

Comparative evaluation of molecular tests that directly detect the nucleic acid of SARS-CoV-2

1 Protocol synopsis

Title	Comparative evaluation of molecular tests that directly detect the nucleic acid of the virus that causes COVID-19/SARS-CoV-2
Short Title	COVID-19 Nucleic Acid Test (NAT) Evaluation
Use case of test	Confirmatory diagnosis of SARS-CoV-2 infection and/or monitoring of infection progression.
Aim and rationale	<p>In recognition of the acute need to provide access to screening of suspect cases and diagnosis, many labs are developing their own molecular testing protocols. In addition, many companies are developing commercial nucleic acid testing (NAT) kits. There is a need for independent, objective data comparing the performance of the commercial kits, in particular, to be able to inform in-country procurement and implementation.</p> <p>To gain an unbiased understanding of the commercially-available nucleic acid testing (NAT) kits that can be used to diagnose SARS-CoV-2 infection, the Foundation for Innovative and New Diagnostics (FIND), launched an expression of interest (EOI) on its website, which was open for over two weeks. This EOI was used to gather standardized information on the test kit components, the stated performance of the kit as per data generated internally by the company, and details about the manufacturing and distribution systems of the applicant. These data were compiled in order to score the applicants to prioritize evaluation of the kits that are likely to be the most accurate, and that are produced by suppliers capable of quality-assured manufacturing to meet the ever-growing demand globally.</p> <p>The objective of this limited evaluation is to verify the claimed analytical sensitivity (limit of detection) and to determine the diagnostic accuracy of the included <i>in vitro</i> diagnostic tests to detect SARS-CoV-2 nucleic acid.</p>
Primary objective(s)	Verify the analytical sensitivity, i.e. lower limit of detection, of commercially-available COVID-19 NAT diagnostic kits using contrived specimens containing known, limited quantities of whole virus.
Secondary objective(s)	Determine the diagnostic performance (sensitivity and specificity) of commercially-available COVID-19 NAT diagnostic kits in previously characterized clinical respiratory samples (SARS-CoV-2 positive and negative).

Exploratory objective(s)	Determine the ease of use and describe the operational characteristics of commercially-available COVID-19 NAT diagnostic kits.
Study design & Participants	<p>LOD verification: Quantified whole virus will be used to create a standardized dilution series to verify the analytical sensitivity, in copies per reaction. The series will include 10 replicates of eight dilutions from 10³ to 1 copy/reaction.</p> <p>Retrospective study: Diagnostic accuracy study using remnant, retrospective clinical samples that have been previously collected from individuals suspected to have, or diagnosed with, the virus that causes COVID-19, and who have consented to have their remnant samples used for research purposes. Blinded, retrospective remnant respiratory specimens and/or RNA extracts from PCR-positive COVID-19 patients and PCR-negative COVID-19 suspected cases will be used. Samples will be either oropharyngeal or nasopharyngeal swabs placed in Universal Transport Media. A total of up to 150 samples will be tested: fifty positive (at minimum 25) and one-hundred negative (at minimum 50). All samples will be labelled with an anonymous ID. Testing operators will be blinded to the original reactivity of the samples.</p> <p>Note: if evaluating the clinical performance of manual kits in which extraction and amplification are separate, can use viral extracts as clinical sample type. If evaluating automated/closed systems in which extraction and amplification are coupled, specimens should be remnant UTM into which swabs have been expressed.</p> <p>There will be no prospective collection of any patient specimens for this study. There is no study-related follow-up. The result of this study will not be used for patient care.</p>
Sample size	<p>50 COVID-19 reference RT-PCR positive samples</p> <p>100 COVID-19 reference RT-PCR negative samples</p>
Index tests	Manual or automated RT-PCR kits/tests
Reference/comparator test	The true positive and true negative reference reactivity will be the original clinical method used for diagnostic purposes. The same reference method will be used for all samples sourced from the same site. Any discordant results will be examined to determine whether the index test is more accurate.
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.