



COVID-19 White Paper

Introduction

Outbreaks with a novel coronavirus disease COVID-19 is caused by the SARS-CoV-2 virus. The virus has shown to be very contagious and has caused a world wide pandemic in the spring of 2020.¹ The SARS-CoV-2 virus is a single strand RNA virus with glycoprotein spikes on its surface. The spike protein (S) contains the receptor-binding domain (RBD) which has affinity to the angiotensin-converting enzyme 2 (ACE2). The interaction between RBD and ACE2 mediates the fusion of the viral and cellular membranes starting the infection process. The S protein has two subunits, S1 and S2. S1 contains the RBD region and the S2 is associated with the membrane of the virus.^{2,3} The nucleoprotein (N) is an abundant protein associated with virus structure and replication. Human ELISA assays have been developed using the S proteins and nucleoprotein of SARS-CoV-2 to detect antibodies to SARS-CoV-2.^{4,5}

Spike Protein Plates

XpressBio provides two plates that utilizes recombinant spike proteins as the antigen on the plates. The S1/S2 plate (SP864C) is a mixture of two recombinant glycoproteins, S1 and S2. The S1 protein contains the amino acids 1-674 with a C-terminal sheep Fc-tag. The S2 protein has amino acids 685-1211 with a C-terminal sheep Fc-tag. Both have been propagated in HEK293 cells and purified with Protein G chromatography. The positive control (SPC864) for the S1/S2 plate is a monoclonal antibody against S2. The conjugate contains anti-mouse antibodies to detect the monoclonal.

Receptor-Binding Domain (RBD) Plates

The RBD (SP866C) plate is coated with a recombinant protein consisting of 234 amino acids from the RBD region of the COVID-19 spike protein. The protein is propagated in baculovirus insect cells, tagged on the C-terminal end with a poly histidine tag for purification by metallic chromatography. The positive control (SPC866) for the RBD plate is also a monoclonal.

Nucleoprotein Plates

The N plates (SP865C) are coated with recombinant phosphoprotein nucleoprotein (amino acids 1-419). The protein is expressed in E. coli and labelled with a C-terminal His tag. The positive control (SPC865) is a mouse monoclonal antibody against Nucleoprotein (N). The conjugate contains anti-mouse antibodies to detect the monoclonal.



Seasonal Coronavirus Plates

Two different seasonal coronavirus plates are available: HCoV-229E (SP867C) and HCoV-NL63 (SP868C). The HCoV-229E can be found at different times around the world. Most of the infections are mild such as the common cold. The HCoV-NL63 continually circulates in the human population in temperate climates. It also uses transmembrane ACE2 as its host cell entry receptor. Both plates are coated with recombinant nucleoprotein produced in *E. coli* with C-terminal His tags. The positive controls are Human positive sera for the seasonal coronavirus: HCoV-229E (PC867) and HCoV-NL63 (PC868).

Positive Predictive Values

Whenever an assay is utilized in a low prevalence population the positive predictive value for the assay will be very low. Since any sample before December 2019 should be negative, past samples will have a positive predictive value of 0%. Therefore, any positive samples will be false positive. There are several reasons for false positive reactions. There could be a cross reaction with another coronavirus. Since coronavirus are seasonal and always circulating, antibodies to coronavirus should be common. Additionally, antibodies to the vector that the recombinant is expressed in can be present. The recombinants are purified. However, vector proteins can be present that co-purify with the protein. The recombinants have a tag engineered into them to make purification easier. Sometimes antibodies can be present to the tag. Finally, some sera have non-specific activity. These types of sera are considered “sticky” and normally cause false positives across several assays. When testing in low prevalence populations, a confirmatory assay is performed on positive results. For instance, when testing for Lyme disease, a positive ELISA result is normally confirmed on a western blot assay. We would suggest testing the samples on the S1 and S2 spike plates first. If a sample is positive, the same sample could be tested on the nucleoprotein plate, RBD plate and the seasonal coronavirus plates. If the sample is positive on the spike plate and negative on the others, it would indicate that the sample has antibodies to the HEK vector or the Fc tag as the others are different vectors and tags. If the sample is positive on all, it could indicate a cross reaction to other coronavirus, otherwise it is a non-specific sera.

Expected Values

The SARS-CoV-2 virus is considered a novel coronavirus meaning that the human population should have very low immunity to it. Its closest relative that infects humans is the Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV). The amino acid sequence homology for the spike proteins is 76%. Therefore, positive results with samples earlier than December 2019 should be predominately negative. We have tested 51 human samples on the spike plates drawn prior to December 2019 and found all to be negative. (Please note that all of XpressBio products are for research use only and not for diagnostic use). We have also tested 21 non-human primate

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(NHP) samples with all negative results. Some (4 NHP laboratories) of our customers have reported their results to us using the spike plates with following results: 170/175 = 97% specificity for an overall specificity of 242/247 = 98%.

Four of the false positives were negative on the RBD, N, and seasonal coronavirus plates indicating that the false positives were due to antibodies against the HEK vector or the Fc tag. One sera was positive on every plate indicating that it was a non-specific serum. By using the algorithm of testing the samples first on the spike plate, and then confirming any positives on the NP plate the specificity can be increased to 246/247 = 99.6%.

The sensitivity of the spike plates are more difficult to assess due to a low number of positive samples available to test. However, all the specific monoclonals, polyclonals and human specimens have been positive on the plates. The nucleoprotein has ~90% homology with other SARS type coronavirus, therefore more cross reactivity with other coronavirus may be seen with this protein. We have tested 50 human samples on the N plates with 50 being negative and 41 NHP sera with 39 being negative for a specificity of 89/91 = 97.8%. The homology of the RBD protein is 73% with SARS-CoV. Therefore, it should have similar specificity as the S plate. We have tested 50 human and 21 NHP with all being negative. The seasonal coronavirus should have more positives as they circulate seasonally. We have tested 20 sera on both and found 3/20 = 15% positive.

References

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