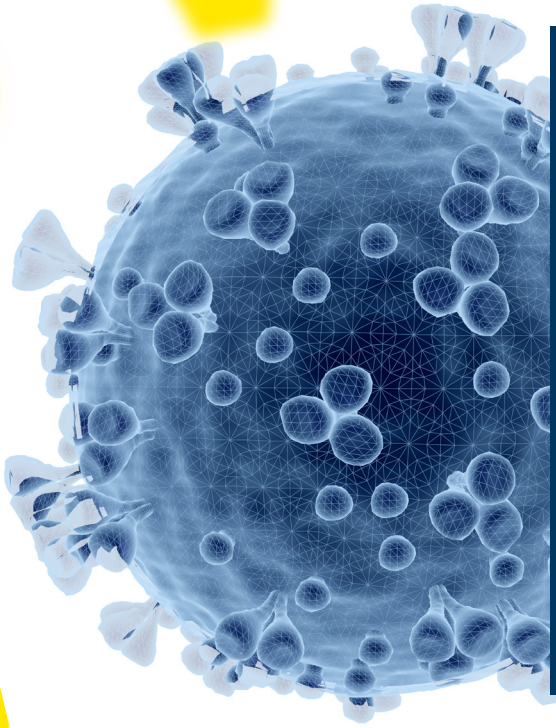
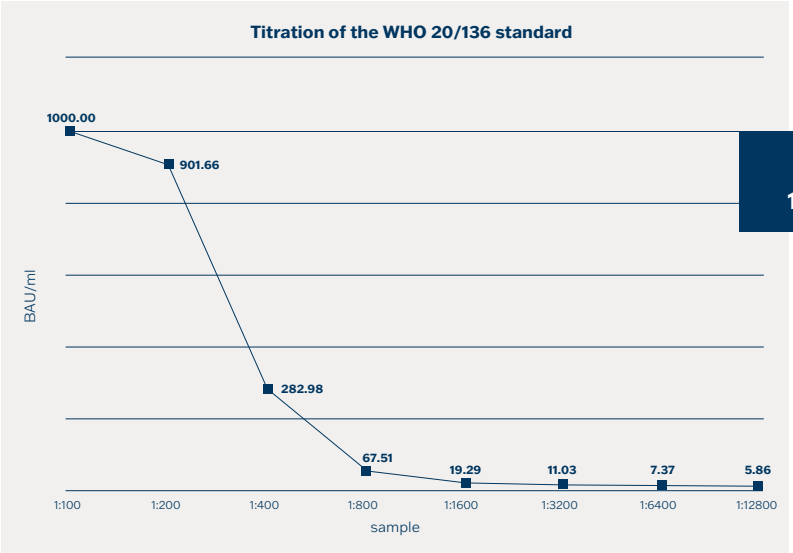


Comparison of TestLine U/ml with BAU/ml for quantitative evaluation of anti-RBD IgG antibodies

The setup of binding antibody units (BAU/ml)

A wide range of immunoassays with different readouts to measure immune responses to SARS-CoV-2 are available globally. Therefore, WHO provided **the First WHO International Standard** (IS) for anti-SARS-CoV-2 immunoglobulin (NIBSC code: 20/136) allowing the accurate calibration of assays to an arbitrary unit, thereby **reducing inter-laboratory variation**, and creating a common language for reporting data.
The Standard is a pool of convalescent plasma from recovered COVID-19 patients, containing high titre of antibodies (Ab) against SARS-CoV-2 with **assigned potency of 250 IU/ampoule** for neutralising Ab activity. **After reconstitution** in 0.25 mL of distilled water, the final concentration of the preparation is 1000 IU/ml. **For binding Ab assays**, an arbitrary unitage of **1000 binding antibody units (BAU)/ml**, can be used to assist the comparison of assays detecting the same class of immunoglobulins with the same specificity (e.g., anti-RBDIgG, anti-NIgM, etc.).

RESULTS: By titrating of the above-mentioned Standard, TestLine (TL) responded to the requirement to accurately determine the level of Ab against SARS-CoV-2 (see the graph below). TL Calibrator 6 (1000 U/ml) in EIA RBD IgG kit was set to 1000 BAU/ml.



TL CAL 6 = 1000 BAU/ml

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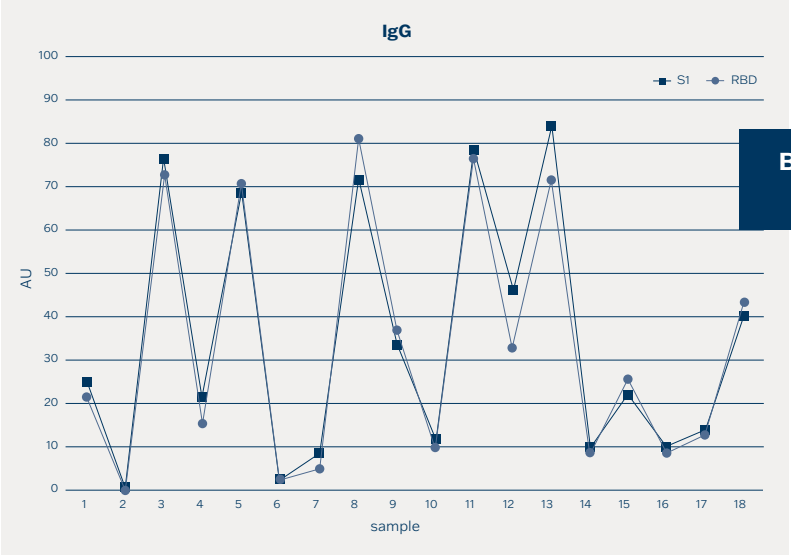
Correlation of VNT and TestLine ELISA kits' results

Binding Ab (BAbs) are defined as Ab that **bind throughout SPIKE** (S) proteins, whereas **neutralizing Ab** (NAbs) are produced only **against the Receptor Binding Domain** (RBD).
According to the latest literature, it is most suitable to use a virus neutralization test (VNT) to determine the protective activity of NAbs. However, the VNT method is time-consuming (2-4 days) and, in addition, requires work with a live virus (BSL3 protection level laboratory).

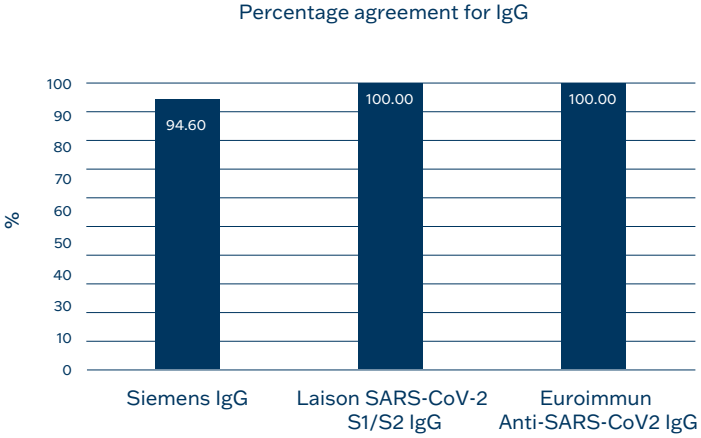
RESULTS: By the correlation study TL confirmed high level of agreement between results obtained by VNT and TL EIA COVID-19 RBD IgG kit. The level of NAbs corresponds with level of anti-RBD IgG Ab and therefore EIA kits developed by TL can be used to determine if concentration of Ab has the protective effect (the full data are available in a separated leaflet). Because RBD is the major immunogenic protein, it was also showed (on TL Microblot-Array COVID-19 IgG kit) that **Ab to other S1 proteins occur together with RBD Ab in 99%** (see the graph below). Therefore, it was concluded that the ratio between the level of BAbs and TL U/ml is 1:1.



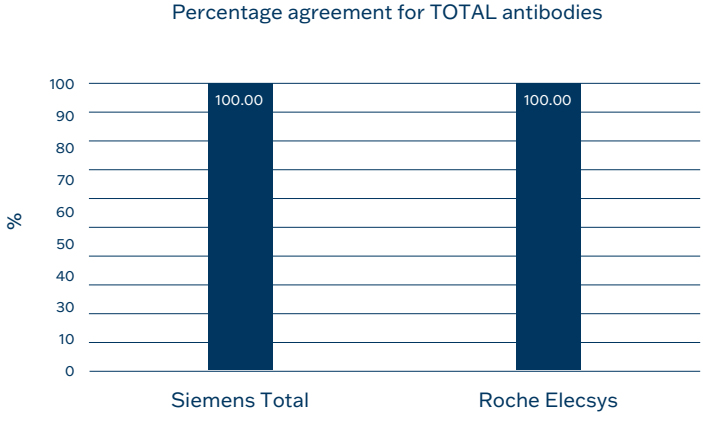
Comparison of TestLine COVID-19 RBD ELISA kits with Verification Panel

Anti-SARS-CoV-Verification Panel for Serology Assays (NIBSC code: 20/B770) is intended for use as a verification to detect of claims by manufacturers on the detection of SARS-CoV-2 Ab and consists of 37 samples (23 positive and 14 negative).

RESULTS: The testing showed that **TL EIA RBD kits** (not only in IgG) **are comparable with other available assays**. **The agreement** in all Ig classes was from **91 to 100%**. The results for EIA RBD IgG kits are showed below.



Antigens used in competitors' kits: Siemens – RBD, Liaison – S1+S2, Euroimmun – S1. TL kits used for the comparison: EIA RBD IgG.



Antigens used in competitors' kits: Siemens – RBD, Roche – RBD. TL kits used for the comparison: EIA RBD IgA, EIA RBD IgG, EIA RBD IgM.

Summary

VNT vs. TL EIA RBD IgG 99% agreement	The results of comparison study showed 99% agreement between EIA RBD IgG and VNT method, hence the kits developed by TL can be used to determine if concentration of Ab in patient's serum has the protective effect.
Suitable for monitoring the Ab levels after vaccination	Thanks to harmonization of TL COVID-19 kits with IS, TL responded to the requirement to accurately determine the level of Ab, sensitivity, and dilution of strongly positive results. Therefore, the kits can be used for monitoring the Ab concentration after vaccination.
TL CAL 6 = 1000 BAU/ml	TL Calibrator 6 in EIA RBD IgG kit was set to 1000 U/ml which corresponds to 1000 BAU/ml (the ratio between BAU/ml and TL U/ml is 1:1).
BAU/ml : TL U/ml 1 : 1	For strongly positive samples with a result higher than 1000 U/ml, the sample can be diluted 1:401 (e.g., 5 µl sample + 2 ml Sample Diluent) for more accurate diagnostic determination of Ab levels and then multiplied 4 times in U/ml.
TL EIA RBD vs. competition 91-100% agreement	TL COVID-19 kits, besides high sensitivity, and specificity, also shows a very good agreement with other commercially available assays.

References:
[1] World Health Organization (2021, January 12). Standardization of vaccines for coronavirus disease (COVID-19). Available from: https://www.who.int/news-room/feature-stories/detail/standardization-of-vaccines-for-coronavirus-disease-covid-19.
[2] The National Institute for Biological Standards and Control. Coronavirus (COVID-19)-related research reagents available from the NIBSC. Available from: https://www.nibsc.org/science_and_research/idd/cfar/covid-19_reagents.aspx.
[3] World Health Organization (2020, December 9-10). Establishment of the WHO International Standard and Reference Panel for anti-SARS-CoV-2 antibody. Available from: https://cdn.who.int/media/docs/default-source/biologicals/ecbs/bs-2020-2403-sars-cov-2-ab-ik-17-nov-2020_4ef4fdae-e1ce-4ba7-b21a-d725c68b152b.pdf?sfvrsn=662b46ae_8&download=true

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