TechNote TN-09

July 2021

Determining the limit of detection of TAmiRNA SARS-CoV-2 IgM/IgG Antibody Rapid Test using the International Standard Anti-SARS-CoV-2 Immunoglobulin

Summary

The TAmiRNA Sars-CoV-2 IgM/IgG Antibody Rapid test enables a fast and qualitative result for the presence of IgG and IgM antibodies directed against the SARS-CoV-2 Spike protein in whole blood and serum samples. As the limit of detection is not established yet, this study is aimed to define the detection threshold and its meaningfulness.

The dilution series with the NIBSC standard in negative serum resulted in a detection limit between 25 and 50 binding antibody units (BAU) per ml. Values above 15 BAU/ml[1] have been correlated to the presence of neutralizing antibodies, thus a positive rapid test result indicates the presence of sufficient neutralizing antibodies.

Introduction

The detection of neutralizing antibodies against the receptor-binding domain of the SARS-CoV-2 virus has had importance throughout the ongoing pandemic and will find use in the future to estimate the antibody status of the population.

The TAmiRNA SARS-CoV-2 IgM/IgG Antibody Rapid Test is an immunochromatographic test for fast (<15 min), qualitative detection of the IgM and IgG antibody against SARS coronavirus 2 (SARS-CoV-2) in human whole blood, serum or plasma. In an independent validation study of the Medical University of Innsbruck with 350 samples, a sensitivity of 98.25% and a specificity of 100% was demonstrated. Thus, the test is comparable to the quality of ELISA tests, with the advantage that the TAmiRNA SARS-CoV-2 IgM/IgG Antibody Rapid Test can be performed on site without laboratory infrastructure. In addition to the detection of severe ongoing or previous infections, the test can also be used to confirm the antibody-based immune response of individuals to vector as well as mRNA vaccines [2].

Aim

The aim of the study was to determine the detection limit of TAmiRNA SARS-CoV-2 IgM/IgG Antibody Rapid test. Therefore, a dilution series using the First WHO International Standard Anti-SARS-CoV-2 Immunoglobulin was prepared and analysed using the rapid test. The established limit of detection was then compared to other tests to estimate the meaningfulness of the result.

[1] Values are adopted from Labros.at (https://www.labors.at/sars-cov-2-antikoerper-testverfahren-2/)

[2] https://www.tamirna.com/wp-content/uploads/2021/04/TN07-technote_internal-anibody-study_v2_DE.pdf

Material and methods

TAmiRNA SARS-CoV-2 IgM/IgG Antibody Rapid Test

This test is based on the principle of immunochromatographic detection of SARS-CoV-2-IgG/IgM antibodies with specificity against the S1 protein in human whole blood, serum and plasma.
The sample is absorbed into the cassette by capillary action after dropping into the sample window, mixes with the SARS-CoV-2 antigen dye conjugate and flows through the pre-coated membrane (see Figure 1).

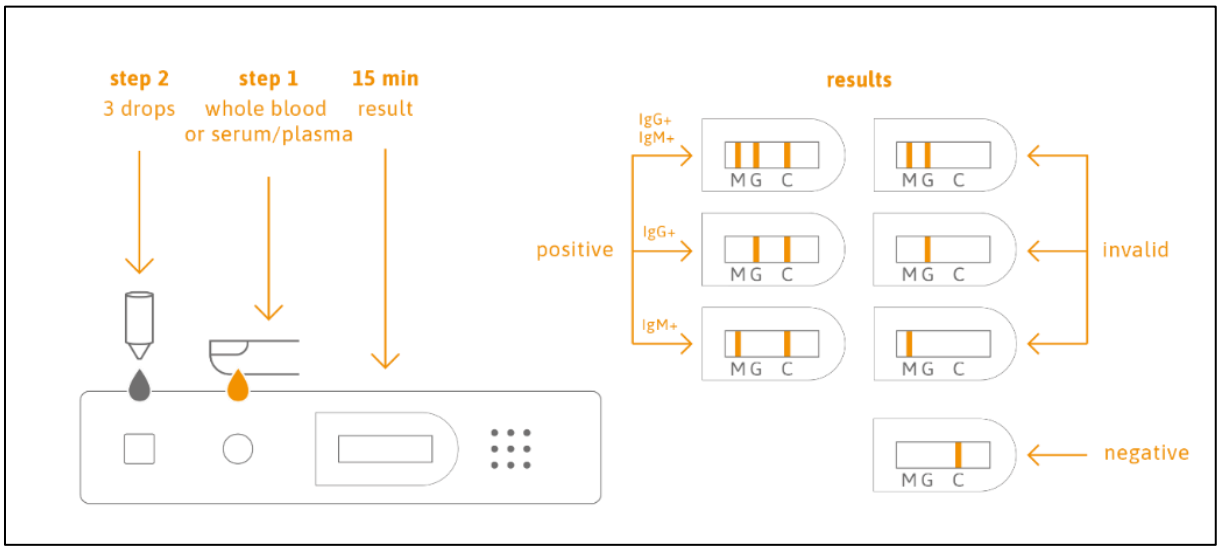


Figure 1: Test method and evaluation of test results

The antibodies of class IgM/IgG, which are formed by the immune system in response to infections with the SARS-CoV-2 virus, react with the recombinant SARS-CoV-2 antigen (S1 spike Protein including the RBD domain) bound to gold particles. This complex travels along the membrane and reaches the test lines to which a monoclonal anti-human IgM/IgG antibody directed against the SARS-CoV-2-IgM/IgG complex is bound. If the SARS-CoV-2 antibody level in the sample is zero or below the target limit, there is no visible colored band. This indicates a negative result.

First WHO International Standard Anti-SARS-CoV-2 Immunoglobulin National Institute of Biological Standards and Control (NIBSC Code: 20/136)

The standard contains the freeze-dried equivalent of 0,25 ml of pooled plasma obtained from eleven individuals recovered from SARS-CoV-2 and has a concentration of 1000 Binding Antibody Units/ml (corresponding to 1000 International Units/ml) after reconstitution.

Dilution series in negative serum

The standard was then diluted in tested negative serum (COVID-19 Remnant Negative Serum Samples, provided by Precision for Medicine, Lot: 29653) to get the following concentrations: 100, 50, 25, 10, 5 and 1 BAU/ml. 10µl of the standards were transferred into the sample window of the test cassette. Afterwards, 120µL (3 drops) sample buffer was applied in the buffer window. Tests were conducted as duplicates. The results were read after 15minutes.

Evaluation

The results of the antibody tests were read, photographed and documented. Only valid test results (with visible C-band) were considered for the evaluation. IgM and IgG bands were interpreted as negative, weak-positive, or positive.

Results

The rapid tests were able to detect concentrations down to 25 BAU/ml in negative serum samples. However, the 25 BAU/ml marks were already faint, thus the limit of detection is seen above 25 BAU/ml, as depicted in figure 2.

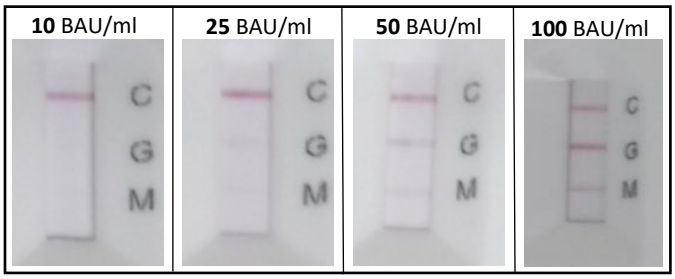
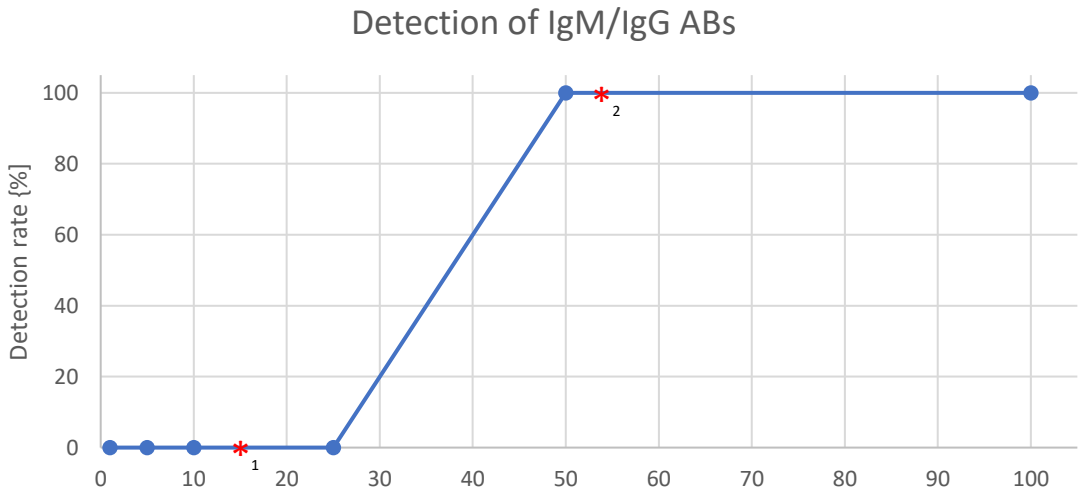


Figure 2: Rapid test results in three different antibody concentrations

Significance

Laboratories in Austria defined different thresholds to indicate immunity for Sars-CoV-2. Generally, a titer of above 0.8 BAU/ml indicates presence of antibodies; however, the limit for neutralizing antibodies is estimated at 15 BAU/ml. A recent study of Khoury et al[3] estimated protective quantity of neutralizing ABs for SARS-CoV-2 above 54 IU/ml. (Figure 3)



*1…15 BAU/ml threshold laboratories
*2…54 BAU/ml proposed threshold

Figure 3: Illustration of the detection rate, the two proposed standards of necessary neutralizing AB presence are highlighted

[3] Khoury, D.S., Cromer, D., Reynaldi, A. et al. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. Nat Med 27, 1205–1211 (2021). https://doi.org/10.1038/s41591-021-01377-8

Discussion

The aim of this study was to narrow down the Limit of detection in rapid tests. It has been demonstrated that the TAmiRNA SARS-CoV-2 IgM/IgG Antibody Rapid Test can detect both IgM and IgG antibodies down to a concentration below 50 BAU/ml (IA/ml) in serum.

On the basis of other austrian laboratories, a sufficient antibody quantity for SARS-CoV-2 is estimated at 15 BAU/ml. Thus, a positive rapid test indicates the presence of neutralizing antibodies, as the limit of detection is above 15 BAU/ml.