SARS-CoV-2: two years of laboratory testing

Maria Rosaria De Cagna, Katia Danza, Susanna D'Oria, Anna Ferretta, Norma Notaristefano, Anna Santoniccolo, Modestina Colecchia, Apollonia Izzo, Giuseppina Angela Portararo, Giovanni Stella, Raffaella Trovato, Elisabetta Morelli, Marilina Tampoia
Clinical Pathology Unit, Covid Laboratory, "SS. Annunziata" Hospital, Taranto, Italy

INTRODUCTION

In December 2019, an outbreak of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) emerged in Wuhan (China) and the infection rapidly spread affecting health worldwide [1], so it has been declared as a pandemic by the World Health Organization (WHO) on March 2020.

SARS-CoV-2 is easily transmitted among humans through aerosol droplets from infected people coughing, speaking or sneezing in close contact with others and it has an incubation period that ranges from 1 to 14 days [2]. As the rapid identification of infected individuals and their isolation is essential to prevent the spread of the disease, it became necessary to develop accurate, easy-to-use diagnostic tests. The aim of this editorial is to review the laboratory assays for SARS-CoV-2 and their fast evolution during the pandemic, with a focus on advantages and disadvantages of each test.

SARS-CoV-2 MOLECULAR TESTS

Real-time reverse transcription-polymerase chain reaction (real-time RT-PCR) is considered the gold standard method to detect SARS-CoV-2 positive cases due to its high specificity and high analytic sensitivity (or low detection limit) [3] and has been approved for diagnostic use under emergency authorization by the US Food and Drug Administration (FDA) [4] and the European Commission (CE) [5].

The first real-time RT-PCR assay protocol, developed outside China, incorporated primers targeting genes of the envelope protein (E), nucleocapsid (N) and RNA polymerase (RdRp). The primers targeting E gene and RdRp gene were the most sensitive and were rapidly and widely adopted in Europe [6]. A point worth mentioning is that reagent design has the most influential role on assay performance, therefore, well-optimized targets are expected to arise from different genomic sequences of SARS-CoV-2 (Fig.1). Currently, numerous primers are designed to target various RNA sequences in six genes of SARS-CoV-2 for diagnostic purposes: *ORF1a/b*, *ORF1b-nsp14* (5′-UTR), *S* (spike protein), *E* (envelope), *N1*/*N2*/*N3* (nucleocapsid) and *RdRp/Hel* (RNA-dependent RNA polymerase/helicase) [7].

It should be noted that other molecular-based diagnostic methods like droplet digital PCR (ddPCR) Reverse Transcriptase Loop-Mediated Isothermal Amplification (RT-LAMP), and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) based techniques have been increasingly developed and, although they have high specificity, their sensitivity is generally lower than real-time RT-PCR assays and their availability is still limited [8].

Despite the high reliability of real-time RT-PCR, certain characteristics undermine its use on a large scale. Although sample processing may take a couple of hours, the logistics of sample collection, transportation, processing, analysis and results delivery can generate bottlenecks delaying the identification and isolation of positive cases. Other drawbacks are its cost and the need for specialized laboratories with specific equipment and trained personnel [9].

In addition, due to its high analytical sensitivity, real-time RT-PCR can be over-sensitive and detect individuals who are in the final phase of the infection when they are no longer contagious [10]

This is due to viral genetic traces that can remain for long periods in the clinical sample, in particular sub-genomic fragments that encode for structural proteins, which are synthesized at high levels during SARS-CoV-2 replicative cycle. These viral mRNAs are suggested to be generated in cytoplasmic double-membrane vesicles during transcription and replication stages and have been observed to persist tightly associated with them for up to 22 days after the onset of symptoms, providing nuclease protection. Consequently, real-time RT-PCR positivity does not necessarily indicate the infective capacity of the individual. In fact, with exceptions, replication-competent viruses cannot be recovered 10 days after symptom onset in patients with mild to moderate COVID-19 [11,12].

In the latter studies, infectivity assays were carried out to assess the potential contagiousness of infected individuals. Unfortunately, these strategies are incompatible to implement in diagnostic schemes due to their complexity, the requirement for biosafety level 3 (BSL-3) facilities and the time required for their analysis [13].

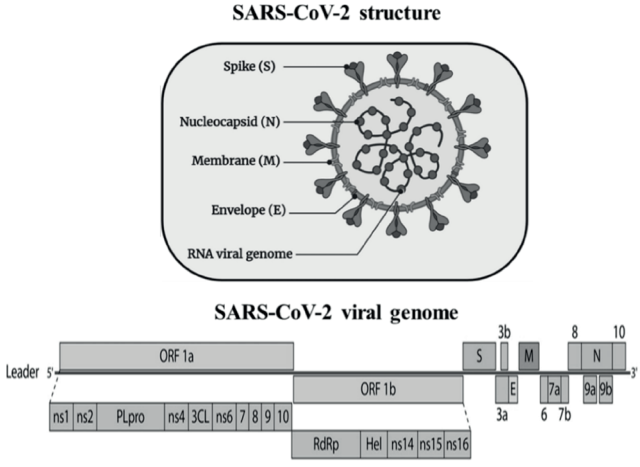

Figure 1
Schematic representation of the SARS-CoV-2 structure and genome architecture (adapted from ref. 6)

The emergence of new viral variants cannot be really considered unexpected or unpredictable, since viruses (thus including those made of single-stranded RNA such as SARS-CoV-2 and Influenza) are subjected to an ecologic and evolutionary pressure and thus tend to continuously mutate and accumulate mutations over time.

Although for some of these variants the biological and clinical characteristics are still unknown or unclear, it is noteworthy that most – if not all – have gradually increased their immune escape potential, though the pathogenicity has not correspondingly increased over time.

At present, the WHO has classified many variants of the SARS-CoV-2, including the variants of concern (VOC), such as the Alpha variant (B.1.1.7), Beta variant (B.1.351), Gamma variant (P.1), Delta variant (B.1.671.2) and Omicron variants (B.1.1.529) [14].

Whether these mutations will enhance pathogenicity and immune evasion of this clade is yet to be definitely proven. It is also noteworthy that at least two different Omicron lineages have been identified so far, called BA.1 and BA.2, which share 21 Spike protein mutations identified in the likely common ancestor. They can be differentiated for the presence of some lineage-specific mutations (12 and 7 unique *S* gene mutations in BA.1 and BA.2, respectively, with another diverging lineage – called BA.3 – that has recently detected in the US). The presence of multiple and different subspecies of the Omicron variants further confirms the considerably high mutational diversity of this virus, especially of its *S* gene [15].

Some mutations that correspond to the target sequences of PCR probe/primer may have significant impact on the detection efficiency. S and N genes, the main targets for real-time RT-PCR detections, have a high probability of mutation, which may have a potential impact on the assay [16].

The diagnostic accuracy in clinical practice of real-time RT-PCR primers recommended by the Centers for Disease Control and Prevention (CDC) or World Health Organization (WHO) remains unproven. Despite the impressive progress made to resolve the pandemic, there is still a need for continuous and active improvement of primers used for diagnosis in clinical practice. For an efficient detection of the newly emerging SARS-CoV-2 variants, the mutations of the real-time RT-PCR target sequences should be closely monitored. The chosen diagnostic targets should be reviewed on a regular basis by manufacturers and by laboratories to ensure efficient primers, binding in currently circulating variants.

SARS-CoV-2 ANTIGEN ASSAYS

The need to rapidly diagnose and isolate infected individuals for containment of COVID-19 outbreaks in communities and hospitals has led to the development of simple and alternative tests to real-time RT-PCR that do not require specialized equipment and skilled technicians but that ensure a good sensitivity and specificity [17].

Antigen tests are based on the detection of the nucleocapsid (N) or the S1 and S2 subunits of the spike (S) protein, using specific antibodies that bind to the S or N proteins and allow the capture of the complete virus or its fragments, respectively [18].

Antigen tests could be designed for quantitative or qualitative analysis. The quantitative test needs more time for the analysis, about 30 minutes, but it can inform about the antigen levels at the moment of the analysis and it could be used to distinguish between the early and late phases of the COVID-19 clinical course.

Among the available tests authorized by FDA or WHO as rapid methods for SARS-CoV-2 patients detection, there are immunochromatographic tests or fluorescence immunoassays, based on SARS-CoV-2 proteins detection in respiratory samples [19]. Immunochromatographic method is a ready to use test that allows a qualitative detection of SARS-CoV-2 antigen in nasopharyngeal secretions after only 15 min. It is based on a membrane technology and uses a colored or fluorescent indicator that allows the change to be detected visually, as the case of the use of colloidal gold, for example. It is a less costly and less time consuming method compared to molecular test, but it is suffering from poor sensitivity [20].

Alternative antigenic test is the automated quantitative chemiluminescent enzyme immunoassay (CLEIA) system, which detects SARS-CoV-2 N protein within 30-60 minutes. It is a not expensive method assay with a good specificity. In contrast with immunochromatographic test, CLEIA system quantitatively measures the antigen levels present in samples and it can reach remarkably high sensitivity if it applied within 1 week after SARS-CoV-2 symptoms or to samples with high viral loads [21].

In summary, antigen tests are particularly suited for point-of-care testing (POCT), as they are easily performed and not require specialized medical personnel with expertise in molecular diagnostic. Moreover, they can typically take 30-60 minutes from the delivery of the patient to obtaining the result, improving turnaround times. On the other hand, antigen tests show some limitations. Unlike real-time RT-PCR, they have low sensitivity and not amplify the information found in the samples tested. Consequently, the amount of antigens present in the sample is sometimes lower than the Limit of detection of the test and this can lead to a false negative result.

SARS-CoV-2 SALIVARY TESTS

The gold standard molecular test assays the viral genome in nasopharyngeal swabs (NPs) [22,23], which continue to be widely used and recommended as the main sample for the respiratory virus diagnosis, including SARS-CoV-2. Because of the disadvantages of nasopharyngeal sampling (trained healthcare workers equipped with protection devices, discomfort for patients, higher risk of viral transmission for the operator), especially in case of serial viral load monitoring or in case of screening strategies [24], several studies have suggested the detection of SARS-CoV-2 in other body fluids such as urine, stool, tears, and saliva [25]. Among these, saliva grabbed both scientific attention and people approval [26].

Saliva is a hypotonic fluid secreted by the parotid, submandibular, sublingual and minor salivary glands, that are distributed throughout the oral cavity. These glands are very permeable and surrounded by blood capillaries, allowing the exchange of molecules and biomarkers, which can be secreted together with saliva. For this reason oral

fluid samples could indicate a viral infection by the presence of viral nucleic acids or antigens [27,28].

The results of studies which used saliva for detection of SARS-CoV-2 RNA by real-time RT-PCR have been variable: some reported it as inappropriate [29,30], while others found it acceptable with regard to sensitivity [24,31]. These differences probably reflect the saliva collection type (oral or posterior oropharyngeal saliva), the sampling procedures (general spitting, drooling, appropriate devices such as *salivette* or *lollisponge*), the time of sampling (within 5 days of the onset of symptoms or later) [32].

Saliva could be considered an option to detect SARS-CoV-2 nucleic acid if NPs cannot be obtained. It could be used for symptomatic people, preferably within the first five days of the onset of symptoms, or for asymptomatic individuals, who are frequently screened for professional reasons, to increase the acceptability of repeated sampling [33]. So, as some studies conducted in school settings reported a high concordance among the results of molecular testing on saliva and NPs [34], saliva is considered a good alternative, especially in screening activities of children involved in "*SARS-CoV-2 circulation Monitoring Plan*". In addition, saliva could apply in the context of scheduled screening for healthcare workers or in frail (symptomatic or asymptomatic) individuals with poor collaboration skills [35]

Regarding the detection of viral antigens in saliva, it has been proposed as a future strategy to achieve earlier diagnosis, reducing the risk of secondary infection caused by the collection procedure of nasopharyngeal specimens [36]. Recent studies evaluated saliva as a possible substrate for chemiluminescence-based antigen tests for automatic laboratory instruments. A sensitivity similar to that observed for nasopharyngeal swabs was found and this kind of assay, as reported by a novel study, may be reliably applied to saliva to identify individuals with high viral loads [37]. On the contrary, when saliva was used as a substrate for rapid lateral flow-based antigen tests, lower sensitivity was reported [38].

In summary, the main advantages of saliva as specimen are: the non-invasive sampling, the possibility of self-collection with almost no discomfort and the decreased risk of exposure for healthcare workers. On the other hand, the drawbacks include: the faster decrease in viral load than NPs, some features of saliva (e.g. mucous or viscous samples) which could make the sample processing difficult [39] and especially the necessity of a careful sampling, which affects the sensitivity of test. For this reason, before collection, patients might be instructed to avoid eating, drinking, smoking, brushing teeth and using chewing gum 30 minutes prior to collect saliva [40].

Finally, the role of saliva as a tool for SARS-CoV-2 detection is highly promising, but a standardization in sampling procedures is needed to date.

SARS-CoV-2 SEROLOGICAL TESTS

During the pandemic, serological tests for the quantitative evaluation of the humoral response have evolved, improving their diagnostic performance. The serological tests now available are very heterogeneous as they differ in classes of immunoglobulins assayed (IgG, IgM, IgA), in type of antigen recognized, in type of test (quantitative, semi-quantitative, qualitative) and in type of analytic method (ELISA, CLEIA or lateral flow immunoassays LFIA) [41].

Since the first half of 2020, the commercial serological tests available highlighted the trend of the antibody response in patients with SARS-CoV-2 infection. It was observed that an initial increase in IgM (within 7-10 days of the onset of symptoms) is rapidly followed by an increase in IgG and IgA (within 2-3 weeks). Then IgM decreases within 8 weeks, while IgG and, to a lesser extent, IgA persist [42]. The almost complete overlap of the IgM and IgG antibodies development led to a nearly exclusive use of IgG or total immunoglobulins for the evaluation of the humoral response. It has been observed that antibodies persist even up to 11 months after infection, whereas memory B lymphocytes persist even after antibody levels decreased.

Serological tests are more readily available and easier to perform compared to molecular assays and they could be an important complementary diagnostic tool in determining the immune status of asymptomatic patients [43]. In addition, serological assays are preferentially used for symptomatic individuals after the acute phase of the illness or for those with a real-time RT-PCR negative result caused by a lower viral load [6,44], so they are particularly useful for the early diagnosis or for confirming the diagnosis of SARS-CoV-2 infections. Moreover, these assays are also used for epidemiological studies.

Among all the structural proteins of SARS-CoV-2, full S or full N proteins, peptides of the N protein and specific domains (S1, S2 or RBD) of the S protein represent the more important antigenic sites for the serological assay [6]. N protein is the most abundant viral protein and it is easy to detect in the early diagnosis of COVID-19. It presents a higher level of conservation among coronavirus infecting human, so it could lead to false positive results through cross-reaction. Despite N protein, S protein is less conserved and it is more specific, so it could be considered a very good target [45,46]. When the SARS-CoV-2 vaccines became available, it was necessary to develop new serological tests targeting the trimeric Spike protein, which is the viral protein induced by vaccination.

In 2021, WHO released a standard preparation (NIBSC - 20/136) obtained from samples of recovered patients in order to standardize the expression of the results obtained with various analytical methods, giving BAU/mL (Binding Antibody Units/mL) as the new unit of measurement [47].

Nowadays, an advantage of SARS-CoV-2 serology is the possibility to use it for: screening and management of clinical patient and close contacts especially the ones with a negative RNA test; population-based serological survey, to understand the actual prevalence and pathogenicity of SARS-CoV-2 infection in different regions and populations; providing scientific guidance for the design and evaluation of vaccines and therapeutic antibodies by analysis of antibody levels and of epitopes spectrum in convalescent people [48].

The appropriate utilization of serological data requires an understanding of its limitations, then greater efforts are being made to increase the correlation of automated serological assays with the gold standard Virus Neutralization Test (VNT).

NEW LABORATORY TESTS

To the wide range of SARS-CoV-2 laboratory tests currently available, two new solutions could be added to support the evaluation of the individual immune response: the surrogate virus neutralization test (sVNT) and the interferon-γ releasing assay (IGRA-test).

Surrogate virus neutralization test (sVNT)

The humoral response through neutralizing antibodies (NAbs) is a key component of the immune response to SARS-CoV-2 and the correlation between the NAbs and total antibodies levels, assessed by generalized and automated serological tests, is essential. It is already known, in fact, that only a small subset of total antibodies are capable of neutralizing viruses and protecting against future infection and disease [49].

The gold standard for evaluation of NAbs is the plaque reduction neutralization test (PRNT) which is technically demanding, needs to be performed in high biosecurity laboratories (BSL-3), has very low throughput and has a long turnaround time [50]. These limitations pushed the development and the validation of alternative methods to assess NAb production.

The sVNT detects NAbs without the need to manipulate live viruses or cells and can be completed in 1 or 2 hours in laboratory. Using purified receptor-binding domain (RBD) from the S protein and the host cell ACE2 receptor, the test is designed to mimic the SARS-CoV-2-host interaction in an ELISA or a FIA immunoassay [51]. This kind of competitive ACE2 test is based on the antibodies-mediated blockade of the interaction between the ACE2 receptor and the receptor-binding domain of S protein (Fig.2). Unlike the classic indirect assays, it is not a quantitative one, has a higher correlation with the PRNT and it is a functional test (Fig.2).

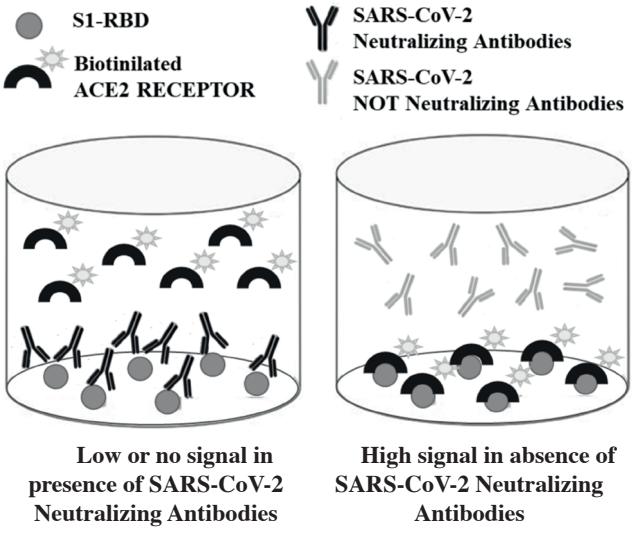

Figure 2
Schematic representation of the SARS-CoV-2 surrogate Virus Neutralization Test (sVNT), in presence or in absence of SARS-CoV-2 Neutralization Antibodies.

At present, sVNT could be a complementary, second-level tool to establish the efficacy of the vaccine in preventing the infections or the development of severe COVID-19. In addition to the well-known role in plasmapheresis, sVNT could have an interesting future application in vaccination campaigns to create a priority criterion for booster doses.

INTERFERON-Γ RELEASING ASSAY (IGRA-TEST)

Nowadays there is an increasing body of evidence surrounding the importance of a T-cell mediated response to SARS-CoV-2 infection [52]. A strong evidence on long-term immunity is lacking to date, but it is likely that a T-cell response is sustained for several months after infection and may last longer than a detectable antibodies production [53]. Furthermore, cellular response could be affected by SARS-CoV-2 variants less than the humoral one [54].

In addition to the important role of T- cell response to natural SARS-CoV-2 infection, several studies focused on its importance after COVID-19 vaccination, not only for immunocompetent people, but especially for immunocompromised patients [55].

The most used method for studying the T- cell response is the IGRA-test, such as Elispot or the Elisa Test for quantification of Interferon-γ. This is a cytokine mainly produced by CD4 + and CD8 + T lymphocytes and it plays a fundamental role in host-protection against viruses and microorganisms.

An IGRA-test consists of more than one step: at first, the lymphocytes of a blood sample are stimulated by pathogen-specific antigens; in the next phase the amount of Interferon-γ, released from stimulated cells, is measured (Fig.3).

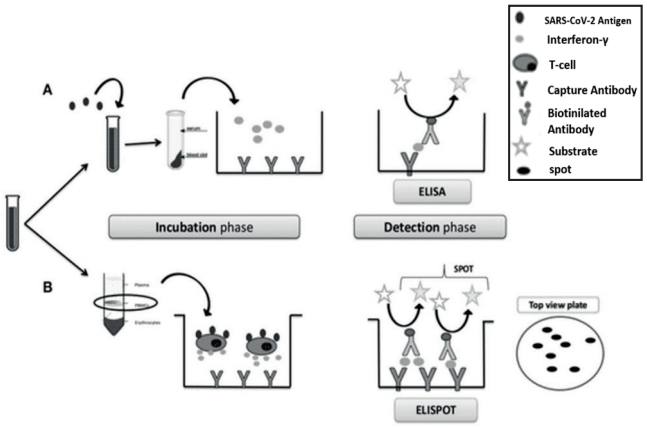

Figure 3
Schematic representation of Interferon-gamma-based in vitro assays (IGRA) in presence or in absence of SARS-CoV-2 Neutralization Antibodies. (adapted from ref. 56)
A) SARS-CoV-2 IFN-γ-release assay with ELISA method. Whole blood is stimulated with a SARS-CoV-2 specific antigen, and the amount of IFN-γ secreted is quantified by an ELISA test.
B) In the ELISPOT method peripheral blood mononuclear cells (PBMCs) are prepared by density gradient centrifugation. A defined number of cells is stimulated with a SARS-CoV-2 specific antigen on plates coated with anti-IFN-γ antibodies. Antigen-responsive cells release IFN-γ, which binds to these antibodies. After removal of the cells, antigens are detected by a second labeled anti-IFN-γ antibody. The number of spots on the plate corresponds to the number of IFN-γ+ cells in the sample .

Nowadays a protective threshold value of Interferon-γ has not been defined, there is not a standardization between different methods and it is not clearly known whether the T- cell response alone is protective, nevertheless cellular assays may be particularly useful in specific high risk populations (e.g. transplant recipients or humoral-immunodeficient patients).

CONCLUSION

In conclusion, though "the long and winding road" to the end of the pandemic is not over yet, this review shows how great was the contribution of Laboratory Medicine to the diagnosis and the spread-containment of SARS-CoV-2 in these two years.

REFERENCES

1. **Wang C, Horby PW, Hayden FG, et al.** A novel coronavirus outbreak of global health concern. Lancet 2020;395:470-3
2. **Xu R, Cui B, Duan X, et al.** Saliva: potential diagnostic value and transmission of 2019-nCoV. Int J Oral Sci 2020;12:11
3. **Arnaout R, Lee RA, Lee GR et al**. The Limit of Detection Matters: The Case for Benchmarking Severe Acute Respiratory Syndrome Coronavirus 2 Testing. Clin Infect Dis 2021;73:e3042-6
4. In vitro diagnostics EUAs FDA (2021), Available online: https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas (Last Access 7th Oct 2021)
5. COVID-19 in vitro diagnostic devices and test methods database (2021), Available online: https://covid-19-diagnostics.jrc.ec.europa.eu/ (Last Access 7th Oct 2021)
6. **Tang Y-W, Schmitz JE, Persing DH, et al.** Laboratory Diagnosis of COVID-19: Current Issues and Challenges. J Clin Microbiol 2020;58:e00512-20
7. **Rabaan AA, Tirupathi R, Sule AA et al**. Viral Dynamics and Real-Time RT-PCR Ct Values Correlation with Disease Severity in COVID-19. Diagnostics (Basel) 2021;11:1091
8. **Abduljalil JM.** Laboratory diagnosis of SARS-CoV-2: available approaches and limitations. New Microbes New Infect 2020;36:100713
9. **Larremore DB, Wilder B, Lester E et al.** Test sensitivity is secondary to frequency and turnaround time for COVID-19 screening. Sci Adv 2021;7:eabd5393.
10. **van Kampen JJA, van de Vijver DAMC, Fraaij PLA et al.** Duration and key determinants of infectious virus shedding in hospitalized patients with coronavirus disease-2019 (COVID-19). Nat Commun 2021;12:267
11. **Wölfel R, Corman VM, Guggemos W et al.** Virological assessment of hospitalized patients with COVID-2019. Nature 2020;581:465-9
12. **Owusu D, Pomeroy MA, Lewis NM et al.** Persistent SARS-CoV-2 RNA Shedding Without Evidence of Infectiousness: A Cohort Study of Individuals With COVID-19. J Infect Dis 2021; 224:1362-71
13. **Ogando NS, Dalebout TJ, Zevenhoven-Dobbe JC et al.** SARS-coronavirus-2 replication in Vero E6 cells: replication kinetics, rapid adaptation and cytopathology. J Gen Virol 2020;101:925-40.
14. Update on Omicron. World Health Organization (WHO). Available on line https://www.who.int/news/item/28-11-2021-update-on-omicron (Last Access 24th Dec 2021)
15. **Salleh MZ, Derrick JP, Deris ZZ.** Structural Evaluation of the Spike Glycoprotein Variants on SARS-CoV-2 Transmission and Immune Evasion. Int J Mol Sci 2021;22:7425
16. **Cao L, Xu T, Liu X et al.** The Impact of Accumulated Mutations in SARS-CoV-2 Variants on the qPCR Detection Efficiency. Front Cell Infect Microbiol 2022;12:823306
17. **Yamayoshi S, Sakai-Tagawa Y, Koga M et al.** Comparison of Rapid Antigen Tests for COVID-19. Viruses 2020;12:E1420
18. **Nguyen NNT, McCarthy C, Lantigua D, et al.** Development of Diagnostic Tests for Detection of SARS-CoV-2. Diagnostics (Basel, Switzerland) 2020;10:E905.
19. **Martín J, Tena N, Asuero AG.** Current state of diagnostic, screening and surveillance testing methods for COVID-19 from an analytical chemistry point of view. Microchem J 2021;167:106305
20. **Scohy A, Anantharajah A, Bodéus M, et al.** Low performance of rapid antigen detection test as frontline testing for COVID-19 diagnosis. J Clin Virol 2020;129:104455
21. **Loconsole D, Centrone F, Morcavallo C et al.** The Challenge of Using an Antigen Test as a Screening Tool for SARS-CoV-2 Infection in an Emergency Department: Experience of a Tertiary Care Hospital in Southern Italy. Biomed Res Int 2021;2021:3893733
22. CDC. Centers for Disease Control and Prevention; 2020. COVID-19 Resources and Guidelines for Labs and Laboratory Workers. https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html (Last access 7th Dec 2021)
23. WHO. Laboratory testing for coronavirus disease 2019 (COVID-19) in suspected human cases: interim guidance, 2 March 2020. Available online https://apps.who.int/iris/handle/10665/331329 (Last Access 7th Dec 2021)
24. **Wyllie AL, Fournier J, Casanovas-Massana A et al.** Saliva or Nasopharyngeal Swab Specimens for Detection of SARS-CoV-2. N Engl J Med 2020; 383: 1283-6
25. **Sun J, Zhu A, Li H et al.** Isolation of infectious SARS-CoV-2 from urine of a COVID-19 patient. Emerg Microbes Infect 2020;9:991-3
26. **Azzi L, Maurino V, Baj A et al.** Diagnostic Salivary Tests for SARS-CoV-2. J Dent Res 2021; 100:115-23.
27. **Medeiros da Silva RC, Nogueira Marinho LC, de Araújo Silva DN, et al.** Saliva as a possible tool for the SARS-CoV-2 detection: A review. Travel Med Infect Dis 2020;38:101920
28. **Corstjens PLAM, Abrams WR, Malamud D.** Saliva and viral infections. Periodontol 2000 2016; 70:93-110
29. **Jamal AJ, Mozafarihashjin M, Coomes E et al.** Sensitivity of Nasopharyngeal Swabs and Saliva for the Detection of Severe Acute Respiratory Syndrome Coronavirus 2. Clin Infect Dis 2021;72:1064-6
30. **Skolimowska K, Rayment M, Jones R, et al.** Non-invasive saliva specimens for the diagnosis of COVID-19: caution in mild outpatient cohorts with low prevalence. Clin Microbiol Infect 2020;26:1711-3
31. **Pasomsub E, Watcharananan SP, Boonyawat K et al.** Saliva sample as a non-invasive specimen for the diagnosis of coronavirus disease 2019: a cross-sectional study. Clin Microbiol Infect. 2021;27:285.e1-285.e4
32. **Lopes JIF, da Costa Silva CA, Cunha RG et al.** A Large Cohort Study of SARS-CoV-2 Detection in Saliva: A Non-Invasive Alternative Diagnostic Test for Patients with Bleeding Disorders. Viruses 2021; 13: 2361.

1. Circular of Italian Health Ministry n.21675 14.05.2021. https://www.certifico.com/news/274-news/14310-circolare-n-21675-del-14-maggio-2021
2. **Oliver J, Tosif S, Lee L-Y et al.** Adding saliva testing to oropharyngeal and deep nasal swab testing increases PCR detection of SARS-CoV-2 in primary care and children. Med J Aust 2021;215:273-8.
3. Circular of Italian Health Ministry n. 43105 24.09.2021. https://www.notiziedellascuola.it/legislazione-e-dottrina/indice-cronologico/2021/settembre/CIRCOLARE_MS_20210924_43105
4. **Azzi L, Baj A, Alberio T et al.** Rapid Salivary Test suitable for a mass screening program to detect SARS-CoV-2: A diagnostic accuracy study. J Infect 2020;81:e75-8.
5. **Amendola A, Sberna G, Lalle E et al**. Saliva is a valid alternative to nasopharyngeal swab in chemiluminescence-based assay for detection of Sars-Cov-2 antigen. J Clin Med 2021;10:1471
6. **Sberna G, Lalle E, Capobianchi MR, et al.** Letter of concern re: "Immunochromatographic test for the detection of SARS-CoV-2 in saliva. J Infect Chemother. 2021 Feb;27(2):384-386. doi: 10.1016/j.jiac.2020.11.016." J Infect Chemother 2021;27:1129-30.
7. T**an SH, Allicock O, Armstrong-Hough M, Wyllie AL**. Saliva as a gold-standard sample for SARS-CoV-2 detection. Lancet Respir Med 2021;9:562-4
8. **Yee R, Truong TT, Pannaraj PS et al.** Saliva is a promising alternative specimen for the detection of sars-cov-2 in children and adults. J Clin Microbiol 2021;59:e02686-20
9. **Nicol T, Lefeuvre C, Serri O et al**. Assessment of SARS-CoV-2 serological tests for the diagnosis of COVID-19 through the evaluation of three immunoassays: Two automated immunoassays (Euroimmun and Abbott) and one rapid lateral flow immunoassay (NG Biotech). J Clin Virol 2020;129:104511
10. **Post N, Eddy D, Huntley C et al.** Antibody response to SARS-CoV-2 infection in humans: A systematic review. PloS One 2020;15:e0244126
11. World Health Organization 2020. Laboratory Biosafety Guidance Related to the Novel Coronavirus (2019-nCoV)https://www.who.int/docs/default-source/coronaviruse/laboratory-biosafety-novel-coronavirus-version-1-1.pdf?sfvrsn=912a9847_2 (Last Access 7th Dec 2021)
12. **Xu Y, Xiao M, Liu X et al.** Significance of serology testing to assist timely diagnosis of SARS-CoV-2 infections: implication from a family cluster. Emerg Microbes Infect 2020;9:924-7
13. **Petherick A.** Developing antibody tests for SARS-CoV-2. Lancet (London, England) 2020; 395:1101-2
14. **Coste AT, Jaton K, Papadimitriou-Olivgeris M, et al.** Comparison of SARS-CoV-2 serological tests with different antigen targets. J Clin Virol 2021;134:104690.
15. WHO/BS.2020.2403 Establishment of the WHO International Standard and Reference Panel for anti-SARS-CoV-2 antibody. Available on line https://www.who.int/publications/m/item/WHO-BS-2020.2403 (Last access 7th Dec 2021)
16. **Sidiq Z, Hanif M, Dwivedi KK, et al.** Benefits and limitations of serological assays in COVID-19 infection. Indian J Tuberc 2020;67:S163-6
17. **Xi Y.** Convalescent plasma therapy for COVID-19: a tried-and-true old strategy? Signal Transduction Target Ther 2020;5:203
18. **Cohen BJ, Doblas D, Andrews N.** Comparison of plaque reduction neutralisation test (PRNT) and measles virus-specific IgG ELISA for assessing immunogenicity of measles vaccination. Vaccine 2008;26:6392-7
19. **Ramos A, Cardoso MJ, Ribeiro L, et al.** Assessing SARS-CoV-2 Neutralizing Antibodies after BNT162b2 Vaccination and Their Correlation with SARS-CoV-2 IgG Anti-S1, Anti-RBD and Anti-S2 Serological Titers. Diagnostics (Basel, Switzerland) 2022;12:205
20. **Jaganathan S, Stieber F, Rao SN et al.** Preliminary Evaluation of QuantiFERON SARS-CoV-2 and QIAreach Anti-SARS-CoV-2 Total Test in Recently Vaccinated Individuals. Infect Dis Ther 2021;10:2765-76.
21. **Zuo J, Dowell AC, Pearce H et al.** Author Correction: Robust SARS-CoV-2-specific T cell immunity is maintained at 6 months following primary infection. Nat Immunol 2021;22:928
22. **Geers D, Shamier MC, Bogers S et al.** SARS-CoV-2 variants of concern partially escape humoral but not T-cell responses in COVID-19 convalescent donors and vaccinees. Sci Immunol 2021;6:eabj1750
23. **Kalimuddin S, Tham CYL, Qui M et al.** Early T cell and binding antibody responses are associated with COVID-19 RNA vaccine efficacy onset. Med (New York, N.Y.) 2021;2:682-8.e4.
24. **Albert-Vega C, Tawfik DM, Trouillet-Assant S et al.** Immune Functional Assays, From Custom to Standardized Tests for Precision Medicine. Front Immunol 2018;9:2367

Corresponding author:
Dott.ssa Marilina Tampoia,
Chief of Clinical Pathology Unit, "SS. Annunziata" Hospital
Via Bruno
74100 Taranto (TA).
Tel.: +39 333 4598457,
e-mail :marilina.tampoia@asl.taranto.it