

Evaluating the Presence of Replication-Competent SARS-CoV-2 from Nursing Home Residents with Persistently Positive RT-PCR Results

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Abstract

Replication-competent virus has not been detected in individuals with mild to moderate COVID-19 more than 10 days after symptom onset. It is unknown whether these findings apply to nursing home residents. Of 273 specimens collected from nursing home residents >10 days from the initial positive test, none were culture positive.

Keywords: COVID-19, SARS-CoV-2, viral culture, nursing home, RT-PCR

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Background

The duration of infectiousness of persons infected with Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has important implications for infection control practice including when transmission-based precautions can be discontinued. Individuals can test positive with a nucleic acid amplification test (NAAT) for weeks after the onset of coronavirus disease 2019 (COVID-19) symptoms [1-3]. However, replication-competent virus has not been detected in individuals with mild to moderate COVID-19 more than 10 days after symptom onset [1, 2, 4, 5]. There are instances of the detection of replication-competent virus between 10-20 days in individuals with severe COVID-19 [6]. There are also reports of severely immunocompromised individuals (e.g., hematopoietic stem-cell transplant recipients) with detectable replication-competent virus beyond 20 days [7]. The Centers for Disease Control and Prevention (CDC) interim guidance on the discontinuation of transmission-based precautions recommends a symptom- and time-based strategy with specific recommendations and considerations based on illness severity and whether the patient is severely immunocompromised [8]. The studies supporting this recommendation have not focused on nursing home residents [1, 2, 5, 6].

Nursing home residents have been significantly impacted by the COVID-19 pandemic and outbreaks with high case fatality rates have been reported [4]. The nursing home resident population is unique for several reasons. Residents experience immunosenescence due to their age, frequently have multiple medical comorbidities, and are often frail. Symptom ascertainment is often unreliable due to dementia or other contributing factors [4, 9]. In addition, SARS-CoV-2 can spread rapidly amongst nursing home residents who often have roommates at high risk for severe disease. Therefore, appropriate initiation and discontinuation of transmission-based precautions in this population are critical. More data

are needed to assess whether the duration of infectiousness with SARS-CoV-2 differs in nursing home residents. In this investigation, we sought to assess whether replication-competent virus could be detected in nursing home residents with persistently positive real-time reverse transcription-polymerase chain reaction (RT-PCR) test results.

Methods

We obtained upper respiratory specimens from nursing home residents in two ways. First, CDC collected specimens by recruiting nursing homes that had experienced prior COVID-19 outbreaks. Six nursing homes from four states participated in this targeted sample. Each facility provided a line list of residents that had previously tested positive, and CDC collected nasopharyngeal swab specimens from these individuals at various time points from their initial positive result. Additional data collected included patient age, sex, and date of initial positive NAAT result. All specimens underwent testing for SARS-CoV-2 at CDC using the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel (CDC) [10]. Virus culture was attempted for all RT-PCR positive specimens with an N1 (nucleocapsid) target cycle threshold (Ct) value <34. The viral culture procedure was described previously[11].

A second group of specimens were collected by the Minnesota Department of Health (DOH). These specimens were collected during COVID-19 outbreak investigations in 93 nursing homes. These investigations included serial testing of test-positive residents, and thus this collection included multiple specimens from the same patient (i.e., the initial positive specimen and subsequent positive specimens). DOH Public Health Laboratory (PHL) queried their laboratory information system to identify cases with repeat positive test results. Data on patient age, sex, date of initial positive NAAT result, and an average of the N1 and N2 Ct values were provided. All specimens that tested positive were subsequently frozen at -20°C

and then provided to CDC where they underwent repeat testing for SARS-CoV-2 using the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel (CDC); specimens with an N1 Ct value <34 underwent attempted viral culture [10].

This activity was reviewed by CDC and was conducted consistent with applicable federal law and CDC policy.¹

Results

CDC collected 108 nasopharyngeal swabs from the targeted sample. There were 63 females (58.3%) and the median patient age was 78 (range: 49 to 98). The time from the initial positive NAAT to the study specimen collection was a median of 25 days (range: 4 to 63 days). There were 61 positive (56.5%), 36 negative (33.3%), and 11 inconclusive (10.2%) results. Of the 61 positive results, 36 (59.0%) had an N1 Ct value <34. Only one specimen was culture positive (0.9%); the specimen was collected 4 days after the initial positive test.

The Minnesota DOH collected and submitted 253 specimens from 73 patients for this study. There were 45 females (61.6%) and the median patient age was 75 (range: 34 to 98). The initial positive specimen was present for 72 of the 73 residents. Including the initial specimen, each resident submitted a median of 3 specimens (range 1 to 6). Excluding the initial positive specimen, the time from initial positive NAAT to the subsequent specimen collection was a median of 23 days (range: 1 to 63 days). Upon retesting at CDC, there were 185 positive (73.1%), 57 negative (22.5%), and 11 inconclusive (4.3%) results. Ct data from the Minnesota DOH NAAT were available for 250 of 253 specimens; the 184 specimens positive at CDC had a mean Ct of 29.7 when tested previously, the 11 specimens

¹ See e.g., 45 C.F.R. part 46.102(l)(2), 21 C.F.R. part 56; 42 U.S.C. §241(d); 5 U.S.C. §552a; 44 U.S.C. §3501 et seq.

inconclusive at CDC had a mean Ct of 35.3 previously, and the 55 specimens negative at CDC had a mean Ct of 36.6 previously. Of the 185 positive results, 116 (62.7%) had an NI Ct value <34; virus culture was attempted in 110 samples (6 had insufficient volume of specimen left). There were 25 specimens that were culture positive; 23 were an initial positive specimen, one was collected 1 day after the initial positive specimen, and one was collected 8 days after the initial positive specimen.

When combining data from specimens collected by the two sources, there were 361 specimens from 181 residents. There were 246 (68.1%) positive specimens; 26 were culture positive (7.2%) (Figure 1). Excluding the initial positive specimens, there were 289 specimens; 177 were RT-PCR positive (61.2%). Of these, 3 were culture positive (1.0%) (1, 4, and 8 days after the initial positive specimen). In total, there were 273 specimens with >10 days between the initial positive NAAT and the study specimen; 163 (59.7%) were RT-PCR positive but none were culture positive.

Discussion

Several studies have evaluated the presence of replication-competent virus with varying durations after symptom onset. Replication-competent virus has only been detected within 10 days of symptom onset for patients with mild to moderate COVID-19; it has been detected beyond 10 days in patients with severe COVID-19 and/or severe immunocompromising conditions [1, 2, 4-7]. We performed a study to determine whether nursing home residents with persistently positive RT-PCR results had replication-competent virus. Of 273 specimens from residents collected beyond 10 days after the initial positive test, none were culture positive.

Our findings support the current CDC interim guidance for the discontinuation of transmission-based precautions and disposition of patients with COVID-19 in healthcare settings which indicates a preference for a strategy based on symptoms and time, and that, just as for the general population, a test-based strategy is not necessary for most nursing home residents [8]. Also, our data support the recommendation that retesting asymptomatic individuals in the 90 days after illness onset is unlikely to yield useful information (i.e., any positive RT-PCR test likely represents persistent shedding of viral RNA rather than a new infection) [12].

There are several limitations to our study. We did not collect symptom or severity information about the initial illness or data on the presence of resident comorbidities. Thus, we could not measure time from symptom onset to the study specimen collection, we were not able to classify residents as having mild, moderate, or severe COVID-19, and it is not known whether any residents had severe immunocompromising conditions. However, symptom data can be difficult to ascertain in nursing home residents [4, 9]. Furthermore, many nursing home residents are undergoing screening testing and thus SARS-CoV-2 is being detected when residents are either asymptomatic or presymptomatic. Thus, we think our findings will likely be applicable to the nursing home resident population more broadly. Another limitation is the use of virus culture as a surrogate for infectiousness. In this study, we recovered virus only from specimens with an N1 Ct <23, whereas in other studies, virus has been recovered from specimens with an N1 Ct of up to 34 [4]. Many factors can contribute to whether virus can be recovered in culture, and therefore it is difficult to determine why virus was not recovered from any particular specimen. We believe that any positive culture indicates the presence of replication-competent virus, and thus likely

infectiousness, although we acknowledge a negative culture does not rule out infectiousness (i.e., loss of viability due to transport, storage, or other factors).

In summary, we tested 361 specimens from nursing home residents, and 273 of these specimens were collected 11 to 63 days after the initial positive specimen. Similarly, to what others have found, persistently positive RT-PCR results beyond 10 days were common [1-3]. However, we found no replication-competent virus amongst any of the specimens beyond 10 days of the initial positive test.

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Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

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Potential Conflicts of Interest

The authors have no reported conflicts of interest.

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Figure Legend

Cycle threshold (Ct) values and culture results for RT-PCR positive specimens (n=246). The dotted line represents a Ct value of 34 which was the cutoff for specimens to undergo attempted viral culture.

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Figure 1

