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REVIEW

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Potential false-positive reasons for SARS-CoV-2 antibody testing and its solution

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Abstract

Coronavirus disease 2019 (COVID-19) has brought a huge impact on global health and the economy. Early diagnosis of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection is essential for epidemic prevention and control. The detection of SARS-CoV-2 antibodies is an important criterion for diagnosing COVID-19. However, SARS-CoV-2 antibody testing also has certain false positives causing confusion in clinical diagnosis. This article summarizes the causes of false-positive detection of SARS-CoV-2 antibodies in clinical practice. The results indicate that the most common endogenous interferences include rheumatoid factor, heterophile antibodies, human anti-animal antibodies, lysozyme, complement, and cross-antigens. The exogenous interference is mainly incomplete coagulation of the specimen, contamination of the specimen, and insufficient optimization of the diagnostic kit's reaction system.

KEYWORDS

antibody, COVID-19, exogenous interference, false positive, intrinsic interference, SARS-CoV-2

1 | INTRODUCTION

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the seventh coronavirus that can infect humans. The virus is highly infectious with a high mortality rate,[1–4] and spread rapidly among humans, gradually sparking a global pandemic. Coronavirus disease 2019 (COVID-19) has brought a huge impact on global health and the economy.[5–13] The current laboratory methods for detecting SARS-CoV-2 include nucleic acid detection and antibody detection.[14] Among them, nucleic acid testing is the gold standard for SARS-CoV-2 testing. The positive rate is as high as 90% at the initial 1–3 days of infection, but the positive rate drops below 80% on the 6th day, and it continues to drop to less than 50% on the 14th day.[14–16] According to research findings, nucleic acid detection's positive rate is higher than the antibody detection at the first 5.5 days after SARS-CoV-2 infection. However, after 5.5 days of the disease, the antibody detection achieves a better true positive rate.[17] The combined detection of SARS-CoV-2 nucleic acid and SARS-CoV-2 antibody can increase the virus's detection rate to 98.6%, which indicates that SARS-CoV-2 antibody detection can be used as a useful complement to SARS-CoV-2 nucleic acid detection.[17–20] However, the detection of the SARS-CoV-2 antibody has false positives, which causes difficulties in clinical diagnosis and treatment. This article discusses the reasons for the false-positive results of the SARS-CoV-2 antibody.

According to their sources, the causes of false-positive detection of SARS-CoV-2 antibody can be divided into endogenous factors and exogenous factors. The details are as follows:

2 | INTRINSIC INTERFERENCE

2.1 | Rheumatoid factors (RF)

There are five types of rheumatoid factor (RF): immunoglobulin G (IgG), immunoglobulin M (IgM), immunoglobulin A (IgA), immunoglobulin D, and immunoglobulin E (IgE), among which IgM is the most common type. RF may appear in the blood of autoimmune diseases, such as rheumatoid arthritis, infectious diseases, and even healthy people.[21–24] In SARS-CoV-2 antibody detection, RF can nonspecifically bind to the specific antibody Fc segment coated on the solid-phase carrier and the labeled antibody Fc segment, resulting in a Nonspecific detection signal, causing false-positive.[23,25] Using the capture method to detect IgM-specific antibodies, the solid-phase carrier-coated antibody is an anti-human μ-chain antibody. Hence IgM-type RF is more likely to bind to the solid phase in large quantities, leading to detection signals and false positives reaction.[26] Studies have shown that if RF > 331IU/ml, IgM antibodies can give false-positive signals. If RF > 981.2IU/ml, both IgG and IgM antibodies can give false-positive signals.[27] Therefore, if the amount of RF in the specimen is high, the false positive caused by RF must be excluded.

In the clinical test, the interference caused by RF can usually be avoided by the following measures: (1) Dilute the specimen to reduce the concentration of RF.[25] Because RF and IgG Fc segments are non-specifically binding, the binding affinity can be reduced by RF dilution, thereby reducing false positives. In the acute pathogen infection period, IgM rises sharply, and this method is particularly useful. (2) Change the enzyme-labeled antibody. The Fc fragment of the coated antibody or/and labeled antibody is digested and removed, leaving only the $F(ab')^2$ part with specific binding function for coating or/and labeling, which can avoid RF interference.[27,28] (3) Enclose and block RF. Before analysis, adding heat-denatured (63°C, 10 min) animal blood (such as rabbit, sheep, etc.) IgG to the sample, or using IgG-coated solid particles to adsorb and detect RF in the sample[29] can reduce RF interference. When only specific IgM antibodies are measured, anti-human IgG can be added to neutralize RF and IgG in the test specimen.[30] (4) Add a certain concentration of urea to the specimen. Urea can dissociate low-affinity bound RF and IgG complex. The nonspecific binding of RF and antibody in the ELISA method can be dissociated by urea with a concentration of 4 mol/L. When the urea concentration increased to 6 mol/L, it can dissociate the nonspecific binding of RF in colloidal gold immunochromatography assay.[31,32] (5) Using polyethylene glycol (PEG) 6000 precipitation before testing.[33] The main component of RF that forms a complex with IgG and interferes with immunoassay is considered to be polyclonal IgM type RF. This large molecule complex is the main component that precipitates with PEG 6000. The monomer RF precipitates very little due to the small molecules.

RF can be detected in many people, and it is the most common endogenous interfering substance in clinical practice. Therefore, when the SARS-CoV-2 antibody test is positive, we must first exclude the influence of RF. The nonspecific binding of RF can be reduced to reduce the false positives by certain measures, including diluting the test sample, using $F(ab')^2$ coated or labeled test reagents, blocking and block RF.

2.2 | Heterophil antibody (HA)

Heterophil antibody (HA) is a type of cross-reactive immunoglobulin secreted by the human immune system, lacking animal serum or animal immunoglobulin stimulation.[34] HA can bind nonspecifically to the Fc or Fab epitopes of various immunoglobulins. Although the affinity is weak, it can stimulate the immune activity of the target antigen in the immune response, attach to the capture antibody and label antibody, and thereby interfere with the measurement result.[32,34]

The following methods can be adopted to avoid the interference of HA: (1) Same as RF, dilute the test sample to reduce the interference of HA. (2) Add excessive animal immunoglobulin (such as normal mouse serum) to the test sample to block possible HA.[35,36] (3) Remove the solid-phase coating antibody's Fc segment, leaving only the $F(ab')^2$ segment as the coating antibody or/and the labeled antibody, which can reduce the nonspecific binding caused by HA.[37,38]

The measures to avoid false positives caused by HA are similar to RF, mainly to reduce its nonspecific binding in the test sample. Such as diluting the sample, blocking the HA in the sample, enzymatically cleave the Fc segment of the coated antibody and/or labeled antibody, etc.

2.3 | Human anti-animal antibody (HAAA)

Human anti-animal antibody (HAAA) can be produced through contact with animals, vaccination, blood transfusion, use of drugs from animal origin, etc.[39] It mainly includes IgG, IgM, and IgA and a small amount of IgE, differing in idiotype and isotype antibodies. It is often sub-classified as a human anti-mouse antibody. The interference mechanism of HAAA is similar to HA, but more specific.[40,41]

The methods to avoid HAAA interference are (1) Consistent with HA, by adding a certain amount of (nonspecific or specific) animal immunoglobulin to the specimen or specimen diluent to block the possible HAAA.[40,41] (2) Use (low reactivity) specific rabbit $F(ab')^2$ fragments as solid-phase antibodies or labeled antibodies[41] to eliminate the interference produced by HAAA by binding to the Fc segment.

Although the interference of HAAA is relatively rare in clinical practice, its binding specificity is more robust than that of RF and HA. Therefore, the effect of the simple dilution of the specimen is not good. Animal immunoglobulins or immunoglobulin $F(ab')^2$ fragment coated reagents are required in the specimen or specimen diluent.

2.4 | Lysozyme

Lysozyme is widely present in various human tissues,[42] with an isoelectric point of pH 11,[43] and has a strong binding ability with substances having a lower isoelectric point. The isoelectric point of immunoglobulin is about 5, so lysozyme can form a bridge between

the labeled IgG and the coated IgG in immunoassays, resulting in false-positive reactions.

The following two methods can be used to avoid lysozyme interference in immunoassays: (1) Studies have shown that CU^{2+} with a concentration of 5×10^{-3} M can effectively block lysozyme,[44] thereby reducing its binding to immunoglobulin. (2) Ovalbumin blocking lysozyme can also achieve good results.

The high isoelectric point of lysozyme resulting in false-positive reactions is mainly due to physical combination. Therefore, neutralizing lysozyme with lower isoelectric point ovalbumin and CU^{2+} ion solution can reduce the interference.

2.5 | Complement

Complement is a serum protein that exists in human and vertebrate serum and tissue fluid. In solid-phase immunoassay, the antibody molecule undergoes allosteric exchange to expose the Fc fragment. The exposed Fc terminal can activate the C1q of complement so that C1q becomes an intermediary to cross-link the antibodies, resulting in an increased false reaction.[45,46]

The complement C1q in the specimen can be inactivated by heating at 56°C for 30 min to reduce the complement's interference. However, it must be verified that heating will not affect the results of specific experiments.

2.6 | Cross antigen

Cross-antigens are similar epitopes between two antigens from different sources. The antibodies produced by certain determinants can bind to the corresponding epitopes on their surface and react with similar epitopes of other antigens. At present, a large number of studies have shown that there are N protein and S protein immune cross-reactions between coronaviruses in the same subgenus or different subgenuses.[47–49] Therefore, false-positive reactions may be caused by cross-reactions of other coronaviruses in the test. Although it is relatively rare in clinical practice, it still cannot be ignored.

3 | EXOGENOUS INTERFERENCE

3.1 | Specimen incomplete coagulation

After the blood is collected according to the standard operating procedure if centrifuging the specimen before complete coagulation, at this time, the blood clot is not completely contracted, and some fibrinogen remains in the separated serum. When these specimens are used for detection, it may be adhesion makes the result false positive.[50,51]

To avoid this kind of error, operation procedures should be strictly followed. After collecting the specimens, the specimens are completely coagulated, and the blood clots are shrunk before centrifuging.

3.2 | Insufficient optimization of the kit system

Some SARS-CoV-2 antibody detection kits are not optimized enough. So, it is recommended that the kit developer pay attention to the interference problem of immunological detection. Such as using rabbit anti-μ chain and/or γ chain antibody $F(ab')^2$ as a solid-phase carrier coating antibody, with a buffer containing nonspecific rabbit IgG, can effectively reduce the endogenous interference.

3.3 | Other

Improper collection of specimens or improper storage of specimens contaminated by bacteria and other substances may also cause false-positive test results. Therefore, it is necessary to strictly abide by the operating procedures and standardize operations and prevent specimen contamination. It is worth noting that experiments have shown that hemolysis, chyle, and jaundice will cause false positives in the enzyme-linked immunosorbent assay and electrochemiluminescence method. However, the impact on the immunochromatographic colloidal gold detection method is minimal and almost negligible.[27]

4 | CONCLUSION

The SARS-CoV-2 antibody detection occupies an indispensable position in clinical testing, but it has a certain false positive. Clinically, nonspecific binding caused by endogenous substances can be excluded by methods, such as dilution, blocking, and restriction digestion. False-positive results caused by exogenous factors can be avoided by strictly following standard operating procedures and optimization of the diagnostic kit's reaction system.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

Qing Ye: conceptualization, writing-original draft preparation and validation. Ting Zhang writing-reviewing and editing. Dezhao Lu: writing- reviewing and editing.

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