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Monitoring the infection of SARS-CoV-2 and the development of diagnostic tools

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Abstract

Several issues are still unclear about the COVID-19 pandemic infection. The spreading of the infection throughout the world shows striking differences. In the present survey is described the prevalence of SARS-CoV-2 infection as reported in internationally updated online registers, comparing reported cases and deaths per million of inhabitants. Analysis of the data reflects a wide range among the continents and within each geographic area there are important differences among different countries. A focus on the Italian regions describes significant differences in terms of cases between North and South Italy in August 2020, a situation that reflects the diffusion of the SARS-CoV-2 infection in the period of February-April 2020. The scenario becomes completely different in October; indeed the number of cases and hospitalized patients shows an increase of 20-fold with respect to August 2020. Tools for the diagnosis of SARS-CoV-2 infection have become pivotal in the efforts to control the infection and monitor infected subjects. In the present report the different tests currently available are described as well as their usefulness in the present situation and their potential usage once the campaign for mass vaccination is effective.

Keywords: COVID-19, Diagnostic Tests, Epidemiology, Infection, SARS-CoV-2

1. Epidemiology of SARS-CoV-2

The SARS-CoV-2 infection has become a major concern worldwide during 2020, capable of seriously affecting economies and causing restrictions in social behaviors aimed at limiting the spreading of the infection, especially to fragile subjects, waiting for a vaccine to be available.

Some elements might be useful to give a picture of the SARS-CoV-2 infection throughout the world:

- on December 31st, 2019 the Health authorities of the municipality of Wuhan, a large city in the Hubei region of the People's Republic of China formally communicated existing cases of "atypical pneumonia";

- on January 9th, 2020 the Chinese authorities determined that the outbreak of atypical pneumonia is caused by a novel coronavirus;
- on January 11th, 2020 the Chinese media reported the first death caused by the new atypical pneumonia;
- on January 13th, 2020 the Thailand government reports the first case of novel coronavirus pneumonitis;
- on January 24th, 2020 France declared the first cases of novel coronavirus pneumonitis;
- on February 11th, 2020 the WHO announced that the disease caused by the novel coronavirus is named COVID-19;
- on March 11th, COVID-19 is officially characterized as a pandemic infection.

On October 15th, from the data reported by the WHO, globally there have been over 38 million diagnosed cases, with more than 280,000 new cases reported in the last 24 hours, and more than 1,080,000 deaths overall (more than 4,000 deaths in the past 24 hours).

The infection is spread all over the world, with cases recorded even in Falkland Islands, or in Greenland, as well in Montserrat where 1 death linked to COVID-19 has been reported. (https://covid19.who.int/table).

If we focus our attention on single countries considering those with at least 5 million inhabitants, the results are shown in Table 1.

A strikingly high incidence is observed in Israel, with a rate of 33,700 cases/moi (million of inhabitants). Besides Israel, countries from South and North America show the highest incidence of infection. In Africa can be observed the specificity of South Africa with an incidence above 18,000 cases/moi. It is also interesting the finding of Saudi Arabia with 9,800 cases/moi, in a geographic area, the Arabic peninsula, showing very high rates in Oman, Bahrein, Qatar, Kuwait, United Arab Emirates as well, although not reported in the present table given a population of lower than 5 moi. Furthermore, the relative low prevalence observed in Australia is quite interesting, and as a whole, in the Far East countries. In this latter region, Japan reports a prevalence of 700 cases/moi and China, where the outburst of the infection took origin, reports 62 cases/moi, which is more than 500 times lower than that registered in Israel.

If the analysis of the data takes into consideration the number of deaths/moi, it is evident that prevalence of infection and deaths do not seem to be correlated. Indeed, the ratio between number of deaths/moi vs. number of cases/moi goes from less than 1% as reported in Israel and Czechia up to 10% or more as recorded in Italy and Mexico. This observation is quite difficult to explain in terms of differences in therapy availability in the different countries. More likely it has to be related to the different ways of classifying deaths correlated to COVID-19 in the different health systems, more precisely, in some countries subjects affected by co-morbidities are not classified as deaths related to COVID-19.

In any case, whatever might be the reason for this striking difference, it raises different perceptions in the population about the consequences of being infected by SARS-CoV-2 and, also, it justifies the different actions that the governments are taking to prevent the spreading of the infection.

It is understandable that in countries where the rate of deaths/prevalence is 10% or higher, the measures to be taken tend to be very restrictive compared to those taken in countries with a risk of death for COVID-19 that is below 1%.

This observation deserves further investigations in order to properly evaluate the real risk of COVID-19 pandemic with respect to the perceived risk.

Country	Cases/moi	Deaths/moi	% Deaths/Cases
Israel	33,700	231	0.685
Peru	25,800	1,011	3.919
Chile	25,300	701	2.771
Brazil	24,000	709	2.954
United States of America	23,300	645	2.768
Argentina	20,000	535	2.675
Spain	18,400	704	3.826
Colombia	18,100	550	3.039
South Africa	18,000	304	1.689
Belgium	15,000	884	5.893
Czechia	12,000	103	0.858
Bolivia	11,900	713	5.992
France	11,200	501	4.473
Netherlands	11,000	387	3.518
Iraq	10,200	248	2.431
Sweden	10,000	584	5.840
Saudi Arabia	9,800	146	1.490
United Kingdom	9,400	634	6.745
Russian Federation	9,200	159	1.728
Belarus	9,000	96	1.067
Ireland	9,000	371	4.122
Ecuador	8,400	694	8.262
Mexico	6,400	651	10.172
Italy	6,000	600	10.000
India	5,300	80	1.509
Canada	4,800	255	5.313
Germany	4,000	116	2.900
Australia	1,070	35	3.271
Japan	700	13	1.857
China	62	3	4.839

Table 1. Prevalence of SARS-CoV-2 infection in sampled countries as of October 15th 2020. Source: Authors' elaboration on data reported by World Health Organization.

2. A look at the situation of SARS-CoV-2 infection in Italy

Analyzing the data recorded in Italy as available from the site of the Italian Ministero della Salute, we compare data obtained in two different windows: the first taken in mid-August, when the first wave of infection was vanishing, and the second taken November 1st, in the presence of the "so called" second wave of infection.

The results are reported in Tables 2 and 3.

The COVID-19 situation in Italy in mid-August showed 260,000 cases, 35,500 deaths and 19,200 currently infected subjects. Most interesting are the observations of cumulative incidence which was remarkably higher in Northern regions compared to Southern Italy.

Overall, the number of known infected subjects was below 20,000, thus suggesting a picture of infection spreading still under control.

Data registered less than three months later show an increase in the number of currently infected subjects by roughly 20-fold and the rate of hospitalized patients is quite similar being just below 19-fold. This scenario has been indicated as the second wave and it is still representing a major concern for the national health system, with the heavy burden of deaths and pressure on emergencies whose reception capability is a limiting factor.

3. Diagnostic tests for SARS-CoV-2 infection

The rapid development of the SARS-CoV-2 pandemic has prompted research to develop diagnostic tools to identify quickly and accurately pathogens in infected patients and asymptomatic subjects.

To date, there are three main types of detection assays relevant for COVID-19 diagnostic testing and screening, which vary according to the type of target to be identified (Brooks and Das, 2020).

1. Nucleic acid test (Real-Time PCR).
2. Antigen test (Immunochromatography test).
3. Antibody test (Immunoenzymatic test).

4. Nucleic acid test (Real-Time PCR)

Nucleic acid tests (molecular test) identify the presence of viral RNA thus indicating that viral infection is currently ongoing. This test is based on Real-time PCR (RT-PCR), a method that detect and amplify a single copy of the specific genomic sequence and therefore, it is extremely sensitive. Although several methods are available to reveal viral presence and activity, the reference method for the diagnosis of acute SARS-CoV-2 infection is RT-PCR (Ishige et al., 2020; Dreyfus, 2018). Samples for SARS-CoV-2 molecular diagnostic tests can be taken from the upper (nasopharyngeal/oropharyngeal swabs or saliva) or lower respiratory tract (sputum or tracheal aspirate or bronchoalveolar lavage), since these compartments are supposed to be the site of active viral infection (Förster et al., 2020). Different steps characterize the RT-PCR method: viral RNA is first extracted from the biological specimen than is purified and converted into a complementary DNA fragment (cDNA) by reverse transcriptase (an RNA-dependent DNA polymerase enzyme). Subsequently, cDNA is copied by the polymerase and the reaction is repeatedly cycled through a series of temperature changes, which allow many copies of the target region to be produced (Saiki et al., 1988). During amplification, generated fragments are labelled with a fluorescent reporter and results are evaluated by an algorithm proportionally related to the amount of the fluorescent signal of the amplified fragments (Bustin et al., 2009). The main steps of this method are reported in Figure 1.

The RT-PCR technology offers several advantages: provide extremely sensitive, specific, and often quantitative detection of the SARS-CoV-2 RNA (Afzal, 2020). However, there are some limitations to this method: RT-PCR is not affordable and results are complex and slow to deliver. In fact, the molecular diagnostics tests are not for end-users but are intended only for qualified clinical laboratory technicians. Furthermore, erroneous RT-PCR results may be caused by inappropriate sample collection, storage, transfer, purification, and processing (Chen et al., 2020; Chan et al., 2020; Wang et al., 2020).

5. Antigen test (Immunochromatography test)

The antigen test detects the presence of a viral antigen, a portion or part of viral protein that is easily recognized by the immune system (Abbas et al., 2018). Most COVID-19 antigen tests target the “spike protein” present on the surface of the coronavirus. Generally, SARS-CoV-2 antigen test uses antibodies coated with a membrane or beads to hunt for proteins embedded in the coronavirus’s surface (Figure 2). Specimens are collected from throat or nose using a swab or by blood fingertips (Chen et al., 2004; Ogata et al., 2020). The swab is washed into a liquid to dissolve the mucus and release the virus antigen/s. Samples containing viral particles are then applied to the test slide surface that is coated with antibodies that “grab onto” any coronavirus proteins. A second mixture of antibodies is then applied to the slide, these antibodies have been conjugated to a chromogen that makes them visible as shown in Figure 2. The gold point of this assay is the speed and ease of use, although this test is not very accurate (Randad et al., 2020; Lieberman et al., 2020). Since antigen testing does not involve any processes of amplification, a swab or a fingertip blood sample may have too little antigen to be detected. Thus, the viral amount is the major limitation of this test and positive results should be confirmed by molecular analysis (Corman, 2020). Given the speed of execution and the low cost of the anti-

gen test, the usefulness of this test would be appropriate in all situations that require a quick response (such as airports, train or bus stations and hospitals).

6. Antibody tests (Immunoenzymatic test)

Serology looks for antibodies against SARS-CoV-2 in the blood to determine if there was a previous infection. Antibodies or immunoglobulin are large, Y-shaped protein produced mainly by plasma cells, used by the immune system to neutralize pathogens such as bacteria and viruses (Abbas, 2018; Dreyfuss et al., 2019). Generally, immune response to viral infection involves three different class of immunoglobulins called IgM, IgG and IgA. Immunoglobulin M (IgM) are the first to appear in response to exposure with the antigen thus indicating a recent infection; immunoglobulin G (IgG) are the most abundant in the antibody response and constitute about 70-75% of the total immunoglobulins present in serum. The presence of specific IgG against viral antigen indicates that a previous or asymptomatic infection occurred between host and pathogen. A third class of immunoglobulin involved in immune response are IgA, mainly present on the mucosal epithelium and representing the first line of protection from pathogens (Chen, 2004; Farina et al., 2016). Anti-SARS-CoV-2 antibodies can be assayed by various methods, using the interaction between antigen and antibody as showed in Figure 3.

As discussed above, the main target of serologic tests is to measure the antibody response. It is important to underline that during infections, antibodies against pathogens are produced over days to weeks after the first contact. Thus, there is a latency gap between primary infection and antibodies production (weeks) that represents a limitation to the serological assay. By the contrary, detection of anti-SARS-CoV-2 antibody (IgG, IgM and IgA) represents a powerful tool in diagnosis and screening of the disease since it can be easily performed by using venous blood samples. Several applications have been proposed for serological tests: first of all, they provide important information about the epidemiology of the SARS-CoV-2 infection. Therefore, antibody tests display a relevant message about seroconversion and seroprevalence in different geographical areas, providing an appropriate assessment of the actual number of subjects infected by the virus. Furthermore, serological tests also offer important data for clinical practice providing quantitative information on the extent of the antibody response and showing the transition from the infectious to the acquired immunity phase. To this regard, the development of "herd immunity", could have a key role in control of viral spread.

To date, numerous SARS-CoV-2 antibody assays have been approved including lateral flow tests (LFA), enzyme-linked immunosorbent assays (ELISA) and fully-automated electrochemiluminescent (ECLIA) or chemiluminescent immunoassays (CLIA) (Brooks and Das, 2020).

A short summary of the three main methods is reported in Table 4.

7. Good practices suggested for diagnosis and monitoring of SARS-CoV-2 infection

To accurately analyze the greatest number of tasks in the shortest time respecting the three main points of laboratory medicine reproducibility, sensibility and cost reduction, automated system represents the best approach.

Comparing costs, advantages and disadvantages of the three analytical methods, Nucleic acid test, Antigen test and Antibody test, we propose an action plan in which we suggest a good practice in relation to the different spectrum of subjects to be evaluated (i.e., affected, suspected, asymptomatic or screening) (Figure 4). This plan might represent a useful tool concerning procedures that should be used.

Region	Cumulative incidence of cases/100,000 inhabitants	Currently known infected subjects	Currently hospitalized patients
Valle d'Aosta	577	11	1
Piedmont	273	1,095	88
Lombardy	261	5,864	168
Veneto	209	2,048	47
FVG	296	291	13
Bozen province	536	172	9
Trento province	141	44	4
Liguria	689	401	22
Emilia Romagna	693	2,139	90
Marche	466	243	13
Tuscany	294	1,015	44
Umbria	187	170	13
Lazio	163	2,151	305
Abruzzo	278	336	35
Molise	61	56	2
Campania	96	1,041	74
Puglia	126	505	85
Basilicata	44	81	7
Calabria	50	167	14
Sicily	80	947	63
Sardinia	100	429	18
Total		19,206	1,115

Table 2. SARS-CoV-2 infection in Italy in Mid-August 2020. Source: Authors' elaboration on data reported by Italian Ministero della Salute.

Region	Cumulative incidence of cases/100,000 inhabitants	Currently known infected subjects	Currently hospitalized patients
Valle d'Aosta	2,686	1,920	173
Piedmont	1,668	34,414	3,023
Lombardy	2,031	90,075	4,664
Veneto	1.208	31,414	964
FVG	925	5,376	218
Bozen province	1,679	5,631	290
Trento province	1,728	2,100	161
Liguria	1,892	8,714	1,143
Emilia Romagna	1,292	24,917	1,399
Marche	971	6,723	369
Tuscany	1,251	29,974	1,279
Umbria	1,241	7,210	341
Lazio	830	36,106	2,240
Abruzzo	842	6,633	423
Molise	588	1,053	23
Campania	1,027	47,178	1,586
Apulia	479	12,032	764
Basilicata	417	1,595	97
Calabria	273	3,362	174
Sicily	457	15,234	1,131
Sardinia	600	6,378	379
Total		378,039	20,841

Table 3. SARS-CoV-2 infection in Italy as of November 1st 2020. Source: Authors' elaboration on data reported by Italian Ministero della Salute.

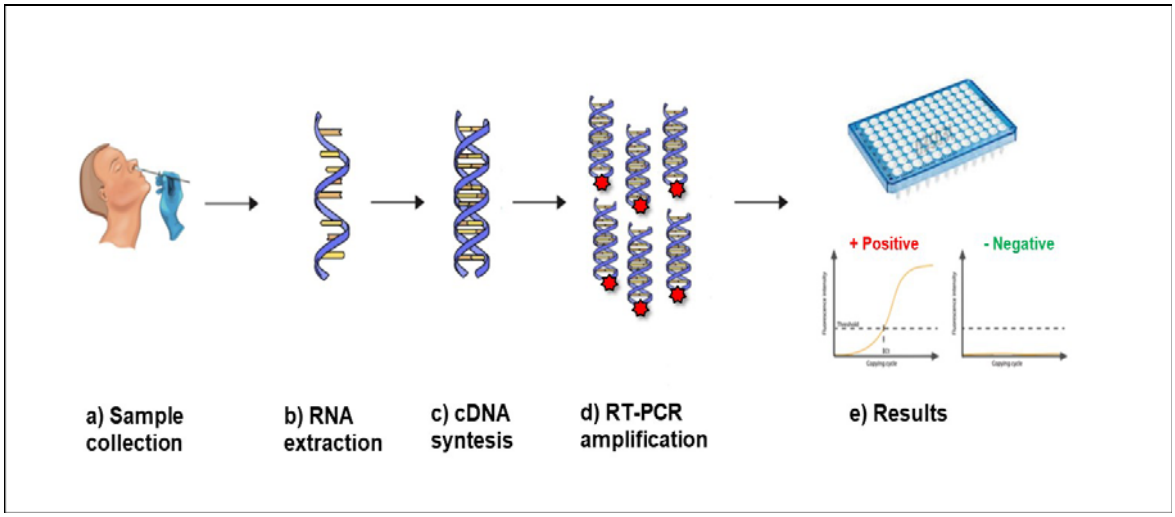

Figure 1. Steps in the RT-PCR test: a) -d) different steps of the RT-PCR method used to amplify and label specific viral RNA target sequence. e) Results are evaluated by computer analysis, an algorithm tracks the amount of fluorescence in the sample after each cycle. The test is positive when the fluorescence level crosses a certain threshold. Source: Authors' elaboration.

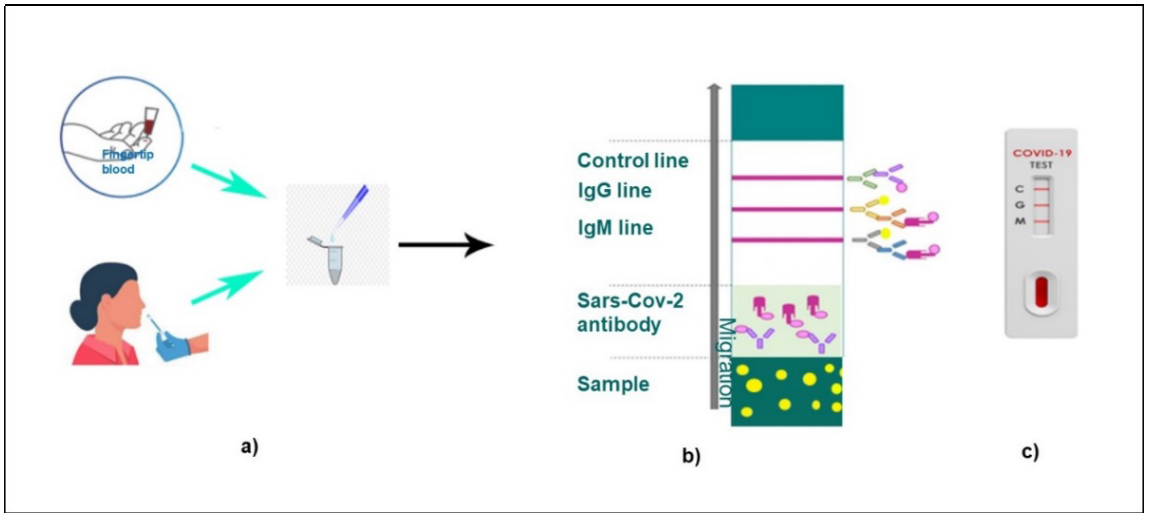

Figure 2. Antigen Test Methods. a) samples are collected from Nasal/Throat swab or fingertip blood and resuspended in diluent Mix. b) The diluted sample is applied on a slide coated with SARS-CoV-2 immunoglobulin (IgM/IgG). The mixture then migrates upwards and reacts with the anti-human IgG and/or IgM in the test line area. c) After migration, SARS-CoV-2 IgG, IgM or both antibodies, will be visualized in the appropriate line through a colorimetric reaction. Source: Authors' elaboration.

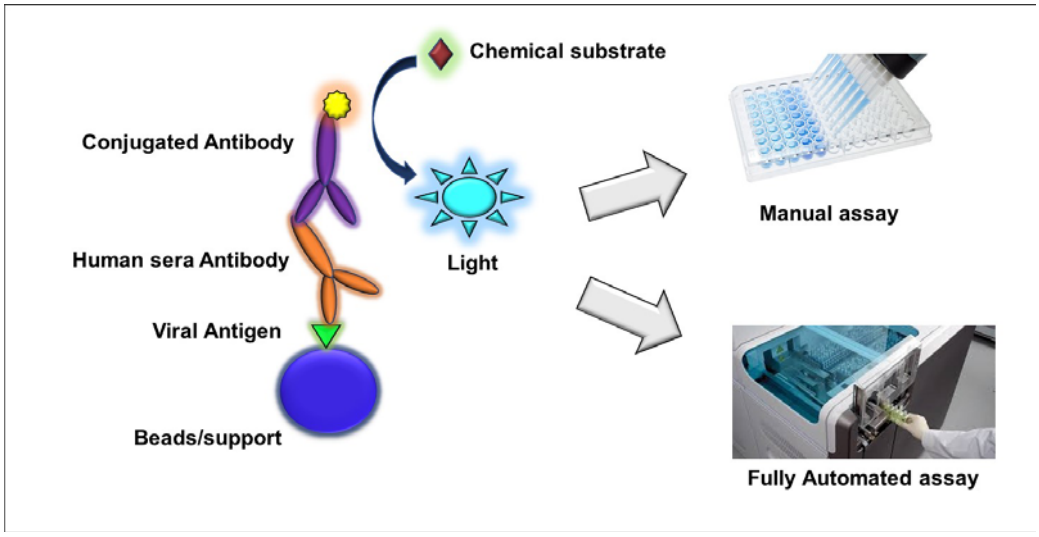

Figure 3. Basic principle of serological tests. Serological tests are all based on the fundamental principles of interaction between antigen (Ag) and antibodies (Ab) of the IgG, IgM or IgA classes. The antigen is generally coated on a support such as magnetic beads or other supports. The primary interaction between an antigen and antibody in vitro cannot be visualized and so serological tests generally employ a secondary indicator system based on the use of a different antibody conjugated with fluorochrome or enzyme directed against human ab. Given that, the interaction between antigen-human (ab)-secondary conjugated (ab) form a sandwich. Following the incubation with a chemical substrate, the conjugated ab produces luminescence that could be detected by manual or fully automated methods. Source: Authors' elaboration.

	Molecular Test	Antigen Test	Antibody Test
Target	Detect Viral Genome (RT-PCR)	Detect Viral Protein	Detect Antibodies against Virus (IgM and IgG)
Sample type	Nasal or Throat swab	Nasal or Throat swab Fingetip Blood	Blood Serum
Advantages	Very Accurate	Fast Less expensive	Accurate Fast Less expensive
Disadvantages	Very Expensive Long Requires high-skill operator	Less accurate Strictly related to the viral load	Strictly related to the IgM/IgG amount and development times

Table 4. Differences between analytical diagnostic procedures. Source: Authors' elaboration.

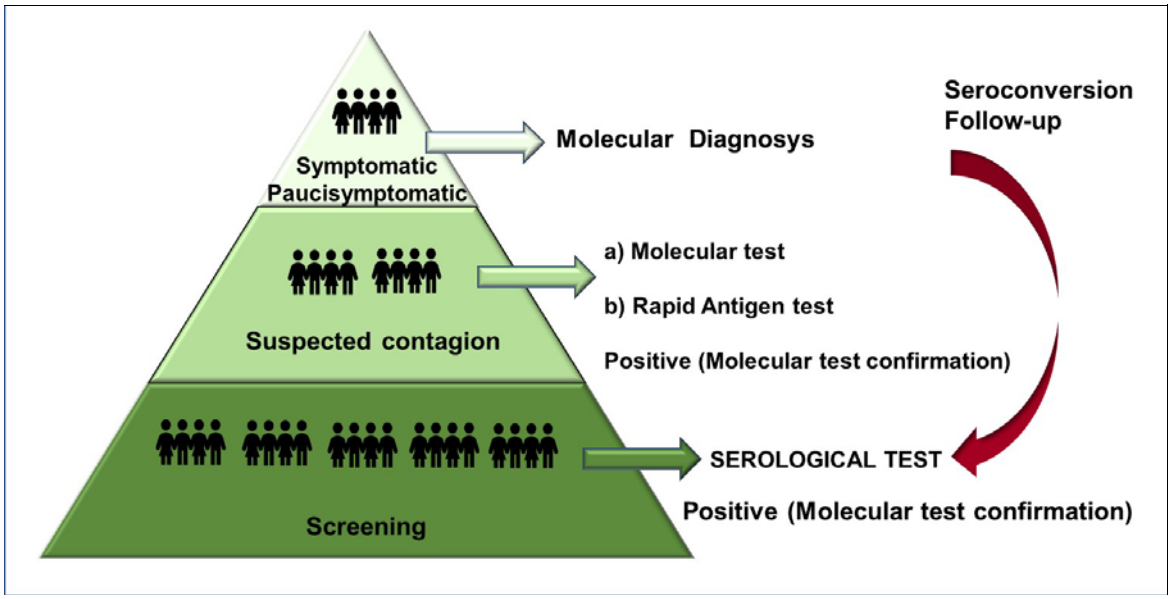

Figure 4. Action Plan for diagnosis and monitoring SARS-CoV-2 infection. Source: Authors' elaboration.

8. Discussion and Conclusion

The scenario described in this review reflects the complex situation triggered by the SARS-CoV-2 pandemic. To date, COVID-19 can be considered a systemic disease where many biochemical activities and physiological functions are disrupted (Gandini et al., 2020; Anastasi et al., 2020). Although many aspects of transmission, infection and treatment remain to be clarified, we can state that current available investigation methods for viral detection, if used appropriately, represent a cornerstone of this complex situation. Certainly, an important factor in tracing subjects infected by SARS-CoV-2 has been the increased availability of laboratory tests that allowed a capillary evaluation of the spreading of the infection. As discussed above, RT-PCR based viral RNA is the current reference standard diagnostic tool for COVID-19 infections, although it is not suitable for a mass screening due to high costs and investigation times. Thus, to not overwhelm specialized laboratories, rapid screening systems such as Antigen test represent powerful detection tools as primary mass screening. In addition, these tests may improve the sensitivity of COVID-19 pathogenic diagnosis when combined with RT–PCR based viral RNA testing. Indeed, these tests are commonly used in major gathering places such as stations, schools, ports and airports. Serological tests are elective methods to assay seroconversion and epidemiology of SARS-CoV-2 and will represent an important instrument to track immune response promoted by future vaccine against this virus.

At the moment, the spreading of SARS-CoV-2 infection reflects different situations: Europe is facing the so-called second wave of infection, whereas in the Americas a continuum of infection has been experienced starting from March 2020, in Far East Asia and Oceania the rate of infection decreased heavily during last spring and remains under control, with no significant second-wave. In Africa, apparently, the spreading of the infection has been very limited with the exceptions of some Mediterranean countries and South-Africa.

This scenario is going to change significantly in the upcoming months, when vaccines will be available. It is evident that a vaccination program aimed at the entire population will represent a very heavy task for the national health systems. Assuming that one or more vaccines will show a capacity to provide an immunological response in 95% of the population, still huge

efforts are needed in terms of organization of the different programs. There is such a need for these vaccines throughout the world that the production and distribution will take several months before reaching the so-called herd immunity in the different countries. Furthermore, it is unclear how long the immune response will last either after viral infection as well as following vaccination. In such uncertainty, monitoring antibodies in seropositive individuals will be important, in order to gain insights about the possibility to prevent "second infection" of the same subjects and to define when and how it will be possible to reduce the restrictions that many countries are carrying out to reduce the spreading of the infection. Therefore, we can easily envisage from a laboratory point of view a shift from nasopharyngeal based tests to serologic tests.

Thus concluding, gaining information on the several aspects of SARS-CoV-2 is crucial to implement proper control measures to help prevent outbreaks or lessen their impact on humans and society. Our ability to handle future outbreaks will rely on the lessons we have learned from the present as well as from previous pandemics.

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