



# The Covid-19 antibody test challenge

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*In the current situation of the Covid-19 pandemic, reliable IgG antibody tests are urgently needed all around the world. Virologists caution against cross-reactivities that could potentially lead to false positives [1,2]. Using ELISAs as an example, we discuss the technical requirements for the development of truly dependable immunological testing methods, since the cross-reactivities debated by many virologists constitute only a small fraction of the actual challenges.*

In the context of Corona-antibody detection assays, virologists rightly caution against cross-reactivities due to the particularities of the Coronavirus family. IgG assays are designed to display a previously completed immunization and to be used on a broad swath of people. The fields of healthcare and public safety are in especially urgent need since immunized workers could potentially resume work without the risk of endangering themselves or others. Step-by-step, however, many other occupational and population groups have to be systematically tested - several times if necessary. Immunity can be conferred by experienced infection, later hopefully by vaccination. In both cases, immunity is characterized by very specific, high affinity IgG antibodies against SARS-CoV-2. It is precisely these antibodies that can be measured by immunoassays in an easy and cost-effective way and on a mass scale - but these immunoassays may also produce false-positive signals, if the assay is not developed, produced, and performed optimally. From a purely technical perspective we here describe established and modern solutions that help to avoid faulty diagnostic results. The illustrated solutions are explained using an anti-IgG-ELISA against SARS-CoV-2 for example, but can be applied to the optimization of serological test systems for infectious diseases for routine diagnostics as well as for accompanying vaccine development. A universal challenge of these testing systems is that many funding agencies of health care systems give particularly low reimbursements for infectious disease testing based on immunoassays and IVD-manufacturers are exposed to an immense cost pressure. This often leads to technical compromises irrespective of patient safety. Hence, many technically valid solutions are rarely used in routine diagnostics, despite demonstrably improving reliability. This is simply because they lead to additional costs of a few cents per sample.

In the context of the Corona pandemic, however, it should be clear to all parties involved, starting with funding agencies, via the laboratory physicians, virologists and epidemiologists and, of course, the IVD-manufacturers, that cost savings of a few cents per sample do not justify even the rarest potential unreliability of this immune status diagnosis against Covid-19. In this kind of tests false positives can lead to a situation in which difficult-to-detect super spreaders are created, because they were wrongly classified as Covid-19 immune and hence non-dangerous. This article aims to help prevent this scenario as much as possible and depicts several parallel technical strategies, none of which should in our opinion be neglected in order to safely reach the aspired goal. Only the combination of all these technical approaches will ensure that reliable IgG antibody tests against SARS-CoV-2 will be available commercially and for research purposes.

## Known causes for “cross-reactivities” and false-positives

Four different causes for false-positives in serological assays are known:

1. Inappropriate capture antigens
2. Cross -reactivities and biochemical interferences
3. Insufficient surface blocking in serological assays
4. Stability of the reagents

A good assay can only be developed if all four aspects are considered. Therefore we discuss all four aspects individually.

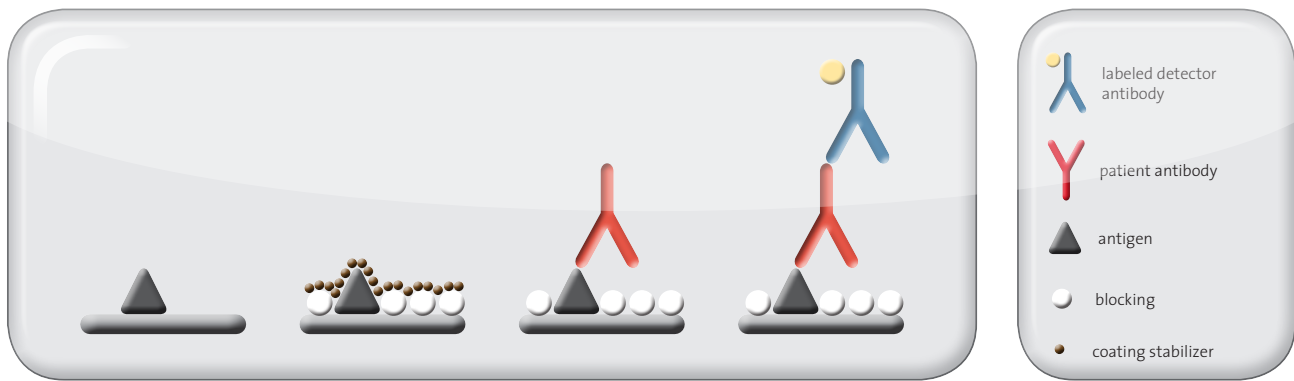


Figure 1 shows an antigen-down-ELISA for the detection of IgG antibodies against SARS-CoV-2

## 1. Inappropriate capture antigens

The most obvious problem for many virologists is the selected capture protein. Among them, this aspect is currently widely discussed and investigated under the heading cross-reactivity, which is why it will not be discussed in detail here [1]. In case of an ELISA, a capture protein, to which the specific IgG from the patient sample is supposed to bind, must be coated on the plate. It is known that a previous infection with other coronaviruses does not reliably protect against Covid-19, i.e. does not confer real immunity. Therefore, an immunity against a different coronavirus must not be detected by a SARS-CoV-2 IgG assay. Since in this case it is not the SARS-CoV-2 with its specific epitopes that is detected, but merely a similar protein epitope, this phenomenon is referred to as cross-reactivity. Cross-reactivity in general describes the binding of an antibody to a structurally similar, but not identical molecule. In the case of different coronaviruses, the source of this structural similarity is their biological kinship [1]. However, purely random or inexplicable cross-reactivities also occur due to a similar 3D structure (structural epitopes), despite the rest of the molecules involved showing no similarities at all. The topic “cross-reactivities” is described in more detail elsewhere, e.g. in the book „Immunoassays“ ed. by A. M. Raem and P. Rauch (Verlag Elsevier 1st edition 2006; 2nd edition in preparation). It is therefore vital to take precautions to ensure that a SARS-CoV-2 IgG assay does not show positive results for patient antibodies that have been generated during infections with related coronaviruses. Otherwise the results would be false positives. In this context it is important to know that it is usually not the protein sequence but the 3D structure including all post-translational modifications, such as glycosylation, phosphorylation, that are recognized by the antibodies [3]. Therefore, for reliable antibody tests, capture proteins must be expressed in a cell line that produces identical or nearly identical modifications to those cells that are actually responsible for the strongest viral expression in a sick patient [2]. Neither an unfavourably chosen capture protein, nor a sub optimally modified protein or protein fragment, nor one that has lost its native fold during coating has a good chance of becoming the basis of a reliable ELISA. In addition, the 3D structure of the coated capture antigen and its modification must be permanently maintained during storage. In contrast to research assays, which are measured directly after production, a diagnostic assay must not only be suitable for mass production, but must also be packable, transportable and permanently storable without the risk of component degradation. In addition, an in vitro diagnostic assay should still be fully functional if it is not measured right after removal from the laboratory refrigerator. The last aspect is also referred to as on-board shelf life in devices such as ELISA processors or high-throughput immunoassay systems and can sometimes be an insufficiently validated detail. All this must be possible without compromising the reliability of the assay due to gradual degradation of the capture antigen. These requirements are completely different from those that have to be met by an assay used in university medical research. The capture protein is hence a central challenge of this type of diagnostics and, depending on its selection, upscaling to a production scale for worldwide supply in the context of the pandemic may become a technical task not to be underestimated. Therefore, the dreaded binding of specific IgGs targeted at related corona viruses is actually not only result of unexpected cross-reactivities in the ELISA, but rather of an unfavourable selection of the presented epitope during the design phase. In this article we will not deal with the selection of the correct epitope, but with the necessity of optimal stabilization, which will be discussed later. Nevertheless, besides the possibility of careful selection, gentle purification, correct treatment and perfect stabilization of the capture antigens, there is a second, completely different approach to battle the dreaded cross-reactivities, which is completely independent of the target and can be used in almost any immunoassay.

## 2. Cross-reactivities and biochemical interferences

Even if the capture molecule is correctly selected and is handled optimally, cross-reactivities are to be expected - even if only in a subset of patients. Some patient antibodies, which are actually able to bind proteins of related coronaviruses with high specificity and affinity due to earlier infections, will therefore also be able to bind the structurally related SARS-CoV-2 proteins, albeit with lower affinity. This may yield a positive test result and thus immitate immunity. So, what exactly is the problem if these patient antibodies can recognise SARS-CoV-2? In simple terms, only highly specific antibodies that bind with high affinity can produce a robust immune response in the body. If an antibody „only“ displays low-affinity binding, it will not hold onto a target in the body „long enough“ (very simplified wording, which is used here for the sake of simplification and shortening) to elicit a full response of the immune system and inactivate enough viruses. However, if the immune system is not activated at all by the medium- or low-affinity antibodies, there is no immune protection or immunity. Nevertheless, a binding would be detected in the assay, which would yield a positive result. In addition, there are many other molecules that are known to occur in sufficiently high concentration only in a few patient samples, but – if present – can lead to interferences with the consequence of false-positive and also false-negative results [4, 5]. These are molecules and antibodies that are not related to earlier coronavirus infections but are nevertheless cross-reactive. Since the more comprehensive research in the field of autoimmunity, it has become increasingly clear that these unknown and – based on our understanding – seemingly illogical antibodies and binding events appear time and time again in patient samples. The actual natural target of the cross-reacting antibodies is often unknown or is not suspected to be an immunogenic target in individual patients. Thus, it is a matter of unexpected, but routinely occurring cross-reactivities and interferences. A field that cannot be examined in detail here due to lack of space are the interferences that arise in immunodiagnostics in relation to rheumatic patients, people with autoimmunity disorders or patients suffering from cancer. In the context of these disorders, interference phenomena have repeatedly been described, the exact cause of which cannot be determined for each individual patient. Worse still, such interferences occur more frequently in particular patient groups, but have also been observed in clinically inconspicuous individuals, albeit much less frequently. This may not be a very serious problem in clinical diagnostics, since only „patients“ are measured. With „patients“ one usually - in addition to the laboratory diagnostics - has a reasonable anamnesis and therefore an „expectation“. Therefore, in case of unclear or unexpected results, other kits or methods can be employed. A good laboratory or attending physician can therefore sometimes compensate for incorrectly measured laboratory diagnostics through experience. In the case of Covid-19, however, screening, which means testing a large number of clinically inconspicuous individuals, is necessary. Even an unknown and unexpected interference occurring only in the per thousand range can have severe effects in this context and must be avoided, but might not be noticed during validation. For example, if a kit has a specificity of 99 %, this sounds very good at first, but is unacceptable in a pandemic with currently low prevalence. Under the optimistic assumption that 5% of the population is already immune against SARS-CoV-2, a positive result means that the person tested only has an 84% probability of actually displaying specific antibodies [6]. This would mean that 16 out of a hundred people labeled as „immunized“ are to be feared as potential super-spreaders after subsequent infection. In relevant areas involving high levels of personal contact, this is not acceptable under any circumstances.

The biochemical interference effects discussed here are usually based on low to medium affinity binding, similar to classic cross-reactivities, and such binding leads to a signal in the ELISA (or, depending on the assay format and type of binding, to the quenching of a signal). This binding must be distinguished from the high-affinity binding that is to be detected in the assay. In our case this is the high-affinity binding of patient antibodies specifically to SARS-CoV-2 antigens and the high-affinity binding of the detector antibody to the interacting patient antibodies. More than one and a half decade ago, the so-called LowCross® technology was introduced to the market and has since been implemented in many immunodiagnostic assays worldwide. The sample dilution buffer can simply be replaced with LowCross-Buffer®. The name LowCross® is derived from „lower cross-reactivities“ because this special diluent helps to avoid cross-reactivities in general, but also many other forms of interference. The use of LowCross-Buffer® allows for very reliable and robust immunoassays. When the antibodies encounter their target in LowCross-Buffer®, all low to medium affinity binding is very reliably and significantly reduced, completely independent of its molecular cause, while high affinity binding events - the actual „true“ signals of the assay - are not affected. True signals are therefore identical with or without the use of LowCross-Buffer® if they are generated by high-affinity antibodies (in the case of SARS-CoV-2 high-affinity antibodies that have been generated as a result of prior infection or vaccination). However, if there is only cross-reactivity by antibodies that were generated in response to other corona viruses, ineffective vaccination or completely different targets, i.e. the SARS-CoV-2 capture protein is only bound with medium to low affinity and thus no protective immune reaction would be triggered in response to a new infection, these patient antibodies will not bind in the assay diluent LowCross-Buffer® and will not generate a signal. LowCross-Buffer® thus helps to avoid the dreaded false positive signals. So if a positive signal is detected with LowCross-Buffer®, it is safe to assume that the patient sample contains antibodies that bind the capture protein - and thus SARS-CoV-2 - with high affinity.

If these specific patient antibodies are present in sufficient concentration, it is generally believed that newly introduced viruses are rendered harmless. The patient is immune. Even though it was not specifically developed for corona virus diagnostics, LowCross-Buffer® thus makes it possible to avoid interferences that can occur with almost all analytes in immunodiagnosics also in case of SARS-CoV-2 and significantly increases the required reliability of an immunoassay. LowCross-Buffer® is ready-to-use, so it is simply used undiluted instead of other diluents to achieve the desired effect. The general increase in reliability of immunoassays thanks to the LowCross® technology, which can be used for almost any ELISA and also for other formats such as lateral flow assays (used as chase or flow buffers), Luminex assays, protein arrays or automated high-throughput immunoassay systems by simply exchanging the assay diluent, has been described many times over the last decade and has become a well-established tool for high-quality immunodiagnosics. Then why is LowCross-Buffer® not used in every immunodiagnostic? The main reasons for the low use in commercial diagnostics, especially in the field of infectious disease serology testing, is the already mentioned cost pressure due to the low valuation of such diagnostics in the health care system. In addition, many diagnostics manufacturers follow a „one-buffer-fits-all“ concept, so that replacing the old diluent with a better one is often technically possible, but would require the simultaneous revalidation of entire assay panels run on automated systems or partially automated ELISA processors. Some manufacturers shy away from this effort. In the context of a Covid-19 pandemic, however, false positives should certainly not be accepted like the many false positives in the established serology. Laboratory physicians and virologists involved in regional and national control measures in the context of the Covid-19 pandemic should consider whether they are willing to make compromises with regard to the reliability of results or whether the respective immunodiagnosics manufacturers should have to certify the use of the LowCross® technology or a comparable alternative strategy - if it really exists - as a precautionary measure prior to decisions on the broad use of IgG assays for Covid-19. If other equivalent or better technologies are known or become known, these could of course also be used for the benefit of the population. It is important to understand that assay diluents, such as those based on PBS, BSA, Tween or similar reagents or, in the worst case, on milk powder or the addition of FCS (fetal calf serum), which are frequently described in the literature, offer no possibility to include the affinity as a criterion for the quality of the antibody interaction into an assay. They therefore do not provide additional protection against cross-reactivities. Even worse, with these formulations, undefined and uncharacterized substances can be introduced into the assay, which may lead to increased cross-reactivities [1].

The LowCross® technology and its use in diagnostics was already discussed in 2005 in an article in IVD Technology and has since then been used primarily in high-quality diagnostics for oncology, for modern biomarker-based diagnostics and, to some extent, in infectiology worldwide [7].

### 3. Surface blocking for serology - an underestimated challenge

A special challenge for all serological ELISA is the blocking of the microplate surface. Good blocking ensures that - irrespective of the presence of an analyte - no assay components can non-specifically bind to the surface. In the antigen down assays discussed here, the blocking reagent must also not cover the epitopes of the capture antigen too strongly and cross-reactivities of the detector with the blocking reagent (possibly mediated by components of the samples), as already observed in commercial antibody tests for SARS-CoV-2 [1, 8], must be prevented. It is generally known that proteins are very well suited for the blocking of ELISA plates. However, it is not only about the primary blocking reaction, meaning the formation of a layer on the surface with as few gaps as possible, but also about preventing the detachment of individual molecules from the blocking layer during the course of the assay. Especially in the case of serology, it is striking that serum and plasma samples of certain patients tend to detach individual molecules from a formerly dense blocking layer and allow the incorporation of other molecules on the blocked surface in an exchange reaction. These are usually patient antibodies, which can be incorporated into the blocking layer by such exchange reactions (the exact mechanism and especially the reasons why this phenomenon occurs more frequently in certain sera are largely obscure). Consequently, high background values are obtained unless the serum samples are diluted sufficiently high. However, high sample dilutions of 1:100 or more have the downside that specific antibodies present in low concentrations, although they may be of high clinical relevance, can become undetectable. The blocking exchange problem in general affects antigen-down assays in which detection is carried out with anti-antibodies, for example the serological assays in infectious disease testing. In addition, various blocking reagents may also cause serum-protein-mediated cross-linking between the blocking layer and the detector antibody. Such a phenomenon has been described in a recent ELISA for the detection of specific patient antibodies against SARS-CoV-2 [1]. The discussed effects are patient- and assay-specific. Therefore, the blocking of ELISA plates for serologic assays requires a high degree of consideration if false positives are to be safely avoided.

Independent of the SARS-CoV-2 IgG-ELISA we would like to add that the one-time testing of the lowest possible „background“ in the absence of an analyte (measurement of a „blank value“) is at best a first preliminary, but certainly not a sufficient step of professional assay development and validation with respect to the choice of the blocker.

Comparing coefficients of variation of different blocking reagents with statistically relevant repetitions show much more clearly the differences in blocking efficiency than a single background value ever could. A simple example of how poorly a BSA blocker performs in contrast to CANDOR's The Blocking Solution is shown in a quite simple comparison test, here using a competitive ELISA (fig. 2). The absolute value of the background (measured as „blank value“) in this assay was indistinguishable.

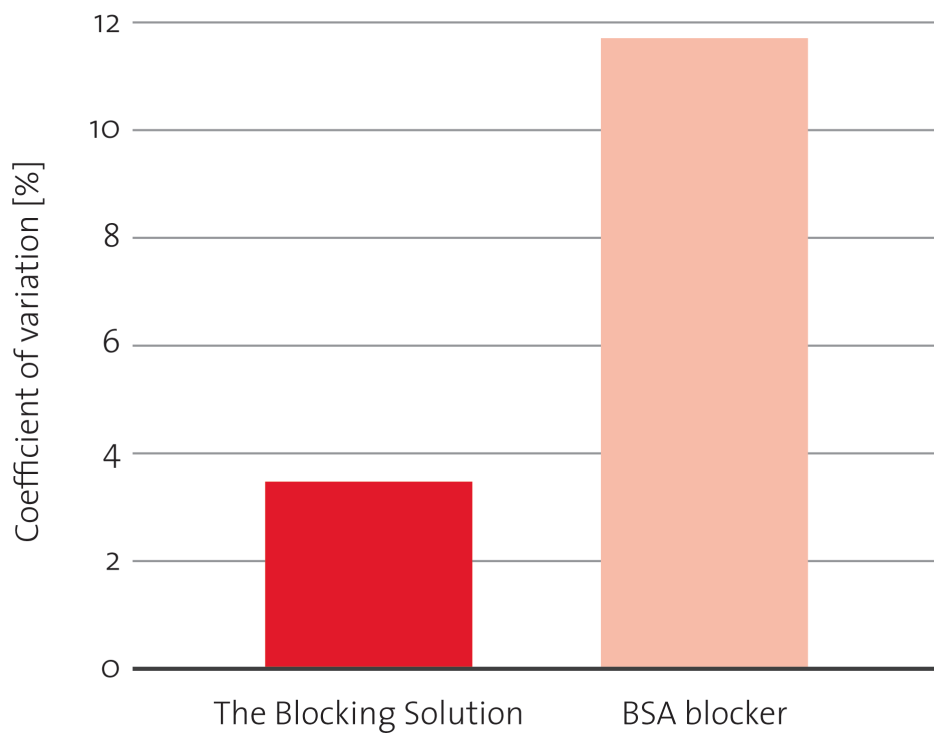


Figure 2: Reduction of the coefficient of variation (CV) with  $n = 96$ , measured at the maximum value  $B_0$ . The improvement by The Blocking Solution can be clearly quantified in an assay validation.

In the context of relevant medical research, the use of undefined foodstuffs such as milk powder as blocking reagent must be rejected entirely. The „purity“, which is arguably an erroneous term here, fluctuates to such an extent that other batch consistency parameters no longer warrant any discussion. Untested and poorly conditioned foodstuff should not find any use and approval in a responsible medical research community, especially since many commercial solutions with high batch consistency from specialized and certified manufacturers are available on the market.

Nevertheless, a lot of research in the university environment is still performed on the basis of milk powder blocking, as can be seen from many - otherwise high-quality - articles. This should in particular be avoided when the reliability of an assay is of critical importance. Nobody would consider using mice captured on a farm for clinical trials. For reasons of reproducibility and comparability, only consistently kept and well characterised laboratory animals are used. The same care should be taken as a scientist when selecting good solutions for designing an immunoassay. Inadequately purified foodstuffs, such as milk powder, should generally be banned from any laboratory. Especially as milk powder is anyway only an acceptable, but by no means a good blocker, as proven by the comparison shown below (s. fig. 4).

Especially due to serology's problem with exchange reactions and the resulting tendencies towards background and outliers in certain rare patient samples, more and more optimized blockers have been developed over the years. In the case of CANDOR, first the universally applicable The Blocking Solution and later SmartBlock™ which - based on our experience - is recommended for particularly small capture antigens. Within the scope of development projects of infection serological assays with established diagnostics manufacturers we tested applicability and reliability. In exemplary product groups it could be shown that the combination of LowCross-Buffer® as assay diluent and SmartBlock™ as surface blocker on standard ELISA plates did find no patient samples that showed false-positive results in the course of the performed validation - in contrast to the formerly used diluents and surface blockers.



Nevertheless, the blocking issue requires an assay-specific solution. The PlateBlock™ is a newly developed, protein-free and animal-free blocker that has been optimized to prevent the above-mentioned exchange reactions in a particularly comprehensive way. Past tests indicate an exceptional applicability in infectious serology (see also fig. 4). Together with partners, CANDOR is currently working on a comprehensive comparison of different blockers for use in Covid-19 IgG diagnostics, showing very good results with PlateBlock™ in preliminary testing phase. We are confident that we will soon be able to make a concrete recommendation for SARS-CoV-2 Anti-IgG ELISAs, in addition to the various options presented here, to ensure that as many diagnostic tests as possible can be optimized for best reliability without much effort. CANDOR's products are available in small volumes for researchers as well as to produce commercial high-volume diagnostics.

#### 4. Stability of reagents

For a commercial test that will be used worldwide in a high volume during a pandemic the stability of the reagents and the associated shelf life of the commercial kits is crucial. In case of an IgG-ELISA for Covid-19, the coated capture molecules and the labeled detector are critical. For the labeled detector a stabilizer based on LowCross-Buffer® (LowCross® HRP-Stab) is available in addition to the well-established HRP-Protector™. If patient-mediated cross-reactivities between the detector and the blocking reagent were to occur, the use of LowCross® HRP-Stab is recommendable. This solution can also be used if the detector conjugate is also to be used as sample diluent, thus reducing the complexity of the protocol. This allows establishing very fast one-step incubation ELISAs, which – thanks to the LowCross® effect – can be as reliable as sequential ELISAs with several washing steps. Currently, however, we assume that a sequential format with washing steps could be advantageous due to the enormous importance of avoiding all incorrect results for SARS-CoV-2. In this case, HRP-Protector™ may be a sufficient solution to guarantee the safety of the detector conjugate for several years or even for international shipping with possibly unreliable cold chains and still enable a rapid detection reaction, since the assay performance of HRP-Protector™ is excellent compared to many other commercial HRP stabilizers. However, the enormous importance of avoiding false positives in the current pandemic in particular and in case of occurring zoonoses with high infectivity in general clearly speaks for the use of the LowCross® technology also in this step of the sequential ELISA protocol. If one compares the interference elimination in an ELISA with a complete airbag system of a car, the use of LowCross® HRP-Stab as diluent for the detector antibody is certainly only a side airbag, but in the more rare case of a side impact it can provide the decisive additional safety. The most important airbag remains the undiluted LowCross-Buffer® as a sample diluent.

For the stabilization of the coated capture antigen it is generally necessary to obtain both a good stability by a closed and evenly formed layer on the capture molecules as well as a good release of the components from the epitopes during the course of the assay. Coating stabilizers are available from various suppliers. The Liquid Plate Sealer® product group was introduced by CANDOR Bioscience about a decade ago and has since been successfully used in millions of samples. In addition to the outstanding stabilization compared to alternative solutions, a special feature of the Liquid Plate Sealer® is the possibility to use it for blocking and stabilization in a very convenient rapid process for classical sandwich ELISAs with capture antibodies. Coat the capture antibody in approx. 50 µl Coating Buffer, allow the capture antibody to bind to the plate in a subsequent incubation step and then add 100 - 150 µl Liquid Plate Sealer® directly without additional washing steps. After a short incubation, the solution can be aspirated and the plate dried and shrink-wrapped. This process is also described accordingly in the product information.

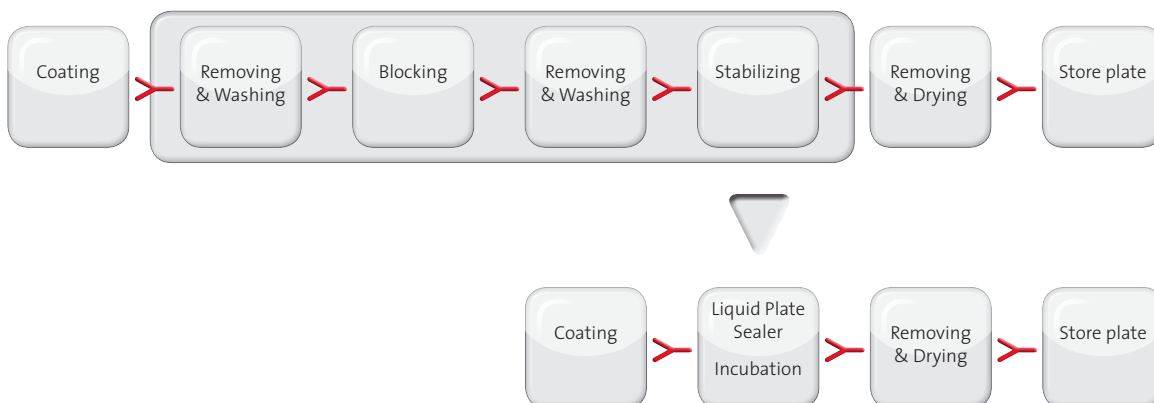


Figure 3: High-speed ELISA plate production with Liquid Plate Sealer® as blocking reagent and coating stabilizer in a single process step is suitable for sandwich ELISA and competitive tests in which no anti-antibody detector is used.

Further developments, namely the Liquid Plate Sealer® animal free specially formulated for veterinary diagnostics and the Liquid Plate Sealer® Plus, suitable for challenging structural epitopes, also allow for this rapid production method without separate blocking.

As described above, blocking in the case of serology, must meet specific and different requirements from those of most other immunodiagnosics, for which the faster production method described here is appropriate and sufficient. We explicitly consider the rapid one-step procedure without separate blocking, independent of the used coating stabilizer, for Covid-19 IgG diagnostics and tests based on anti-antibodies as detector molecules in general, not recommendable! In a simple test setup for blocking reagents, resistance to exchange reactions is tested by blocking the plate, incubating with human pool serum diluted in assay diluent and detecting it with a secondary antibody after washing the plate. If the blocking reagent is able to prevent serum IgGs from attaching to the plate or lodging into the blocking layer, no signal is visible. The results show clear differences in the suitability of the different blockers for antigen down assays (fig. 4). Completely unsuitable for this assay format in serology are both milk powder and coating stabilizers (exemplified here with Liquid Plate Sealer®, which was used in this test in the one-step procedure without additional blocking). This also applies to one-step coating stabilizers from other providers (data not shown). In this test, PlateBlock™ was clearly the best candidate.

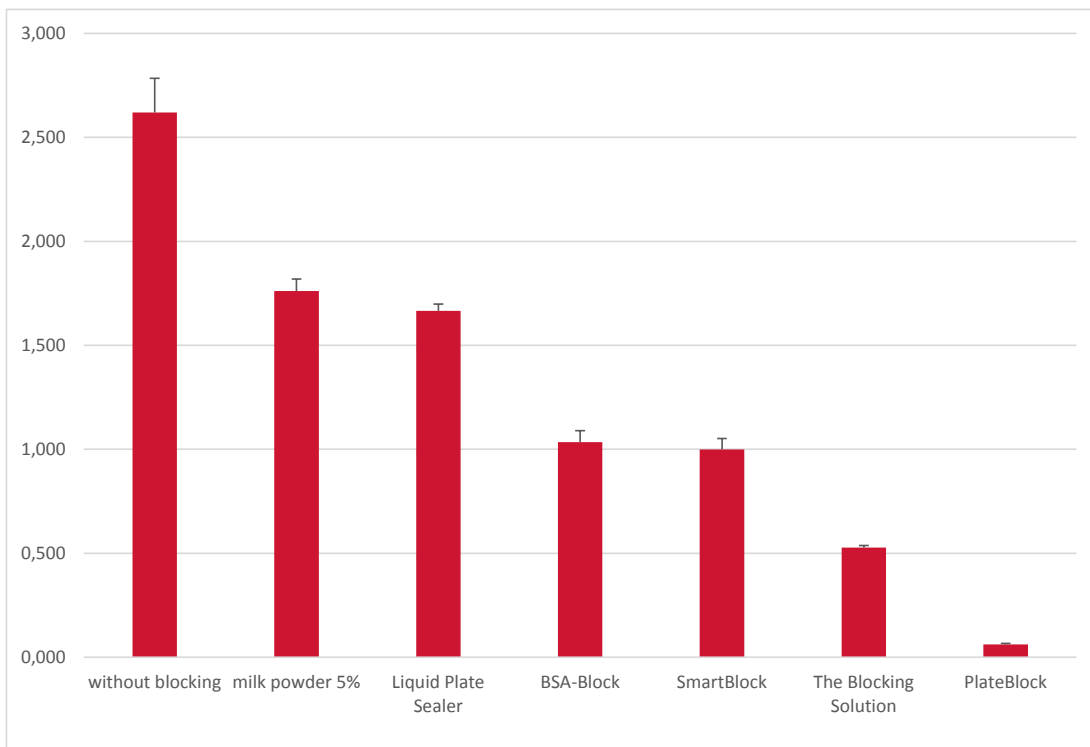


Figure 4: Comparison of blocking reagents for serology: a Nunc MaxiSorp plate was saturated with different surface blockers and then incubated with a human pool serum diluted 1:10 in CANDOR's assay diluent Sample Buffer on the plate (n = 4 each; error bars correspond to one standard deviation). After washing, the accumulation of antibodies from the serum is detected with a peroxidase-labeled anti-human-antibody.

If stabilization is omitted, it is safe to assume that - as is the case with many coated proteins - the native fold of the molecules will gradually degrade during storage or a possibly not optimally cooled transport or handling in the laboratory. With the use of an optimal coating stabilizer this can be prevented so effectively that, due to the resulting exceptional stability of the ELISA plates, one could even abandon cold chains during transport. The stabilizers of the Liquid Plate Sealer® product group are ideally suited in combination with prior blocking by PlateBlock™ or other blockers, meaning they offer excellent protection for serological assays.

## Ideal ELISA setup in short

1. coating of the carefully selected and produced capture antigen
2. aspirate or tap the plate (do not wash)
3. blocking with PlateBlock™ followed by stabilization with Liquid Plate Sealer® (depending on the selected capture epitope probably rather with Liquid Plate Sealer® Plus)
4. aspirate or tap the plate (do not wash), dry and store
5. dilute the patient sample (1:10 to 1:50) in LowCross-Buffer® and incubate on the plate
6. after washing, incubate with detector conjugate stored in LowCross® HRP-Stab (or HRP-Protector™)
7. detect with substrate after further washing.

Notes:

Wash steps during the assay protocol should not be omitted in favour of faster ELISA execution.

The serum sample should not be too highly diluted

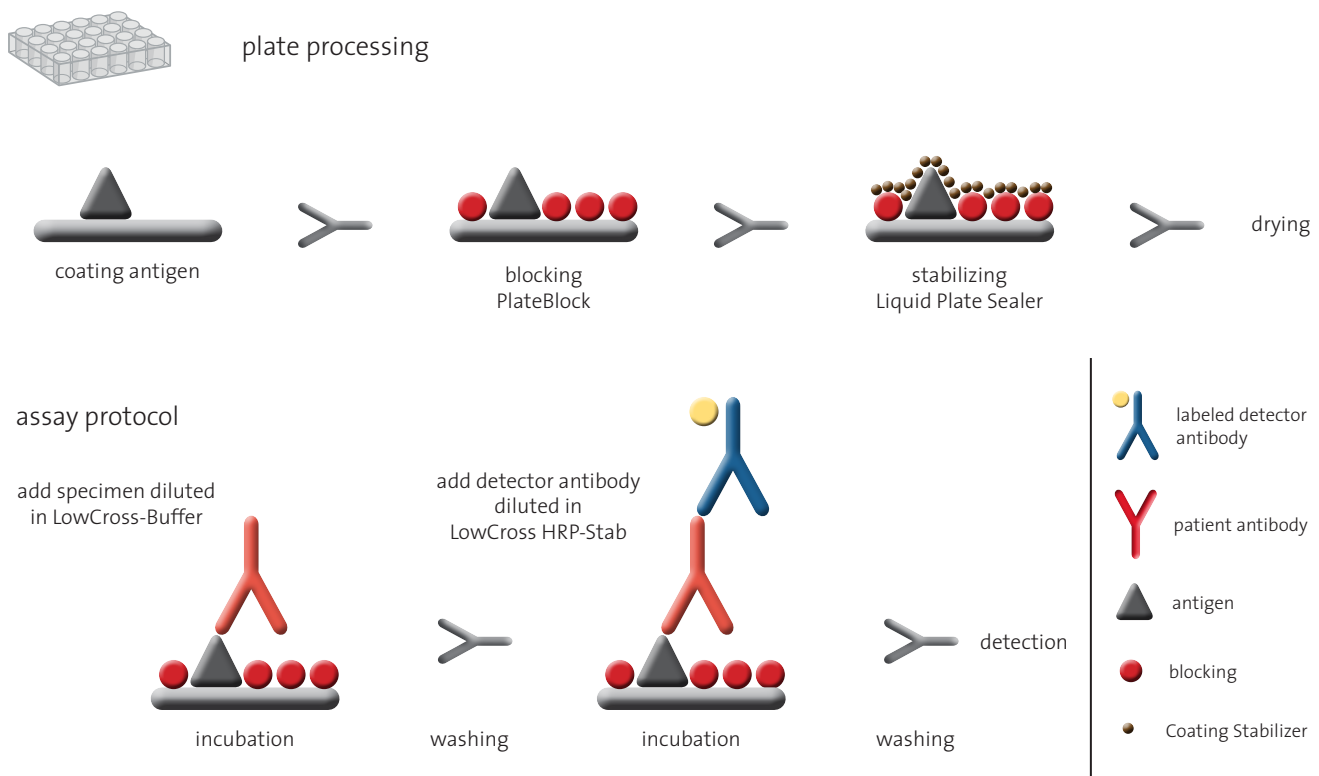


Figure 5: optimum plate production and assay protocol for serological ELISA

## Conclusion

The development of reliable immunodiagnosics for Covid-19 is a complex challenge that can benefit not only from the expertise of virologists, but also from experienced practitioners in the field of immunoassay optimization. Reliability can only be achieved by combining the best tools against all potential sources of misdiagnosis.

We expect that really reliable antibody tests, equipped with the most modern tools of immunodiagnosics, will soon be available for Covid-19 as well.



As several suboptimal assays have already been published and are in use and first commercial diagnostics do not appear to be significantly better optimized, we decided to publish this article to broaden the technical knowledge for optimizing immunoassays also in the virological community. CANDOR Bioscience supports all interested parties in the optimization of immunoassays, also with personal advice. For more than 15 years CANDOR Bioscience has successfully pursued the goal of progressively making immunodiagnosics and immunoassays for research use more reliable. Here we showed some simple steps - which can also be used by diagnostics manufacturers to significantly improve their first commercially available tests, which are already in the market. This way we hope to make our modest contribution to overcome the crisis caused by Covid-19.

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