Coronavirus Disease 2019 (COVID-19)

Common Investigation Protocol for Investigating Suspected SARS-CoV-2 Reinfection

Updated Oct. 27, 2020 Print

Protocol summary: This protocol is designed to support a common public health investigation into suspected SARS-CoV-2 reinfection cases across jurisdictions. Confirming SARS-CoV-2 reinfection requires advanced laboratory diagnostic support built upon advanced planning to implement this protocol, or a locally adapted version, with referral of specimens to supporting laboratory networks. Data collected with this protocol will identify potential cases of reinfection, advance understanding of SARS-CoV-2 epidemiology, and inform public health response.

Introduction

Current state of knowledge: A gold-standard confirmation of SARS-CoV-2 reinfection will require confirmation of initial infection and virus detection across two distinct time periods with genetic sequencing data needed to support a conclusion of high probability that reinfection has occurred. Possible SARS-CoV-2 reinfection could be differentiated from persistent viral carriage through a variety of laboratory-based parameters, patient symptomology, and/or epidemiologic links[1]. However, reinfection cannot be confirmed if clinical specimens from the initial coronavirus disease 2019 (COVID-19) illness are not available.

Reinfection is known to occur with other human coronaviruses (HCoVs)[2]. A study in Kenya found that 4%–21% of people infected with endemic coronaviruses (HCoV-229E, NCoV-NL63, and HCoV-OC43) had two or more episodes of infection with the same virus species during a six-month period[3]. Another study of HCoVs that used an antibody increase as a proxy for reinfection found that reinfections occurred at a median of 30 months but could occur as early as 6 months following the first infection[4]. However, immunologic data on durability of immunity for SARS-CoV-2 are limited[6]. Of note, South Korea has documented RT-PCR-confirmed COVID-19 cases that became undetectable by RT-PCR, then subsequently tested positive again by RT-PCR within 35 days due to detection of presumable incomplete (defective) viral genomes, suggesting that reinfection was not detected during that time frame[5].

CDC is aware of recent scientific and media reports of cases of suspected SARS-CoV-2 reinfection among persons who were previously diagnosed with COVID-19[7–9]. However, these reports use different testing methods to ascertain reinfection. Because of the need for a common understanding of what constitutes reinfection, CDC proposes this common investigation protocol for identifying cases with a high index of suspicion for reinfection and suggests paired specimen testing using the following approaches.

Justification: Detecting confirmed or suspected SARS-CoV-2 reinfections is critical to public health control and related risk assessments. The possibility of reinfection could present challenges to controlling viral transmission within communities or within specific vulnerable populations. A better understanding of reinfection and the immune response to SARS-CoV-2 is also needed to inform vaccine planning efforts.

Intended use of study findings: Findings on the likelihood of reinfection will be used to guide future public health surveillance and prevention guidance for COVID-19. Additionally, confirmed or suspected SARS-CoV-2 reinfection case detection can inform future research into SARS-CoV-2 host immunity and vaccine development.

Study design: This protocol describes the use of public health surveillance of suspected SARS-CoV-2 reinfection cases to systematically investigate these cases and guide public health response. The protocol can be used to investigate both passively reported cases and those detected through routine queries on case-based surveillance data in which individuals with multiple test results are tracked over time. The protocol includes diagnostic testing of available specimens from distinct episodes of SARS-CoV-2 RT-PCR positivity as well as laboratory guidance and quality standards for genomic analysis.

Objectives: 1. Determine the frequency at which SARS-CoV-2 reinfection occurs among persons who appear to have recovered clinically from COVID-19. 2. Characterize suspected SARS-CoV-2 reinfection cases and resulting laboratory evidence to better understand the natural history of SARS-CoV-2 infection and guide public health response. 3. Determine the time interval from initial illness to reinfection.

Questions: What is the frequency with which SARS-CoV-2 reinfection occurs in humans? What is the interval between initial infection and reinfection, and what is the clinical course? Among confirmed reinfection cases, what is the duration of RT-PCR positivity and shedding of replication-competent virus? What is the serologic response to reinfection?

General approach: Descriptive epidemiology paired with genomic testing might be used to identify or support SARS-CoV-2 reinfection. Serial antibody determination and evidence of active viral replication might be used to provide additional support for and further characterize SARS-CoV-2 reinfections.

Procedures/Methods

DESIGN

Statement of purpose: This toolkit is designed to provide state and local health departments with the tools needed to investigate suspected cases of SARS-CoV-2 reinfection.

How investigational design meets objectives: This toolkit can be used in conjunction with surveillance (passive or active) for suspected cases of SARS-CoV-2 reinfection. Once the study population is identified, chart abstraction and reviews of existing surveillance reporting will be used to characterize suspected cases. Additionally, paired specimens might undergo confirmatory RT-PCR, viral culture, sgmRNA, and genomic sequencing to provide evidence of reinfection.

Description of risks: This research involves little to no risk to participants. Adherence to the HIPAA Privacy Rule and deidentification of collected data will ensure participant anonymity. If additional nasal wash specimens are collected, adverse effects are expected to be mild but could include nosebleeds and nasal irritation. If additional serum is collected, adverse effects are expected to be mild but could include hematoma or bruising. There is also minimal risk to the medical professionals. For sub-studies pursuing additional specimen collection we recommend following universal precautions and COVID-19 guidance on specimen collection and transport (Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens for COVID-19).

Description of anticipated benefits to the research participant: We anticipate that research participants will benefit from the improved COVID-19 prevention guidelines that will result from this research.

Description of the potential risks to anticipated benefit ratio: The potential risks posed by specimen collection are outweighed by the societal and individual benefit of enhanced surveillance and improved prevention guidelines that could reduce transmission of SARS-CoV-2 within communities.

STUDY POPULATION

Description and source of study population: The study population can include all individuals with a suspected or confirmed case of COVID-19 within the surveillance catchment area or the health department's jurisdiction.

Investigative criteria:

Prioritize persons with detected SARS-CoV-2 RNA ≥90 days since first SARS-CoV-2 infection:

Persons with detected SARS-CoV-2 RNA* ≥90 days after the first detection of SARS-CoV-2 RNA, whether or not symptoms were present

AND

Paired respiratory specimens (one from each infection episode) are available

*If detected by RT-PCR, only include if Ct value <33 or if Ct value unavailable

Consider persons with COVID-19–like symptoms and detection of SARS-CoV-2 RNA 45–89 days since first SARS-CoV-2 infection:

Persons with detection of SARS-CoV-2 RNA* ≥45 days after the first detection of SARS-CoV-2 RNA

AND

With a symptomatic second episode and no obvious alternate etiology for COVID-19–like symptoms **OR** close contact with a person known to have laboratory-confirmed COVID-19

AND

Paired respiratory specimens (one from each infection episode) are available

*If detected by RT-PCR, only include if Ct value <33 or if Ct value unavailable

Adaptation considerations:

- If resources are limited, further prioritize the sampling of persons in high-risk groups (e.g. healthcare workers).
- If investigating suspected reinfection cases among severely immunocompromised persons, consider a prospective study dedicated to this population, as results will not be generalizable to the general population.

Participant exclusion criteria:

- Laboratory specimen from either first or second illness episode is unavailable.

Estimated number of participants: The estimated monthly enrollment is expected to vary by jurisdiction, duration of local outbreak intensity, and referral testing operational factors. Consider taking these factors, as well as prior number of suspected SARS-CoV-2 cases reported, into account during local protocol adaptation.

Sampling: No *a priori* sampling will be undertaken; instead all suspected cases reported will be investigated per protocol. When necessary, eligibility criteria may be narrowed per adaptation considerations provided in this common investigation protocol.

Recruitment and Enrollment: Options for enrollment are as follow:

1. Passive surveillance: Cases reported to the health department that meet eligibility criteria
2. Active surveillance: Routinely analyze RT-PCR data with individual unique IDs over time to identify those with recurrent positive tests beyond the given time intervals
3. Once cases are identified, optionally enroll case-patients in a sub-study to characterize the clinical course of reinfection events.
4. If interested in investigating duration of viral shedding, presence of replication-competent virus, and serologic response to suspected reinfection, optionally enroll case-patients in a sub-study to collect serial respiratory and serum specimens.

Description and justification of reimbursements or incentives that will be used: Any reimbursements or incentives provided to participants are at the discretion of the institution using this protocol.

Statement of extra costs to participants due to involvement in the study: Participants may incur extra costs in the form of travel expenses and time lost to interviews. These costs will only be incurred if participants consent to the collection of additional nasal specimens and follow up interviews.

Procedures for implementing and documenting informed consent: Whenever appropriate, obtain informed consent from participants that require interviews for data collection, complete 14-day symptom logs, or enroll them in a sub-study for subsequent respiratory and serum specimen collection.

VARIABLES/INTERVENTIONS

Variables:

Demographics: Age (years), sex, race, ethnicity, occupation, and residence

Medical history: Immunomodulating agents and conditions, comorbidities, medications received for first episode and subsequent episode

Clinical course: Date of initial illness onset, date of initial clinical resolution, date of symptom onset or positive test for suspected reinfection, level of care received, duration of isolation, and complications

Diagnostic test results: Dates, type of testing, platform or laboratory assay used, site of specimen collection, and results (including Ct value) for all SARS-CoV-2 diagnostic tests

Epidemiologic data: Exposure history and residing in or visiting congregate settings

Extract these data from medical records, public health surveillance records, or interviews, and use descriptive epidemiology to characterize the suspected cases of reinfection

Specimen Collection:

Consider serial collection of respiratory specimens and sera for suspected cases of reinfection, detailed below.

Serial respiratory specimen collection: If participant is enrolled in a sub-study to investigate viral shedding and transmissibility, collect respiratory specimens daily for 7 days and then every other day for 7 additional days following the date of symptom recurrence or RT-PCR positive diagnosis of suspected reinfection (if asymptomatic).

Serial serum collection: Collect stored sera from first episode, any sera available between first and second episode, and sera available at the time of suspected reinfection. Collect sera at 3 days, 7 days, 14 days, 21 days and 6 weeks following suspected reinfection.

Study instruments:

Case report form (CRF) and data dictionary: Provided to facilitate systematic data collection [Appendix 1].

Training for all study personnel:

Prior to using the CRF, review the corresponding data dictionary to ensure that all data are collected properly.

DATA HANDLING AND ANALYSIS

Data analysis plan: Investigate all reported suspected cases, collect medical records for enrollees, abstract medical records using the attached CRF, and request the submission of paired specimens for each suspected case of reinfection. Data can be abstracted from medical records, existing surveillance data, or patient interviews. The CRF should be completed by trained state/local health department staff or clinical and academic partners. Regarding personal identifiable information (PII), the institution using this protocol should follow its institutional rules on how to collect, receive, store, and transmit this data to protect individuals' privacy. Descriptive epidemiology should be used to characterize the clinical course of primary infection and reinfection, as well as the interval between episodes/diagnoses.

Data collection: The CRF in Appendix 1 should be used for chart reviews. The CRF can be printed and filled out by hand, or it can be built into an electronic data collection platform (EpiInfo, REDCap, Microsoft Access, etc.). If data is collected by hand, data entry into an electronic database will be necessary.

Information management and analysis software: Data management and analysis software may include EpiInfo, REDCap, Microsoft Access, Microsoft Excel, SAS, SPSS, STATA, Python, R, or others.

Bias in data collection, measurement and analysis: Bias can be introduced into this protocol when data are collected by different data abstractors or institutions. Providing training on the proper use of the CRF and data dictionary for all data collection staff prior to implementing this protocol will facilitate systematic data collection. Abstracting records from different medical systems might introduce bias in record quality or medical management between facilities. Stratifying by data abstractor and medical system will help to assess and control for these potential biases.

Limitations of study: This protocol will be limited by the exclusion of individuals who remain asymptomatic or experience mild symptoms and never seek testing for SARS-CoV-2. Another major limitation is the availability of paired specimens in a retrospective framework, as specimens might not be regularly stored >3 months. This protocol might not be able to identify people who sought care in different medical facilities for their distinct episodes of COVID-19. The quality of data collected on clinical course will also be dependent upon the quality of the medical records. The use of this protocol to facilitate a case series will likely result in a small sample size from a convenience sample and will not provide a representative sample for examining risk factors for reinfection. Lastly, the protocol does not include the collection of specimens that would allow for examination of shedding and transmissibility during reinfection.

Anticipated products: We anticipate that the data collected using this toolkit will be used to inform the public health response efforts to the COVID-19 pandemic.

LABORATORY TESTING & INTERPRETATION

Laboratory testing:

Respiratory specimens should be tested by RT-PCR or other nucleic acid amplification tests to detect viral RNA (Ct values reported) and genomic sequencing to compare strains across episodes. Viral culture and sgmRNA can be used to determine the presence or absence of replication-competent virus. If serum is available, also consider serologic testing to determine the immunologic response to initial infection and to suspected reinfection.

If interested in investigating cases in which the initial illness specimen is not available, consider the same laboratory testing, with the exception of genomic sequencing. Genomic sequencing of the suspected reinfection specimen, in the absence of a paired respiratory specimen or detailed knowledge of the circulating SARS-CoV-2 strains during the first SARS-CoV-2 illness or infection, is not recommended.

Genomic sequencing of paired specimens—that meet the quality criteria below—is needed to investigate reinfection. Single nucleotide polymorphism analysis alone might not be sufficient to distinguish reinfection from long-term shedding, as intra-host variation in the mutation rate of SARS-CoV-2 is poorly understood. However, identification of paired specimens from distinct lineages (as defined in Nextstrain or GISAID) serves as higher quality evidence for SARS-CoV-2 reinfection. The quality criteria for testing and levels of evidence are described in more detail below.

Genomic testing should meet the following quality criteria for investigation for reinfection with SARS-CoV-2:

- Genome coverage >100/per base position is recommended for consensus generation
- Q score of consensus >30 with 99% of the genome covered
- 1000x average genome coverage recommended for analysis of minor variation
- Removal of amplicon primer contamination from assembly
- Use of high-fidelity sequencing platforms (Q score per read >30) preferred for consensus generation
- If low fidelity sequencing platforms (Q score per read <30) are used, verification of SNPs via alternate sequencing method is encouraged

Support for but not definitive evidence of reinfection can be provided by other information, such as culture or sub-genomic mRNA analysis (to detect the presence of replication-competent virus) or serology, which could be useful to document a serologic response to SARS-CoV-2. Aside from laboratory evidence, other supporting evidence for reinfection could include clinical course (COVID-19–like symptoms) and epidemiologic links to a confirmed case.

Laboratory evidence:

Levels of evidence for reinfections using genomic data are as follows:

Best evidence

Differing clades as defined in Nextstrain and GISAID of SARS-CoV-2 between the first and second infection, ideally coupled with other evidence of actual infection (e.g., high viral titers in each sample or positive for sgRNA, and culture)

Moderate evidence

>2 nucleotide differences per month* in consensus between sequences that meet quality metrics above, ideally coupled with other evidence of actual infection (e.g., high viral titers in each sample or positive for sgmRNA, and culture)

Poor evidence but possible

≤2 nucleotide differences per month* in consensus between sequences that meet quality metrics above or >2 nucleotide differences per month* in consensus between sequences that do not meet quality metrics above, ideally coupled with other evidence of actual infection (e.g., high viral titers in each sample or positive for sgmRNA, and culture)

* The mutation rate of SARS-CoV-2 is estimated at 2 nucleotide differences per month, therefore if suspected reinfection occurs 90 days after initial infection, moderate evidence would require >6 nucleotide differences.

Guidance for Isolation Recommendations

Please refer to local public health authority guidance and consider referring to CDC guidelines at: https://www.cdc.gov/coronavirus/2019-ncov/hcp/duration-isolation.html

Appendix 1

SARS-CoV-2 Reinfection Case Investigation Form

COVID-19 Data Dictionary: Common Investigation Protocol

References

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Last Updated Oct. 27, 2020

Content source: National Center for Immunization and Respiratory Diseases (NCIRD), Division of Viral Diseases