# Comparative evaluation of lateral flow assay (LFA) and ELISA tests that detect human antibodies specific to SARS-CoV-2 to support COVID-19 case management

## 1 Protocol synopsis

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| **Title** | Comparative evaluation of lateral flow assay (LFA) and ELISA tests that detect human antibodies specific to SARS-CoV-2 to support COVID-19 case management |
| **Short title** | COVID-19 Immunoassay Evaluation |
| **Use case of test** | Detection of seroconversion status to determine exposure to COVID-19, intended for 1) triage of COVID-suspected* patients, 2) aid in diagnosis of COVID-suspected* patients, and 3) assessment of recovery in COVID-19-convalescent patients.<br>*as defined by country or WHO case definitions |
| **Rationale and background** | This study is designed to independently evaluate immunoassay tests for COVID-19 to generate unbiased performance data to help inform test intended use, utility, and public health decisions for COVID-19. Index tests will be automated or manual benchtop enzyme-linked immunosorbent assays (ELISAs) or lateral flow assays (LFAs), which can enable case management in resource-limited and point of care (POC) settings.<br>SARS-CoV-2 specific antibodies are part of the immune response to infection, and may be detected during the early, late, convalescent, and post-recovery phases of disease. Detection of a SARS-CoV-2 antibody response may be useful to inform triage and management in cases of active infection, to detect convalescence, and to provide evidence of prior exposure. |
| **Primary objective(s)** | 1.1 To evaluate the clinical sensitivity of novel COVID-19 antibody tests (index test) using serum/plasma specimens from COVID-19 confirmed specimens (RT-PCR positive test result).<br>1.2 Evaluate the clinical specificity of index tests using serum/plasma specimens from COVID-19 negative specimens (defined below). |
| **Secondary objective(s)** | 2.1. Evaluate the performance of index test compared to a reference ELISA antibody test (in-house or commercially-available, defined through site-specific validation).<br>2.2. Determine the association of index test sensitivity with disease stage (day from symptom onset) and symptom severity.<br>2.3. Determine index test specificity (cross-reactivity) for other alpha/beta-human coronaviruses, influenza, EBV and/or CMV (dependent on site-specific availability). |
| **Study design** | This is a retrospective, multi-centre performance evaluation study of novel COVID-19 serological assays. All index test results are for research use only, and will not be reported for patient care. |

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| **Index tests** | ELISA or LFA that detect antibodies specific to recombinant SARS-CoV-2 proteins; antibody detection can include IgA, IgM, IgG or a combination thereof. Tests to be evaluated can be found at the below link (list may evolve overtime).<br>https://www.finddx.org/covid-19/sarscov2-eval-immuno/ |
| **Reference test(s)** | • All COVID-19 positive samples will be characterized using a validated RT-PCR test results (site-specific)<br>• All specimens will be evaluated against a validated ELISA/automated IA comparator test<br>• TBD: All participating laboratories may be sent a serological EQA panel if possible, to benchmark performance across study sites and tests. |
| **Study sites/setting** | The sample size may be achieved by one site or distributed across more than one site for each index test (likely one site will evaluate each index ELISA format test and at least two sites will evaluate each index LFA format test.) |
| **Study Samples** | Study samples will be sourced from de-identified, remnant samples of patient serum or plasma.<br>COVID-19 positivity will be determined by documentation of a patient-matched positive RT-PCR test using a respiratory specimen such as a nasopharyngeal (NP) or oropharyngeal (OP) swab or sputum.<br>COVID-19 negative samples will be defined as archived specimens that are unlikely to have any exposure to SARS-CoV-2, preferentially obtained in 2018 or earlier, prior to the introduction of SARS-CoV-2 (Nov 2019 or earlier is possible). The COVID-19 negatives can include specimens that were confirmed for infection with other syndromic respiratory diseases to evaluate index test specificity (cross-reactivity). Negative specimens may also have been obtained from patients who were suspected to have COVID-19 but had a negative RT-PCR test result.<br>Clinical data associated with each sample will be extracted from site-specific databases. |
| **Sample size** | The number of samples used to evaluate each test should be minimally 100 COVID-19 positives and up to 300 COVID-19 negatives (at minimum 100 negatives):<br>• The 100 positives should approximately fall into sub-categories for day from symptom onset (d.f.s.o), as is possible:<br>➢ N = 10 for Day 0-3, N = 20 for Day 4-7, N = 30 for Day 8-14, N = 20 for Day 15-28, N = 20 for Day 29+<br>• The sample size may be achieved by one site (ELISA) or distributed across more than one site (LFA).<br>Barring discordant results, each sample will be tested once per LFA index test per site, and in duplicate for each ELISA index test per site. |
| **Ethics** | All clinical studies will be performed on samples in which individuals provided informed consent for additional or archived/remnant samples to be used for research purposes. |