**Kinetics of SARS-CoV-2 Shedding in *N*ursing *H*om*e* Staff and *R*esid*ent*s (INHERENT) Study**

The INHERENT study was conducted as part of the Nursing Home Public Health Response Network (NH PHRN). The NH PHRN serves as a national public health research and surveillance network and is composed of a diverse network of academic sites that have nursing homes across the United States (U.S.) within their network. Established in September 2022, the NH PHRN includes eight U.S.-based academic institutions (study sites) with expertise in NH research. Working within their existing NH research networks, each study site identified NHs willing to participate in the national NH PHRN. There are currently eighty-seven nursing homes participating in the NH PHRN network, though not all nursing homes participated in the INHERENT study. The NH PHRN is funded by the U.S. Centers for Disease Control and Prevention (CDC; contract number 75D30121D12704).

The studies objectives were:

1. ***Primary objective*:** Characterize the kinetics of SARS-CoV-2 shedding including proliferation, peak, and clearance using qRT-PCR, antigen testing, genetic sequencing, and culture among NH residents and staff using different testing approaches and examine the influence of different factors including participant characteristics (e.g., vaccination status, prior infection, symptom status, receipt of treatment) and circulating variant.
2. ***Sub-study objective:*** Assess the long-term (>14 days) duration of SARS-CoV-2 positivity using qRT-PCR, antigen testing, genetic sequencing, and culture.

Enrollment

Each of the eight study sites identified nursing homes to participate in the network. There are currently eighty-seven nursing homes participating in the full NH PHRN network. A subset of the nursing homes in the full NH PHRN network were recruited to participate in the INHERENT study (twenty-four total).

Residents [or their legally authorized representatives (LAR)] and staff were recruited, screened, consented, and enrolled into the INHERENT study prior to or at the first detection of positive tests in the facility. Potential participants were excluded if they had impaired decision-making capacity and no legally authorized representative or were unable to communicate in English.

Following consent, study staff used a standardized REDCap form to abstract demographic, general health, and clinical information (including SARS-CoV-2 infection, treatment, and vaccination history) from NH records for residents. Study staff administered a questionnaire to the residents (or LAR) to obtain key information not readily available from the NH record. Participating NH staff received a short questionnaire via email or text to self-complete. Alternatively, NH staff completed a paper form, or the study staff aided in form completion including administration of the survey. The NH staff questionnaire included demographic information, presence of clinical conditions, SARS-CoV-2 infection and treatment history, SARS-CoV-2 vaccination history, information related to role, work hours, and work at other facilities.

The report a new outbreak, nursing home staff at participating NHs notified the site study team within 24 hours of identifying a positive case of SARS-CoV-2 in any resident or staff of the facility. The study team assisted the facility with outbreak testing on the affected unit(s), which included testing of NH residents and staff with both SARS-CoV-2 antigen and qualitative and quantitative RT-PCR for SARS-CoV-2 on days 1, 3, and 5 after exposure per public health recommendations. However, some NH chose to do their own testing using their standard approach which in most cases, was only antigen testing. Roommates of infected residents were tested daily with both antigen and RT-PCR from days 1-7 after exposure. Additional participants, including residents or staff who tested positive as part of nursing home surveillance or during an outbreak response who were not already consented, were recruited, screened, consented, and enrolled during outbreak testing.

SARS-CoV-2 Testing Approach Using a “Tiered approach”

Ongoing, systematic collection, analysis, and interpretation of SARS-CoV-2-related data were collected as part of four tiers (Tier 0 through Tier 3) (Figure 1).

**Figure 1. Diagram of study tiers and testing at each tier.**

A screenshot of a computer screen

Description automatically generated

**Tier 0** – Consisted of routine testing of NH residents and staff performed by NH staff based on current NH policies and procedures (e.g., testing done for all newly admitted residents, symptomatic residents or staff, or asymptomatic screening of staff during high community transmission). NHs notified the site study team within 24 hours of any positive test in a NH resident or staff member. Study staff then visit the affected facility within 24 hours of notification.

**Tier 1** - Consisted of rapid antigen and RT-PCR testing conducted on alternate days for all potentially exposed residents and staff in the affected unit (or the facility, if appropriate based on exposures) for 5 days (i.e., on days 1, 3, and 5). Site study staff supported Tier 1 testing, which occurred regardless of consent in line with CDC guidance for potentially exposed individuals.1 Testing with both antigen and RT-PCR was conducted to ensure rapid identification of new cases and to maximize sensitivity to detect new infections. Some NHs elected to conduct their own outbreak testing (usually antigen only testing). Data on antigen test results and presence of any symptoms suggestive of SARS-CoV-2 were collected at each testing occurrence. Site study staff also collect daily swabs from consented roommates of residents who test positive for rapid antigen test and RT-PCR for 7 days to ensure rapid identification of new cases among those at highest risk of being infected.

**Tier 2** – Consisted of antigen and RT-PCR testing for consented participants with either a positive test identified in Tiers 0 or 1**.** During Tier 2, site study staff collected specimens daily from participants for 14 days. Select specimens also underwent genetic sequencing and culture (described below). At the end of the Tier 2 period, study staff asked enrolled tier 2 participants to consent to continue participation in **Tier 3,**

**Tier 3** - For those who continued into Tier 3, site study staff collect biweekly (approximately every 3-4 days) swabs for antigen and RT-PCR testing from days 15-30 and then weekly from days 31-90 after the first positive test. A questionnaire documenting treatment and hospitalization was performed every two weeks for participants in Tiers 2 and 3 using the same administration approaches as described for the enrollment questionnaire.

Specimen Collection and Testing

During each testing occurrence, two anterior or mid-turbinate nasal swabs were collected using standard methods. Site study staff collected the swabs from NH residents and used one of the swabs to conduct on-site antigen testing per the manufacturer instructions and recorded the result in a REDCap data entry form. Enrolled NH staff self-collected their own swabs and performed antigen testing themselves using the manufacturer instructions and then entered their result directly into the REDCap web form that was sent via text message or email.

For both NH residents and staff, the other nasal swab was shipped to Marshfield Clinic Research Institute. The second nasal swab was tested by RT-PCR assay using the TaqPath COVID-19, FluA, FluB Combo Kit on QuantStudio 7 Pro which provides results for SARS-CoV-2, influenza A, and influenza B. Specimens with a SARS-CoV-2 Ct value less than 30 undergo qRT-PCR using the CDC Influenza SARS-CoV-2 (Flu SC2) Multiplex Assay on ABT 7500 Fast Dx.

For NH residents participating in Tier 2 (ongoing virologic monitoring on days 1-14), viral culture was performed on one RT-PCR positive specimen with a Ct value less than 30 collected from each of set of days 1-2, 3-5, 6-8, 9-11, and 12-14. If there were multiple samples that were positive with a cycle threshold (Ct) value less than 30 in one time period, culture was performed on the specimen with the highest viral load, as determined by qRT-PCR. For NH residents and staff in Tier 3 (long-term virologic monitoring), viral culture was performed on any RT-PCR positive specimen from days 31-90. Viral culture was performed by St. Jude Children’s Research Hospital (Memphis, TN).

Specimens with an RT-PCR Ct value less than 30 were also eligible for whole genome sequencing (WGS). For enrolled NH residents and staff participating in daily testing (Tier 2), the first specimen with an RT-PCR Ct value less than 25 underwent WGS. If no specimen from Tier 2 testing had a Ct value less than 25, but at least one specimen had a Ct value lower than 30, the specimen with the Ct value closest to 25 was sequenced. For those in Tier 3, the last positive specimen with a Ct value less than 30 received WGS and additional positive specimens were tested. Additionally, WGS was repeated if there is a concern that the participant has a new infection based on clinical (e.g., development of new COVID-19 symptoms) or epidemiological (e.g., new exposure) factors. The remaining aliquots of specimens were stored for potential future testing.

Data Management and Quality

The REDCap project used several approaches to ensure high-quality data including branching logic, data validation, range checks, and automated skip patterns. Additionally, study staff performed regular, frequent data quality checks and study sites reviewed the results of these checks and verified or corrected the data if necessary.

Ethical Approval and Considerations

The study protocol has been reviewed and approved by the Abt IRB, which is the IRB of record for each study site. All individuals participating in Tiers 2 and 3 provide informed consent. Small stipends were given to these participants for completing study activities. Stipend amounts and format were determined on a site-specific basis so that study sites could ensure that they were context-appropriate and complied with institutional- and state-specific policies and regulations regarding compensation from participating in research studies.