The total testing process harmonization: the case study of SARS-CoV-2 serological tests

**Opinion Paper**

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**Abstract:** The total testing process harmonization is central to laboratory medicine, leading to the laboratory test's effectiveness. In this opinion paper the five phases of the TTP are analyzed, describing, and summarizing the critical issues that emerged in each phase of the TTP with the SARS-CoV-2 serological tests that have affected their effectiveness. Testing and screening the population was essential for defining seropositivity and, thus, driving public health policies in the management of the COVID-19 pandemic. However, the many differences in terminology, the unit of measurement, reference ranges and parameters for interpreting results make analytical results difficult to compare, leading to the general confusion that affects or completely precludes the comparability of data. Starting from these considerations related to SARS-CoV-2 serological tests, through interdisciplinary work, the authors have highlighted the most critical points and formulated proposals to make total testing process harmonization effective, positively impacting the diagnostic effectiveness of laboratory tests.

**Keywords:** harmonization; SARS-CoV-2 serological tests; total testing process

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## Introduction

The COVID-19 pandemic represented one of the biggest public health challenges. Despite the ongoing debate on the best strategy for managing and controlling SARS-CoV-2 infection, testing, and screening the population remain essential for defining seropositivity and, thus, driving public health policies. Therefore, laboratory tests play a crucial role by responding to two different needs: (1) to detect the presence of the virus through the identification of viral RNA (molecular tests) or its proteins (antigenic tests); indeed, these tests should be required to confirm the diagnostic suspicion in symptomatic subjects and to identify asymptomatic subjects; (2) to recognize the existing antibodies in the serum (serological test) of patients who have developed an immune response against the virus (natural or induced by vaccination). These tests should be required for monitoring the progress of the immune response in the subject and for large-scale epidemiological investigations. Several serological tests are on the market with different characteristics regarding detection method, antigenic target, and isotype of the detected antibodies (Table 1).

Besides the differences related to the selected method or approach, several differences exist in terms of terminology, measurement unit, reference ranges, and parameters for results interpretation. These differences make the analytical results of different serological tests hardly comparable, leading to a general confusion that affects or fully precludes data comparability [1].

To improve results comparability the so-called "total testing process" (TTP) harmonization have now a central role in laboratory medicine [2]. "Harmonization" is defined as "the process of recognizing, understanding, and explaining differences while taking steps to achieve uniformity of results, or at a minimum, a means of the conversion of results such that different groups can use the data obtained from assays interchangeably" [3].

Harmonization allows to provide the same result and interpretation for the sample of a given patient, regardless of the laboratory that produced the result. The concept refers to

**Table 1:** Detection method, antigenic target, and isotype of the detected antibodies by the most common serological tests analyzed within the COVIDIAGNOSTIX project [1].

| Test | Developer | Technology | Target | Antibody | Cut-off |
|---|---|---|---|---|---|
| Elecsys Anti-SARS-CoV-2 | Roche Diagnostics | High throughput ECLIA (qualitative) | Nucleocapsid | Pan-Ig | 1.0 |
| Elecsys Anti-SARS-CoV-2 S | Roche Diagnostics | High throughput ECLIA (qualitative and semi-quantitative) | Spike (RBD) | Pan-Ig | <0.80 U/mL: negative<br>≥0.80 U/mL≤250 U/mL: positive<br>>250[a]: positive (numeric value as "> 250 U/mL") |
| LIAISON SARS-CoV-2 S1/S2 IgG | DiaSorin | High throughput CMIA (qualitative) | Spike (S1–S2) | IgG | <15.0 AU/mL: negative<br>≥15.0 AU/mL: positive |
| LIAISON SARS-CoV-2 TrimericS IgG | DiaSorin | High throughput CLIA (qualitative and semi-quantitative) | Spike | IgG | <13.0 AU/mL: negative<br>≥13.0 AU/mL: positive<br>>800[a] AU/mL: positive not reported |
| SARS-COV-2 ELISA (IgG) | EUROIMMUN | ELISA (qualitative) | Spike (S1) | IgG | OD ratio<0.8: negative<br>OD ratio≥0.8<1.1 borderline<br>OD ratio≥1.1 positive |
| ADVIA Centaur SARS-CoV-2 IgG (sCOVG) | Siemens Healthcare Diagnostics | High throughput CLIA (qualitative and semi-quantitative) | Spike (S1 RBD) | IgG | 1.00 index |
| CHORUS SARS-CoV-2 "NEUTRALIZING" Ab | DIESSE | Immunoenzymatic method (quantitative) | Spike (S1) | Pan-Ig | >50.0 BAU/mL: positive<br><20.0 BAU/mL: negative<br>20.0–50.0 BAU/mL: equivocal |

[a]Values above the measuring range are reported as >250 U/mL (or, on 1:10 diluted samples, up to 2500 U/mL).

the entire process of laboratory medicine [4, 5] and should not be confused with standardization, more properly referred to the analytical phase. Table 2 lists some key terms that are sometimes misused, contributing to the difficulty of completing the harmonization process and the different phases of the TTP, also mentioned in Table 2, are described below.

TTP is a stepwise process in which five phases are distinguished: (i) the pre-pre-analytical phase, where the clinician requests the test; (ii) the pre-analytical phase, related to the sample processing before the analytical phase; (iii) the analytical phase, where the analyte is measured, the result is validated and released; (iv) the post-analytical phase, where results are recorded and reported to the physician; (v) the post-post-analytical phase, where the physician interprets the test results.

Harmonization in laboratory testing is the key to the quality of laboratory data, leading to the diagnosis's effectiveness and involving all aspects of the TTP scheme (Figure 1). Pre-analytical, analytical, and post-analytical aspects must be considered to ensure that results are comparable regardless of the test used and where/when the test is performed [10]. Standardizing the analytical phase can be utterly frustrating if everything that comes before and after the measurement of analytes is not adequately standardized [11]. Furthermore, the extra-analytical phases represent an important source of error, as they are more difficult to standardize and control [12].

Therefore, differences in examination results, terminology, units of measurement, reference intervals, and decision-making levels can cause confusion and potentially harm the patient. Significant differences between laboratories compromise the value of guidelines and interpretation of results based on recommended decision-making levels and preclude the transferability of data reported in electronic repositories. The lack of harmonization negatively affects results comparability obtained in different laboratories/hospitals or even the same laboratory/hospital. The situation is further exacerbated by increased population migration and mobility [12].

The National Cancer Institute (NCI) Serological Sciences Network (SeroNet) has made an effort towards the harmonization process. Among the objectives of this network, the main one is "to develop, validate, improve, and implement serological testing and associated technologies". SeroNet has also identified as a critical point the need for assay harmonization and standardized reporting units to compare the results of different studies [13].

This opinion paper describes, highlights, and summarizes the critical issues that emerged in each phase of the TTP

**Table 2:** Definitions of the terms relevant in the harmonization process.

| | Definition | Phase |
|---|---|---|
| Harmonization [6] | The process of recognizing, understanding, and explaining differences while taking steps to achieve uniformity of results, or at a minimum, a means of the conversion of results such that different groups can use the data obtained from laboratory tests interchangeably | TTP |
| Standardization [7] | A process in which the values assigned to hierarchically lower order standards are systematically determined either by a direct comparison to the highest order reference standard available, or indirectly, by comparison with an intermediate (lower order) reference standard | Analytical phase |
| Reference materials [8] | Material, sufficiently homogeneous and stable with reference to one or more specified properties, which has been established to be fit for its intended use in measurement or examination | |
| Quality control materials | Substance, material, or article used to verify the performance characteristics of an *in vitro* diagnostic medical device (ISO 15198) [9]. It can be a reference material without an assigned quantity value [8]. | |
| Calibrators [8] | Measurement standard is used routinely to calibrate or verify measuring instruments or systems. | |
| Commutability of a reference material [8] | Property of a reference material, demonstrated by the closeness of agreement between the relations among the measurement results for a stated quantity in this material, obtained according to measurement procedures for which the material is intended for use, and the relation obtained among the measurement results for other specified materials. | |

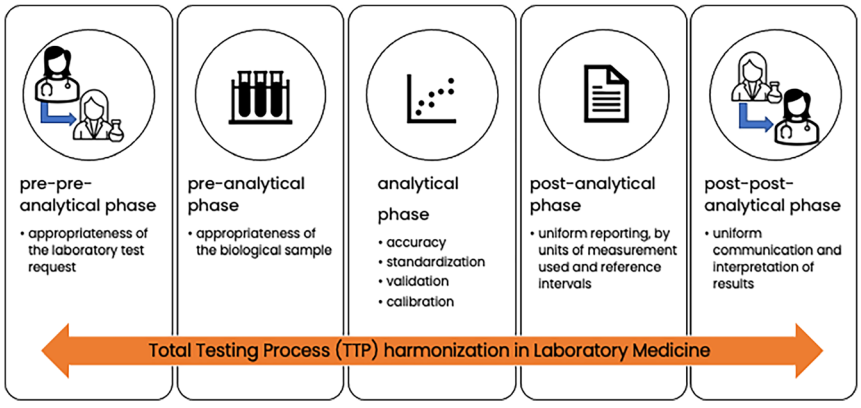


**Figure 1:** Critical points of total testing process (TTP) harmonization in laboratory medicine.

with the SARS-CoV-2 serological tests and that have affected their effectiveness. Subsequently, the common factors influencing laboratory test harmonization were identified and, therefore, processes were suggested that could be employed to improve it.

## Lack of SARS-CoV-2 serological testing harmonization phase-by-phase

### Pre-pre-analytical phase

The pre-pre-analytical phase is characterized by the appropriateness of test requesting and is related to the clinician knowledge and experience [14, 15]. Laboratory tests should only be requested if their results will be used to influence subsequent patient management decisions. Therefore, it is essential to define the analytical target in accordance with its clinical use. An appropriate prescription is critical to direct not only towards effective laboratory tests for a correct diagnosis (effectiveness) but also to contribute to the correct use of laboratory resources (efficiency), with the final aim to move towards the best outcome for the patient [15]. The clinician decides which test to require based on his knowledge and experience [16].

In the contingency of the COVID-19 pandemic, SARS-CoV-2 serological tests have been prescribed without prior experience and with limited possibility for developers to disseminate information (through workshops, congresses, and one-to-one meetings). A further difficulty is related to the heterogeneous nature of SARS-CoV-2 serological tests: they are a set of tests, some of which are summarized in Table 1. They are based on both qualitative and quantitative methods, and they can reveal IgA, IgM, and IgG can be directed against the S proteins and N proteins of SARS-CoV-2, the major immunogenic components produced either in

proportion to viral load (Figure 2A) or response to vaccination (Figure 2B). The intrinsic aspects that impact prescribing appropriateness and that must be investigated for their correct clinical governance are related to the production and persistence times of the different classes of antibodies, as specified in the CDC's Interim Guidelines for COVID-19 Antibody Testing in Clinical and Public Health Settings [17]. In particular, the first antibodies developed (1–4 weeks after infection) are secretory immunoglobulin A (IgA), which forms in the mucosal tissues of the nasal and intestines, and humoral immunoglobulin M (IgM), which has a peak between 2 and 5 weeks and then decreases. Humoral immunoglobulin G (IgG) is formed later (2 weeks) than IgM but has a higher specificity and guarantees long-term protection (peak between 3 and 5 weeks). On the contrary, the BNT162b2 COVID-19 mRNA vaccine induces antibody responses in healthy subjects. In individuals with prior SARS-CoV-2 infection, one single dose promotes a similar or even higher immune response than in natural seropositive receiving two-dose immunization [18]. In particular, several reports have assessed the generation of vaccine-induced IgG antibodies in the serum of immunized individuals [19] (Figure 2B).

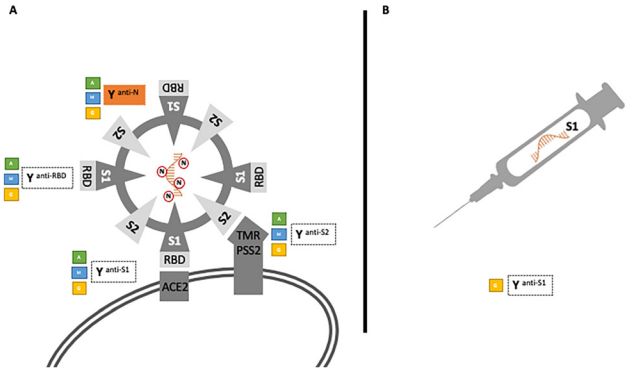


**Figure 2:** (A) SARS-CoV-2 infection-induced antibodies. SarS-CoV-2 is an enveloped, single-stranded RNA virus belonging to the β coronavirus cluster. The virus enters the host cells by receptor-mediated endocytosis through the high-affinity binding of the trimeric spike (S) protein and the surface receptor ACE2 (angiotensin-converting enzyme 2) present in the lungs, kidneys, blood vessels, and heart. S protein binds ACE2 through the receptor-binding domain (RBD) of the sub-unit 1 (S1). Once the receptor binds to the cell, the transmembrane serine protease TMPRSS2 proteolytically cuts the S protein, allowing the endocytosis of the viral genome. Therefore, the S1 and S2 functional sub-units have distinct functions: S1 is responsible for receptor binding through the RBD, and S2 is responsible for the fusion of the virus's membrane with the host cell. The single-stranded RNA genome is associated with the nucleocapsid (N) protein, which increases its stability. Protein N is involved in the transcription and replication of the viral genome and is highly conserved in all coronaviruses. (B) BNT162b2 COVID-19 mRNA vaccine-induced IgG antibodies anti-S protein.

Therefore, to maximize the informativeness of the serological tests, anti-N IgG could be prescribed to identify or exclude Sars-CoV-2 infection in naïve and vaccinated subjects [20], as N-specific IgG concentrations were not affected by vaccination [21]. Also, anti-IgA indicates mucosal immunity and is present in both naïve and vaccinated subjects shortly after infection [22].

Instead, an absolute anti-S1 IgG value can monitor the BNT162b2 COVID-19 mRNA vaccine-induced response [23, 24]. In conclusion, the serological assessment may help to define the potential incidence and prevalence of community infections (i.e., "seropositivity") and, thus, drive public health policies. However, this concept no longer seems as simple as the currently available immunoassays have been manufactured using antigen(s) derived from the SARS-CoV-2 identified and sequenced in Wuhan in 2019 [25]. Since then, the viral genome has undergone such a huge number of mutations; consequently, the antibodies generated against the new Omicron sublineages or during periods of the prevalence of previous SARS-CoV-2 variants would not be recognized, underestimating the serum levels of anti-SARS-CoV-2 antibodies [20].

From what has been said, a deep and up-to-date knowledge of the tests is necessary to optimize their diagnostic potential. Rapid technological innovation and the continuous proposal of new diagnostic tests require updating knowledge on tests and new organizational approaches. However, the logic of the silo system (i.e., disjointed work processes, compartmentalizes mentality, barriers to communication), widespread and difficult to eradicate in medicine, does not favor the spread of communication. To overcome this problem, the laboratory professional and the clinician must interact to choose the most appropriate test in a specific clinical and epidemiological context to provide a critical tool informing care.

## Pre-analytical phase

It is the phase related to a series of processes from the identification of the patient to the correct transport and storage, and the preparation of the samples before the analysis [26, 27]. The choice of the sample type should be driven by the diagnostic setting and not only by the organizational aspects of the patient's journey.

There are two macro-types of SARS-CoV-2 serological tests on the market: classic and rapid. The classic tests are carried out on a serum sample obtained from a venous blood sample; they detect qualitatively and quantitatively the presence of antibodies of the various immunoglobulin classes (IgA, IgM, and IgG) [1]. Instead, the so-called

"rapid tests" detect the presence of IgM and IgG in capillary blood samples or saliva through immuno-chromatographic methods; being purely qualitative, they only indicate the presence or absence of antibodies [28].

SARS-CoV-2 antibodies in saliva serve as the first line of defense against the virus. They are present in the mucosa, more precisely in saliva, after a recovered infection. Reportedly, antibody persistence in plasma and saliva was shown for up to 15 months after mild COVID-19 [29].

Anti-SARS-CoV-2 antibodies were measured in the cerebrospinal fluid (CSF) in assessing neurological disease associated with COVID-19 and long-COVID. Evidence from CSF profiling in COVID-19 with neurological symptoms mainly suggests blood-brain barrier disruption compatible with cerebrospinal endotheliopathy [30].

## Analytical phase

The analytical phase is related to the measurement quality [31]. This phase focuses on the experimental conditions under which the determination is performed (measuring, reference procedure, primary and secondary reference materials). Since one of the primary objectives of laboratory medicine is to produce equivalent results regardless of the laboratory and method used, laboratory professionals have made great efforts to implement the harmonization and standardization of measurements.

The frequent lack of comparability of the results and a consensus on each analytical phase adopted in the laboratories has forced the international community towards harmonization of tests. Results comparability is based on the traceability of test results to reference materials and measurement procedures. This is achievable only when the method and the measurand (i.e., the entity to be measured) can be defined. Metrological traceability is an essential tool to standardize and harmonize measurements in laboratory medicine: it is defined as the property of a measurement result whereby the result can be related to a reference through a documented unbroken chain of calibrations, each contributing to the measurement uncertainty [32]. At the base of the concept of metrological traceability, there are factors related to the analytical target, the calibrators, the measurement methodology and the measurement procedure.

The unambiguous definition of the analytical target is a priority requirement. While for chemically well-defined analytes belonging to "classical" clinical chemistry, there are usually no problems of definition, for analytes of more heterogeneous structure, such as many of the proteins determined in laboratory medicine, the definition of what is measured can be much more problematic (e.g., whole or part(s) of the molecule, activity/reactivity of the molecule, etc.). Once this aspect has been defined, commercially available methods should be able to selectively measure the measurand to achieve a good level of homogeneity between the measurements and the results obtained.

In the case of serological tests for SARS-CoV-2, problems have occurred related to the analysis of analytes of heterogeneous structure (the trimeric spike protein; the receptor-binding domain of the sub-unit 1; the sub-unit 2 responsible for the fusion of the virus's membrane with the host cell) and the analysis of heterogeneous mixtures (Ig A, IgM, IgG). The SARS-CoV-2 serological test efficiency depends on its specificity and sensitivity. Sensitivity is the probability that a test result will be positive when the disease is present (true positive rate), and specificity is the probability that a test result will be negative when the disease is not present (true negative rate). There are no legal obligations in this regard. Still, it is preferable to use tests with a specificity of at least 95 % and a sensitivity of at least 90 % to reduce the number of false-positive and false-negative results. The high sensitivity of the test is essential. Still, it must not be at the expense of specificity because, as mentioned, antibody tests aim not to diagnose an active infection. In this sense, the choice of the antigen to identify is crucial since several factors must be considered, including concentration, specificity, and availability. For example, most antibodies are produced against N and S proteins. Therefore, there are methods on the market to quantify total antibodies, IgM, or IgG directed against N or S protein (monomeric, trimeric, or specific for its RBD domain). Furthermore, using an antigen with highly conserved epitopes can cause cross-reactivity, detecting specific antibodies to previous infections, such as SARS-CoV or other Coronaviruses. Consequently, in the particular SARS-CoV-2 case, serological tests could welcome using a single target Ig antibody directed against a single specific binding protein (S or N) to help and facilitate measurement comparability.

As far as calibrators are concerned, manufacturers of serological tests for SARS-CoV-2 usually prepare calibrators for their commercial methods, which are frequently different from those used by other manufacturers of diagnostic kits, and this is the basis of the substantial disagreement often detected between the results obtained with the various methods commercially available for the measurement of the same analyte (Table 3). The calibration of the measurement instrument used to perform the test is essential to assess the instrument's proper functioning and determine the threshold value to evaluate the test sample's positivity. To this aim, the results obtained from the analysis of sera taken from subjects who have

**Table 3:** Detection method, standard, and calibrators of the most common serological tests analyzed within the COVIDIAGNOSTIX project [1].

| Test | Developer | Standard | Calibration |
|---|---|---|---|
| Elecsys anti-SARS-CoV-2 | Roche Diagnostics | No international standard was available for anti-SARS-CoV-2 | Positive calibrator consisting of human serum, reactive for anti-SARS-CoV-2 antibodies |
| Elecsys anti-SARS-CoV-2 S | Roche Diagnostics | No international standard was available for anti-SARS-CoV-2-S | This method has been standardized against the internal Roche standard for anti-SARS-CoV-2-S. This standard consists of an equimolar mixture of 2 monoclonal antibodies that bind Spike-1 RBD at 2 different epitopes. 1 nM of these antibodies corresponds to 20 U/mL of the Elecsys Anti-SARS--CoV-2 S assay |
| LIAISON SARS-CoV-2 S1/S2 IgG | DiaSorin | No international standard was available for anti-SARS-CoV-2-S | Test assay-specific calibrators allow the detected relative light unit (RLU) values to adjust the assigned master curve |
| LIAISON SARS-CoV-2 TrimericS IgG | DiaSorin | No international standard was available for anti-SARS-CoV-2-S | Individual LIAISON SARS-CoV-2 TrimericS IgG reagent Integrals contain specific information for instrument calibration based on the reagent Integral lot used. Test assay-specific calibrators allow the detected relative light units (RLU) values to adjust the assigned master curve |
| SARS-COV-2 ELISA (IgG) | EUROIMMUN | No international standard was available for anti-SARS-CoV-2-S | The results were expressed as ratios, which are a relative measure of the concentration of antibodies in serum or plasma |
| ADVIA Centaur SARS-CoV-2 IgG (sCOVG) | Siemens Healthcare Diagnostics | No international standard was available for anti-SARS-CoV-2-S | The calibrators provided with each kit were used to set up the parameters for the ADVIA Centaur sCOVG assay |
| CHORUS SARS-CoV-2 “NEUTRALIZING” Ab | DIESSE | The Chorus TRIO instrument expresses the result in binding antibody units, BAU/mL (first international standard WHO 20/136 for anti-SARS-CoV-2), calculated based on a batch-dependent curve stored in the instrument | Positive calibrator consisting of protein solution containing specific antibodies capable of binding the antigen present on the microplate |

developed an antibody reaction against SARS-CoV-2 are compared with those obtained from pre-pandemic sera.

To harmonize the measurements and to compare results from different serological tests with the final aim of reducing inter-laboratory variability, an international standard (IS) has been made by World Health Organization (WHO) (20/136), issued upon specific request by the National Institute for Biological Standards and Control (NIBSC) which is the UK’s National Control Laboratory. The standard, called here “WHO-IS”, consists of 250 μL (1000 BAU (Binding Antibody Unit)/mL) of a pool of 11 sera from patients recovering from COVID-19, collected 28 days after the onset of symptoms [33]. A qualitative analysis of the WHO-IS has been done by Colombini et al. [24] and revealed the presence of IgA, IgG, and IgM anti-N, S1, S2, and RBD. WHO-IS standard is very similar to “natural seropositive” in composition. It is suggested to produce a new international standard containing predominantly anti-S1 IgG and RBD to monitor vaccines more effectively.

Therefore, the first problem for WHO-IS production lies in the scarcity of available materials. A proposal to overcome this problem might be the preparation of a Working Standards (WS) starting from the sera of vaccinated subjects (10 sera obtained from vaccinated subjects, 21 days after administration, all-natural seropositive). Despite this, Ferrari et al. did not find a good correlation between the methods using this strategy, calculating the conversion factors for WS, similarly to what was done for the WHO-IS, and converting the data. This has led to the conclusion that WS (reproducible and available in large quantities) can be used as a secondary reagent after calibration with the WHO-IS to harmonize the results if using the Roche assay, but not to harmonize the results of serological assays obtained with the other commonly used serological methods [34].

Finally, the combination of different serum compositions in the different subjects and the different specificity of the various assays in recognizing different antibodies leads to the failure to harmonize the methods using a standard. Moreover, it should not be forgotten that the biological variability linked to the fluctuation of antibody titers in different clinical conditions is added to the analytical variability.

In addition, three other different types of protein Certified Reference Materials (CRMs) were developed by the National Institute of Metrology (NIM) China to support the development of new serological tests and the evaluation of existing serological test kits: Nucleocapsid (N) protein CRM (GBW(E)091097), Human IgG monoclonal antibody (GBW(E)091109-GBW(E)091110), and IgM CRM.

In conclusion, the harmonization and standardization of infectious disease serology, in general, are largely unmet goals [35]. Regardless of the availability of new international standards, such as the 1st International Standard 2022 Antibodies to SARS-CoV-2 variants of concern (WHO 21/338, National Institute for Biological Standards and Control (NIBSC), Potters Bar, Hertfordshire, EN6 3QG, UK), it is impossible to compare measures of anti-SARS-CoV-2 antibodies obtained in different clinical laboratories using different methods.

### Post-analytical phase

In the post-analytical phase, results are recorded and reported to the physician [1, 2].

Age, sex, and hormonal differences make the intra-individual variability of serological test results high [18]. For this reason, it is essential to consider the critical difference to evaluate the longitudinal trend of the antibody response in the patient. Moreover, the serological assessment may help define the immunization status of the community [36] and thus driving public health policies; therefore, the factors to consider when establishing the importance of appropriate translation of such evidence to local practice are the prevalence of the condition, health care setting, patient demographics, comparability of clinical pathways, availability of adequate treatment and further diagnostic options. The serological test result, indicating the immunization of the subject and the community, can be evaluated in the light of the patient's response to infection (morbidity and mortality) or to treatment, to improve the patient's management [36].

### Post-post-analytical phase

The post-post-analytical phase is when the physician interprets the test results [2]. As showed in Table 1, results are provided as positive, negative, or indeterminate (qualitative test), using a test specific scale not comparable to other assays (semi-quantitative), or providing a measured and scaled assessment of antibody levels (quantitative). Furthermore, the impossibility of establishing a threshold or a reference interval and harmonizing the results of serological tests obtained from different laboratories makes identifying the correct diagnostic setting mandatory: serological testing for the monitoring of antibody response to SARS-CoV-2 should be used to determine if a person has COVID-19 antibodies, which suggests past infection or vaccination, and, as a community epidemiological tool, to monitor and evaluate population levels of immunity [36].

Table 4 summarizes the main critical points of each TPP phase and the key potential solutions to improve the harmonization of SARS-CoV-2 serological tests.

## Harmonization in laboratory tests: a compass to find the way

The analysis of the TTP of SARS-CoV-2 serological tests has brought out some main critical issues that have affected the effectiveness of these tests. However, a broad discussion, as the one proposed here, can allowed to identify the critical points and formulate some suggestions to improve the harmonization of laboratory tests.

Communication is a common factor in extra-analytical phases. Clinical laboratory initiatives to harmonize laboratory tests are doomed to fail without good communication among all relevant stakeholders.

Laboratory tests provide a crucial tool informing care when warranted and indicated: efficiency of test-ordering practice could reduce the share of low-value (i.e., unnecessary, unindicated, or potentially harmful) tests, which is now estimated at 20–30 % [37]. Effective methods to involve non-laboratory physicians include (1) ensuring adequate clinical laboratory representation on hospital standing committees, (2) encouraging collegial relationships with solid communication between clinical laboratory faculty/staff/trainees and other clinical partners, (3) creating a laboratory test formulary committee to address test stewardship [38], and (4) close partnership in the creation of protocols with interventionists who perform complicated sampling procedures [39].

**Table 4:** Main critical issues and possible key interventions to be implemented in each phase of the analytical cycle to promote the harmonization of SARS-CoV-2 serological tests.

| | Critical issues | Key interventions |
|---|---|---|
| Pre-pre-analytical phase | Silo system communication | The laboratory professional must act as an interface between the clinician and the patient to improve the integration of diagnostic results |
| | Fast technological innovation and ongoing proposals for new diagnostic tests | Foster interaction between clinicians and laboratory professionals to update knowledge on novel diagnostic tests and workflow to improve test-ordering practice |
| Pre-analytical phase | Appropriateness of the type and timing of sampling | To improve effective methods to involve non-laboratory physicians in the pre-analytical process |
| | Appropriateness of transport and the storage of the sample | |
| Analytical phase | Measurement of different analytes, based on the type of test in use, that generally claims high specificity over a single target. | Clearly determine and define which analyte can be measured. Increasing the test's specificity for the target analyte by standardizing the measurement positively impacts the comparability of results between different methods |
| | Biologically heterogeneous analytes present problems in the univocal definition of the analytical target | The analytical target must be defined unambiguously and related to the clinical use of the measurement |
| | Accuracy | Specificity and sensitivity clearly defined by FDA or EMA |
| | Calibration | Using calibrators provided for each specific diagnostic kit or, even better, from WHO or certified reference materials producers |
| | Standardization | Use of certified reference material and high order measurement procedures traceable to the international system of units |
| Post-analytical phase | Intra-individual variability | Assessment of critical difference |
| Post-post-analytical phase | Establishment of a threshold and harmonization | To improve the actionability of test results |

In an ideal world, any lab should get the same result when testing the same measurand. While there have been many efforts and significant advances in this direction, clinical laboratories have not yet achieved this level of performance. There are many reasons why different laboratories produce different values for the same measurement, including the use of different calibrators from different manufacturers, as demonstrated by experience with SARS-CoV-2 serology testing. Standardization of measurements in laboratory medicine is very complex and one of the reasons is that the measurements often involve complex and variable mixtures. So many confounding factors influence the result of a measurand (matrix effect) that achieving high comparability of results remains a challenge even when metrological traceability is respected.

One way to reduce the variability of laboratory test results is to ensure that methods are traceable to reference materials and methods, so a traceable method has to meet the following criteria: (i) must have an unbroken chain from specific reference material and/or method to the final result; (ii) must include an associated measurement uncertainty; (iii) must be validated and, if possible, the commutability of each reference material should be demonstrated [40].

Under the hypothesis that tests already available on the market are reliable and validated, each *in vitro* diagnostics (IVD) test should ensure performance at least equivalent to those already available on the market. Unfortunately, this is not sufficient to ensure that the quality of new tests, in addition to those already on the market, is comparable to a "gold standard" method. In the specific case of serological tests for SARS-CoV-2, the global emergency has necessarily forced rapid and often qualitatively dubious solutions. To minimize the difference between the results produced by different laboratories, using external quality assurance (EQA) systems is a valuable aid in pursuing the goal of harmonization. Another key aspect emerging from the data on SARS-CoV-2 serological tests is the importance of the "commutability" of reference material, i.e., the ability to produce an analytical response that mimics that of patient samples.

In 2002, the Joint Committee for Traceability in Laboratory Medicine (JCTLM) was formed to achieve the standardization goal. The JCTLM is an international consortium sponsored by the Bureau International des Poids et Mesures (BIPM), the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC), and the International Laboratory Accreditation Cooperation (ILAC),

joining together government agencies, clinical laboratories, and industries. The JCTLM aims to support the comparability and traceability of measurement results in clinical laboratories with regulatory bodies and IVD manufacturers. With the help of this consortium, clinical laboratories remain responsible for selecting methods that meet the clinical standards necessary to ensure correct performance. The metrology community, i.e., the National Metrology Institutes (NMIs) and Designated Institutes (DIs), is sharing and joining competences and efforts in providing traceability for the *in vitro* diagnostics. NMIs and DIs from all over the world are contributing to the JCTLM by producing reference materials and certified reference materials and by developing traceable methods, all listed in the JCTLM database. In addition, the Consultative Committee for Amount of Substance: Metrology in Chemistry and Biology (CCQM) within the Meter Convention has a working group, the Protein Analysis Working Group (PAWG) dedicated to establishing SI traceability for protein quantification including antibodies. Among the PAWG members, for example, an inter-laboratory comparison, described by Mi et al. [41] was organized to contribute at the development of measurement standards for the antibody detection of SARS-CoV-2. A SARS-CoV-2 monoclonal antibody reference material developed by the Chinese NMI was used as a model system to optimize methods for mass fraction (mg/kg) of amino acids and peptides in antibody quantification, with associated uncertainty estimation. In conclusion, in order to improve the actionability of test results, a harmonization process covering each phase of the test procedures is needed and should be addressed. To this aim, ISO standards, such as the ISO 21151:2020 "*In vitro* diagnostic medical devices – Requirements for international harmonization protocols establishing metrological traceability of values assigned to calibrators and human samples", could be guidance to establish harmonization protocols for metrological traceability in Laboratory Medicine.

Moreover, producing serological tests specific for detecting a single class of antibodies against a unique epitope will improve the tests' clinical usefulness and life cycle, this characteristic could improve dramatically the comparability of the results between tests from different producers thanks to their inner specificities and lack of other antibodies classes cross interferences [42]. It will also help to calibrate the tests with specific monoclonal antibody reference materials or sera international standards, or simple working standards produced in clinical laboratories for internal studies.

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