translated article
original published in |transkript 2.2020

SARS-COV-2 ANTIBODY TESTS

In the CoViD-19 pandemic, reliable antibody detection is urgently needed worldwide. Since many of the available assays are not performing adequately[1], the technical requirements for the development of truly dependable antibody detection assays are explained here.

Sebastian M. Richter, Manuel Hecht & Peter Rauch; CANDOR Bioscience

Immunity against SARS-CoV-2 is characterized by highly specific and high-affinity IgG antibodies[2]. Assays for the detection of these antibodies are intended to indicate immunization and to be widely used. For mass testing, however, an extremely high specificity, i.e. a low rate of false positive results, is essential. Even with an apparently good specificity of 99 % and an optimistically estimated antibody prevalence of 5 %, a positive result means that the person tested has only an 84 % probability of actually having specific antibodies against SARS-CoV-2[3]. Such poor certainty is simply unacceptable for tested individuals working in critical areas. Using ELISAs as an example, we here describe the reasons for the low reliability of many available infection diagnostics assays and the technical requirements for the development and mass production of truly reliable test procedures using established and modern solutions. After all, cost pressure should not be the justification for compromises at the expense of safety.

Four different causes of false positives in serology are known: 1) unfavorably selected capture molecules, 2) cross-reactivities and biochemical interference, 3) insufficient surface blocking and 4) stability of the reagents. Regarding 1) it should only be mentioned here that the RBD of SARS-CoV-2 is currently emerging as the most promising antigen[4].

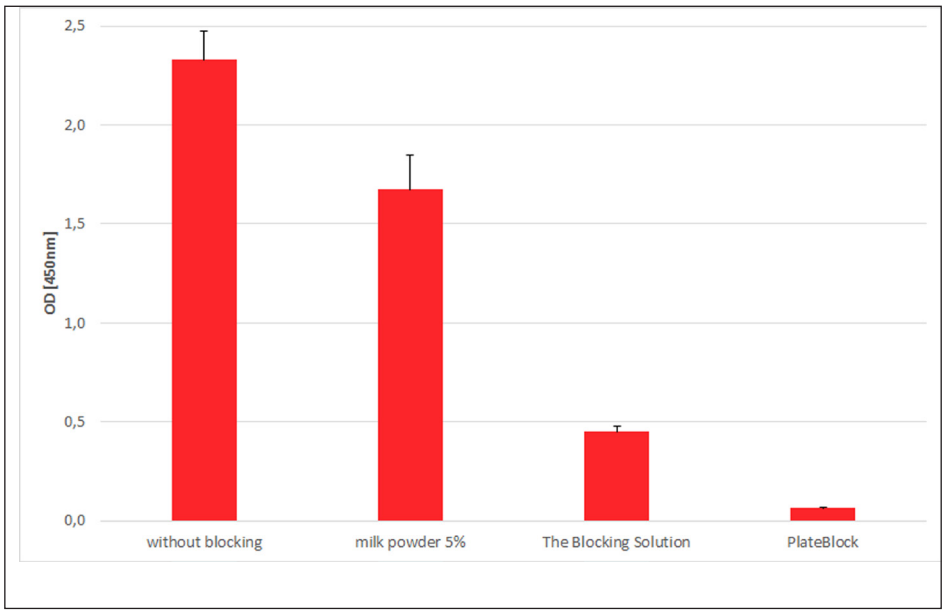

Fig. 1: Comparison of blocking against 1:10 diluted serum samples. Detection of nonspecifically bound antibodies with anti-human antibody. PlateBlock™ very effectively prevents the unwanted background.

CROSS-REACTIVITIES

Even with optimal capture molecules, cross-reactivities and interferences are always to be expected. As an example, antibodies against other corona viruses may also bind to related epitopes of SARS-CoV-2 and lead to false positives. These cross-reactivities are based on low to medium affinity antibody interactions and are not sufficient for immunity against CoViD-19. Many potentially fatal false positives can be prevented by using the LowCross technology[5]. LowCross-Buffer® replaces the sample dilution buffer and very reliably and independently of their molecular causes reduces low to medium affinity binding significantly, while high affinity binding events - the „true“ signals of the assay - are not affected. The LowCross technology reduces cross-reactivities as well as many other forms of interference and has helped to optimize many diagnostic assays worldwide since its introduction. LowCross-Buffer® is ready-to-use and replaces assay diluents not only in ELISAs but also in lateral flow assays (used as chase or flow buffer), Luminex

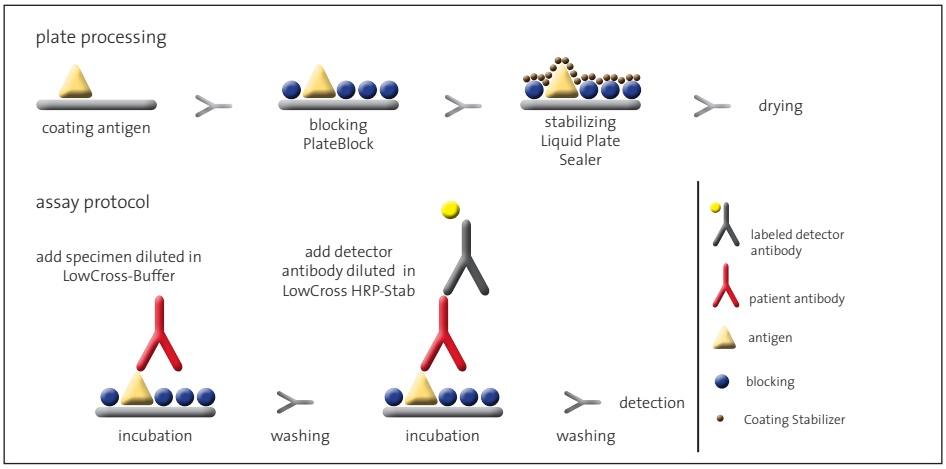

Fig. 2: Ideal ELISA setup for serology.

assays, protein arrays, automated high-throughput immunoassay systems and can be used in many other formats. In a pandemic, false positives should not be accepted as easily as in routine serology and LowCross-Buffer® can make an important contribution to the development of reliable diagnostic tools for SARS-CoV-2 antibodies.

BLOCKERS FOR SEROLOGY

Surface blocking is a particular challenge for all serological assays. It is intended to ensure that false positives do not occur - independent of the presence of an analyte - due to nonspecific binding of molecules to the surface. A serology specific problem is that some serum and plasma samples can detach individual molecules from a previously dense blocking layer and allow the attachment of unspecific antibodies to the surface in an exchange reaction. The results are high background values if the samples are not sufficiently diluted. However, high sample dilutions of 1:100 or more have the disadvantage that clinically relevant, specific antibodies present in low concentrations may no longer be detectable. The use of undefined blockers such as milk powder or FCS is always at the expense of assay performance (Fig. 1). Even solutions for the plate production of sandwich ELISAs, with which blocking and coating stabilization can be performed in one step, such as Liquid Plate Sealer®, are not sufficient in serological assays[6] if they are not supplemented with a serological blocker. For these critical assays CANDOR has developed the PlateBlock™. The protein-free and animal-free PlateBlock™ was optimized to prevent these exchange reactions in a most comprehensive way. Experiments demonstrate a very good applicability in serology (Fig. 1). In combination with LowCross-Buffer® as sample diluent, PlateBlock™ shows very good performance in ELISA tests for neutralizing SARS-CoV-2 antibodies.

REAGENT STABILITY

For a commercial test that can be used worldwide with high volumes during a pandemic, the stability of the reagents and the associated kit shelf life is crucial. For the labeled detector, a stabilizer based on LowCross® (LowCross® HRP-Stab) is available in addition to the well-established HRP-Protector™. Both solutions allow shelf lives of several years, reduce the dependence on cold chains and show better assay performance compared to other commercial HRP stabilizers. Equally essential are coating stabilizers for the capture molecule to prevent the loss of native protein folding during storage and transport and thus to avoid misidentifications. CANDOR's Liquid Plate Sealer® product group offers outstanding stabilization compared to alternative solutions and has already been used on millions of samples. In combination with PlateBlock™, Liquid Plate Sealer® enables excellently blocked and stabilized serological assays.

IDEAL SEROLOGICAL ELISA

1) Coating of the capture molecule.
2) Aspiration of the plate (do not wash).
3) Blocking with PlateBlock™.
4) Stabilization with Liquid Plate Sealer®.
5) Aspiration of the plate (do not wash), dry and store.
6) Dilution of the patient sample (1:10 to 1:50) in LowCross-Buffer® and incubation.
7) After washing, incubation with detector conjugate stored in LowCross® HRP-Stab (or HRP-Protector™).
8) Detection after washing.

The development of reliable diagnostics for CoViD-19 is a complex challenge. CANDOR Bioscience is happy to provide advice to all interested parties because the current crisis can only be mastered together.

REFERENCES:

1. Lassaunière, R et al. (2020). Evaluation of nine commercial SARS-CoV-2 immunoassays. *Preprint at medRxiv*
2. Long, Q et al. (2020). Antibody responses to SARS-CoV-2 in patients with COVID-19. *Nat Med*
3. Richter SM (2020). Specificity, positive predictive value and validation statistics in the context of CoViD-19; free for download on *www.candor-bioscience.de; 4-2020*
4. Chee, WT et al. (2020). A SARS-CoV-2 surrogate virus neutralization test based on antibody-mediated blockage of ACE2-spike (RBD) protein-protein interaction. *Preprint at Research Square*
5. Polifke T & Rauch P (2009). Affinity discrimination to avoid interference in assays. *IVD Technology.*
6. Polifke T & Rauch P (2020). The Covid-19 antibody test challenge. *www.bionity.com*