Environmental sampling for SARS-CoV-2 in long term care facilities: lessons from a pilot study [version 2; peer review: 2 approved]

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First published: 15 Sep 2021, 6:235
https://doi.org/10.12688/wellcomeopenres.17047.1
Latest published: 28 Jun 2023, 6:235
https://doi.org/10.12688/wellcomeopenres.17047.2

Abstract

Background: The SARS-CoV-2 pandemic has highlighted the risk of infection in long-term care facilities (LTCF) and the vulnerability of residents to severe outcomes. Environmental surveillance may help detect pathogens early and inform Infection Prevention and Control (IPC) measures in these settings.

Methods: Upon notification of SARS-CoV-2 outbreaks, LTCF within a local authority in South West England were approached to take part in this pilot study. Investigators visited to swab common touch-points and elevated ‘non-touch’ surfaces (>1.5m above ground level) and samples were analysed for presence of SARS-CoV-2 genetic material (RNA). Data were collected regarding LTCF infrastructure, staff behaviours, clinical and epidemiological risk factors for infection (staff and residents), and IPC measures.

Criteria for success were: recruitment of three LTCF; detection of SARS-CoV-2 RNA; variation in proportion of SARS-CoV-2 positive surfaces by sampling zone; and collection of clinical and epidemiological data for context.

Results: Three LTCFs were recruited, ranging in size and resident demographics. Outbreaks lasted 63, 50 and 30 days with resident attack rates of 53%, 40% and 8%, respectively. The proportion of
sample sites on which SARS-CoV-2 was detected was highest in rooms occupied by infected residents and varied elsewhere in the LTCF, with low levels in a facility implementing enhanced IPC measures. The heterogeneity of settings and difficulty obtaining data made it unfeasible to assess association between environmental contamination and infection. A greater proportion of elevated surfaces tested positive for SARS-CoV-2 RNA than common touch-points.

Conclusions: SARS-CoV-2 RNA can be detected in a variety of LTCF outbreak settings, both on common-touch items and in elevated sites out of reach. This suggests that further work is justified, to assess feasibility and utility of environmental sampling for infection surveillance in LTCF.

Keywords
infection control; infectious disease transmission; environmental exposure; fomites; disease outbreaks; long-term care; epidemiologic methods
Introduction

Long term care facilities (LTCF) are inadvertently ideal environments for the spread of pathogens. (Strausbaugh et al., 2003) Residents are often susceptible to infection or colonisation, and in frequent and close contact with staff who have links to the wider community. Outbreaks of infectious diseases are common in these settings (Inns et al., 2017; Inns et al., 2019) and the coronavirus 2019 (COVID-19) pandemic, caused by SARS-CoV-2 coronavirus, has highlighted the vulnerability of people in LTCF to infectious disease threats: almost 30,000 excess deaths are thought to have occurred among LTCF residents in England over the first 23 weeks of the epidemic. (Morciano et al., 2021)

If detected early enough transmission of infections within the LTCF can be curbed, (Inns et al., 2018) however SARS-CoV-2 infections are often asymptomatic or paucisymptomatic leading to large outbreaks. Regular clinical testing of residents and staff helps identify cases early but is resource-intensive and unpleasant for frail individuals, so non-invasive surveillance strategies may be more sustainable. Respiratory pathogens have been identified from surfaces in various healthcare and non-healthcare settings: typically on objects that have been contaminated by touch, but also surfaces such as window ledges, ventilation grilles and ventilation filters which airborne pathogens will settle on. (Goyal et al., 2011; Moore et al., 2021; Santarpia et al., 2020). The authors also identified SARS-CoV-2 RNA on the top of staff lockers in a hospital setting and a university workshop in the early phases of the pandemic (unpublished data). Swabbing common-touch and non-touch surfaces within LTCF could signal the presence of infection as well as providing insights into how the virus is transmitted, which can inform infection prevention and control (IPC) measures. According to a World Health Organization scientific brief on transmission of SARS-CoV-2 (2020), direct (droplet) transmission and indirect spread via fomites (contaminated surfaces) and long-distance aerosols are thought to occur; however there is no conclusive evidence for indirect transmission in LTCF. (Ben-Shmuel et al., 2020; Greenhalgh et al., 2021; Ong et al., 2020; Ye et al., 2020)

COVID-19: Detecting Indirect Transmission in Facilities for Enhanced Care sTudy (COVID-19: DISinFECT) aims to investigate the role of indirect transmission of SARS-CoV-2 in LTCF and assess whether environmental surveillance could inform IPC measures in these settings. The idea was to conduct environmental sampling (surface swabs, air sampling and wastewater) during outbreaks of SARS-CoV-2 infection in LTCFs and assess environmental dispersal of SARS-CoV-2 RNA in the context of a) infections identified through enhanced clinical testing of residents and staff; b) clinical and epidemiological risk factors for infection; and c) different infection prevention and control measures. We present findings from a pilot conducted between 14th January and 28th March 2021, during the second epidemic wave in South West England.

Methods

The full DISinFECT protocol can be accessed online. (Kwiatkowska & Ready, 2021) LTCFs were eligible for inclusion if they provided residential care for older adults (>65 years), were within the boundaries of a selected local authority in the UKHSA (UK Health Security Agency, formerly Public Health England South West region, and experienced a COVID-19 outbreak: defined as two or more laboratory-confirmed cases among staff and/or residents within a 14-day period.

Recruitment

On notification of an outbreak, investigators contacted the LTCF manager with information about DISinFECT and offered environmental sampling as part of outbreak management. If managers expressed an interest, they were asked to complete a written consent form permitting the study team to conduct telephone interviews, collect information from LTCF records, sample the LTCF environment and approach residents and staff for involvement. Prior to the sampling visit, residents and staff were provided with written and pictorial leaflets describing the purpose of the investigations, sampling procedures and how their information would be processed. Each of the residents selected for sampling was consulted to make sure they understood this information and were happy to provide samples. Sampling was not carried out if the individual lacked mental capacity to complete a consent form. Staff were asked for consent to participate prior to accessing the electronic questionnaire.

Sampling

Approach. Settings varied in size and layout but sampling was done systematically, with a focus on a) common touch points (for example: door handles, light switches, television remote controls) and b) elevated surfaces above 1.5m in height, which were unlikely to be touched or regularly cleaned, onto which airborne virus might settle (for example: door sills, tops of wall-mounted cabinets).

Within each LTCF, sampling sites were categorised in to three ‘zones’: 1) rooms occupied by residents isolating with active SARS-CoV-2 infection, or equipment used by them, 2) areas/equipment used by both staff and residents such as lounges and dining areas, shared kitchen equipment, and 3) staff-only areas/equipment such as offices, recreation areas, and key cabinets.
Materials. The majority of surfaces were sampled using Sterillium flocked swabs, supplied in tubes containing 2mL of universal transport medium which was used to wet the swab prior to sampling. Larger areas (e.g., benches/ tabletops) were sampled using pre-moistened TSC Enviroscreen sterile cellulose sponges measuring 50cm², supplied in stomacher pouches containing 10mL neutralising buffer. Wetted swabs were also used to sample from the fingertips of residents in isolation rooms. All samples were transported to a public health laboratory specialising in aerobiology, biocontainment and biosafety measures.

The full DISinFECT protocol includes sampling stool and saliva from resident cases and air and wastewater sampling from the facility. For logistical reasons, stool and saliva could not be taken during this pilot. Likewise, no air sampling was conducted, and wastewater was collected from only one facility; results will be reported separately.

Laboratory analysis

Extraction. From each swab/ sponge container, we took a 140μL aliquot of the transport medium/ neutralising buffer. Viral RNA was extracted using a QIAamp Viral RNA Mini Kit (Qiagen Ltd, Manchester, UK), according to the manufacturer’s instructions. From 60μL eluate, 5μL extracts were used for analysis. Each set of around 15 extractions included at least one negative (ethanol) extraction control.

Analysis. Each sample was analysed in duplicate using a Reverse Transcription Polymerase Chain Reaction (RT-PCR) assay targeting both the N gene and the ORF1ab gene of SARS-CoV-2 (Viasure, CerTest Biotec, Zaragoza). We used an Applied Biosystems QuantStudio 5 thermocycler.

RT-PCR results were reported in cycle threshold (Ct) numbers. A sample was classified as ‘positive’ for SARS-CoV-2 if amplification of one or both targets was detected in both duplicates; ‘suspect’ if detected in only one duplicate and ‘negative’ if no amplification was detected for either gene target. A Ct value of 39 was used as the cut-off point, as in similar publications and sampling studies in hospitals and care homes. (Dumont-Leblond et al., 2021; Moore et al., 2021; Nelson et al., 2021).

RT-PCR was repeated (in duplicate) for ‘suspect’ samples. If repeat RT-PCR detected amplification of a gene target in both duplicates, then the ‘suspect’ sample was reclassified as ‘positive’.

A PCR reaction efficiency internal control was included in the qPCR mastermix, to assess for inhibition. If duplicate Ct values of the internal control had a standard deviation (SD) of >0.5, qPCR was repeated.

Quantification was carried out for swab samples; methods and results are available with underlying data (see below). This paper will focus only on detection or absence of SARS-CoV-2 RNA.

Whole Genome Sequencing

Whole genome sequencing (WGS) was conducted on a subset of clinical isolates from nose/ throat swabs, to identify SARS-CoV-2 variants and mutations of interest or concern.

Epidemiological data collection

In addition to sampling, the study team recorded details of LTCF layout, cleaning and IPC measures, and staff and resident behaviours. Clinical test results (nasopharyngeal swabs analysed with PCR) were obtained from the regional public health laboratory and LTCF managers provided additional information about clinical case notifications, resident risk factors for infection and IPC measures. Finally, staff members were sent an electronic questionnaire asking questions about exposures and risk factors for infection.

Criteria for success in the pilot are presented in Box 1.

Box 1. DISinFECT pilot criteria for success

- a) Recruitment of three LTCF and consent to participate from at least one resident per facility;
- b) Detection of SARS-CoV-2 RNA from surface sampling;
- c) Variation by sampling zone in the proportion of sample sites testing positive for SARS-CoV-2 RNA;
- d) Potential to assess transmission risk from environmental contamination, in the context of individual risk factors for infection.

Ethical considerations

These investigations were carried out as part of a public health response to the SARS-CoV-2 pandemic. Ethical approval was granted on 14th January 2021 by the Public Health England Research Ethics and Governance Group (PHE REGG: RD 415).

Results

Four LTCFs were approached on notification of an outbreak: one declined to participate as they did not have the capacity to support recruitment and sampling. Three LTCFs were sampled between 2nd February and 10th March 2021 and a total of 84 environmental swabs were taken (56 from common touch points, 28 from elevated sites). One LTCF had two sampling visits, 14 days apart. For simplicity, we have labelled the L A, B and C in order of sampling dates. Table 1 contains the full list of sampling sites.

Settings and population

LTCF sizes ranged in size: there were 40 beds in facility A, which was a self-contained unit within an 80-bedded LTCF, 16 beds in facility B and 13 beds in facility C. The number of occupants was 30 (A), 15 (B) and 12 (C) on the date of onset of the first case. Facility A was a short stay residential unit with clients aged between 55 and 98 years; facility B a residential LTCF for older adults (65 and over) with and without dementia; facility C a residential LTCF for adults with learning difficulties aged between 35 and 88 years. All residents were included in the epidemiological analysis, regardless of age. All residents slept in single occupancy rooms; residents...
| Facility A: sampling sites | * | Ct (N) | SARS-CoV-2 | Facility B: sampling sites | * | Ct (N) | 2nd visit | SARS-CoV-2 | 2nd visit | Facility C: sampling sites | * | Ct (N) | SARS-CoV-2 |
|---------------------------|---|-------|------------|---------------------------|---|-------|----------|------------|----------|---------------------------|---|-------|------------|
| Female staff changing room |   |       |            | Staff toilet              |   |       |          |            |          | Staff office               |   |       |            |
| 1. Top of lockers (next to door) | E | ND    | Negative   | 1. Air vent/ extractor    | E | ND    | ND       | Negative   | ND       | 1. Top of key cupboard   | E | ND    | Negative   |
| 2. Toilet door: inside handle and lock | T | ND    | Negative   | 2. Soap dispenser lever   | T | ND    | ND       | Negative   | ND       | 2. Computer mouse         | T | ND    | Negative   |
| 3. Air vent/ extractor (toilet) | E | ND    | Negative   | 3. Door ledge (outer)     | E | 36.80, 36.15 | ND       | Positive   | Negative   | 3. Top of message board | E | ND    | Negative   |
| 4. Bench top               | T | ND    | Negative   | Manager’s office          |   |       |          |            |          | 4. Telephone receiver     | T | ND    | Negative   |
| 5. Soap dispenser lever    | T | 39.06 | Negative   | 4. Computer mouse         | T | ND    | ND       | Negative   | Negative   | Shared toilet (staff/ residents) |   |       |            |
| Dining room (staff)        |   |       |            | Laundry room              |   |       |          |            |          |                           |   |       |            |
| 6. Top of TV               | E | ND    | Negative   | 5. Air vent/ extractor    | E | ND    | ND       | Negative   | ND       | 5. Air vent/ extractor    | E | ND    | Negative   |
| 7. Top of trolley (for lunch trays) | E | ND    | Negative   | 6. Glove box (size small) | T | ND    | N/A      | Negative   | N/A      | Reception area            |   |       |            |
| 8. Radio dials             | T | ND    | Negative   | Shared toilet (staff/residents) |   |       |          |            |          |                           |   |       |            |
| Sluice room                |   |       |            | 7. Toilet booster handles | T | ND    | ND       | Negative   | ND       | 7. Front door keypad (inside) | T | ND    | Negative   |
| 9. Code pad (door exterior) | T | ND    | Negative   | 8. Air vent/ extractor    | E | 35.97, 36.46 | ND       | Positive   | Negative   | 8. Top of kitchen door frame (outside) | E | ND    | Negative   |
| Breakout room (staff)      |   |       |            | 9. Toilet booster underside | T | ND    | ND       | Negative   | ND       | 9. Air vent/ extractor    | E | ND    | Negative   |
| 10. Chair arms             | T | ND    | Negative   | Dining area (residents & staff) |   |       |          |            |          | 10. Fire exit sign        | E | ND    | Negative   |
| 11. Air vent/ extractor    | E | ND    | Negative   | 10. Tablecloth            | T | 36.99 | ND       | Suspect    | Negative   | 11. Door handle (exit to reception) | T | ND    | Negative   |
| 12. Light switch           | T | ND    | Negative   | 11. Tea trolley handles   | T | ND    | ND       | Negative   | Negative   | 12. Bannister             | T | ND    | Negative   |
| Corridor                   |   |       |            | 12. Armchair handles      | T | ND    | ND       | Negative   | Negative   | Lounge/ shared kitchen area |   |       |            |
| 13. UV cabinet handle      | T | ND    | Negative   | 13. Top of picture frame | E | 37.05 | ND       | Suspect    | Negative   | 13. Top of kitchen hatch  | E | ND    | Negative   |
| Photocopier room           |   |       |            | 14. TV remote control     | T | 37.45, 37.62 | ND       | Positive   | Negative   | 14. Chair arms            | T | ND    | Negative   |
| 14. Photocopier digital pad | T | ND    | Negative   | Bedroom/ bathroom         |   |       |          |            |          | 15. Kettle handle & switch | T | ND    | Negative   |
| Bedroom/ bathroom #1       |   |       |            | 15. Top of wardrobe       | E | 36.67, 33.93, 35.00, 35.50 | Positive | Positive   | 16. Microwave handle & dial | T | ND    | Negative   |
| 15. Bedrails (unoccupied)  | T | 37.43 | Suspect    | 16. Fingertips (L hand)  | - | ND    | ND       | Negative   | Negative   | 17. Air vent/ extractor    | E | ND    | Negative   |
| 16. Fingertips (R hand)    | - | ND    | Negative   | 17. Fingertips (R hand)  | - | ND    | ND       | Negative   | Negative   | Corridor                  |   |       |            |
| Facility A: sampling sites | * Ct (N)† | SARS-CoV-2 | Facility B: sampling sites | * Ct (N)† | 2nd visit | SARS-CoV-2 | 2nd visit | Facility C: sampling sites | * Ct (N)† | SARS-CoV-2 |
|---------------------------|-----------|-----------|---------------------------|-----------|-----------|-----------|-----------|---------------------------|-----------|-----------|
| 17. Hoist handle          | T         | ND        | Negative                  | 18. TV remote | T         | 31.79, 31.71 | ND        | Positive                  | 18. Glove box – size small | T         | ND        | Negative |
| 18. Chair seat (occupied) | T         | ND        | Negative                  | 19. Tabletop (swab) | T         | ND         | ND        | Negative                  | 19. Lip of PPE cabinet | T         | ND        | Negative |
| 19. Air vent/ extractor (bedroom) | E      | ND        | Negative                  | 20. Tabletop (sponge) | T         | 37.24, 36.17 | ND        | Positive                  | 20. Air vent/ extractor | E         | ND        | Negative |
| 20. Catheter stand (bathroom) | T       | ND        | Negative                  | 21. Chair arms | T         | 34.90, 34.93 | ND        | Positive                  | 21. Hand rail | T         | ND        | Negative |
| Bedroom/ bathroom #2      | 22. Walking frame handles | T         | ND        | Negative                  | 22. Wardrobe handle | T         | 37.50       | ND        | Positive                  | 22. Wardrobe handle | T         | ND        | Negative |
| 21. Bed remote control (occupied) | T       | 37.06, 38.13 | Positive                  | 23. Bed head | T         | 37.58       | 37.37     | Suspect                  | 23. Bed head | T         | ND        | Negative |
| 22. Air vent/ extractor (bedroom) | E      | ND        | Negative                  | 24. Wardrobe handle | T         | 37.50       | ND        | Positive                  | 24. Table top | T         | ND        | Negative |
| 23. TV control (in use)   | T         | 34.99, 35.48 | Positive                  | 25. Bathroom door handle (outer) | T         | 37.40       | ND        | Suspect                  | 25. Bathroom door handle (inner) | T         | 37.02     | ND        | Negative |
| 24. Top of TV             | E         | 35.96, 35.38 | Positive                  | 26. Bathroom door handle (inner) | T         | 37.02       | ND        | Suspect                  | 26. Chair arms | T         | ND        | Negative |
| 25. Top of clock          | E         | ND        | Negative                  | 27. Air vent/ extractor (bathroom) | E         | 34.64, 34.65 | 36.60     | Positive                  | 27. Air vent/ extractor (bathroom) | E         | 37.75     | Suspect |
| 26. Commode seat (bathroom) | T         | 37.93, 37.63 | Positive                  | 28. Toilet seat booster | T         | ND         | ND        | Negative                  | 28. Toilet seat booster | T         | ND        | Negative |
| 27. Fingertips (both hands) | -       | ND        | Negative                  | Corridor    | T         | ND         | ND        | Negative                  | 28. Walking frame handles | T         | ND        | Negative |
| 28. Top of light above bathroom sink | E      | 35.59, 36.86 | Positive                  | 29. Bannister outside laundry | T         | ND         | ND        | Negative                  | 29. Bannister outside laundry | T         | ND        | Negative |
| 29. Oxygen saturations probe (outside room) | T       | 35.99, 35.95 | Positive                  | Staff office | T         | ND         | ND        | Negative                  | Staff office | T         | ND        | Negative |
| 30. Top of key cabinet    | E         | 37.97, 37.54 | 37.34 Positive            | 31. Air vent/ extractor (bathroom) | E         | 37.75       | Suspect |
| 31. Air vent/ extractor (bathroom) | E       | 37.75       | Suspect                  | 32. Hoist rail (bathroom) | T         | ND        | ND        | Negative                  | 32. Hoist rail (bathroom) | T         | ND        | Negative |
| 33. Toilet handle         | T         | ND        | Negative                  | 33. Toilet handle | T         | ND        | ND        | Negative                  | 33. Toilet handle | T         | ND        | Negative |

ND = Not Detected.

* Designated common touch point (T) or elevated site (E).
† Samples are analysed in duplicate: a positive result means the target gene is detected in both replicates (reported as Cycle threshold (Ct) numbers). If the target gene is identified in only one sample, it is reported as suspect. Ct values reported for N gene target: the higher the Ct value, the lower the concentration of viral RNA. Limit of detection set at Ct 39.

NB on the second visit to facility B, some sites were not re-sampled (labelled N/A). Others were combined in one swab (merged cells in the table).
in facilities A and C all had private bathrooms and 13/16 rooms in facility B were en-suite. Characteristics of the three facilities are summarised in Table 2.

### Outbreak trajectories

SARS-CoV-2 attack rates among residents were highest in facility A: 16/30 (53%), of which 15/16 (94%) were symptomatic, followed by facility B: 6/15 (40%, of which 3/6 (50%) symptomatic). Only one resident tested positive in LTCF C (8%): this individual was asymptomatic and had received the first vaccine dose four weeks beforehand. They also had a history of laboratory-confirmed COVID-19 a year previously. A repeat sample taken 10 days after the most recent diagnosis was PCR negative for SARS-CoV-2, nonetheless the individual remained in isolation for 14 days as a precaution. Non-agency staff attack rates were also highest in facility A: 16/60 (27%) followed by facility B: 4/22 (18%) and C: 1/35 (3%). Numbers of agency staff were not available.

Duration of outbreak (calculated from the date of first illness onset to 28 days after onset of the final case) was 63, 50 and 30 days for facilities A, B and C respectively. Facilities A and B had residents admitted to hospital (n=5 symptomatic cases and n=3 of which one was symptomatic, respectively). Sadly, there were three COVID-19-related deaths among residents (facility A: n=2, both receiving end-of-life care, one hospitalised; facility B: n=1, hospitalised). Figure 1 illustrates outbreak trajectories.

### Outbreak control measures

Only facility A (the short stay unit) was effectively isolating all residents within their rooms at the time of the sampling visit. Facilities A and C were cohorting staff, allocating them to all residents within their rooms at the time of the sampling and not moving them. However, due to the shorter length of stay of residents in facility A compared to B and C, the cohorting was easier to achieve. In facility A, the cohorting was effective from the day of admission, whereas in facilities B and C the cohorting started after a few days. In facility A, 83% of residents had received the first dose of vaccine but just four days before onset of the outbreak. In facility A, 80% of non-agency staff were reported to have received a vaccine, though these figures related to the wider facility and exact vaccination dates were not provided. Facility B reported 55% and facility C 96% of non-agency staff vaccinated with at least one dose more than two weeks prior to the outbreak.

**Table 2. Characteristics of DISinFECT pilot LTCFs and resident populations.**

| ID | CQC rating | No. residents/ no. beds (% occupancy) | No. floors | Private/ shared bathroom | Care provision | Agency staff | Dependent/ independent | Age range (years) | Walk with purpose* | Prevalence comorbidities* | No. (%) positive sample sites |
|----|------------|----------------------------------------|-------------|--------------------------|----------------|-------------|-----------------------|-------------------|----------------------|---------------------------|-----------------------------|
| A  | O          | 30/40 (75.0%)                          | 1           | Private                  | Residential: short stay | Yes - block  | Mixed                  | 55-98             | No                   | 23/30 (76.7%)            | 6/27 (22%)                  |
| B  | G          | 15/16 (93.8%)                         | 2           | Mixed                    | Residential: older adults (+/- dementia) | Yes          | Mixed                  | 71-97             | Yes                  | 13/15 (86.7%)           | 17/28 (61%)                 |
| C  | G          | 12/13 (92.3%)                          | 2           | Private                  | Residential: adults with Learning Difficulties | No           | Mixed                  | 35-88             | Yes                  | data unavailable         | 1/31 (3%)                  |

*registered occupancy at time of sampling visit; †CQC = Care Quality Commission, O = Outstanding, G = Good; ‘one or more resident unable to adhere to self-isolation within private room; ‘presence of one or more chronic condition (overweight/obese; chronic respiratory disease; chronic heart disease; dementia; diabetes; hypertension; immunocompromised/ cancer) among registered occupants over the course of the outbreak; Dependent/ independent relates to resident mobility.
Facility B was an older building, once a large house. Residents’ rooms were small and somewhat cluttered with several sampling sites visibly soiled. Several residents were observed using the dining area and lounge (unmasked); staff wore their own clothing.

Facility C was a relatively modern building; rooms were small but uncluttered with fewer soft furnishings than LTCFs A and B. Two residents were observed walking with purpose (unmasked), accompanied by carers; staff wore their own clothing.

Staff wore surgical masks in all three facilities.

Proportion of sites testing positive and distribution of SARS-CoV-2 RNA

Facility B had the highest proportion of sampling sites testing positive/ suspect for SARS-CoV-2 RNA (PCR positive on one or both duplicates): 17/28 (61%), followed by LTCF A: 6/27 (22%). In facility C, all environmental swabs were negative for SARS-CoV-2 except for one suspect positive from an air extractor in the index case’s bathroom: positivity 1/31 (3%).

A repeat visit to facility B two weeks after the initial sampling visit yielded a much lower proportion of SARS-CoV-2 positive/ suspect sampling sites (4/19; 21%).

The lowest Ct value was 31.71, and only 20% (10/ 51) of values were below Ct 35. Amplification was above the Ct cut-off (39) for one sample (see Table 1).

SARS-CoV-2 positive/ suspect surfaces were most common in zone 1 (rooms occupied by residents with active SARS-CoV-2 infection, and equipment used by them), as illustrated in Figure 2.

Proportion of SARS-CoV-2 positive sites in proximity to a COVID-19 case

Within zone 1, there was significant variation in the proportion of sample sites testing positive/ suspect for SARS-CoV-2 RNA. For example, in facility A two residents’ rooms were sampled: in the first room, 1/5 (20%) of sample sites was ‘suspect positive’ for SARS-CoV-2 RNA and in the second room 6/8 (75%) of sample sites tested positive. Both rooms were similar in size and layout, and subject to the same cleaning
| ID | Outbreak metrics | Attack rate | Attack rate | Duration (days) | COVID-19 deaths | Hospital admissions | Infection Prevention & Control | COVID-19 vaccine |
|----|-----------------|-------------|-------------|-----------------|-----------------|---------------------|-----------------------------|-----------------|
|    |                 | residents   | staff        |                 |                 |                     | Residents isolating      | Staff cohorted | Staff tested | Closed to visitors | Enhanced cleaning | PPE available | Uptake residents | Uptake staff |
| A  |                 | 16/30 (53%) | 16/60 (27%) | 63              | 2               | 5                   | All                        | Yes            | Yes          | Yes†            | Yes            | Yes          | 25/30 (83%)      | 147/183 (80%) |
| B  |                 | 6/15 (40%)  | 4/22 (18%)  | 50              | 1               | 3                   | Cases                      | No             | Yes          | Yes            | Yes            | Yes          | 15/15 (100%)     | 12/22 (55%)   |
| C  |                 | 1/12 (8%)   | 1/35 (3%)   | 30              | 0               | 0                   | Cases                      | Yes            | Yes          | Yes            | Yes            | Yes          | 12/12 (100%)     | 24/25 (96%)   |

*Approximate staff attack rates, based on numbers of non-agency staff. Period between onset/swab dates of index case, and 28 days after illness onset for the final case; With the exception of end of life visits; *as per PHE guidelines: daily Lateral Flow Tests (LFT) for at least 5 days followed by twice weekly LFT and once weekly PCR testing; *Personal Protective Equipment; †Receipt of 1 or more dose >=14 days prior to onset in the index case; ‡Staff vaccination figures provided for whole facility, not just the short stay unit. No dates reported so figures may not accurately represent vaccine-induced immunity.
There were no zone 2 areas to sample in LTCF A, since all residents were confined to their rooms. The first room was occupied by an individual who was bed/chair bound, had tested positive for the virus 11 days previously and had fever and a slight cough. The occupant of the second room spent much of their time confined to bed, though was mobile with a wheelchair. This individual tested positive for SARS-CoV-2 infection seven days prior to the visit and had a cough. Figure 3 shows the environmental sample site positivity in relation to the time from onset of illness for the room occupant.

Proportion of common touch points vs elevated surfaces testing positive for SARS-CoV-2

Overall, the proportion of common touch points testing positive/ suspect for SARS-CoV-2 RNA (15/56; 27%) was
Figure 3. Proportion of sample sites that tested positive for SARS-CoV-2 RNA within the rooms of infected residents. Resident A1 had fever and slight cough and was bed/chair-bound; A2 had a cough and was mobile with a wheelchair; B reported only lethargy, and was independently mobile; C was asymptomatic and independently mobile; this individual's positive PCR result was unexpected given history of prior infection and vaccination.

Table 4. Proportion of common touch point and elevated sampling sites testing positive for SARS-CoV-2 RNA, by LTCF.

| LTCF | No. (proportion) common touch sites positive for SARS-CoV-2 | No. (proportion) elevated sites positive for SARS-CoV-2 |
|------|-----------------------------------------------------------|---------------------------------------------------------|
| A    | 5/17 (29%)                                                | 2/10 (20%)                                              |
| B    | 10/20 (50%)                                               | 6/7 (86%)                                               |
| C    | 0/19 (0%)                                                 | 1/10 (10%)                                              |
| Total| 15/56 (27%)                                               | 10/28 (36%)                                             |

Discussion
The greatest proportions of SARS-CoV-2 positive/ suspect sample sites were found in the immediate vicinity of laboratory-confirmed COVID-19 cases, which is consistent with findings from other studies. (Onakpoya et al., 2021; Ong et al., 2020; Stevenson et al., 2005) The proportion of SARS-CoV-2 positive/ suspect sites within this sampling zone varied considerably however, even between two rooms.
with similar layouts and cleaning regimes occupied by individuals with comparable symptom profiles. Levels of environmental contamination around COVID-19 patients evacuated from the Diamond Princess cruise ship and in hospital isolation rooms in Singapore did not correlate with clinical signs of illness either. (Chia et al., 2020; Santarpia et al., 2020) The timing of our samples (7 versus 11 days after symptom onset) may explain some of this variation: Chia et al. found more surface contamination in patient isolation rooms during the first week of illness, and associated with lower Ct values from clinical samples, (Chia et al., 2020) whereas Nelson et al. report no relationship between timing of sampling and proportion of contaminated surfaces in LTCF. (Nelson et al., 2021) Other studies suggest that there are a myriad of environmental, clinical, behavioural and pathogen factors affecting dispersion of the virus, which must be controlled for in any analysis of infection risk by indirect transmission. (Moore et al., 2021; Onakpoya et al., 2021)

The majority of our positive samples had Ct values >35 which is higher than the average from routine clinical sampling. (Singanayagam et al., 2020) There is limited value in comparing studies with different protocols, but for context environmental samples from LTCF in British Columbia yielded Ct values of >37.4, (Nelson et al., 2021) and a systematic review of studies assessing fomitic transmission of SARS-CoV-2 reported surface sample Ct values between 20.8 and 44.1. (Onakpoya et al., 2021)

The limit of detection of the Viasure assay is 10 copies/reaction and we consistently detected 5 copies in our standard curve. Since we saw no amplification in our negative extract samples or qPCR no-template controls we are confident the late amplification we detected in COVID-19 outbreak environmental samples is genuine detection of SARS-CoV-2 RNA

The fact that fingertip swabs were all PCR negative for SARS-CoV-2, despite observing infected residents touching contaminated areas, was surprising: people typically touch their nose, eyes and mouth more than 20 times per hour and an experimental study suggested that the virus can persist on skin for at least 8 hours at body temperature. (Harbourt et al., 2020; Kwok et al., 2015) Sampling may have occurred too late to detect viral shedding, since participants were between seven and 14 days of diagnosis, and the dispersal of virus on surfaces will not have been uniform. It is also possible that residents applied hand sanitiser unobserved, that the sampler did not apply sufficient pressure or friction to pick up viral RNA, or that flocked swabs have a very low recovery efficiency on skin. (Mbithi et al., 1992)

Notably, facility A experienced the highest attack rates despite implementing more comprehensive IPC and cleaning measures compared to facilities B and C. Residents in facility A were more susceptible to infection than those in facilities B and C, having only received the first dose of vaccine shortly before the onset of the outbreak. (Shrotri et al., 2021; Tenforde et al., 2021) Facility A also had a higher rate of admissions from the local hospitals and these factors, as well as the relatively large size of the facility, may have increased the probability of multiple introductions of the virus to the premises. (Burton et al., 2020; Shallcross et al., 2021) Poor response rates to the staff survey, reflecting extreme work pressures, meant that analysis of individual-level infection risk factors was not possible.

High attack rates may also have reflected community case rates, which peaked at the end of December and remained high through January (see Figure 1 for pilot LTCF outbreak trajectories). This surge in case rates was fuelled by emergence of the more transmissible B.1.1.7 Alpha variant, which quickly entered English LTCFs. (Krutikov et al., 2021) At this time a substitution at the E484K location of the receptor binding domain also appeared, raising concerns that the virus might evade the host immune response. (Wise, 2021) Six clinical samples were sequenced and were all of the B.1.1.7 Alpha variant with no E484K substitution, however we cannot exclude the possibility that multiple strains of the virus contributed to these outbreaks.

Results from surface swabbing provided some reassurance that facility A (with staff cohorting and enhanced IPC measures) was effectively containing the environmental spread of the virus, in contrast to LTCF B (without cohorting) in which viral RNA was widely distributed. Repeat sampling 14 days after the initial visit to facility B yielded a much lower proportion of SARS-CoV-2 positive/suspect sites. Since the first visit corresponded with the end of the final case’s infectious period and no further cases of COVID-19 were identified it seems unlikely that anyone in the facility was actively shedding virus at the second sampling visit. Our observations could reflect the effectiveness of cleaning protocols introduced between sampling visits or of swabbing at the first round of sampling, and/or degradation of viral RNA over a 14 day period. (Onakpoya et al., 2021; van Doremalen et al., 2020) Facility C was the only one to isolate its resident case on a separate floor/wing which may have helped reduce egress to other areas within the LTCF. However, this individual’s history of vaccination and prior infection, and a negative repeat PCR test suggest that the diagnosis was a false positive and they were not shedding SARS-CoV-2 at the time of sampling.

Elevated sampling sites, being cleaned less regularly, may be a more pragmatic means of SARS-CoV-2 detection than common touch points: of the four sites that remained positive/suspect for SARS-CoV-2 on a repeat visit to facility B, three were elevated. Reactive sampling, as applied in this pilot, will not distinguish between historic and current viral shedding but there is evidence that levels of surface contamination with SARS-CoV-2 RNA mirror contemporaneous levels of airborne SARS-CoV-2 RNA. (Cherrie et al., 2021; Dumont-Leblond et al., 2021; Zhou et al., 2021) Air vents may be useful sentinel sampling points since three of four air vents in facility B tested positive for SARS-CoV-2 RNA, and in facility C the air vent was the only sampling site that tested suspect positive. Similar observations are reported from sampling ventilation grates in the Diamond Princess COVID-19 quarantine rooms.
and in COVID-19 isolation rooms in Nebraska, and respiratory viruses have been isolated from air filters in aeroplanes and large public buildings. (Goyal et al., 2011; Korves et al., 2011; Santarpia et al., 2020) It is interesting that none of the air vents sampled from facility A tested positive for SARS-CoV-2 RNA, including one in a room that was otherwise quite heavily contaminated. This could have been an artefact of different sampling techniques, or reflect the design of the air vents, which were circular with a single ring opening rather than a slatted grate, though the vent that tested suspect positive in facility C was of the same circular design. Facility A may also have been better ventilated than the other facilities, which has been associated with lower levels of surface contamination, (Dumont-Leblond et al., 2021) however this seems unlikely given positive results in other elevated sites in the building.

The uncluttered environment in facility A, in reducing build-up of dust, may have helped limit environmental spread of SARS-CoV-2: there is some evidence that respiratory droplets containing SARS-CoV-2 are adsorbed to dust and particulate matter. (Andree, 2020; Conticini et al., 2020; Qu et al., 2020; Remninger et al., 2021; Setti et al., 2020; Travaglio et al., 2021). The presence of dust can also indicate poor ventilation, which increases the likelihood of aerosol deposition; (Dumont-Leblond et al., 2021) – this merits further investigation.

Limitations
This pilot has limitations, including small sample size, lack of control sites and heterogeneity of sample sites. Our interpretation of results is speculative and intended to generate hypotheses rather than answer questions.

Between-site variation in layout and infrastructure means that sampling frames cannot be entirely standardised. In addition, there may be a tendency to oversample areas that are visibly soiled; a weak correlation was found between the number of samples taken from COVID-19 isolation rooms in Nebraska and the proportion of samples that tested positive for SARS-CoV-2 RNA. (Santarpia et al., 2020). Technique may also vary between samplers, though only two individuals conducted sampling in this study which should have minimised discrepancies. We did not capture timings for cleaning activity which could explain some of the variation we saw in environmental contamination, though variation was also observed between elevated sites which were unlikely to have been cleaned as part of daily routine. Results from surface swabs represent a snapshot in time and cover a small fraction of the LTCF environment so we may not have accurately captured overall levels of environmental contamination. Among other things these may have been influenced by trends in community prevalence of COVID-19 (affecting risk of importation), expansion of new variants, and the effects of vaccination (impacting viral shedding and transmissibility).

We chose to use flocked swabs and sponges based on a laboratory-based comparison of recovery efficiencies from surfaces inoculated with low concentrations of SARS-CoV-2 RNA (Evaluation of SARS-CoV-2 environmental surface sampling protocols conducted by UKHSA for the PROTECT COVID-19 National Core Study on transmission and environment). We did not assess recovery efficiency in the field so do not know how sensitive our sampling methods were when applied in LTCF. More work is needed to validate limits of detection and assess sensitivity of environmental sampling for SARS-CoV-2 in LTCF.

We did not include field controls in our sampling strategy and cannot exclude the possibility that there was contamination or cross-contamination of samples.

We were unable to confirm whether the diagnostic test for the single resident case in facility C was a true positive, therefore the SARS-CoV-2 RNA detected in this individual’s rooms may have been residual from previous occupants or their careers. This bias also applies to the other facilities to some extent since all were likely to have been exposed to the virus at some point prior to the outbreak.

Sequencing data were only available for a minority of outbreak samples therefore we were unable to assess whether new or multiple strains contributed to these outbreaks.

**Conclusions and recommendations**
We have successfully recruited three pilot LTCFs to the DISinFEcT pilot and recovered SARS-CoV-2 RNA from a high proportion of surfaces around individuals with a laboratory-confirmed infection. Elevated surfaces had relatively high positivity so sampling these areas may be a simple way to detect pathogens that have been airborne, though we cannot infer infection risk from environmental results. Our experiences highlight that LTCF staff can be overburdened with information requests during outbreaks. There is therefore a need for efficient and parsimonious data collection tools to support the interpretation of environmental surveillance data.

In summary: there is a need for tools to help detect outbreaks in LTCF and to optimise IPC in these settings. This pilot suggests that further work is justified to assess the feasibility and utility of environmental sampling for infectious disease surveillance in LTCF.

**Data availability**
**Underlying data**
To preserve anonymity of LTCF residents and staff, the study data are stored on a secure drive hosted by the UK Health Security Agency (UKHSA) Field Services South West. Access to personal identifiable data is restricted to personnel responsible for outbreak investigations. PCR results from environmental sampling are available via the Open Science Framework via the following link: https://doi.org/10.17605/OSF.IO/DE2ZF.

**Extended data**
Open Science Framework: COVID-19: Detecting Indirect Spread in Facilities for Enhanced Care sTudy (COVID-19: DISinFECT). Investigating environmental epidemiology of
SARS-CoV-2 in long term care facilities in England. Protocol v4.1. https://doi.org/10.17605/OSF.IO/3QNOZ (Kwiatkowska & Ready, 2021)

This project contains the following files:
- DISinFECT_ProtoCol_OSF.pdf. The protocol for this research study.
- DISinFECT_staffsurvey.pdf. The electronic survey distributed to staff to collect information on epidemiological and clinical risk factors for SARS-CoV-2 infection.
- DISinFECT_Tools_line_lst_v6.xlsx. The data collection template for LTCF residents: clinical and epidemiological risk factors for SARS-CoV-2 infection.
- DISinFECT_Tools_setting_log_v2.xlsx. The data collection template for details of LTCF layout and staffing arrangements.

Data are available under the terms of the Creative Commons Attribution 4.0 International license (CC-BY 4.0).

Acknowledgements
The authors would like to express thanks to the staff and residents of LTCFs who supported these investigations during a very challenging time. Also, to acknowledge the hard work of regional Health Protection Teams and local authorities throughout the SARS-CoV-2 epidemic, along with laboratory staff without whom this work would not have been possible.

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The authors report on SARS-CoV-2 surface contamination status during the COVID-19 outbreak in three LTCFs. Environmental surveillance by wiping surfaces in the facility could be a potential tool for monitoring infectious diseases as a non-invasive method. It could support testing by specimen collection from residents and staff within the LTCF. As SARS-CoV-2 is a respiratory infection, information on which environmental surfaces in the facility are contaminated with virus in droplets would be useful for infection control.

The results of the survey showed that the viral genome could be detected in less touched, elevated areas as well as in areas touched by people, suggesting that these less touched areas are potential candidates as sites for identifying the presence of an infected person, unaffected by daily surface cleaning in the LTCF.

At the same time, the results also indicated that aerosol control from infected individuals would be important. However, as they pointed out in the limitations section, even if the wipe tests were positive, it could not imply infectivity. In addition, because Ct value in most positive cases was high, the results would be interpreted with various speculations, e.g. it might have simply been tracking past contamination residues.

In other words, it is difficult to confirm indirect transmission from these data.

However, the presence of viral genome on various surfaces in the LTCF reaffirms the need for infection control measures corresponding to aerosol exposure and close contact infection. As the authors mentioned, thus survey would increase workload for staff in LTCF during the outbreak. If possible, it would be valuable to indicate how the survey results had influenced infection control measures in the facility.

Below minor points could be clarified by the authors:

Sampling:
Although the authors mentioned two staffs were in charge for sampling, this information would move to "sampling" in M&M section. The authors should mention for measures against cross-contamination for PCR test in sampling.
Outbreak control measures:
In this study, most PCR tests indicate high number of Ct value. How is the interval between sampling and surface cleaning at facilities A, B, and C? If records not available, please discuss whether “enhanced cleaning” would relate to detect viral genome by PCR test or not in the limitation section.

Result:
After the results of the environmental sampling were returned to each LTCF, what kinds of action was taken by LTCF? If possible, could you describe it.

Table 1:
LTCF B. Line7. 2nd visit. “Negative” is correct?

Please check again whether numbers of samples in Table are same as those in result section.

Figure 1:
The result for environmental sampling could be helpful to understand a prevalence of contaminated surface at LTCF. Could the authors indicate positive rate in each sampling date in figure?

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Not applicable

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: environmental surveillance

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.
The authors have thoughtfully and rigorously updated the methods to clarify processes and results, and modified discussions to align interpretation with the data. The result is a manuscript that reaffirms that environmental sampling of surfaces can be used to detect pathogens, including SARS-CoV-2. Further, environmental sampling in long term care facilities can offer a non-invasive surveillance approach for the emergence or spread of a disease in the facility. The result is a comprehensive case study offering both methodologies and insights into fomite-mediated disease surveillance.

Notably, the authors also investigated a variety of surface types, including sites elevated (>1.5m) off the ground and note more frequent detection at these sites. This finding offers additional insights into the potential aerosolized spread of SARS-CoV-2 prior to deposition, and suggests non-traditional sampling of surfaces beyond those frequently contacted may be beneficial when surface testing is focused on surveillance, as opposed to assessing potential for transmission risks.

The authors highlight the need for continued work on this subject, and point to specific areas where advances would be warranted. Quantification of pathogens from surfaces is once such area they note, as quantity drives transmission risks, though may be less important to accurately determine for surveillance purposes where presence/absence measured here may be sufficiently informative.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Environmental Microbiology, Environmental Virology, Exposure Assessment, Risk Assessment

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.
Additional improvements (or discussion at least) of a lack of a standard curve is warranted, given that it hinders experimental interpretation, particularly discussions of concentrations of SARS-CoV-2 on surfaces and the associated risks of contacts. Synthetic SARS-CoV-2 RNA is commercially available, and would provide a sufficient standard for quantification of samples.

An additional update is that authors should consider modifying the manuscript discussion and conclusions to more closely align with the results. A specific example is that the study investigated three LTCFs and attempts to draw conclusions on results as a function of cleaning regime: such inference would require a larger sample size with multiple facilities with variable cleaning and quarantining regimes to rule out location-specific effects not associated with cleaning.

Detailed Comments:

Abstract:
- “potential to assess infection risk from SARS-CoV-2 positive surfaces” - should be connected to methods for this approach, unclear how this would be done.
- Define “elevated” here and in Methods.
- “could be associated with implementation” – this is speculation, and was not specifically tested, so suggest authors remove this from the abstract.

Introduction:
- “touch-points and elevated surfaces” – how did authors conclude they should test elevated surfaces? Is prior literature available here that could be referenced?
- Author discussion of COVID-19: DISinFECT suggests a project larger than the study results presented here, but it is unclear. Author clarification on the project DISinFECT and its relationships to this study would be helpful and interesting.

Methods:
- “complete a consent form” – suggest “written consent form”.
- Substantially more information is recommended on collecting samples. Detection/Non-detection of viral RNA on surfaces is influenced by the sensitivity of the methods, which includes sample collection, RNA extraction, and RNA detection. Authors should consider reporting material used (not only swabs, but also eluent volume and material, volume of material subsequently processed, RNA extraction methods, including volumes eluted (if applicable), and full details of the QPCR methodology. Inhibition may also influence sample detection, and some indication of qPCR inhibition testing should be included. Indeed, less frequently contacted surfaces (like elevated surfaces) may have more dust and thus may be more inhibited?

- Bustin et al. 2009 MIQE Guidelines\(^1\) provide a motivation and framework for reporting on data relying on qPCR. Additionally, authors should report all controls used, including positive controls (source and concentration), field blanks, extraction blanks, and no template controls. Authors should also conduct, or report on previously studies that conducted, SARS-CoV-2 or surrogate virus RNA from surfaces to provide an estimate of recovery efficiency. Environmental sampling relying on detection of low concentrations
should demonstrate sufficient use of controls to rule out contamination and cross contamination of samples, and recovery efficiency to provide insights into relative magnitude of surface contamination.

- How was a Ct value of 39 chosen as a cut-off? How does this value compare to no template controls? See MIQE guidelines\(^1\) for guidance on Limits of Detection. Authors could consider motivating why no standard curve was included to estimate concentrations.

- On mention of WGS, it was unclear in the methods that this meant clinical isolates and not environmental samples. Suggest authors clarify. Also, authors should specify directly, for example through referencing, what protocols were followed, what primers sets were used, for sequencing.

- Figure 1 provides minimal novel insights into the study, authors could consider replacing with more descriptive figure covering all specific locations tested, with further information on their classification (elevated or not).

**Results**

- Term “home” is used and could be replaced with LCTF.

- Table 1 – in one negative sample, a threshold Ct value is reported. Similar, positive Ct is reported for all samples. Consider reporting Ct for all samples as a separate column.

- Table 2 – a helpful table for comparing locations, is it possible to add the percent of surfaces that were positive at each site?

- Figure 2- why is C not included in this trajectory?

- “Concentrations of SARS-CoV-2 RNA” – in the absence of standard curves and recovery efficiency experiments, there is an obscure relationship between Ct values and true (unobserved) concentrations on surfaces. Suggest authors avoid discussing or inferring concentrations and instead focus on detection/non-detection.

- “lower than the proportion of sites” – should this say elevated sites?

- Fingertip samples – repeat how many samples were tested here.

- Figure 3 – suggest updating legend descriptions from Zone 1-3 to more detailed descriptions provided in legend to make it easier to read.

- Figure 4 – unclear why there are two y-axes here since there are only four data points. Suggest authors place days since illness as a number above the bar charts. An alternative, if the point is to visualize the relationship (or lack of) between days since illness and positivity rate, is to plot a scatter plot with days since illness on x-axis and positivity rate on y-axis and label each point with A1, A2, B, or C with text labels.

**Discussion**

- “with findings from other studies” – authors should reference these studies in this sentence, only one study is currently referenced.
“can detect the presence of an infected individual” – although conceptually the authors demonstrate detection in rooms with infected patients, the study does not evaluate the specificity or sensitivity of the approach: how often in an infected patients room or other building area is SARS-CoV-2 detected in the room? How often is SARS-CoV-2 detected in uninfected patients rooms or other building areas? What are the false negative/false positive rates? These values are necessary to determine the feasibility of this approach in environmental surveillance.

“Consequently, there was insufficient epidemiological…” – I am a bit skeptical that survey results could provide insight into the role of fomites vs. long range aerosol vs. close contact in disease transmission. Can the authors further describe how this could be done?

“The fact that fingertip swabs…”, could also be very low methodological recovery. This should be tested or at least discussed in the context of other work on recovery efficiency. An alternative is spatial heterogeneity (in addition to temporal heterogeneity discussed by authors).

“may have been residual” – or analytical false negatives.

“This pilot demonstrates…” - the efficacy of surface swabbing to determine efficacy of IPC measures should rely on substantially more than three LCTFs, given that the variation in site-specific contamination may be caused by factors other than IPC. The sample size is too small to clearly demonstrate this. Suggest authors rephrase this point to highlight that future studies could use these methodology to explore impacts of IPC on surface contamination.

Comments on specific elements of the review:

Technically Sound / Methods and Analysis:
- As discussed, more data on methods and associated results of controls would be beneficial.

Statistical Analysis:
- The analysis presented here is primarily qualitative given the pilot nature, small sample size, and heterogeneity in results.

Data Availability:
- The authors present much of their data in the manuscript, and offer the rest (non-identifiable) on contact. Although this is generally sound, suggest authors make non-identifiable data freely available, in particular a modified version of Table 1 that includes all Ct values (not the mean, SD).

References
1. Bustin SA, Benes V, Garson JA, Hellemans J, et al.: The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clin Chem. 2009; 55 (4): 611-22 PubMed Abstract | Publisher Full Text

Is the work clearly and accurately presented and does it cite the current literature? Yes

Is the study design appropriate and is the work technically sound?
Partly

**Are sufficient details of methods and analysis provided to allow replication by others?**

Partly

**If applicable, is the statistical analysis and its interpretation appropriate?**
Not applicable

**Are all the source data underlying the results available to ensure full reproducibility?**
No

**Are the conclusions drawn adequately supported by the results?**
Partly

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Environmental Microbiology, Environmental Virology, Exposure Assessment, Risk Assessment

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

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**Author Response 27 Jun 2023**

**Rachel Kwiatkowska**

Thank you for the thorough and constructive review, please see our responses below. Hopefully, the amended manuscript will clarify our methods. Considering the limitations of the study, we have modified the discussion and conclusions to flag points of interest/further investigation without drawing too much inference from this small number of observations.

**Abstract**

1. “potential to assess infection risk from SARS-CoV-2 positive surfaces“ - should be connected to methods for this approach, unclear how this would be done.
   - Thanks for pointing this out. We’ve updated the wording to: ‘Collection of clinical and epidemiological data to provide context for environmental findings.’
2. Define “elevated” here and in Methods.
   - Defined as a surface >1.5m above ground, see amended abstract and methods > sampling > approach.
3. “could be associated with implementation” – this is speculation, and was not specifically tested, so suggest authors remove this from the abstract.
   - Agreed – we have removed this statement.

**Introduction:** 4. “touch-points and elevated surfaces“ – how did authors conclude they should test elevated surfaces? Is prior literature available here that could be referenced?
This decision was based on our own observations from sampling in healthcare settings, and findings from other studies. We've added this information to paragraph 2 of the introduction.

5. Author discussion of COVID-19: DISinFECT suggests a project larger than the study results presented here, but it is unclear. Author clarification on the project DISinFECT and its relationships to this study would be helpful and interesting.

○ Thanks, we've added additional detail to the final paragraph of the introduction.

Methods:

6. “complete a consent form” – suggest “written consent form”

○ Amended.

7. Substantially more information is recommended on collecting samples. Detection/Non-detection of viral RNA on surfaces is influenced by the sensitivity of the methods, which includes sample collection, RNA extraction, and RNA detection. Authors should consider reporting material used .. swabs,

○ Please see ‘sampling’ section, paragraph 2.

.. eluent volume and material, volume of material subsequently processed, RNA extraction methods, including volumes eluted (if applicable)

○ Added to the beginning of ‘laboratory analysis’ section.

.. full details of the QPCR methodology.

○ Added to ‘laboratory methods’, second paragraph. We did not perform qPCR on sponge samples, and have added this to our limitations.

8. Inhibition may also influence sample detection, and some indication of qPCR inhibition testing should be included. Indeed, less frequently contacted surfaces (like elevated surfaces) may have more dust and thus may be more inhibited?

○ An important point, thank you. See details of inhibition testing in ‘laboratory methods’, paragraph 4. We detected no evidence of inhibition. Interesting point regarding dust as an inhibitor: colleagues have processed quite a lot of samples from heavily soiled areas in public transport, and still detected viral RNA.

○ Please see ‘laboratory methods’ section for details of positive controls, extraction blanks and no template controls. Field controls/ blanks were not used - we have listed this among the study limitations.

9. Authors should also conduct, or report on previously studies that conducted, SARS-CoV-2 or surrogate virus RNA from surfaces to provide an estimate of recovery efficiency.

○ See discussion - we have referenced (unpublished) UKHSA data on recovery efficiency of flocked swabs and sponges, which informed our choice of sampling materials. We did not attempt to calculate recovery efficiency in the field and have added this to the study limitations.

10. Environmental sampling relying on detection of low concentrations should demonstrate sufficient use of controls to rule out contamination and cross contamination of samples, and recovery efficiency to provide insights into relative magnitude of surface contamination

○ Thank you for the detailed explanation and reference to MIQE guidelines. We hope that the additional methodological detail and inclusion of caveats improves the quality of the paper.

11. How was a Ct value of 39 chosen as a cut-off? How does this value compare to no template controls? See MIQE guidelines1 for guidance on Limits of Detection. Authors could consider motivating why no standard curve was included to estimate concentrations.
We followed the example of other environmental sampling studies in healthcare and LTCF when defining the cut-off at Ct 39 – we've referenced these in methods > laboratory analysis > analysis.

12. On mention of WGS, it was unclear in the methods that this meant clinical isolates and not environmental samples. Suggest authors clarify. Also, authors should specify directly, for example through referencing, what protocols were followed, what primers sets were used, for sequencing.
   ○ Thanks, we've clarified that these were clinical isolates. Sequencing was carried out externally, through a consortium of laboratories operating in the COVID-19 response; unfortunately we do not have access to the protocols used.

13. Figure 1 provides minimal novel insights into the study, authors could consider replacing with more descriptive figure covering all specific locations tested, with further information on their classification (elevated or not).
   ○ We have decided to leave this figure out, having attempted a more comprehensive visual which got quite messy.

Results

14. Term “home” is used and could be replaced with LCTF.
   ○ All references to (care) home corrected to LTCF.

15. Table 1 – in one negative sample, a threshold Ct value is reported. Similar, positive Ct is reported for all samples. Consider reporting Ct for all samples as a separate column.
   ○ N gene Ct values added for all samples.

16. Table 2 – a helpful table for comparing locations, is it possible to add the percent of surfaces that were positive at each site?
   ○ Good idea, added.

17. Figure 2- why is C not included in this trajectory?
   ○ We have now added the two data points for facility C.

18. “Concentrations of SARS-CoV-2 RNA“ – in the absence of standard curves and recovery efficiency experiments, there is an obscure relationship between Ct values and true (unobserved) concentrations on surfaces. Suggest authors avoid discussing or inferring concentrations and instead focus on detection/non-detection.
   ○ Noted. We have simply commented on high Ct values without inference re: viral/ RNA concentrations.

19. “lower than the proportion of sites” – should this say elevated sites?
   ○ Yes. Corrected.

20. Fingertip samples – repeat how many samples were tested here.
   ○ Amended.

21. Figure 3 – suggest updating legend descriptions from Zone 1-3 to more detailed descriptions provided in legend to make it easier to read.
   ○ Legend updated.

22. Figure 4 – unclear why there are two y-axes here since there are only four data points. Suggest authors place days since illness as a number above the bar charts. An alternative, if the point is to visualize the relationship (or lack of) between days since illness and positivity rate, is to plot a scatter plot with days since illness on x-axis and positivity rate on y-axis and label each point with A1, A2, B, or C with text labels.
   ○ Good suggestion – we've reproduced this as a scatter plot.

Discussion

23. “with findings from other studies” – authors should reference these studies
in this sentence, only one study is currently referenced.
  ○ Additional references added.

24. “can detect the presence of an infected individual” – although conceptually the authors
demonstrate detection in rooms with infected patients, the study does not evaluate the
specificity or sensitivity of the approach: how often in an infected patients room or other
building area is SARS-CoV-2 detected in the room? How often is SARS-CoV-2 detected in
uninfected patients rooms or other building areas? What are the false negative/false
positive rates? These values are necessary to determine the feasibility of this approach in
environmental surveillance.
  ○ You're right, more work needed – we've removed this sentence and added to the
limitations: ‘More work is needed to validate limits of detection and assess sensitivity and
specificity of environmental sampling for SARS-CoV-2 in LTCF’

25. “Consequently, there was insufficient epidemiological...” – I am a bit skeptical that
survey results could provide insight into the role of fomites vs. long range aerosol vs. close
contact in disease transmission. Can the authors further describe how this could be done?
  ○ We have deleted this sentence since we did not intend to conduct statistical analyses
as part of the DISinFECT study.

26. “The fact that fingertip swabs...”, could also be very low methodological recovery. This
should be tested or at least discussed in the context of other work on recovery efficiency. An
alternative is spatial heterogeneity (in addition to temporal heterogeneity discussed by
authors).
  ○ Thanks, yes. We mention recovery efficiency here and discuss in the limitations. Re:
spatial heterogeneity, the next sentence now reads: ‘Sampling may have occurred too
late to detect viral shedding, since participants were between seven and 14 days of
diagnosis, and the distribution of virus on surfaces will not have been uniform’

27. “may have been residual” – or analytical false negatives.
  ○ Do you mean a false positive environmental swab, or false negative from the room's
occupant? We've implied the latter.

28. “This pilot demonstrates...” - the efficacy of surface swabbing to determine efficacy of
IPC measures should rely on substantially more than three LCTFs, given that the variation in
site-specific contamination may be caused by factors other than IPC. The sample size is too
small to clearly demonstrate this. Suggest authors rephrase this point to highlight that
future studies could use these methodology to explore impacts of IPC on surface
contamination.
  ○ Thanks, we've modified this paragraph as suggested.

**Competing Interests:** No competing interests were disclosed.