GSD/VIROTECH/NovaLisa® SARS-CoV-2 ELISA

Scientific status update on COVID-19 serology / N protein (nucleocapsid protein) significance – 04/30/2020

Kai Schuchmann, David Hartmann, Adelheid Bursen - VIROTECH Diagnostics

The GSD/VIROTECH/NovaLisa® SARS-CoV-2 ELISA uses the SARS-CoV-2 nucleocapsid protein (N protein) as antigen for the detection of antibodies to SARS-CoV-2. The N protein is a structural component of the helical nucleocapsid and plays an important role in viral pathogenesis, replication and RNA packaging. Furthermore, this protein is more conserved than other proteins of the virus, such as spike and membrane glycoproteins [1-3]. It is well known that S1 (part of the spike protein) and N proteins are the dominant antigens of SARS-CoV and SARS-CoV-2 that elicit IgG, IgM and IgA antibodies, and antibody response against N protein is usually stronger [4]. Studies have demonstrated for SARS-CoV that among all coronavirus proteins, the N protein is the most abundant throughout infection and the most reactive antigen [2][5].

In a study of patients infected with SARS-CoV, the antibody response was most frequently directed against the N protein (89% of assayed patients). Only 63% of these patients had antibody response targeting the spike protein [6].

A study from April 2020 led by scientists from the National Institutes of Health, USA, using an immunoprecipitation assay with either the N protein or the S protein demonstrated that the N protein is more sensitive. Fifteen or more days after symptom onset, antibodies against SARS-CoV-2 N protein showed 100% sensitivity and 100% specificity, while antibodies to the S protein were detected with 91% sensitivity and 100% specificity [7].

Regarding the timing of the antibody response, seroconversion of antibodies directed against the N or full-S protein seem to be comparable, with the N response being slightly more rapid, whereas the seroconversion against the S1 fraction is delayed [8]. Depending on the study, seroconversion for IgG against the N protein is detectable as soon as 5 to 14 days post symptom onset. Timing of IgM and IgA antibody response is not well established yet but is expected to start around the same time [9][10]. In one study, detection of antibodies was more efficient than PCR testing 5.5 days after onset of symptoms due to the decreasing virus load in respiratory specimens of patients in the course of the disease [9]. A closer look into disease severity and antibody response indicates that anti-SARS-CoV-2 antibody titers correlate with disease severity, likely reflecting higher viral replication rates and/or immune activation in patients with severe outcome [8] [10].

A strong indicator for immunity to viral infections is the presence of antibodies able to neutralize the virus. Neutralizing antibodies have a major role in preventing reinfections for many viral diseases. Currently considered as gold standard for SARS-CoV-2 serology are neutralization assays (plaque reduction neutralization test, PRNT) that can quantify neutralizing antibodies. However, these tests are very labor intensive and require BSL3 facilities. It is important that a serological assay that is more feasible for routine testing such as an ELISA has a high correlation to the result of PRNT assays. All available studies including one led by scientists from the Institut Pasteur, France, demonstrate a high correlation between the presence of antibodies targeted against the N protein and antibodies that can neutralize SARS-CoV-2 [8] [11] [12]. One study comparing ELISAs using the spike RBD, S1 and N antigens demonstrated that the RBD and N ELISAs were more sensitive than S1 ELISA in detecting antibodies in mildly infected patients and showed stronger correlation with PRNT titers [12].

At the beginning of the emergence of SARS-CoV-2 it was assumed that the N protein is not the optimal antigen for serological assays because significant cross reactivity was expected. This is based on the observation that the N protein is closely related to N proteins of other coronaviruses including common cold coronaviruses that have a high prevalence in the population. All available studies refuted these concerns. A study using a microarray with SARS-CoV-2 antigens observed no cross reactivity of the N protein with common cold or MERS coronaviruses. The only significant cross reactivity is observed with SARS-CoV [13]. However, SARS-CoV has not circulated in the human population since 2003, i.e. 17 years ago, and an earlier study reported waning of SARS-CoV-specific antibodies which made them undetectable in serum samples tested 6 years following infection [14]. It is therefore unlikely that antibodies to this virus are present in the population and thus there is hardly a chance that false positives result from SARS-CoV-antibodies reactivity [12]. Two other studies using a SARS-CoV-2 N protein ELISA or Western Blots confirmed that there was no cross reactivity of the N protein with seasonal coronaviruses associated to the "common cold" (such as HCoV-OC43, HCoV-229E, HCoV-HKU1 or HCoV-NL63) [8][9]. Experiments performed for the validation of the GSD/VIROTECH/NovaLisa® SARS-CoV-2 ELISA with samples positive for HCoV-OC43, -NL63, -229E or -HKU1 did not show any cross reactivity as well.

Most cases of SARS-CoV-2 infections are associated with only mild or even no detectable symptoms. Most of these patients develop only low levels of antibodies to SARS-CoV-2 as has been observed earlier for MERS-CoV [12]. A study screening 175 PCR confirmed COVID-19 recovered patients with mild symptoms for neutralizing antibodies identified 10 patients (6%) that had not developed detectable neutralizing antibodies [15]. All but one of these patients were younger than 40 years. This indicates that PCR confirmed cases that have a negative serological result should be regarded as true negative. Currently, it is not established which antibody titers are indicative for immunity. Besides neutralizing antibodies, the cellular immunity carried out by T cells could play an important role. A study led by the Charité, Germany, demonstrated SARS-CoV-2 reactive T cells in 34% of healthy donors that were negative for antibodies to SARS-CoV-2. This opens the possibility that a background immunity based on cross reactive cellular immunity exists, probably acquired as a result of previous exposure to common HCoVs [16].

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