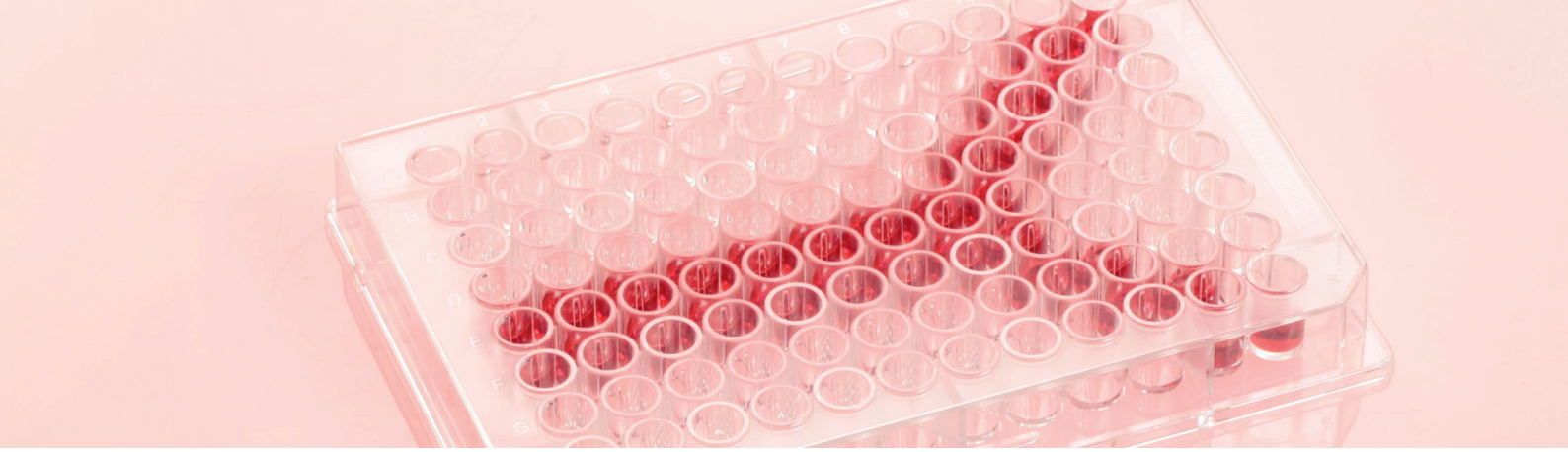

# Lessons learned from CoViD-19
# A guide for dependable immunoassays

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## 1. Surface Blocking - the underestimated challenge

Surface Blocking is especially difficult for serologic assays for antibody detection. These antigen-down assays require very efficient surface blockers, like PlateBlock™ (Fig. 1a). For the detection of the virus itself, antigen tests with low detection limits are essential. A low coefficient of variation is a prerequisite for achieving this goal (Fig. 1b).
Read our article *Surface Blockers in serology: Some background about background* for more details.

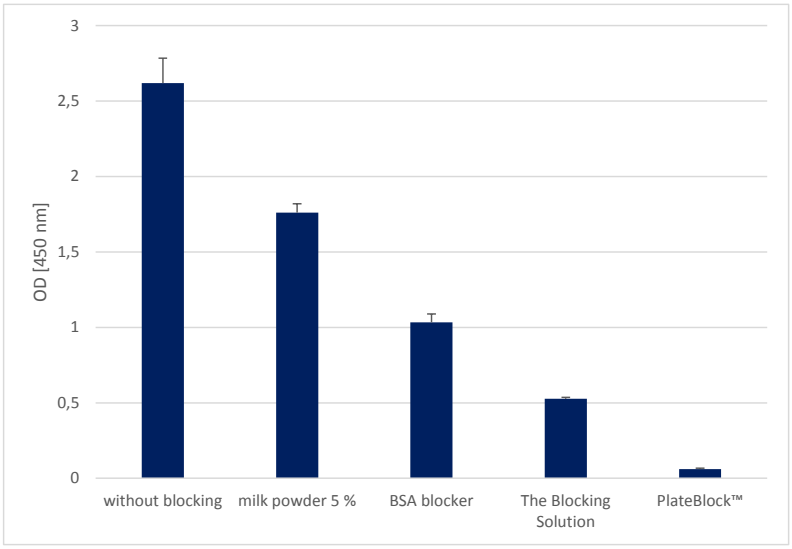


Fig. 1a: PlateBlock™ is a very efficient surface blocker for serologic assays. A Nunc MaxiSorp plate was saturated with different surface blockers and then incubated with a human pool serum diluted 1:10 in CANDOR's assay diluent Sample Buffer on the plate (n = 4 each; error bars correspond to one standard deviation). After washing, antibodies from the serum were detected with a peroxidase-labeled anti-human-antibody.

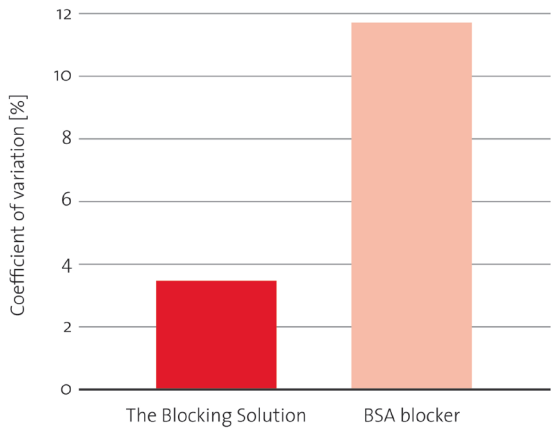


Fig. 1b: State-of-the-art surface blocking allows for low coefficients of variation. Reduction of the coefficient of variation (CV) with n = 96, measured at the maximum value Bo. The improvement by The Blocking Solution can be clearly quantified in a thorough assay validation.

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## 2. Stabilization: Making sure that a good assay stays a good assay

Plate stabilization is essential for retaining functionality after worldwide distribution and continued storage of immunoassays (Fig. 2a). RBD and Nucleocapsid, used in antigen down assays to detect antibodies, are very unstable capture proteins and require state-of-the-art stabilization (Fig. 2b). This can already be achieved very fast with just 2 min of incubation with Liquid Plate Sealer (Fig. 2c) during production of the plates. Liquid Plate Sealer works well with surface blockers, like PlateBlock™ (Fig. 2d). Read our article *Stabilization of ELISA plates – technology comparison* for more details.

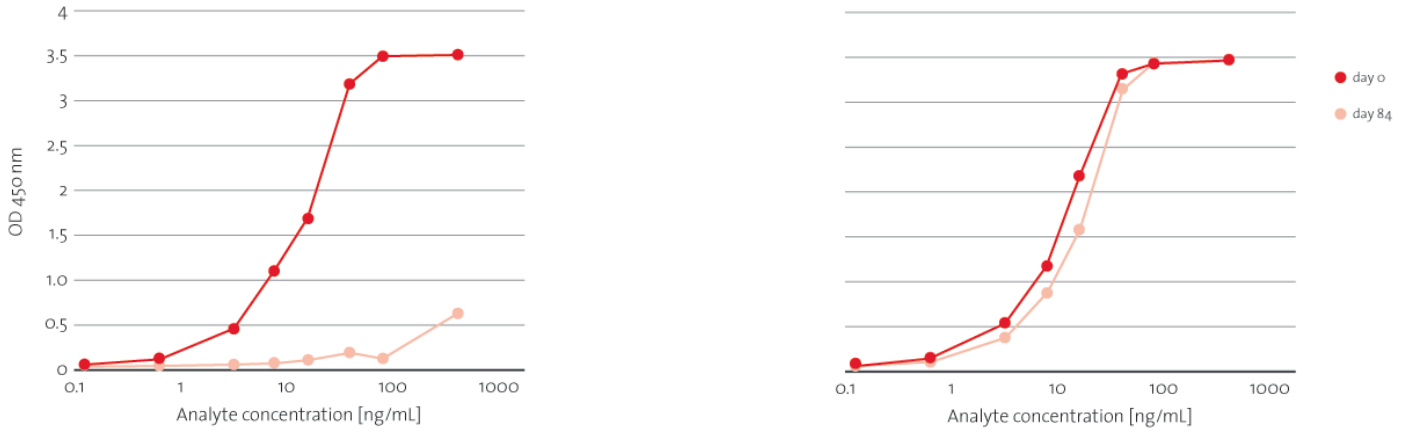


Fig. 2a: State-of-the-art surface blocking allows for low coefficients of variation. Calibration curves of an ELISA that was either blocked only with BSA (left panel) or blocked with BSA and additionally stabilized with Liquid Plate Sealer® (right panel). Calibration curves at the starting day are identical. After 84 days at 37 °C, no remaining binding activity of the capture antibodies is detectable in the unstabilized plate. In contrast, the stabilized plate does not show a significant decrease in sensitivity even after 84 days of incubation at 37°C.

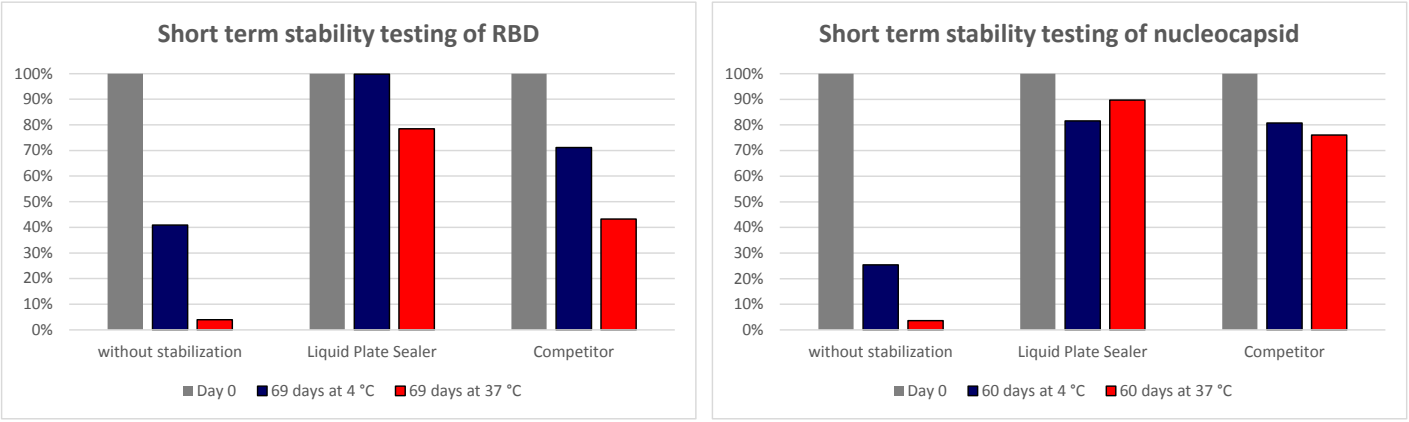


Fig. 2b: SARS-CoV-2 antigens require excellent stabilization by Liquid Plate Sealer®. Stabilization of the Receptor-binding domain (RBD, Trenzyme) (left panel) or the nucleocapsid (Acro Biosystems) (right panel) of SARS-CoV-2: a Nunc MaxiSorp plate was coated with 100 ng antigen and saturated with PlateBlock™. A subset of wells was stabilized with Liquid Plate Sealer® or the stabilizer of a leading competitor brand for 60 min. Plates were stored for 69 days (RBD) or 60 days (nucleocapsid) at 4 °C or 37 °C and then incubated with human serum samples. After washing, captured antibodies from the serum are detected with a peroxidase-labeled anti-human-IgG-antibody. Values are normalized to the maximum value at day zero.

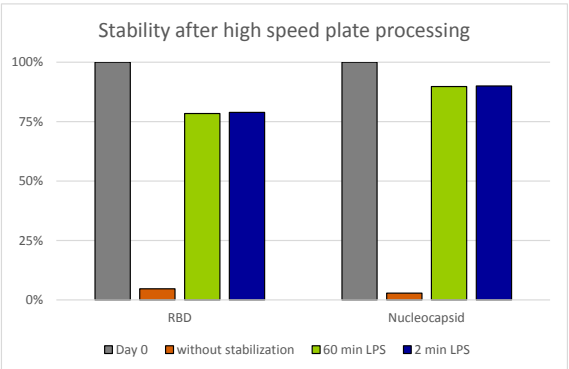


Fig. 2c: Two minutes of incubation with Liquid Plate Sealer® are sufficient for stabilizing the RBD or the nucleocapsid of SARS-CoV-2. Stabilization of the Receptor-binding domain (RBD, Trenzyme) (left panel) or the nucleocapsid (Acro Biosystems) (right panel) of SARS-CoV-2: a Nunc MaxiSorp plate was coated with 100 ng antigen and saturated with PlateBlock™. Wells were stabilized with Liquid Plate Sealer® for 60 min or for only 2 min. Plates were stored for 69 days (RBD) or 60 days (nucleocapsid) at 37 °C and then incubated with human serum samples. After washing, captured antibodies from the serum are detected with a peroxidase-labeled anti-human-IgG-antibody. Values are normalized to the maximum value at day zero.

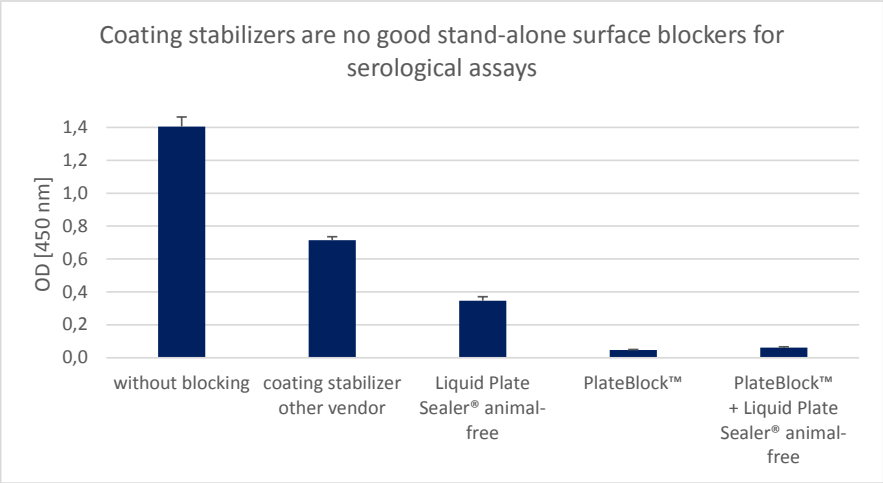


Fig. 2d: Liquid Plate Sealer® performs well in combination with PlateBlock™. A Nunc MaxiSorp plate was saturated with different surface blockers and then incubated with a human pool serum diluted 1:100 in CANDOR's assay diluent Sample Buffer on the plate (n = 4 each; error bars correspond to one standard deviation). After washing, antibodies from the serum are detected with a peroxidase-labeled anti-human-antibody.

In case of using PlateBlock™ for antibody tests, washing steps should be saved for easier plate processing (Fig. 2e).


Standard plate processing


Plate processing with PlateBlock™

Fig. 2e

In case of antigen tests, plate processing can be greatly accelerated if Liquid Plate Sealer® is used as one-step blocker and stabilizer (Fig. 2f). Read our article *The Covid-19 antibody test challenge* for more details.

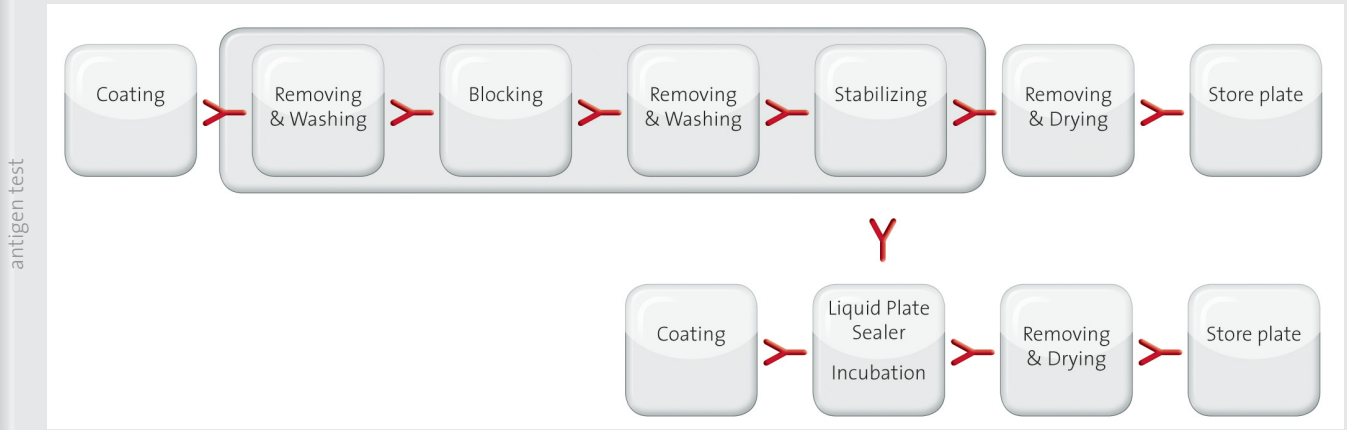


Fig. 2f: Plate processing for antigen tests can be accelerated using Liquid Plate Sealer®. High-speed ELISA plate production with Liquid Plate Sealer® as blocking reagent and coating stabilizer in a single process step is suitable for sandwich ELISA and competitive tests in which no anti-antibody detector is used.

## 3. Sample dilution: Affinity discrimination prevents false results

Cross-reactivities with antigens or antibodies against related corona viruses are problematic for assay reliability. Moreover, interfering substances in the serum or saliva can lead to faulty results (Fig. 3a and 3c). These phenomena are caused by low-affinity binding reactions and lead to increased background. LowCross-Buffer® selectively suppresses low-affinity binding by affinity discrimination and eliminates background and faulty results (Fig. 3b and 3d).
Read our article *SARS-CoV-2 Immunoassays: Interference elimination by affinity discrimination* for more details.

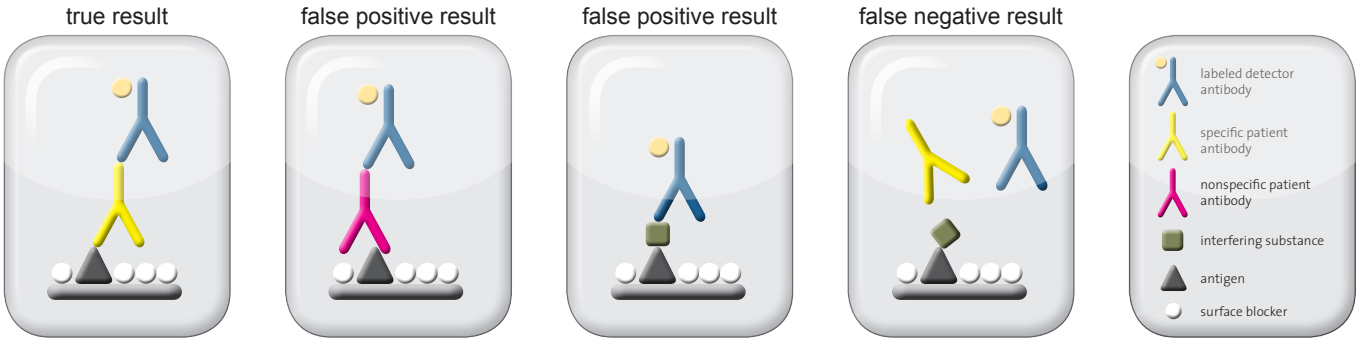


Fig. 3a: Interfering antibodies or other components and proteins from the patient sample can result in false positives or false negatives in SARS-CoV-2 antibody assays

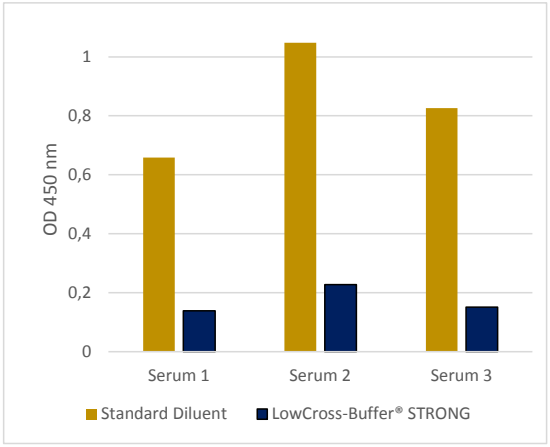


Fig. 3b: Interferences in antigen-down assays can be eliminated by LowCross-Buffer® STRONG. An antigen-coated microwell plate was incubated with human cancer patient sera diluted in a standard diluent (yellow columns) or CANDOR's LowCross-Buffer® STRONG (blue columns). An HRP-coupled donkey-anti-human antibody was used as detector. Despite none of the sera 1 -3 containing antibodies against the coated antigen, they showed faultily high readings in standard diluent. CANDOR's LowCross-Buffer® STRONG eliminated these false positive readings and greatly improved assay specificity and reliability.

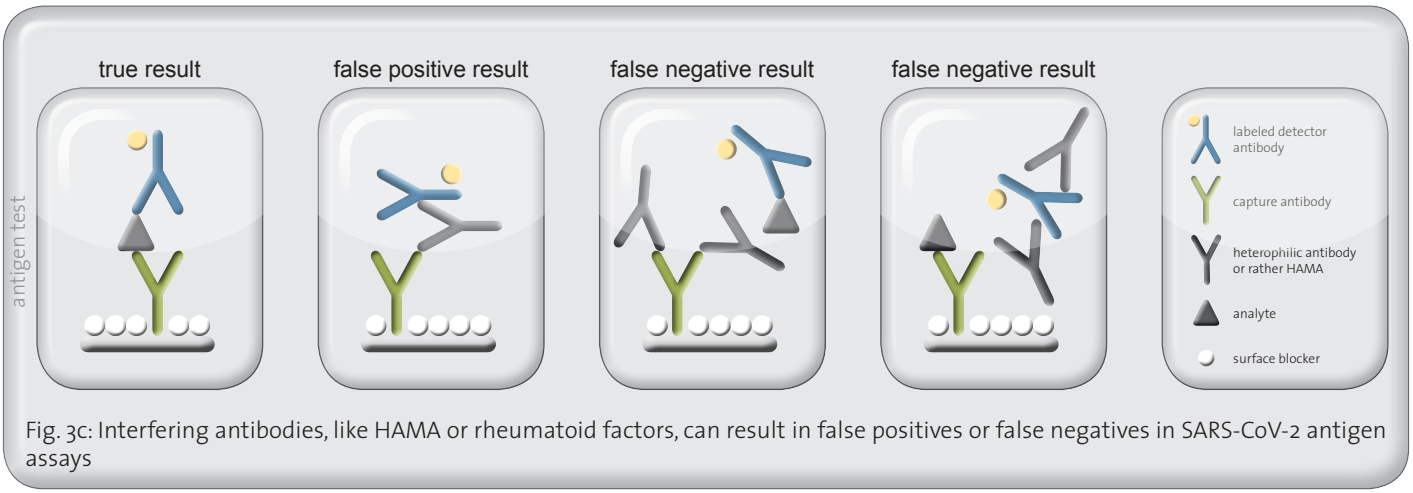


Fig. 3c: Interfering antibodies, like HAMA or rheumatoid factors, can result in false positives or false negatives in SARS-CoV-2 antigen assays

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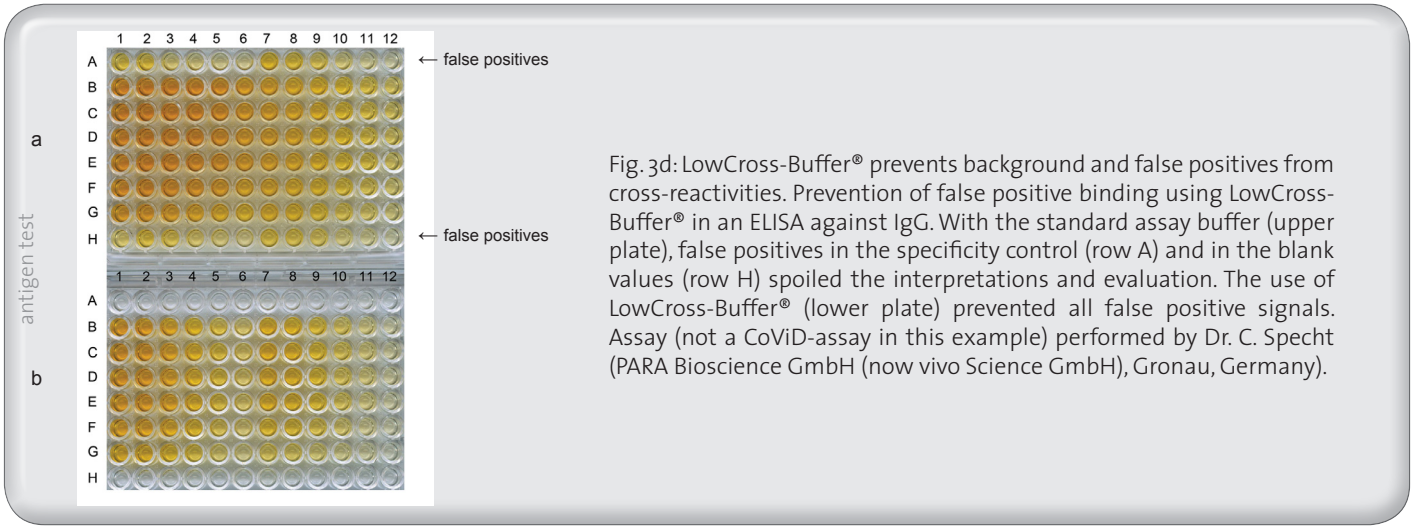


Fig. 3d: LowCross-Buffer® prevents background and false positives from cross-reactivities. Prevention of false positive binding using LowCross-Buffer® in an ELISA against IgG. With the standard assay buffer (upper plate), false positives in the specificity control (row A) and in the blank values (row H) spoiled the interpretations and evaluation. The use of LowCross-Buffer® (lower plate) prevented all false positive signals. Assay (not a CoViD-assay in this example) performed by Dr. C. Specht (PARA Bioscience GmbH (now vivo Science GmbH), Gronau, Germany).

## 4. Conjugate Stabilization - Essential for stable and reliable immunoassays

Antigen- and antibody-testing relies on the continued functionality of detector antibodies and conjugated enzymes when distributed worldwide - even under stress conditions. Under these circumstances, functionality is protected by conjugate stabilizers, such as HRP-Protector™ (Fig. 4).

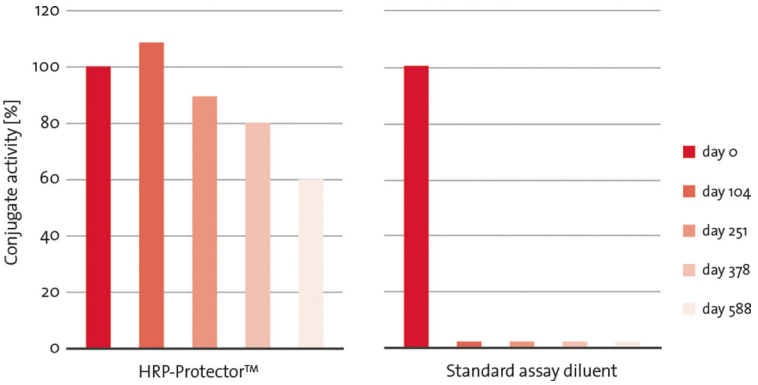


Fig. 4: HRP-conjugated antibodies are stabilized by HRP-Protector™. Shelf life of an HRP-labeled antibody was measured as signal (= antibody binding + enzymatic activity) in an ELSA after storage under heat stress at 45 °C. The concentration of the stored antibody is 400 ng/mL

## 5. Conclusion - How to do better?

In combination, all the reagents described above can greatly improve the performance of SARS-CoV-2 antibody assays in comparison to assays performed with a standard assay buffer (Fig. 5a). The following scheme gives an overview on how such a superior antibody assay can be assembled (Fig. 5b).
CANDOR products are "Made in Germany" in an EN ISO 9001 and EN ISO 13485 certified production facility and meet highest quality standards.

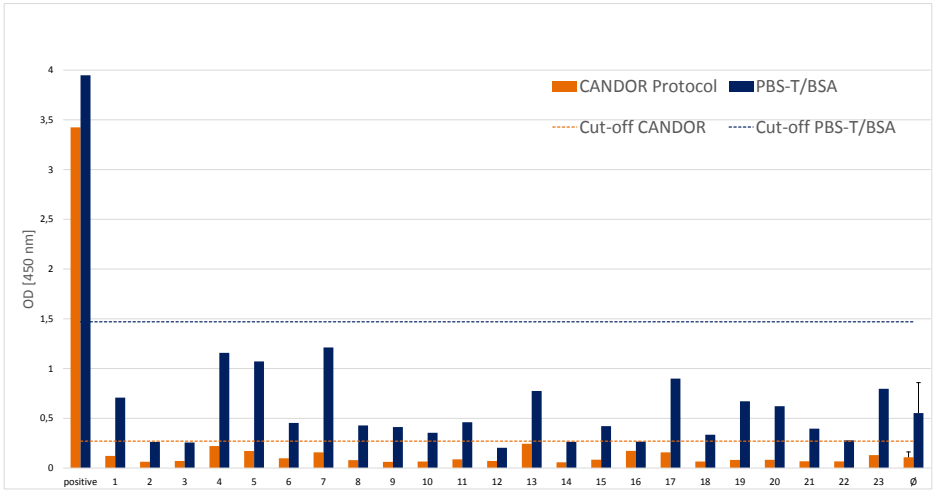


Fig. 5a: Superior immunoassays using CANDOR's state-of-the-art buffer solutions. Comparison of negative samples (# 1 –23; Ø is arithmetic mean of 1-23) CANDOR protocol vs. PBS-T/BSA. Samples were analyzed either using the CANDOR protocol (s. Fig. 5b) or a standard PBS-T/BSA protocol. The latter was performed with a BSA-based surface blocker and a standard PBS buffer supplemented with Tween and BSA (PBS-T/BSA) for sample and conjugate dilution. The CANDOR solutions allow for less average background, less sample-to-sample variation, a better signal-to-noise-ratio, and a better detection limit due to a decreased cut-off.

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Assay protocol:

1. Coating of 0.5 µg/ml RBD of SARS-CoV-2 (trenzyme, Germany) in Coating Buffer pH 7.4
2. Aspirate or tap the plate (do not wash)
3. Blocking with 200 µl PlateBlock™ for 2 h
4. Aspirate or tap the plate (do not wash)
5. Stabilization with Liquid Plate Sealer® animal-free for 2 - 15 min
6. Aspirate or tap the plate (do not wash), dry and store
7. Dilute the patient sample (1:100) in LowCross-Buffer® STRONG and incubate (2 h) 100 µl per well on the plate
8. Wash three times with 300 µl 1-fold Washing Buffer TRIS per well
9. Incubate (1 h) with detector conjugate stored in HRP-Protector™
10. Detect with substrate after further washing.

Notes:

- Avoid using detergents in all steps prior to application of the diluted sample when blocking with PlateBlock™
- If the plates are used directly after blocking, stabilization is not necessary and steps 5 and 6 can be omitted

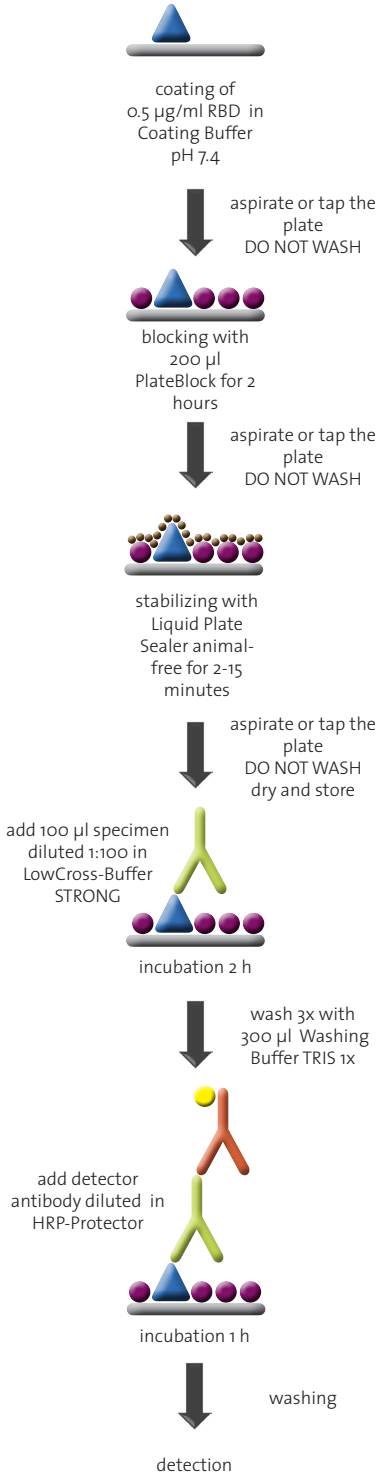


Fig. 5b: The quick guide to reliable antibody assays

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