TECHNICAL NOTE

IMMUNO-COV™ ASSAY SENSITIVITY AND SPECIFICITY

Based on assay validation and verification data obtained in April and May, 2020

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What are specificity and sensitivity?

Assay specificity and sensitivity are measures of an assay's accuracy. For these types of assays, sensitivity is a test's ability to correctly identify samples with SARS-CoV-2-neutralizing antibodies. Specificity is a test's ability to correctly identify samples that do not contain neutralizing antibodies. High sensitivity means fewer false negatives, while high specificity means fewer false positives.

What is the sensitivity and specificity of IMMUNO-COV™?

IMMUNO-COV™ exhibited **100% specificity** and at least **98.6% sensitivity** in validation and verification studies. See description below for details.

What matters more, sensitivity or specificity?

Both are important for determining a test's accuracy. If an assay has poor specificity, some individuals without neutralizing antibodies will receive false positive results, and think they have some protection against future infection. In contrast, if an assay has poor sensitivity, false negatives will make some individuals with neutralizing antibodies think they have no protection against future infection*.

*It is not currently known what constitutes a protective response for SARS-CoV-2.

To evaluate the sensitivity and specificity of IMMUNO-COV™, 197 serum samples were tested in a single-blinded approach (Table 1). Two test cohorts were used: positive and negative. Samples in the negative cohort had previously tested negative for SARS-CoV-2 antibodies by ELISA or were collected from individuals who had not been exposed to, or shown any symptoms of, COVID-19. Samples in the positive cohort had previously tested positive for SARS-CoV-2 antibodies by ELISA or were collected from individuals who self-reported as testing positive for SARS-CoV-2 by PCR test.

All 125 samples in the negative cohort were negative in the IMMUNO-COV™ assay. Thus, no false positives were detected. From the positive cohort, all samples that previously tested positive by ELISA also tested positive for neutralizing antibodies in the IMMUNO-COV™ assay. The majority of samples (31 out of 34) collected from individuals who had previously tested positive for SARS-CoV-2 infection by PCR were positive for neutralizing antibodies in the IMMUNO-COV™ test. Moreover, upon further analyses, two of the three samples that tested negative were also negative by plaque reduction neutralization titer (PRNT) or ELISA, and therefore were excluded from sensitivity analyses. Taken together, SARS-CoV-2-neutralizing antibodies were detected in 69 out of 70 samples presumed positive for SARS-CoV-2 antibodies, corresponding to an assay specificity of at least 98.6%.

Table 1: Summary of IMMUNO-COV™ blinded testing

	Total tested	Number Negative Results	Number Positive Results
Negatives	**125**	**125**	**0**
Presumptive (not tested)	83	83	0
Negative by ELISA*	42	42	0
Positives	**72**	**3†**	**69**
Positive by PCR	34	3†	31
Positive by ELISA*	38	0	38

* Samples also tested by EPITOPE IgG ELISA (clinically validated at Mayo Clinic).
† Two of these samples were subsequently found negative by other serological assay.