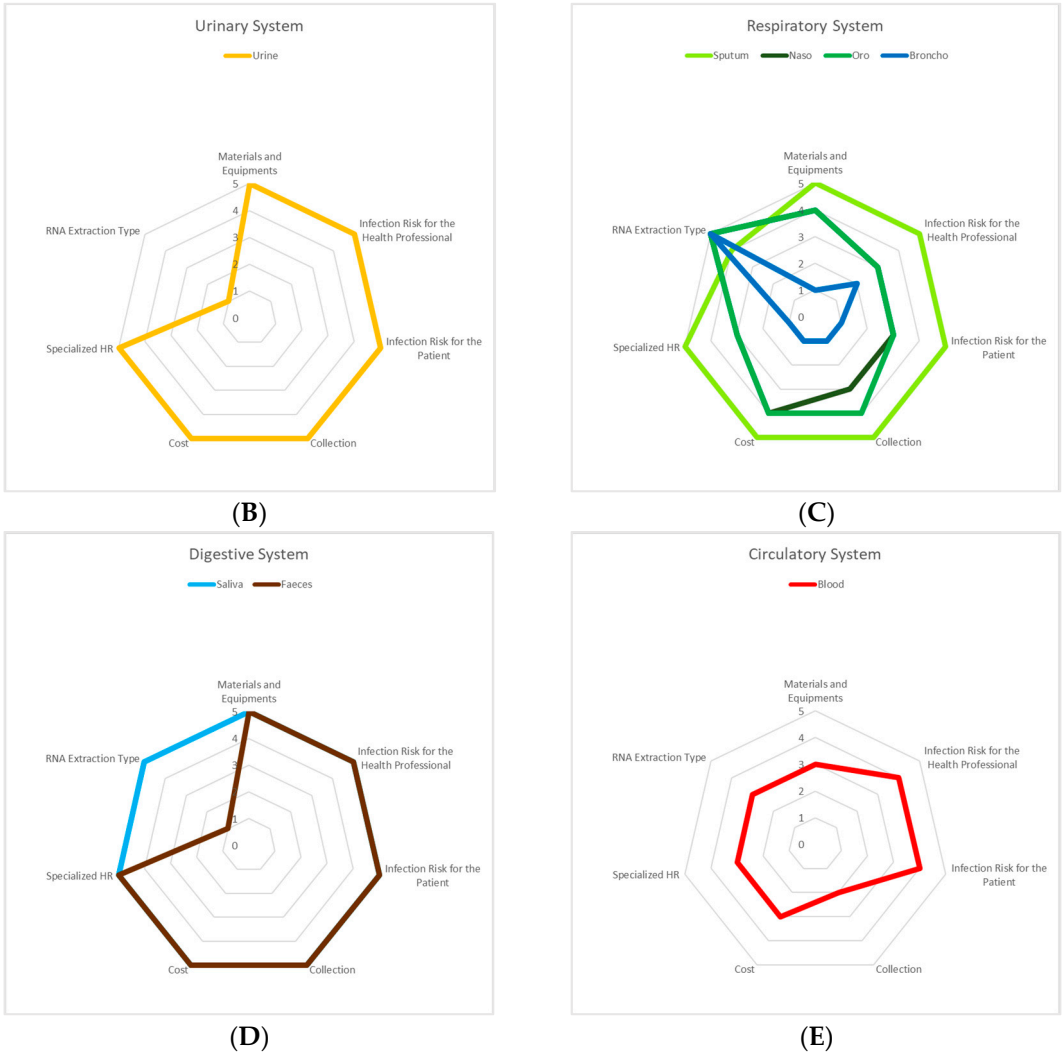

Figure 3. (**A**) Score of the selected criteria according to each studied specimen. (**B**–**E**) present the scores developed for urinary, respiratory, digestive, and circulatory systems, respectively.

3.1. Urinary System

Regarding urine specimen collection, data analysis emphasizes the RNA extraction, which is characterized as the minimum level (1.0), due to its complexity and procedure difficulties, as demonstrated in Figure 3B. The extraction of nucleic acid from urine samples can be performed using, for example, the Extraction NucleoSpin Dx Virus kit (Macherey Nagel, Duren, Germany) [18,19] or the automated NucliSENS easyMAG (bioMerieux, Marcyl'Etoile, France) [18]. The procedures for each piece of equipment are similar and, briefly, urine specimens are firstly lysed in the presence of chaotropic salts [20], in this case, lysis buffer, which is designed to disrupt viral particles and inactivate any nucleases upon addition of specimen [21]. Next, ethanol is added to take initiate the binding of nucleic acids to the silica membrane of the equipment. The following procedure involves three washing steps to decrease possible contamination from substances such as salts, metabolites, and soluble macromolecular cellular components [20]. Lastly, the nucleic acids are eluted in a low-salt buffer or RNase-free water. As a result of these techniques, the viral RNA is isolated and purified and ready to be used for PCR techniques [20].

Contrary to RNA, all other criteria had a score of 5.0. The collection of urine samples is quite simple as it involves using a sterile plastic cup with a lid, and the patient must be requested to cleanse the urethral area, void the first portion of the urine stream into the toilet, and then hold the cup and collect a midstream urine sample to reduce cellular and microbial contamination [22]. No help from the healthcare professional is required, so there

are no additional costs and no potential risks of infection, as said earlier regarding sputum. The risk of infection for the patient is also minimal, and it is advisable after the collection to once again clean the genital area with soap and water to avoid any infection.

3.2. Respiratory System

Published RT-qPCR assays have demonstrated excellent analytical sensitivity and specificity for respiratory samples. However, false-negative tests remain a concern, especially in patients who have distinctive symptoms of SARS-CoV-2 infection in high-prevalence settings or who have consistent findings on chest imaging that lead to the suspicion of viral pneumonia. In the case of NPSs, a false negative result can be a consequence of several factors, such as poor quality of collection, test performance during the incubation period, or sample processing errors [23].

As previously mentioned in the introduction, the specimens currently recommended and used for the diagnosis of COVID-19 are from the upper respiratory system, in particular NPSs and/or OPS. The collection of these types of samples requires a proximity between the patient and the healthcare professional that naturally increases the risk of infection for both [8], having a score of 3.0 assigned in these two parameters. In addition, the two types of samples are considered invasive, and the collection may imply several adverse effects to the patient that may span from discomfort to more serious complications.

As far as OPS is concerned, it consists of collecting biological material from the region of the palatine tonsils and the posterior wall of the oropharynx [24]. For this purpose, the patient must completely remove their mask and open their mouth wide enough [8,25]. Besides being uncomfortable for the patient, the collection may induce the gag reflex, leading to reduced participation and compliance [8,26]. In addition, completely removing the mask implies an increased risk for the healthcare professional, as not only does the collection require proximity but it also involves the removal of personal protective equipment on one side. Unlike the OPS, in the nasopharyngeal specimen collection, it is possible for the patient to partially remove the mask, exposing only the nose [25]. However, even with this advantage, this collection is still considered more invasive when compared to the OPS, essentially due to the complications to which is associated: not only does it trigger some reflexes, such as sneezing and/or coughing, but it may also cause epistaxis and other minor adverse effects, such as nasal discomfort, headache, earache and rhinorrhoea [27,28]. So, because of that, when it comes to the collection criteria, NPS is rated as 3.0 and OPS as 4.0, as is seen in Table 1. These complications are due to nasal mucosa being extremely fragile, sensitive, and very vascularized; therefore, there is a tendency for mild bleeding as a consequence of superficial trauma [28]. In addition to this natural sensitivity of the mucosa, patients infected with SARS-CoV-2 also have greater fragility, since the upper respiratory system is inflamed, leading to more prominent bleeding, as may happen in patients with rhinitis and sinusitis [28,29]. In both samples, the reflexes induced by the collection may originate droplets, possibly endangering the healthcare professional responsible for the procedure; this transmission is favoured by the total or partial removal of the patient's mask [30]. However, according to Murphy (2020), several studies have shown that there is a more prolonged detection by RT-qPCR in specimens from the lower than from the upper respiratory system [23]. This would lead us to choose a collection from the lower respiratory tract, but several factors need to be meticulously evaluated to conclude which type of sample best suits the patient and the workflow of each hospital and laboratory. Among the many factors that influence the choice of the specimen are the invasiveness of the collection and the risk of infection to the patient and healthcare worker. Several studies have looked for methods to decrease all associated risks, searching for a balance between a less invasive collection procedure and the obtainment of high-quality samples. It should be noted that, although some interactions can be reduced, there will always be a minimal risk involved, especially for the healthcare professional (e.g., during the receipt and handling of the samples).

Among the types of samples addressed in this article, the one with the highest risk is bronchoalveolar lavage fluid, as shown in Table 1 with a total score of 1.7. This fact is essentially due to the high complexity of the collection and the conditions under which it is performed justifying level 1.0 in most criteria. Briefly, it consists of the instillation of a sterile saline solution in a subsegment of the lung, with subsequent suction and collection of the sample; this process is performed with the aid of a bronchoscope [13,31]. Due to the nature of this procedure, aerosols are formed, increasing the risk of transmission to healthcare professionals (score 2.0 in Table 1 for this topic) and allowing the occurrence of nosocomial infections. Therefore, it is essential that the professionals, who should be previously trained, perform this procedure properly equipped and in an adequately ventilated room [31]. Other measures can be adopted to decrease the risk of infection, such as the use of general anaesthesia or an appropriate sedative, as these decrease the induction of coughing during the collection, also reducing the formation of aerosols and allowing greater patient compliance during the process [32]. In addition, the distance between the operator and the patient can be preserved by positioning the operator to the rear of the patient's head, also reducing the risk of infection [33]. Although this sample may, in some cases, increase the sensitivity of molecular assays, this type of biological material is usually collected only in patients who are critically ill or on mechanical ventilation; it is not recommended for patients who are not under these conditions, due to the complexity of collection and its significant invasiveness [13,33,34]. Therefore, the collection of this type of material is not advised as a first-line test in the diagnosis of SARS-CoV-2 infection for the general population, since, besides being invasive, bronchoscopy carries risks for infected patients and may lead to the worsening of ventilatory dynamics and hypoxemia [35]. The fact that this collection is mostly performed on debilitated patients requires additional attention and diligence from the healthcare workers.

Another type of sample from the lower respiratory tract is sputum. The collection of this biological material carries some risk, especially for the healthcare professional if it is carried out with their assistance. This risk is increased especially in patients who are not intubated, as it happens in the collection of bronchoalveolar fluid, and it is associated with the fact that the collection of this type of material may generate aerosols [23,32]. In certain cases, this source of transmission could be avoided, since not all patients produce sputum, as in the case of elderly patients or during the early phase of the disease [8,31,35]. In these cases, the obtainment of the sample is usually performed by inducing sputum, using a hypertonic saline solution [14]. The performance of this procedure is controversial since several articles state that this induction does not carry associated problems and is therefore recommended, while others do not endorse this practice [8,14,33,34]. However, each case is unique, and it is therefore necessary to evaluate each patient individually, considering, if possible, whether the benefits outweigh the associated risks [36]. However, even though there may be some risks for the healthcare professionals that assist in the procedure, sputum collection has an advantage that greatly reduces the aforementioned risks: unlike the other samples, it can be self-collected [37]. This method is not only simple and fast but also reduces the risk for the professionals, since there is no need for their involvement during collection; moreover, it is beneficial in situations where there is a shortage of personal protective equipment [37]. In addition, this method also reduces risks for the patient by avoiding the gathering of crowds, since there is no need for patients to go to a specific place to collect the sample [38]. Regarding collection, among all the specimens of the respiratory system, sputum is the least invasive. Criteria like Collection, Materials and Equipment, Specialized HR, Infection Risk for the Health Professional and Patient were assigned as 5.0. Despite the higher complexity related to obtaining this sample (since not all infected patients produce sputum), the fact that no object is used for the collection itself, such as a swab in the case of NPSs and OPS collections, makes it less invasive. However, the induction procedure sometimes performed to collect the sample may have some consequences for the patient, since it leads to stimulation of the respiratory tract [14]. Thus, the primary effect of this induction is essentially the development of a moderate

cough, in addition to the sputum production itself. Nevertheless, these symptoms are quickly mitigated and there are no significant adverse effects in most cases, thus being considered a safe procedure for the patient [14,34].

Another essential factor when it comes to choosing one type of sample over others is the complexity related to RNA extraction. Regarding respiratory tract samples, such complexity is essentially similar apart from sputum. This specimen frequently contains mucoid or mucopurulent material, thus causing some inconveniences for the RNA extraction process [31,32,39]. These complications are mainly because the sample constitution hinders the access of the reagents used in this step, which consequently leads to a low yield of extracted RNA [40]. Therefore, to reduce its viscosity, a pre-treatment is usually necessary, thus facilitating this procedure, particularly when using automatic equipment [31,32]. For this step, several reagents may be used to perform the liquification, such as Proteinase K (PK), dithiothreitol (DTT) and Nacetyl-L-cysteine (NALC). Due to the importance of this procedure, it is essential to mention that not performing the pre-treatment may lead to multiple consequences such as cross-contamination in the case of using automatic equipment, pipetting errors, clot formation, and a lack of amplification [40].

All the factors mentioned above are extremely important, but due to the current pandemic situation, the costs related to performing the collection of biological material need to be considered and minimized. Thus, before choosing the most adequate specimen, it is also necessary to evaluate the costs, which include specialized human resources, materials and equipment, and the facilities needed for the collection to take place.

In general, it can be concluded that of all the samples presented in this article, the most expensive is bronchoalveolar lavage fluid. This is due to the need for an inherently extensive team, although composed only of the essential healthcare professionals for the various duties required, such as a bronchologist, nurse, operational assistant, anaesthesiologist and anaesthesia nurse [41,42]. It is also important to mention that this team should mainly include the most experienced professionals, to reduce the duration of the procedure and to guarantee an efficient resolution of possible complications [41]. Moreover, the cost of this type of sample also increases due to the number of materials and equipment needed during the procedure: briefly, a saline solution for washing, a sterile container for collecting the sample, a bronchoscope, lubricating gel, and an aesthetic compound such as lidocaine [43,44]. The bronchoscope should preferably be disposable; however, if it is not possible to use this type of bronchoscope, a high-level disinfection of the devices should be performed, meeting all safety requirements [42]. Depending on the severity of the patient's condition, other materials may be needed, further increasing the costs of the collection. It is also important to mention that adequate personal protective equipment must be required for the entire team, as well as various hospital devices, such as a stretcher, and a monitor for controlling the procedure, among others [42,44]. Finally, the required facilities may also lead to a more expensive collection, since it is advised to perform this procedure in a negative-pressure room with good ventilation and strict isolation [41]. However, if this type of room is not available, the procedure may be performed in a specific room with adequate natural ventilation [41]. All these requirements are essential given the debilitated condition in which the patients subject to this type of collection are expected to be, requiring reinforced care to ensure their safety.

Regarding the collection of NPSs and OPSs, the costs presented are similar between themselves (with a given score of 4.0), since both demand the same level of necessary materials, health professionals and facilities. In short, both collections are performed by a properly equipped and trained professional, using a swab and a tube with an inactivation medium [24,45]. A tongue depressor may also be used to assist in oropharyngeal specimen collection; however, it is not essential [45]. As far as the facilities are concerned, they do not require any specificity, only that they should be properly ventilated. Furthermore, regarding sputum, although it has a significant cost resulting from the need for pre-treatment; the possibility of self-collection leads to a substantial cost reduction. This is because it is no longer necessary for a healthcare professional to be present during the collection procedure,

thus reducing the use of personal protective equipment, since there is no contact between the patient and the professional. It also leads to cost reduction in facilities because there is no need for a specialized place with the appropriate and regulated conditions to perform the collection. In addition, the material required for the collection will not increase costs, since only one sterile container is needed [45].

3.3. Digestive System

Recent research findings indicate that certain individuals diagnosed with COVID-19 have reported gastrointestinal symptoms such as diarrhoea and vomiting. Additionally, studies have shown the presence of SARS-CoV-2 RNA in stool specimens, raising concerns about the potential for faecal contamination to contribute to the spread of the disease [16,46]. The most common method for diagnosing SARS-CoV-2 is through airways, by oral swats, and this has been shown not to be particularly effective since oropharyngeal specimens are testing negative while live viruses can still be detected in faeces [16]. Regarding the extraction of viral RNA in stool samples, the method is like the one used for urine specimens, so the value assigned is the same as the one used for (1.0). Before applying PCR, the samples require that the RNA to be prepared [47]. For this treatment, it can be used with EasyMag (bioMerieux France, Craponne, France); the method is explained in the section related to urine specimens [48]. Another option is the QIAamp Viral RNA Mini Kit (Jant Pharmacal Corporation, Encino, CA, USA), which simplifies purification of viral RNA from cell-free body fluids [49,50]. The processes within this apparatus begin with subjecting the samples to lysis under highly denaturing conditions, primarily to deactivate RNases and facilitate the isolation of intact viral RNA. Following this, the buffering conditions are adjusted to promote the binding of RNA to the QIAamp membrane, and the samples are subsequently loaded onto the QIAamp Mini spin column [47]. The contaminants are then washed away in two steps using two different wash buffers. Finally, RNA is eluted in a special RNase-free buffer resulting in a purified RNA free of protein, nucleases and other contaminants and inhibitors [47]. Collecting stool samples is quite easy to perform, and there is no need for intervention by a health professional and therefore no risk for them. It only requires a clean, dry, leak-proof screw cap container and tape with 5 mL of liquid or 5 g of solid (pea-size) freshly passed stool [45]. Therefore, in addition to RNA, all other criteria had a score of 5.0.

Another type of specimen that can be used for COVID-19 diagnosis is saliva [2,51], a biological material rich in multiple biological markers (e.g., DNA, RNA, proteins) [51].

Angiotensin-converting enzyme II (ACE2)-expressing cells are likely to be the major target cell type which are vulnerable to SARS-CoV-2 infection [51], and this enzyme is also expressed in high levels in the oral mucosa, with particularly high expression on the tongue [52,53]. Thus, there is biological plausibility that samples collected from the oral cavity are an adequate specimen type [53] since possible routes of entry for SARS-CoV-2 are provided [51].

Regarding transmission and symptoms, salivary droplets are already identified as the main source of SARS-CoV-2 infection through human-to-human transmission below a social distance limit of 2 m [6], and people infected with the disease may present oral symptoms including taste loss and dry mouth [51]. Some studies have reported that as SARS-CoV-2 infection progresses, the chances of viral particles appearing in the saliva increase [51], meaning that the higher viral loads are seen in patients with more severe disease [54].

The collection of saliva is a very simple procedure as patients are asked to pool saliva in their mouth and then gently spit into a sterile container, which is the only material needed to collect the sample [6]. Therefore, for both Materials and Equipment and Collection criteria, the score rated was 5.0. It should be noted that saliva samples remain stable for several days at various temperatures but may be less optimal when not a first morning collection, for asymptomatic individuals [54,55]. The collection of saliva can be easily performed with non-invasive procedures [6,51,54] presenting fewer risks and less discomfort for the patient, this

parameter being classified with a score of 5.0. Since it also can be self-collected [2,6,56] and there is no need for trained personnel to perform it, the risks for the healthcare professionals are reduced [6], as is the amount of personal protective equipment needed [54]. This means that the use of saliva specimens for diagnosis of COVID-19 implies a reduction in the risk for both the professional healthcare and patients [57]. This justifies the score 5.0 attributed to Specialized RH criteria, Infection Risk for the Health Professional criteria, and Infection Risk for the Patient criteria. Thus, saliva has many advantages compared to NPSs and OPS sampling including a decrease in risk to healthcare professionals, a reduction in patient discomfort, and no requirements for specialized materials [53]. There is also no need for trained healthcare workers or personal protective equipment [6,53]. Another advantage is that saliva can provide a larger amount of test material than upper respiratory swabs [52]. Additionally, several reports indicated that SARS-CoV-2 was detected in saliva specimens of equally or higher sensitivity than the NPSs [57] and that the sensitivity to detect SARS-CoV-2 using saliva is high, as demonstrated in Table 3.

Table 3. Sensitivity obtained at detection of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) using saliva. CI: confidence interval. COVID-19: Coronavirus Disease 2019. CT: cycle threshold. ddPCR: droplet digital PCR. NM: not mentioned. NPS: nasopharyngeal swab. NPA: negative percent agreement. OPS: oropharyngeal swab. PPA: positive percent agreement. RT-qPCR: quantitative reverse transcription PCR. SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2.

N Total. Saliva Positive	Sensitivity (Positive Agreement %) and Notes	Type of Molecular Test	Reference
74 samples. 53/58 positives	91.37% positive agreement. The CT value for detection of the SARS-CoV-2 ORF1 gene tendentially lower in saliva than that of NPSs (mean 27.07; 95% CI, 25.62 to 28.52 vs. mean 28.24; 95% CI, 26.62 to 29.85, although nonsignificant statistically ($p > 0.05$).	RNA extraction + RT-qPCR	[5]
522 samples. 33/39 positives	84.6% positive agreement (33/39 patients, 95% CI 70.0–93.1%) in opposition to NPSs than only had 39/522 SARS-CoV-2 positive cases (6.3%; 95% CI 4.6–8.5%). The median CT value was lower in NPSs compared with saliva and was correlated with the days from symptom onset in both samples.	RNA extraction + RT-qPCR	[58]
166 samples. 16/16 positives	93.75% positive agreement using ddPCR (15 out of 16 samples) and 100% using RT-qPCR (16 out of 16 samples).	RNA extraction + RT-qPCR and ddPCR	[52]
459 samples. 31/37 positives	81.1% positive agreement, and 90.0% in samples with moderate to high viral load. The mean CT values were on higher in saliva compared with NPSs.	RNA extraction + RT-qPCR	[54]
31 samples. 3/31 positives 3/4 critically ill patients	23.08% positive agreement and 75% positive agreement in critically ill patients	RT-qPCR	[51]
58 samples. 49/58 positives	84.5% positive agreement.	Xpert Xpress SARS-CoV-2 assay	[59]
185 samples. 6/37 positives	16.22% positive agreement. The mean viral load was 5677 ± 13,647 compared to 16,224 ± 67,507 at NPSs. Many patients in the recovery period negative for SARS-CoV-2 in NPSs were positive with other specimens, particularly in anal swabs.	RNA extraction + ddPCR	[60]

Table 3. *Cont.*

N Total. Saliva Positive	Sensitivity (Positive Agreement %) and Notes	Type of Molecular Test	Reference
5 samples. 5/5 positives	100% positive agreement (first saliva in the morning compared to NPSs). Exception was in children by days 8–10 after the onset of symptoms that were positive for SARS-CoV-2 in NPSs but not in saliva.	RNA extraction + RT-qPCR	[2]
401 samples. 20/35 positives	The virus was detected in 6.5% (n/N = 26/401) cases by swab and 7.0% (n/N = 28/401) by saliva. For saliva the sensitivity was 73.1% (95% CI 52.2–88.4%), the specificity was 97.6% (95% CI 95.5–98.9%), the positive predictive value was 67.9% (95% CI 51.5–80.8%) and the negative predictive value was 98.1% (95% CI 96.5–99.0%).	RNA extraction + RT-qPCR	[6]
28 samples. 19/23 positives	82.61% positive agreement. In 3 convalescent patients, the virus was detected in saliva in opposition to NPSs, at 4 time points.	RNA extraction + RT-PCR	[56]
36 samples. 20/27positives	86% positive agreement. The values of PPA and NPA were 85.0% and 28.6%, respectively. Compared with NPSs, OPS, and sputum, saliva had higher detection performance and less false negatives.	RT-dPCR	[61]
432 samples. 46/53 positives	98.4% positive agreement.	RNA extraction + RT-PCR	[55]
NM	Almost equal to or higher viral loads in saliva than in NPSs/OPS (saliva 1.07 ± 0.34–1.65 ± 0.46 log10 copies/mL, versus NPSs/OPS 1.18 ± 0.12–1.34 ± 0.30 log10 copies/mL)	RNA extraction + RT-qPCR	[62]
16 samples. 4/7 positives	Detection of viral RNA in 7 out of 16 saliva specimens (43.8%) and viral antigen in 4 out of 7 positive saliva specimens (47.8%).	Rapid immunochromato-graphic test	[57]

During the ongoing pandemic, diagnostic testing is crucial to suppress the spread of COVID-19 [2], but there are adversities such as the cost of sample collection devices with transport media and challenges with supply logistics [6]. These aspects can impact the screening capability of healthcare systems, particularly in resource-limited settings [6]. To solve the logistic problems associated with specimen collection, there is a need for an easily collected specimen type that does not limit community screening [6,59]. Therefore, a non-invasive specimen that can be self-collected by the patient without the need for complex materials and supervision by trained personnel such as saliva can be useful for public health surveillance [6]. Potential shortages of both swabs and personal protective equipment could also reduce the cost of sample collection (which is classified with the score of 5.0. in Table 1) and increase the volume of tests performed [5,52]. Furthermore, the use of saliva as a diagnostic specimen may present opportunities for SARS-CoV-2 RT-PCR testing in remote and low-resource settings, as well as allowing scalable population-level screening [53].

3.4. Circulatory System

As previously mentioned, blood serves as a viable specimen for COVID-19 diagnosis. Presently, only a limited number of studies indicate the detectability of viral RNA in plasma or serum samples from COVID-19 patients [12]. Conversely, some studies suggest that the current approach for viral RNA detection using oral swabs may not be optimal, as instances exist where the virus is present in anal swabs or blood, yielding negative results in oral swab tests [12,16]. Additionally, in challenging situations such as comatose patients, obtaining an oropharyngeal or nasopharyngeal sample may be difficult, making blood collection a potential alternative [12]. It is noteworthy that despite the identification of

SARS-CoV-2 RNA in infected patients' plasma or serum, there is currently no evidence of SARS-CoV-2 transmission through blood transfusion [12]. In what concerns the risk of infection for the health professional, the blood tests have less harm than the tests where respiratory swaps are used [12] since the patient does not need to remove the mask. This fact justifies the score given to the Infection Risk for the Health Professional criteria (4.0).

According to the WHO (2000), the material necessary for a blood sample collection includes a skin disinfection solution (70% alcohol or 10% povidone iodine), swabs, gauze pads, a band-aid, a tourniquet, a vacutainer, and a monovette or disposable syringes and needles and blood culture bottles. All the described material justifies the score of 3.0 in the criteria Materials and Equipment. When performing the collection, the tourniquet is placed above the venepuncture site and, after swabbing the skin concentrically from the centre of the venepuncture site outwards with the skin disinfectant solution, the venepuncture is executed. There are different stipulated amounts of blood that can be taken according to the age group of the patient and the equipment used to withdraw the blood. The specimen is then transferred to cap transport tubes and culture bottles [45]. Taking these facts into account, the score given to the collection criteria was 2.0. It should be noted that the separation of serum from blood requires additional materials and procedures, which means that more specialized human resources are needed, making the given score 3.0 in this parameter.

Regarding the costs, all the material and diversity of health professionals needed to perform the sampling and analysis of this specimen make it more expensive than all other specimens, as shown in Table 1, where this parameter was rated with 3.0. There are some adversities when using this specimen for COVID-19 diagnosis related to the identification of viral RNA. The small amounts of RNA in plasma or serum samples combined with the fact that large amounts of background RNA can mask the low abundance of viral RNA fragments can originate false-negative outcomes [12]. The mentioned facts show that the RNA Extraction on this type of specimen is more difficult to perform, which explains the score 3.0 given in Table 1.

3.5. Viral Load

Despite the biological sample origin and detection methods, SARS-CoV-2 viral load is a key parameter for virus detection and infectiousness evaluation and risk assessment.

Previous studies observed that SARS-CoV-2 viral load is significantly higher in sputum when compared to throat swabs and nasal swabs (17,429 $\pm$ 6920 copies/test, 2552 $\pm$ 1965 copies/test, $p < 0.001$ and 651 $\pm$ 501 copies/test, $p < 0.001$, respectively) and that viral loads detected in the early and progressive stages of the infection were significantly increased than that in the recovery stage [63]. It is described that viral load kinetics as well as viral shedding duration are key determinants for disease transmission and must be taken into consideration when selecting a biological sampling method. Previous studies have reported that the peak of SARS-CoV-2 titres in the upper respiratory tract occurs in the first week of illness. On the other hand, despite the fact that SARS-CoV-2 RNA shedding in respiratory and stool samples can be prolonged, virus viability may be brief [64].

On the other hand, Wang W. et al. demonstrated that in SARS-CoV-2 RT-PCR detection the mean cycle threshold values for blood, sputum, faeces, urine, bronchoalveolar lavage fluid and fibro bronchoscope brush biopsy were more than 30 (<2.6×10^4 copies/mL), while for nasal swabs authors obtained a mean cycle threshold value of 24.3 (1.4×10^6 copies/mL), which is indicative of a higher viral load. Thus, it is clear that the testing of specimens from multiple sites is preferable in order to increase the test sensitivity and decrease false-negative results [65].

Furthermore, reports regarding rapid antigen tests (RATs) for COVID-19 based on lateral flow technology demonstrated that even the most sensitive RAT was not able to detect SARS-CoV-2 in the samples containing small amounts of virus [66]. Additionally, despite the fact that for the RT-PCR methodology, the sensitivity of rectal stools/swab, urine, and plasma is inferior to sputum and that RT-PCR remains the gold standard for

the diagnosis of SARS-CoV-2, the combination of different diagnostic tests has been highly recommended to obtain proper sensitivity and specificity [67]. Currently, SARS-CoV-2 detection can be performed utilizing techniques such as reverse-transcription loop-mediated isothermal amplification (RT-LAMP), enzyme-linked immunosorbent assay (ELISA), lateral flow assay (LFA), chemiluminescent immunoassay (CLIA), and neutralization assay and new technologies such as sensor-based or CRISPR applications are also been developed for the rapid detection of SARS-CoV-2 [68]

Overall, our results demonstrated that NPSs, OPS, and BAL have the maximum score in RNA Extraction Type. However, BAL has high associated costs. Concerning sputum and saliva, all the aspects were evaluated with a score of 5.0 except for RNA Extraction Type in sputum. Regarding the total scores of the multiple specimens, the lowest corresponds to BAL with a score of 1.7, followed by blood with 3.1 and NPSs and OPSs with 3.6 and 3.7, respectively. Urine and faeces have the same value, 4.4, sputum has 4.9, and the highest and maximum possible corresponds to saliva with 5.0, making this last specimen the most suitable one in all aspects to proceed COVID-19 diagnosis. Nevertheless, it is important to note that this biological sample also imposes risks for health personnel with occupational direct contact [69].

Importantly, even though SARS-CoV-2 testing has currently decreased, other respiratory viruses are still very active, particularly in children, including respiratory syncytial virus (RSV), influenza and parainfluenza viruses, and adenoviruses, among others. Even though these infections are usually diagnosed clinically based on symptoms and local epidemiology, the identification of the specific pathogen may affect clinical management and be crucial for containing potential outbreaks [70]. The evaluation of the most cost/efficient biological sample suitable for these infections is of the utmost importance to decrease costs, false results, and time to diagnose.

4. Conclusions

Although OPSs and NPSs are the most widely used specimens for SARS-CoV-2 diagnosis, this work suggests that there can be better alternatives. Blood is the only specimen that did not achieve the maximum score in any parameter and urine, sputum and faeces did not reach the maximum score due to RNA extraction type criteria. Among all the assessed respiratory system specimens, sputum had the highest rate according to the utilized index score. The BAL score indicates that this specimen is less suitable for SARS-CoV-2 diagnosis. On the other hand, the saliva score demonstrated that this biological specimen can be the best option to perform SARS-CoV-2 diagnosis. Despite the fact that these infections are usually diagnosed clinically based on symptoms and local epidemiology, the identification of the specific pathogen may affect clinical management and be crucial for containing potential outbreaks.

This comprehensive analysis allowed the development of an indexed score to identify the most suitable biological material to perform SARS-CoV-2 diagnostics, provides scientific support and urges the development of further studies focusing on different biological samples and diagnosis methodology, robustly supporting the decision-makers.

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Abbreviations

Full name's	Acronym
Angiotensin-converting enzyme II	ACE2
Bronchoalveolar	BAL
Chemiluminescent immunoassay	CLIA
Clustered regularly interspaced short palindromic repeats	CRISPR
Confidence interval	CI
Coronavirus Disease 2019	COVID-19
Cycle threshold	CT
Dithiothreitol	DTT
droplet digital polymerase chain reaction	ddPCR
Enzyme-linked immunosorbent assay	ELISA
Lateral flow assay	LFA
Nacetyl-L-cysteine	NALC
Nasopharyngeal	NPS
Negative percent agreement	NPA
Not mentioned	NM
Oropharyngeal	OPS
Personal protective equipment	PPE
Positive percent agreement	PPA
Proteinase K	PK
Quantitative reverse transcription PCR	RT-qPCR
Rapid antigen tests	RATs
Respiratory syncytial virus	RSV
Reverse-transcription loop-mediated isothermal amplification	RT-LAMP
Ribonucleic acid	RNA
Severe Acute Respiratory Syndrome Coronavirus 2	SARS-CoV-2
World Health Organization	WHO

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