Comparative evaluation of lateral flow assay tests that directly detect antigens of SARS-CoV-2

1 Protocol synopsis

Title	Comparative evaluation of lateral flow assay tests that directly detect antigens of SARS-CoV-2 and can be interpreted visually or through the assistance of a reader
Short title	COVID-19 Antigen RDT Evaluation
Use case of test	Rapid, point-of-care (POC) detection of active infection in adults with suspected COVID-19 infection.
Rationale and background	The aim of this study is to independently evaluate the performance of novel, rapid, point-of-care (POC) lateral flow assays for the direct detection of SARS-CoV-2 antigens (Ag) in comparison to the current gold standard for testing, RT-PCR. This protocol synopsis covers two approaches to assess the performance of index RDTs: 1) a lab-based approach using contrived respiratory specimens[1]; 2) a prospective clinical approach using fresh respiratory swabs collected in assay-specific buffer from individuals confirmed or suspected COVID-19, as defined by national or WHO case definitions. If rapid diagnostic tests (RDTs) that detect SARS-CoV-2 antigen are shown to have sufficient accuracy and sensitivity, then their use could facilitate rapid clinical decision-making, as these tests are very simple to perform and the turnaround time for results is typically < 30 minutes [1]Note: if lab-based studies demonstrate equivalency between fresh and frozen samples, or supplier-provided information is provided showing equivalence, a retrospective, clinical study design can be considered.
Primary objective(s)	1.1 [Lab Evaluation]To determine the relative analytical sensitivity of COVID-19 antigen RDTs (index test) using contrived specimens: respiratory swab samples spiked with known quantities of cultured viral isolate. 1.2 [Clinical Evaluation] To determine the diagnostic accuracy of COVID-19 antigen RDTs in patients presenting with influenza-like illness using upper respiratory tract specimens compared to gold-standard RT-PCR.
Secondary objective(s)	2.1 [Clinical Evaluation] To determine the association of positive index test results with disease stage (days since symptom onset, e.g. acute, early, late) and symptom severity
Exploratory objective(s)	3.1 [Lab Evaluation] To compare the relative analytical sensitivity of COVID-19 antigen RDTs (index test) using different swab preparation methods of contrived specimens (e.g. fresh swab in proprietary buffer, in UTM and in UTM that is frozen and thawed) 3.2 To assess the feasibility, ease of use of the index test (NP swabs and processing with RDT)
Study design	**Lab Evaluation:** This is a **lab-based performance evaluation study** of novel COVID-19 Antigen assays to determine comparative sensitivity across multiple tests using contrived samples that are swabs spiked with cultured virus isolate. The master dilution stock used for each dilution series will be tested by RT-PCR and the Ct values will be

	compare with the index test reactivity. Optionally, off-label swab preparations, i.e. using universal transport media will also be compared. **Clinical Evaluation:** This is a **prospective, multicentre, blinded performance evaluation** study of SARS-CoV-2 Ag RDTs. All index test results will be compared to RT-PCR results, are for research use only, and will not be reported for patient care. **Clinical Evaluation – Archived specimens:** *should the lab evaluation demonstrate equivalence between fresh swabs in the proprietary buffer and the frozen UTM samples for the index test, then remnant, frozen swabs* can be used to in a **retrospective, multicentre, blinded performance evaluation** study of SARS-CoV-2 Ag RDTs. Briefly, up to 100 COVID-19 PCR positive remnant, archived remnant swab samples and at least 100 COVID-19 PCR negative remnant, archived remnant swab samples should be assessed per test; operators will be blinded to sample reactivity.
Index Tests	Rapid diagnostic tests that detect antigens of the SARS-CoV-2 virus. Tests to be evaluated can be found at the below link (list may evolve overtime). https://www.finddx.org/covid-19/sarscov2-eval-immuno/
Reference test(s)	• RT-PCR (lab validated, site-specific) • Known viral titer, dilution series
Study sites/setting	The sample size may be achieved by one site or distributed across more than one site for each index test.
Study Samples	**Lab Evaluation:** Standard dilution series will be prepared (expressed in TCID50) using cultured virus, and will be used to spike swabs which will then be placed in the proprietary buffer for testing as per manufacturer's instructions. In addition, at least two other, off-label sample types may be analyzed: 1) swabs will be placed directly in UTM and the required volume will be added to each test, according to the instructions for use; 2) swabs will be placed into UTM, then frozen overnight, thawed and then the required volume will be added to each test, according to the manufacturer's instructions for use. **Clinical Evaluation**: (prospective) Individuals suspected to have COVID-19 (as per national or WHO guidelines) presenting at the testing site(s) will be invited to participate. Each participant will have two respiratory swabs collected: one for RT-PCR testing and diagnosis, paired with a second swab to be used on the index RDT. Enrollment will continue until 100 positive samples are obtained for each test (volumes could be combined across sites should multiple sites be evaluating the same test). Alternatively, if swabs placed in UTM are shown to be equivalent in the lab study, then only one swab will be needed per patient to be used across both RT-PCR and index testing. **Clinical Evaluation**: (retrospective) samples will be sources from de-identified, remnant swab specimens which have been collected from individuals suspected to have COVID-19 who provided informed consent for their remnant sample to be stored and used for future research purposes. All samples will have documented RT-PCR results. If possible, a range of samples across days from symptom onset and severity of symptoms should be included. A standardized CRF will be provided to each site to guide needed clinical information for each included sample.

Inclusion/ exclusion criteria	**Clinical Evaluation**: (prospective) • **Inclusion**: adult (≥ 18), recently confirmed or suspect COVID-19, understands and signs the consent form, agrees to provide medical history and NP specimens • **Exclusion**: does not meet inclusion criteria
Sample size	• For prospective study: a minimum of 100 COVID-19 RT-PCR positives; a minimum of 100 COVID-19 RT-PCR negatives (300 preferred) • For retrospective study: a minimum of 100 COVID-19 RT-PCR positive and 100 PCR negative samples. • Each sample will be tested once per Ag RDT 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.