Health Information and Quality Authority

An tÚdarás Um Fhaisnéis agus Cáilíocht Sláinte

Duration of immunity (protection from reinfection) following SARS-CoV-2 infection

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About the Health Information and Quality Authority

The Health Information and Quality Authority (HIQA) is an independent statutory authority established to promote safety and quality in the provision of health and social care services for the benefit of the health and welfare of the public.

HIQA's mandate to date extends across a wide range of public, private and voluntary sector services. Reporting to the Minister for Health and engaging with the Minister for Children, Equality, Disability, Integration and Youth, HIQA has responsibility for the following:

- **Setting standards for health and social care services** — Developing person-centred standards and guidance, based on evidence and international best practice, for health and social care services in Ireland.

- **Regulating social care services** — The Chief Inspector within HIQA is responsible for registering and inspecting residential services for older people and people with a disability, and children's special care units.

- **Regulating health services —** Regulating medical exposure to ionising radiation.

- **Monitoring services** — Monitoring the safety and quality of health services and children's social services, and investigating as necessary serious concerns about the health and welfare of people who use these services.

- **Health technology assessment** — Evaluating the clinical and cost-effectiveness of health programmes, policies, medicines, medical equipment, diagnostic and surgical techniques, health promotion and protection activities, and providing advice to enable the best use of resources and the best outcomes for people who use our health service.

- **Health information** — Advising on the efficient and secure collection and sharing of health information, setting standards, evaluating information resources and publishing information on the delivery and performance of Ireland's health and social care services.

- **National Care Experience Programme** — Carrying out national service-user experience surveys across a range of health services, in conjunction with the Department of Health and the HSE.

List of abbreviations used in this report

CI	confidence interval
COVID-19	Coronavirus disease 2019
C_t	cycle threshold
HIQA	Health Information and Quality Authority
HSE	Health Service Executive
IgA	immunoglobulin A
IgM	immunoglobulin M
IgG	immunoglobulin G
NAAT	nucleic acid amplification test
NPHET	National Public Health Emergency Team
NCP	nucleocapsid protein
RBD	receptor-binding domain
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
S protein	spike protein
WGS	whole genome sequencing
WHO	World Health Organization

Glossary of terms/explanatory notes

Antibody	An antibody is a protein produced by the immune system that binds specifically to a particular substance (its antigen). Each antibody molecule has a unique structure that enables it to bind specifically to its corresponding antigen, but all antibodies have a similar overall structure and are known collectively as immunoglobulins or Igs. Antibodies are produced by plasma cells in response to infection or vaccination, and bind to and may neutralise pathogens (invading microorganisms) or prepare them for uptake and destruction by phagocytes (cells that destroy pathogens). Antibodies do not enter cells, and can only play a protective role before the virus enters the cell.
B cell	A B cell, or B lymphocyte, is one of the two major types of lymphocyte. On activation by an antigen, B cells differentiate into plasma cells, which produce antibody molecules.
CD4 and CD4 T cells	CD4 is a cell-surface protein important for recognition by T-cells. CD4 T cells are T cells that carry the co-receptor protein CD4, and play a central role in the immune system, acting as 'helper' T cells, providing essential help for B cells and other T cells.
Cell-mediated immunity (or cellular immunity)	Cell-mediated immunity, or a cell-mediated immune response, describes any adaptive immune response in which antigen-specific T cells have the main role in protection. Once a virus enters a cell, cell-mediated immunity is the only effective immune response.
Convalescent period	The convalescent period is the time during which an individual has recovered from an infectious disease (e.g. COVID-19) and during which blood serum may contain antibodies against the infectious agent of the disease.
Cycle threshold (Ct)	In RT-PCR, a positive reaction is detected by accumulation of a fluorescent signal. The Ct (cycle threshold) is defined as the number of cycles required for the fluorescent signal to cross the threshold (therefore exceed background level). The lower the Ct level, the greater the amount of target nucleic acid in the sample.
Genome	The genetic material of an organism.
Humoral immunity	Humoral immunity is another term for antibody-mediated immunity and the term 'humoral immune response' refers to the antibody response to a specific antigen.
Immunoglobulins	All antibody molecules belong to a family of plasma proteins called immunoglobulins (Ig). Membrane-bound immunoglobulin serves as the specific antigen receptor on B lymphocytes.

IgG	IgG is the class of immunoglobulin characterised by γ heavy chains. It is the most abundant class of immunoglobulin found in the plasma and is also found in tissues.
Immunity	Immunity is the ability to resist infection.
Lineage	Descent in a line from a common ancestor. Viruses can be grouped into lineages (families), based on the evolutionary trajectories of the virions and their production mechanisms.
Memory cells	Memory cells are the lymphocytes that facilitate immunological memory. They are more sensitive to antigen than naive lymphocytes and respond rapidly on re-exposure to the antigen that originally induced them. Both memory B cells and memory T cells have been defined.
Mucosal immunity	Mucosal immunity is the study of the immune system associated with mucosal sites, such as the lining of the respiratory and gastrointestinal tracts.
Neutralising antibodies (NAb)	A neutralising antibody (NAb) is an antibody that is responsible for defending cells from pathogens, which are organisms that cause disease. They are produced naturally by the body as part of its immune response, and their production is triggered by both infections and vaccinations against infections. Specific pathogen proteins bind to proteins on human cells, which act as receptors. Neutralising antibodies usually bind the pathogen protein, which binds the receptor.
Pathogen	Pathogens are microorganisms that can cause disease when they infect a host.
Receptor-binding domain (RBD)	A receptor-binding domain (RBD) is part of a virus, located on its 'spike' domain, which allows it to dock to body receptors to gain entry into cells and lead to infection. In the case of coronaviruses, the RBD is found on the 'spike' domain.
Reverse transcriptase–polymerase chain reaction	The reverse transcriptase–polymerase chain reaction (RT-PCR) is used to amplify RNA sequences. The enzyme reverse transcriptase is used to convert an RNA sequence into a cDNA sequence, which is then amplified by PCR.
Seroconversion	Seroconversion timing refers to the first time an individual tests positive for antibodies (based on serial serological samples).
Seropositive	When someone has a blood test (serologic test) and detectable antibodies against a specific antigen are found.
Seronegative	When someone has a blood test (serologic test) and detectable antibodies against a specific antigen are not found.

Single nucleotide polymorphisms (SNPs)	Single nucleotide polymorphisms (SNPs) are the most common type of genetic variation among people or organisms. Each SNP represents a difference in a single DNA building block, called a nucleotide.
T cells	T cells, or T lymphocytes, are a subset of lymphocytes defined by their development in the thymus (organ). T cells play a key role in co-ordinating the immune response, and protection against viruses and fungi.
Titre(s)	The strength of a solution or the concentration of a substance in solution as determined by titration.
Whole genome sequencing (WGS)	Whole-genome sequencing (WGS) is the analysis of the entire genomic DNA sequence of a cell at a single time, providing the most comprehensive characterisation of the genome.

Version History

Version number	Date	Details
V1.0	13 May 2020	
V2.0	9 June 2020	Updated search with 35 new studies
V3.0	6 August 2020	Updated search with 28 new studies
V4.0	11 November 2020	Refined search with 28 new studies
V5.0	5 March 2021	Refined search with 5 new reinfection studies and scoping review on the long-term duration of immune response following SARS-CoV-2 infection
V6.0	8 April 2021	Updated search with 6 new reinfection studies

Duration of immunity (protection from reinfection) following SARS-CoV-2 infection

Key points

- A systematic search was conducted to identify studies that investigated the risk of SARS-CoV-2 reinfection in previously infected individuals over time.
- Eleven observational cohort studies were identified that met the inclusion criteria. Six general population studies were identified, of which two were conducted in the US and one each was conducted in Austria, Denmark, Israel and Qatar. Three studies that enrolled healthcare workers (HCW) and two studies that enrolled staff and residents of care homes were identified, all five were conducted in the UK.
- Across studies, the total number of PCR- or antibody-positive participants at baseline was 615,777. The median follow-up of individuals within studies was 4.4 months (range of medians: 1.8 to 7 months), with a maximum follow-up of over 10 months in three studies. Reinfection was a rare event (median PCR-confirmed reinfection rate: 0.27%, range: 0% to 1.1%), with no study reporting an increase in the risk of reinfection over time.
- Of the six general population studies, only one study estimated the population-level risk of reinfection based on whole genome sequencing. Sequencing was undertaken in a subset of participants with clinical evidence of reinfection from a larger cohort of 43,044 anti-SARS-CoV-2 nucleocapsid antibody positive participants at baseline. The estimated risk of reinfection was (0.1% [95% CI: 0.08 to 0.11%]), with no evidence of waning immunity for up to seven months.
- One study reported the relative risk of reinfection by age group. In individuals aged 65 years or more, the adjusted relative risk was 0.529 (95% CI: 0.372 to 0.753), compared with 0.173, 0.199 and 0.187 in individuals aged 0-34 years, 35-49 years and 50-64 years, respectively. However, one UK study that enrolled elderly residents of care homes (median age ≥84 years) reported a low relative risk of reinfection (adjusted Hazard Ratio [aHR] of 0.15).
- Three UK studies estimated the risk of reinfection based on PCR testing among HCWs (median follow-up ranged from 4.6 to 6.7 months):
    - The first study detected no symptomatic infections out of 1,038 HCWs with evidence of previous infection (0%, 95% CI: 0–0.4%), compared with 290 out of 10,137 HCWs without evidence of prior infection (2.9%, 95% CI: 2.6–3.2%, p<0.0001).

- The second study detected two asymptomatic infections (and no symptomatic infections) out of 1,265 seropositive HCWs, compared with 223 infections (100 asymptomatic and 123 symptomatic) out of 11,364 seronegative HCWs; the adjusted incidence rate ratio in HCWs who were seropositive at baseline was 0.11 (95% CI: 0.03 to 0.44) (adjusted for age, gender and month of testing).
- The third study reported 44 reinfections (15 of which were symptomatic) out of 6,614 seropositive HCWs, compared with 318 new PCR positive infections (249 of which were symptomatic) and 94 antibody seroconversions in the seronegative cohort of 14,173 individuals. The adjusted odds ratio was 0.17 in HCWs who were seropositive at baseline for all reinfections (95% CI: 0.13 to 0.24) and 0.08 (95% CI 0.05-0.13) for symptomatic reinfections.

- Two UK studies were identified that investigated the risk of reinfection in staff and residents of care homes.
  - In the first study, the relative risk of reinfection in two London care homes (with median ages of 84 and 85, respectively) was very low in the seropositive group (RR=0.038; 95% CI: 0.005 to 0.273), and the protection against reinfection after four months was estimated at 96.2% (95% CI: 72.7 to 99.5%).
  - In the second study, a sample of staff and residents (N=2,111) across 100 care homes in England were followed between October 2020 and February 2021. The estimated adjusted hazard ratio for reinfection, stratified by care home, was 0.15 (95% CI: 0.05 to 0.44) in residents (with a median age of 86) and 0.39 (95% CI: 0.19 to 0.82) in staff.
- As all studies were observational in nature, they cannot be used to demonstrate causality. Therefore, only longitudinal associations between prior infection and protective immunity can be measured.
- There are limitations relating to the applicability and generalisability of identified studies. Specifically:
  - No study reported the risk or relative risk of reinfection in paediatric populations.
  - Only two studies included data from after December 2020. The first study from Israel recorded higher counts of reinfection in January 2021 compared with March-December 2020. The second study followed care home residents and staff in the UK during a period of high community prevalence of SARS-CoV-2, associated with the rapid emergence of the

B.1.1.7 variant (October 2020 to February 2021). The adjusted relative hazard of infection comparing seropositive and seronegative groups was relatively low (aHR 0.15 in residents and 0.39 in staff). Sequencing data were not available for either study. Overall, there are insufficient data to evaluate the effectiveness of prior infection to prevent reinfection with new variants.

  - The applicability of the findings to vaccinated populations is unknown. All studies preceded vaccine roll-out, apart from one study that removed vaccinated individuals from the study 12 days after vaccination.

- While the clinical characteristics of reinfected cases were poorly reported across studies, reinfection events were generally not associated with severe disease.
- A scoping review was conducted to evaluate the long-term duration of immune responses following SARS-CoV-2 infection. Five studies were identified that investigated immune responses at ≥6 months post-infection, including two studies at ≥8 months post-infection. In general, studies reported a waning of antibody responses in the late convalescent period (3-6 months post-infection). However, T-cell and memory B-cell responses were still present, and in many cases increased, up to eight months post-infection in all study participants.
- In conclusion, 11 studies were identified that reported low rates of SARS-CoV-2 reinfection up to ten months following initial infection. Additionally, a scoping review of the long-term duration of immune responses found that while there may be a waning of antibody responses over time, T- and B-cell responses persist for up to eight months post-infection.

Duration of protective immunity (protection from reinfection) following SARS-CoV-2 infection

Background

The Health Information and Quality Authority (HIQA) has developed a series of evidence syntheses to inform advice from HIQA to the National Public Health Emergency Team (NPHET). The advice takes into account expert interpretation of the evidence by HIQA's COVID-19 Expert Advisory Group.

The following specific research question was developed and forms the basis of this evidence summary:

> How long does protective immunity (that is, prevention of antigen or RT-PCR confirmed reinfection) last in individuals who were previously infected with SARS-CoV-2 and subsequently recovered?

This evidence summary is expected to inform a range of policy questions relating to the duration of protective immunity following infection with SARS-CoV-2. Relevant policy questions include the following:

1. How long can asymptomatic individuals (including healthcare workers) who have recovered from a prior SARS-CoV-2 infection be exempted from restriction of movement policies if they become a close contact of a confirmed COVID-19 case?
2. How long can asymptomatic individuals who have recovered from a prior SARS-CoV-2 infection be exempted from serial testing, for example serial testing in indoor settings where social distancing is difficult (such as food processing facilities)?
3. How long can asymptomatic patients who have recovered from a prior SARS-CoV-2 infection be exempted from the requirement for testing prior to scheduled admission to hospital or inter institutional transfer?

The present review is an update of a review published on 8 March 2021 (https://www.hiqa.ie/sites/default/files/2021-03/Duration-of-protective-immunity_Evidence-Summary.pdf). Prior to this, four evidence summaries relating to immunity following SARS-CoV-2 infection were published by HIQA (13 May 2020, 9 June 2020, 6 August 2020 and 11 November 2020).

In the November update,[1] the following research questions were addressed:

> 1) Is reinfection with SARS-CoV-2 possible following recovery?

2) What is the long-term duration of the antibody response (≥2 months)?

The November 2020 update concluded that SARS-CoV-2 reinfection is possible, although a rarely reported event, and that antibody-mediated immune responses can be detected in most patients beyond two months and up to six months post-symptom onset. These data were limited by the longest duration of follow-up in identified studies, and cell-mediated responses were not considered. Due to the evolving nature of these data, a scoping review of the long term (≥6 months) duration of antibody-mediated (humoral) and cell-mediated immunity, including the development of immune memory, was also undertaken for the current review.

Sections below report both components to this review:

1) a systematic search of databases to identify cohort studies that estimated the risk of reinfection over time (updated to 19 March 2021)
2) a scoping review of the long-term duration of humoral and cellular responses following SARS-CoV-2 infection.

Part 1: Evidence summary – prevention of reinfection

Methods – systematic search

The processes outlined in HIQA's protocol for this review (www.hiqa.ie) were followed. Databases (PubMed, Embase and EuropePMC) were searched on 19 March 2021.

Table 1 outlines the Population Outcome Study design (POS) criteria for study selection relating to the systematic search.

Table 1. Population Outcome Study design (POS) criteria for systematic search

Population	Individuals (of any age) with evidence of prior SARS-CoV-2 infection, who subsequently recovered.* Evidence of prior infection includes diagnosis by RT-PCR or antigen testing, or evidence of an immune response through antibody detection (seropositivity). Subgroups include healthcare workers, age groups and high risk/very high risk groups (HSE definitions**)
Outcomes	**Prevention of reinfection** Primary outcomes: 1. Relative risk of RT-PCR or antigen-confirmed SARS-CoV-2 reinfection, comparing populations with evidence of prior infection with populations with no prior evidence of infection, at specified time points 2. Risk of RT-PCR or antigen-confirmed SARS-CoV-2 reinfection over time 3. Time interval between first and second infections 4. RT-PCR cycle threshold (Ct) results, if reported 5. Whole genome sequencing (WGS) results of reinfected cases comparing first and second infections, if reported
Types of studies	**Include:** ▪ Observational studies (prospective or retrospective) **Exclude:** ▪ Cohort studies that enrolled fewer than 100 participants unless the study reported comparative WGS on all reinfection cases (comparing first and second infections) ▪ Studies with durations of follow-up of less than 3 months ▪ Animal studies.

*'Recovered' refers to molecular or clinical evidence of viral clearance following initial infection; definitions of recovery in primary studies will be used. Common definitions include two consecutive negative respiratory RT-PCR tests 24 hours apart and WHO clinical criteria of viral clearance (27 May 2020).[2] **Definitions used by HSE[3]

Results – systematic search

The collective database search resulted in 1,893 citations, with four citations retrieved from other sources (grey literature search). Following removal of duplicates, 1,771 citations were screened for relevance. This resulted in 105 studies eligible for full text review (Figure 1), where a further 94 studies were excluded (Appendix 1).

Eleven studies were identified that met the inclusion criteria.[4-14] Five studies were conducted in the UK,[5, 6, 8, 10, 11] of which three enrolled healthcare workers[5, 6, 8] and two enrolled both staff and residents of elderly care homes.[10, 11] The remaining six studies were all general population studies, conducted in Austria,[13] Denmark,[14] Israel,[9] Qatar[4] and the US.[7, 12] Six studies are currently published as preprints.[4, 5, 7, 9, 11, 12] Across studies, the total number of PCR- or antibody-positive participants at baseline was 615,777 (median: 8,845; range: 88 to 378,606). No cases were identified on the basis of antigen testing. The longest duration of follow-up was not stated in all studies, or was provided only as an approximate estimate. When not stated, duration of follow-up was inferred from figures or tables within the study. The median follow-up of individuals within studies was 131 days (4.4 months), (range of medians: 54-210 days), with a maximum follow-up of ≥300 days (ten months) in three studies.[9, 11, 13] Studies reported a range of primary endpoints (Table 2 and Appendix 2).

Figure 1. PRISMA diagram of study selection

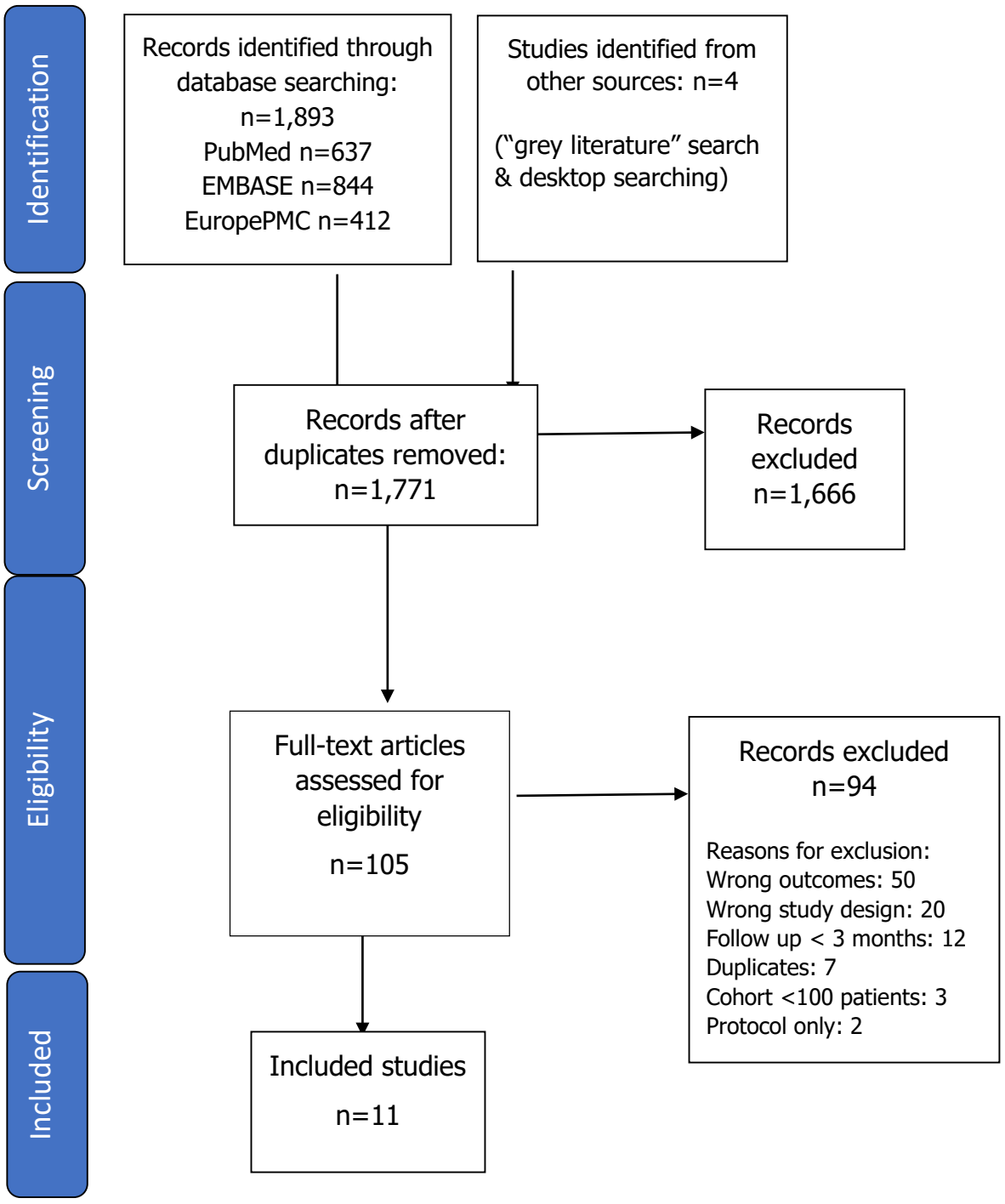


Table 2 Summary of included studies and primary outcome results

First author Country Population	Participants[a] Follow-up	Author reported primary outcomes	Quality appraisal
Abu-Raddad 2021[4] **Qatar** **General population**	N=43,044 **Median f/u:** 114 days (3.8 months) **Maximum f/u:** 242 days (8.1 months)	**Risk of reinfection (confirmed by WGS)[b]**: 0.10% (95% CI: 0.08 to 0.11%) **Risk over time:** Incidence rate of reinfection by month of follow-up did not show any evidence of waning of immunity over seven months of follow-up	'Fair' quality
Hall 2021[5] **UK** **HCWs**	N=6,614 **Median f/u:** 202 days (6.7 months) **Maximum f/u:** 227 days (7.6 months)	**Adjusted odds ratio of reinfection comparing antibody or PCR-positive group with negative group** ▪ 'Probable' reinfection[c]: aOR: 0.01 (95% CI 0.00-0.03) ▪ All 'possible' and 'probable' reinfections: aOR: 0.17 (95% CI: 0.13 to 0.24) ▪ Symptomatic reinfection: aOR: 0.08 (95% CI 0.05-0.13)	'Good' quality
Hanrath 2020[6] **UK** **HCWs**	N=1,038 **Median f/u:** 173 days (5.8 months) **Maximum f/u:** 229 days (7.6 months)	**Symptomatic reinfection:** A positive PCR test was returned in 0/1,038 (0% [95% CI: 0–0.4) of those with previous infection, compared with 290/10,137 (2.9% [95% CI: 2.6–3.2) of those without (P<0.0001 $\chi 2$ test).	'Fair' quality
Hansen 2021[14] **Denmark** **General population**	N= 11,068 **Median f/u:** 122 days (4.1 months) **Maximum f/u:** 295 days (9.8 months)	**Main analysis:** **Adjusted rate ratio (aRR) of reinfection**=0.20 (0.16–0.25) This represents 72 reinfections out of 1,346,920 person-days in PCR positive group, compared with 16,819 new infections out of 62,151,056 person-days in PCR negative group. **Additional cohort analysis (that includes all infection periods):** aRR=0.21 (0.18–0.25) By age group: ▪ 0-34 years: aRR=0.17 (0.13–0.23) ▪ 35–49 years: aRR=0.20 (0.14–0.28) ▪ 50–64 years: aRR=0.19 (0.13–0.27) ▪ ≥65: years: aRR=0.53 (0.37–0.75)	'Good' quality
Harvey 2020[7] **USA** **General population**	N=378,606 Median f/u: 54 days (1.8 months) Maximum f/u: 92 days (3.1 months)	Ratio of positive NAAT results (comparing patients who had a positive antibody test at index versus those without)[d]: 2.85 (95% CI: 2.73 to 2.97) at 0-30 days; 0.67 (95% CI: 0.6 to 0.74) at 31-60 days ; 0.29 (95% CI: 0.24 to 0.35) at 60-90 days; 0.10 (95% CI: 0.05 to 0.19) at >90 days	'Poor' quality

Jeffery-Smith 2021[10] **UK** **Staff & residents at care homes**	N=88 **Mean f/u:** 120 days (4 months) **Maximum f/u:** unclear	**Relative Risk:** 0.04 (95% CI: 0.005–0.27) This represents 1 reinfection out of 88 in seropositive group compared with 22/73 in seronegative group.	'Fair' quality
Krutikov 2021[11] **UK** **Staff & residents at care homes**	N=634 **Median f/u:** 79 days (2.6 months) **Maximum f/u:** 300 days (10 months)	**Relative adjusted hazard ratios for reinfection:** Residents of care home: aHR=0.15 (0.05-0.44)[e] Staff of care home: aHR=0.39 (0.19-0.82)[e]	'Good' quality
Lumley 2020[8] **UK** **HCWs**	N=1,265 **Median f/u:** 139 days (4.6 months) **Maximum f/u:** 217 days (7.2 months)	**Incidence rate ratio** (IRR[f])**:** 0.12 (95% CI, 0.03 to 0.47; p=0.002); 2/1,265 seropositive (both asymptomatic reinfections) and N=223/11,364 seronegative had positive PCR **Adjusted IRR[g]:** 0.11 (95% CI, 0.03 to 0.44; p=0.002)	'Good' quality
Perez 2021[9] **Israel** **General population**	N=149,735 **Median f/u:** 165 days (5.5 months) **Maximum f/u:** Approx. 325 days[h] (10.8 months)	**Overall reinfection risk:** 0.1% (at any time between March 2020 and January 2021) This represents 154 individuals who had two positive tests at least 100 days apart out of 149,735 individuals with a record of a prior positive PCR test.	'Fair' quality
Pilz 2021[13] **Austria** **General population**	N=14,840 **Median f/u:** 210 days (7 months) **Maximum f/u:** 300 days (10 months)	**Odds Ratio:** 0.09 (95% CI: 0.07 to 0.13) This represents 40 reinfections out of 14,840 individuals PCR positive in the first wave (0.27%) compared with 253,581 infections out of 8,885,640 (2.85%) in the remaining general population.	'Fair' quality
Sheehan 2021[12] **USA** **General population**	N=8,845 **Median f/u:** 131 days (4.4 months) **Maximum f/u:** 269 days (9 months)	Protective effectiveness against any reinfection: 78.5% (95% CI: 72.0% to 83.5%)[i] Protective effectiveness against symptomatic infection: 83.1% (95% CI: 75.1% to 88.5%)	'Fair' quality

Key: aHR – adjusted hazard ratio; aOR – adjusted odds ratio (adjusted for week group); CI – confidence interval; f/u – follow-up; HCW – healthcare worker; NAAT – nucleic acid amplification test; WGS – whole genome sequencing. Numbers rounded to two decimal points.

[a]In the baseline antibody and or PCR positive group ('seropositive' or prior positive cohort)

[b]Based on cases with WGS confirming the first and second infections were from different viral strains (N=16)

[c]'Possible' reinfection was defined as a participant with two PCR positive samples ≥90days apart with available genomic data, or an antibody positive participant with a new positive PCR at least four weeks after the first antibody positive result. A 'probable' case additionally required supportive quantitative serological data and or supportive viral genomic data from confirmatory samples

[d]NAAT used as proxy; includes all symptomatic reinfections and prolonged viral shedding, comparing patients who had a positive antibody test at index versus those with a negative antibody

[e]Multivariate analysis of risk of PCR positive infection by baseline antibody status, stratified by LTCF and adjusted for sex and age

[f]IRR is the relative incidence of subsequent positive SARS-CoV-2 PCR tests and symptomatic infections comparing antibody-positive and antibody-negative groups at baseline

[g]After adjustment for age, gender, and month of testing or calendar time as a continuous variable.

[g]Based on National Institutes of Health (NIH) quality appraisal criteria

[h]The midpoint of a range of follow-up dates was taken (300-349 days)

[i]Authors report effectiveness with the following calculation: 1-((56/8845)/(4163/141480))

Due to heterogeneity in outcome measures and populations, meta-analysis of data was not considered appropriate. The following sections narratively report the findings of included studies by population group (general population, healthcare workers, and residents and staff of care homes).

General population studies

Six studies were identified that investigated reinfection in the general population. Two studies were conducted in the US,[7, 12] and one each was conducted in Austria,[13] Denmark,[14] Israel[9] and Qatar.[4]

Austria

In the study by Pilz et al.,[13] national SARS-CoV-2 infection data from the Austrian epidemiological reporting system was used to investigate potential reinfection events. The primary outcome was the odds of PCR positivity in individuals who recovered from a confirmed SARS-CoV-2 infection during the first wave (February to 30 April 2020) compared with the odds of first infections in the remainder of the general population during the second wave (from 1 September to 30 November 2020).

In total, 40 possible reinfections were recorded out of 14,840 individuals with a history of prior infection during the first wave (0.27%), compared with 253,581 infections out of 8,885,640 individuals of the remaining general population (2.85%). This translated into an odds ratio of 0.09 (95% CI: 0.07 to 0.13).

Of the 40 possible reinfections, 62.5% were women and the median age was 39.8 years (range: 15.4 to 93.8). There were eight hospitalisations relating to the first infection and five hospitalisations relating to the second infection. Four patients were hospitalised during both infections. One death occurred which was not causally associated with reinfection. Detailed clinical or demographic information was not captured by the dataset. Cycle threshold values were not reported and whole genome sequencing was not performed.

Denmark

In the study by Hansen et al.,[14] individual-level data were collected on patients who had been tested in Denmark in 2020 from the Danish Microbiology Database. Infection rates were analysed during the second wave of the COVID-19 epidemic, from 1 September 2020 to 31 December 2020, comparing PCR-positive individuals with PCR-negative individuals during the first wave (March to May 2020). For the main analysis, people who tested positive for the first time between the two waves and those who died before the second wave were excluded. In an alternative cohort

analysis, infection rates were compared throughout the year, irrespective of date. In addition, infection rates by age category were reported in the alternative cohort analysis.

During the first wave (prior to June 2020), 533,381 people were tested, of whom 11,727 (2.2%) were PCR positive; 525,339 were eligible for follow-up in the second wave, of whom 11,068 (2.11%) had tested positive during the first wave. Among eligible PCR-positive individuals from the first wave, 72 (0.65%, 95% CI: 0.51 to 0.82%) tested positive again during the second wave compared with 16,819 of 514,271 (3.27%, 95% CI: 3.22 to 3.32%) who tested negative during the first wave. The daily rate of infection during the second wave was 5.35 positive tests per 100,000 people among those who had previously tested positive versus 27.1 per 100,000 people among those who previously tested negative. After adjusting for sex, age group, and test frequency, the adjusted RR (aRR) of reinfection was 0.20 (95% CI: 0.16 to 0.25). Protection against repeat infection was estimated at 80.5% (95% CI: 75.4 to 84.5).

In the alternative cohort analysis, the relative risk was similar (aRR of 0.21, 95% CI: 0.18 to 0.25, estimated protection 78.8%), however there was variation in the aRR by age group:

- 0–34 years: aRR=0.17 (0.13–0.23)
- 35–49 years: aRR=0.20 (0.14–0.28)
- 50–64 years: aRR=0.19 (0.13–0.27)
- ≥65: years: aRR=0.53 (0.37–0.75).

Among those aged 65 years and older, the observed protection against repeat infection was substantially lower, at 47.1% (95% CI: 24.7 to 62.8%). There was no difference in estimated protection against repeat infection by sex (male 78.4% versus female 79.1%). There was no evidence of waning protection over time (3–6 months of follow-up: 79.3% protection [95% CI: 74.4 to 83.3] versus ≥7 months of follow-up: 77.7% [95% CI: 70.9 to 82.9]). Clinical information on cases was not captured by the dataset. Cycle threshold values were not reported and whole genome sequencing was not performed.

Israel

In the preliminary preprint report by Perez et al.,[9] reinfection rates within the members of a large healthcare provider (Maccabi Healthcare Services) in Israel were reported. This healthcare provider has more than 2.5 million members (approximately 25% of the population) and is a representative sample of the Israeli population.

A total of 149,735 individuals had a recorded positive PCR test between March 2020 and January 2021. Among them, 154 members had two positive PCR tests at least 100 days apart and were included in this study. The reinfection rate was estimated at approximately 0.1%. In this cohort, 73 individuals (47.4%) had symptoms at both PCR positive events.

In terms of age distribution, reinfections were seen in small numbers across all age groups, with the highest absolute reinfection count observed among individuals aged 10 to 19 years. The first reinfection occurred in July 2020 and reinfection counts peaked in January 2021 (99 members). In terms of the time interval between infection events, 30 individuals had a second positive PCR test more than 200 days following their first positive PCR test. Cycle threshold values were not reported and whole genome sequencing was not performed.

Qatar

In the study by Abu-Raddad et al., 43,044 anti-SARS-CoV-2 nucleocapsid antibody positive participants were followed for a median of 3.8 months (maximum follow-up: 8.1 months) for evidence of reinfection.[4] This retrospective cohort was identified from a database that covers all serological testing for SARS-CoV-2 conducted in Qatar.

'Suspected cases' of reinfection included all SARS-CoV-2 antibody-positive individuals with at least one PCR positive swab that occurred ≥14 days after the first positive antibody test. These were further classified as showing either 'good' evidence, 'some' evidence, or 'weak'/'no' evidence of reinfection based on cycle threshold (Ct) and epidemiological criteria. Only 314 individuals had a PCR positive swab ≥14 days after the first-positive antibody test, and thus qualified for inclusion in the analysis. There were 1,099 swabs (551 positive and 548 negative) collected from these 314 individuals after the first positive antibody test. Investigation of these 314 suspected cases of reinfection yielded 32 cases with good evidence for reinfection (Ct≤30 for reinfection swab), 97 cases with some evidence (Ct>30 for reinfection swab), while evidence was weak for the remaining 185 cases.

Individuals with good or some evidence of reinfection had a median age of 37 years (range: <1 to 72 years) and included 92 men (71.3%). The median interval between the first positive antibody test and the reinfection swab was 52 days (range: 15 to 212 days). The median Ct value of the reinfection swab was 32.9 (range: 13.9 to 38.3). A third of cases were diagnosed based on clinical suspicion (n=34; 26.4%) or individual request (n=9; 7.0%), while the rest (n=86) were identified incidentally either through random PCR-testing campaigns/surveys (n=47; 36.4%), healthcare routine testing (n=18; 14.0%), contact tracing (n=15; 11.6%), or at a port of entry (n=6; 4.7%). At the time of reinfection, eight cases had records in the severity

database. One of these was classified as "severe" and two as "moderate", while the other five were classified as "asymptomatic." At time of primary infection, 14 cases had records in the severity database, one of whom was classified as "critical", three as "severe", five as "moderate", two as "mild", and three as "asymptomatic."

Among the 129 cases with good or some evidence for reinfection, 62 had records indicating prior diagnosis of a primary infection. Of these, viral genome sequencing evidence was available for 16 cases. Five of these 16 cases were confirmed as reinfections (confirmation rate: 31.3%). For one pair, there were few changes of allele frequency offering supporting evidence for reinfection. For the four other pairs, there were multiple clear changes of allele frequency indicating strong evidence for reinfection. One of the latter pairs also documented the presence of the D614G mutation (23403bp A>G) at the reinfection swab, a variant that has progressively replaced the original D614 form. For seven additional pairs, while there were one to several changes of allele frequency indicative of a shifting balance of quasi-species, there was no evidence for reinfection. For four pairs, there was strong evidence for *no* reinfection as both genomes were of high quality, yet no differences were found. Three of these four cases had a Ct<30 for the reinfection swab, indicating persistent active infection.

Applying the confirmation rate obtained through viral genome sequencing, the risk of documented reinfection was 0.1% (95% CI: 0.08 to 0.11%); that is, 31.3% of the suspected 129 reinfections in the cohort of 42,272 anti-SARS-CoV-2 positive participants (followed for 610,832 person-weeks). The incidence rate of documented reinfection was estimated at 0.66 per 10,000 person-weeks (95% CI: 0.56 to 0.78). There was evidence of a decreasing trend in the incidence rate of reinfection with each additional month of follow-up from the first month (incidence rate: 0.97 per 10,000; 52 cases per 167,149 person-weeks) to the sixth month (zero cases per 19,148 person-weeks) (Mantel-Haenszel trend analysis p-value: <0.001). There was an increase at ≥7 months, however this was only based on one case of reinfection (per 3,094 person-weeks).

These reinfections were compared to a cohort of 149,923 antibody-negative individuals followed for a median of 17 weeks (range: 0-45.6 weeks). Risk of infection was estimated at 2.15% (95% CI: 2.08-2.22%) and the incidence rate of infection was estimated at 13.69 per 10,000 person-weeks (95% CI: 13.22-14.14). The efficacy of natural infection against reinfection was estimated at 95.2% (95% CI: 94.1-96.0%).

USA

Two US studies were identified. In the first study, a retrospective database analysis of electronic health records was used to determine the risk of nucleic acid

amplification test (NAAT) positivity, a proxy for reinfection, in a cohort of antibody-positive versus antibody-negative individuals (Harvey et al.[7]). NAAT was used as a proxy for new infections or continued viral shedding.

A total of 3,257,478 unique patients with an index antibody test were identified after excluding 132 patients with discordant antibody tests on the index day. Of these, 2,876,773 (88.3%) had a negative index antibody result (seronegatives), 378,606 (11.6%) had a positive index antibody result (seropositives), and 2,099 (0.1%) had an inconclusive index antibody result (sero-uncertain). The linked data permitted individual longitudinal follow-up for a median of 47 days for the seronegative group (interquartile range (IQR): 8 to 88 days) and a median of 54 days for the seropositive group (IQR: 17 to 92 days).

Among patients with a positive index antibody result, 3,226 (11.3%) had a positive diagnostic NAAT during follow-up that occurred within 30 days of index, decreasing consistently to 2.7% from 31-60 days, 1.1% from 61-90 days, and 0.3% at >90 days. For the seronegative patients, 5,638 (3.9%) showed a positive NAAT result within 30 days. That proportion remained relatively consistent at ~3.0% over all subsequent periods of observation, including at >90 days. The ratio of positive NAAT results among patients who had a positive antibody test at index versus those with a negative antibody test at index declined from 2.85 (95% CI: 2.73 to 2.97) at 0-30 days; to 0.67 (95% CI: 0.6 to 0.74) at 31-60 days; to 0.29 (95% CI: 0.24 to 0.35) at 60-90 days; and to 0.10 (95% CI: 0.05 to 0.19) at >90 days. Cycle threshold values were not reported and whole genome sequencing was not performed.

In the second study (Sheehan et al.[12]), all 150,325 patients who underwent RT-PCR testing from 12 March 2020 to 30 August 2020 in one multi-hospital health system in Ohio and Florida were investigated. Tests on healthcare workers were excluded. The main outcome was reinfection, defined as RT-PCR positivity ≥90 days after initial testing. Secondary outcomes were symptomatic infection and protective effectiveness of prior infection. Infection rates were determined for distinct periods following the initial test: 4-5 months, 6-7 months and ≥8 months. Protective effectiveness of prior infection was calculated as one minus the ratio of infection rate for positive patients divided by the infection rate for negative patients.

In total, 150,325 (45.1%) patients had tests performed before 30 August 2020, of whom 8,845 (5.9%) tested positive and 141,480 (94.1%) tested negative. After at least 90 days, 974 (11%) of the positive patients were retested and 57 (5.9%) were reviewed for possible reinfection. One patient had an immediate negative test and was excluded due to a presumed false positive test. Of the 56 reinfections, 26 were symptomatic. Seventeen symptomatic patients were hospitalised within 30 days of the positive test, five with symptoms considered possibly related to COVID-19 (none required intensive care or needed mechanical ventilation).

Of those with negative initial tests, 22.8% (32,208/141,480) were retested and 4,163 (12.9%) were positive; 1,703 (40.9%) of these positive tests were performed for pre-procedural screening or had an asymptomatic indication. The protective effectiveness of prior infection against reinfection was estimated at 78.5% (95% CI: 72.0 to 83.5), and 83.1% (95% CI: 75.1 to 88.5) against symptomatic reinfection. Risk of reinfection was greatest just after 90 days and declined thereafter. Cycle threshold values were not reported and whole genome sequencing was not performed.

Of note, while this study included tests performed between 12 March 2020 and 7 January 2021, no disaggregated data are presented by specific time periods or calendar months.

Healthcare workers

Three UK studies were identified that exclusively enrolled healthcare workers. Additionally, a further two studies were identified that enrolled both staff and residents of elderly care homes (see next section).

In the first study by Hall et al.,[5] interim results from Public Health England's 'SIREN' study are reported. In total, 20,787 hospital staff (including healthcare workers, support staff and administrative staff) were followed between 18 June and 9 November 2020 for evidence of reinfection. Of these, 32% (n=6,614) were assigned to the positive cohort (antibody or PCR positive) and 68% (n=14,173) to the negative cohort (antibody negative, not previously known to be PCR or antibody positive). Enrolment began on 1 February 2020 with data censorship on 24 November 2020. Questionnaires and PCR testing was undertaken every two weeks and antibody testing every four weeks. In total, 1,339,078 days of follow-up data was analysed from the baseline positive cohort.

A 'possible' reinfection was defined as a participant with two PCR positive samples 90 or more days apart with available genomic data, or an antibody positive participant with a new positive PCR at least four weeks after the first antibody positive result. A 'probable' case additionally required supportive quantitative serological data and or supportive viral genomic data from confirmatory samples. The median interval between primary infection and reinfection beyond 90 days was 172 days (range: 90-227).

In total, 44 reinfections (2 probable, 42 possible) were detected in the baseline positive cohort (15 of which were symptomatic), compared with 318 new PCR positive infections (249 of which were symptomatic) and 94 antibody seroconversions in the negative cohort. The incidence density per 100,000 person

days was 3.3 reinfections in the positive cohort compared with 22.4 new PCR confirmed infections in the negative cohort.

The adjusted odds ratio was 0.17 for all reinfections ('possible' or 'probable'; 95% CI: 0.13 to 0.24). Restricting reinfections to probable reinfections only, participants in the positive cohort had a 99% lower odds of probable reinfection, adjusted odds ratio (aOR) 0.01 (95% CI 0.00-0.03). Restricting reinfections to those who were symptomatic, investigators estimated that participants in the positive cohort had an aOR of 0.08 (95% CI 0.05-0.13).

The two probable reinfections from this cohort are described in a separate paper that is awaiting publication.[15] Both of these cases were symptomatic with high viral loads and there was a boosted antibody response. Genome sequencing demonstrated phylogenetic relatedness to concurrently circulating strains.

In the second study by Hanrath et al.,[6] symptomatic reinfection in UK healthcare workers during the second wave of the UK pandemic was investigated, comparing those who had evidence of prior SARS-CoV-2 infection from the first wave with those who had no evidence of prior infection. In the first wave (10 March to 6 July 2020), 481/3,338 symptomatic healthcare workers tested positive for SARS-CoV-2 by PCR, while SARS-CoV-2 IgG was detected in 937/11,103 (8.4%). From these, 1,038 healthcare workers were identified with evidence of previous infection (PCR and or antibody positive) and 10,137 without (negative antibody and PCR). The primary endpoint for analysis was symptomatic SARS-CoV-2 infection, defined as a positive PCR for SARS-CoV-2 from a combined nasopharyngeal/oropharyngeal swab taken as part of a symptomatic staff testing programme in the period from 7 July 2020 to 20 November 2020.

During the second time period, 2,243 symptomatic healthcare workers underwent PCR testing; 128 of these had previous confirmed SARS-CoV-2 infection while 2,115 had not. In those previously infected, there was a median of 173 (IQR: 162–229) days from the date of first positive PCR or antibody result to the end of the analysis period. Test positivity rates were 0% (0/128 [95% CI: 0–2.9]) in those with previous infection compared to 13.7% (290/2,115 [95% CI: 12.3–15.2]) in those without ($p<0.0001$, $\chi 2$ test). Considering the population as a whole, a positive PCR test was returned in 0% (0/1,038 [95% CI: 0–0.4%]) of those with previous infection, compared to 2.9% (290/10,137 [95% CI: 2.6–3.2]) of those without ($p<0.0001$, $\chi 2$ test).

Fewer healthcare workers in the previous infection group presented for symptomatic testing in the second period: 128/1,038 (12.3% [95% CI: 10.5–14.5]) compared with 2,115/10,137 (20.8% [95% CI: 20.1–21.6]) in the group without previous infection ($p<0.0001$ $\chi 2$ test). Asymptomatic PCR screening was undertaken on a

pilot basis in an additional 481 healthcare workers, 106 with past infection and 375 without. These healthcare workers were distinct from the study population. There were similarly no positive results in the group with previous infection, 0/106 (0% [95% CI: 0–3.5]), compared with 22/375 (5.9% [95% CI: 3.9–8.7], p=0.011) positive PCR results in the group without previous infection, consistent with results of symptomatic testing.

In summary, there were no reinfection events in healthcare workers with prior evidence of infection (compared with 2.9% positivity in those without evidence of prior infection). Additionally, in a separate population, there were no asymptomatic reinfections in healthcare workers with evidence of prior infection (compared with 5.9% positivity in those without evidence of prior infection).

In the third study by Lumley et al.,[8] a cohort of 12,541 UK healthcare workers were followed for up to 31 weeks (from 23 April to 30 November 2020) to compare the incidence of SARS-CoV-2 infection in the group that was antibody seropositive versus seronegative at baseline. After initial assessment of antibody status, the researchers tracked the presence of viral RNA using PCR over time. Baseline antibody status was determined by anti-spike (primary analysis) and anti-nucleocapsid IgG assays. PCR testing was undertaken in those who became symptomatic; asymptomatic screening (serial testing) was also undertaken (PCR testing every two weeks and serological testing every two months).

In total, 12,541 healthcare workers participated and had anti-spike IgG measured; 11,364 were followed up after negative antibody results and 1,265 after positive results, including 88 in whom seroconversion occurred during follow-up. A total of 223 anti-spike seronegative healthcare workers had a positive PCR test (1.09 per 10,000 days at risk), 100 during screening while they were asymptomatic and 123 while symptomatic, whereas two anti-spike seropositive healthcare workers had a positive PCR test (0.13 per 10,000 days at risk); both workers were asymptomatic when tested. Incidence varied by calendar time, reflecting the first (March through April) and second (October and November) waves of the pandemic in the UK, and was consistently higher in seronegative healthcare workers.

After adjustment for age, gender, and month of testing or calendar time as a continuous variable, the incidence rate ratio in seropositive workers was 0.11 (95% CI: 0.03 to 0.44) compared with those who were seronegative at baseline.

Parallel testing for antibodies against another SARS-CoV-2 antigen, the nucleocapsid protein, revealed similar results. Taking 'any' antibody positive cases, three healthcare workers subsequently had PCR positive tests (one with anti-spike IgG only, one with anti-nucleocapsid IgG only, and one with both antibodies). The time between initial symptoms or seropositivity and subsequent positive PCR testing

ranged from 160 to 199 days. Only the healthcare worker with both antibodies had a history of PCR-confirmed symptomatic infection that preceded serologic testing; after five negative PCR tests, this worker had one positive PCR test (low viral load: cycle number, 21 [approximate equivalent cycle threshold: 31]) at day 190 after infection while the worker was asymptomatic, with subsequent negative PCR tests 2 and 4 days later and no subsequent rise in antibody titres. Whole genome sequencing was not performed.

Residents and staff of elderly care homes

Two studies were identified that enrolled both residents and staff at UK care homes.[10, 11]

In the first study (Jeffery-Smith et al.[10]), the risk of reinfection according to antibody seropositivity was investigated following outbreaks in two London care homes[10, 16] with high rates of SARS-CoV-2 seropositivity after outbreaks in the first wave of the pandemic. In the first care home, serological investigations in June 2020 identified 50% as seropositive after the first outbreak (18/32 residents; 15/34 staff), and in the second care home, serological investigation in May 2020 identified 50.4% as seropositive (26/52 residents; 33/65 staff).

In total, 88 individuals with evidence of prior infection were investigated for evidence of reinfection (antibody positive N=87; RT-PCR positive N=1). The reinfection rate in this cohort was 1/88 (1.1%), and this reinfection event was observed in a staff member. By comparison, infection risk in the seronegative cohort was 30.1% (22/73, including four people diagnosed by seroconversion). The RR was estimated at 0.038 (95% CI: 0.005 to 0.273). The protection against reinfection after four months in seropositive group was estimated at 96.2% (95% CI: 72.7 to 99.5%).

In terms of whole genome sequencing, the second COVID-19 outbreaks experienced by both care homes were due to SARS-CoV-2 strains that were genetically distinct from their respective first outbreaks (Appendix 2), and fatal cases in residents had identical viral genomes to surviving residents. Ct values were not reported.

In the second study, staff and residents in 100 long term care facilities (LTCFs) in England were followed between October 2020 and February 2021 (Krutikov et al.[11]). In total, 2,111 individuals were enrolled (682 residents and 1,429 staff). The median age of residents was 86 years (IQR: 79-91) and 47 years for staff (IQR range: 34-56). Blood sampling was offered to all participants at three time points separated by 6-8 week intervals in June, August and October 2020. Samples were tested for IgG antibodies to nucleocapsid and spike protein. PCR testing for SARS-CoV-2 was undertaken weekly in staff and monthly in residents. The time-at-risk

('entry time') for participants was 1 October 2020 or 28 days after their first available antibody test, whichever was later. The primary analysis estimated the adjusted hazard ratio (aHR) of a PCR-positive test by baseline antibody status (Cox regression adjusted for age and gender, and stratified by LTCF). Discrepancies were noted in this study, whereby the results of the Cox regression were reported differently in the abstract and results sections. The findings presented in this review reflect those in the study's results section only.

Baseline IgG antibodies to nucleocapsid were detected in 226 residents (33%) and 408 staff (29%). Staff and residents contributed 3,749 and 1,809 months of follow-up time, respectively. There were 93 PCR-positive tests in seronegative residents (0.054 per month at risk) compared with four in seropositive residents (0.007 per month at risk). There were 111 PCR-positive tests in seronegative staff (0.042 per month at risk) compared with 10 in seropositive staff (0.009 per month at risk). Controlling for the potential confounding effect of individual LTCFs, the relative aHRs for PCR positive infection were 0.15 (95% CI: 0.05 to 0.44) and 0.39 (95% CI: 0.19 to 0.82) comparing seropositive versus seronegative residents and staff, respectively.

Of 12 reinfected participants with data on symptoms, 11 were symptomatic. None of the reinfection cases were admitted to hospital or died as a result of their infection. Ct values were retrieved for 13/14 reinfection samples; the median Ct value for reinfection cases was 36. Antibody titres to spike and nucleocapsid were comparable in PCR-positive and PCR-negative cases. Whole genome sequencing was not performed.

Study authors concluded that the presence of IgG antibodies to nucleocapsid was associated with substantially reduced risk of reinfection in staff and residents for up to 10 months after primary infection, assuming that the earliest infections occurred in March 2020.

Quality of included studies

The National Heart, Lung and Blood Institute (NIH) quality assessment tools was used for appraisal of observational cohort studies.[17] Ten studies were considered of 'good' or 'fair' methodological quality (Appendix 3), with one study[7] that used a proxy measure for outcomes (NAAT positivity) considered to be of poor quality. The baseline exposure ('any' antibody) testing and subsequent reinfection events (NAAT positivity) in this study were derived from a database analysis and the specific tests used, and the validity of these tests, cannot be evaluated. The clinical characteristics of seropositive individuals who subsequently tested positive by NAAT, and the course of disease, could not be determined. The reason for NAAT testing (screening or

symptomatic testing) is unknown. Additionally, the follow-up was not considered long enough to adequately capture reinfection events (median 1.8 months).

A number of studies were downgraded due to lack of controlling for confounders (n=7 studies). In these studies, potential confounding variables were either not assessed or not measured appropriately, or the statistical analysis was not adequately described (Appendix 3). As all studies were observational in nature, they cannot be used to demonstrate causality. Therefore, only associations between prior infection and reinfection risk can be measured. While estimates of the effectiveness of natural infection to prevent reinfection were reported in a number of studies, such measures cannot be reliably estimated on the basis of these data. Observational studies are prone to bias and confounding. For example, individuals who are aware of their infection status may have altered testing behaviour, introducing potential ascertainment bias. Over half of included studies (8 of 11) were retrospective in nature.

Six studies are currently published as preprints,[4, 5, 7, 9, 11, 12] so have not yet been formally peer-reviewed, raising additional concerns about overall quality and the potential for results to change prior to formal publication.

Each of the ten studies of 'good' (n=4) or 'fair' (n=6) methodological quality were considered large enough to adequately capture reinfection events in their respective populations. While studies followed individuals for a prolonged duration of time, it is notable that all but two studies[9, 11] preceded the widespread emergence and spread of a number of new viral strains of international concern (for example, variant 202012/01 from the UK and 501Y.V2 from South Africa, both identified in December 2020[18]). Therefore, the applicability of these studies to populations that are experiencing the emergence and spread of new variants of concern is unknown.

Discussion – systematic search

Summary of findings

Eleven cohort studies estimated the risk or relative risk of SARS-CoV-2 reinfection in individuals who were either antibody-positive or who had a history of PCR-confirmed COVID-19 at baseline, compared with those who did not, for up to ten months. Across studies, the total number of PCR- or antibody-positive participants at baseline was 615,777, with a maximum follow-up of over ten months in three studies. Reinfection was a rare event (median PCR-confirmed reinfection rate: 0.27%, range: 0% to 1.1%), with no study reporting an increase in the risk of reinfection over time. Apart from risk of reinfection, a range of other primary outcome measures were reported, including odds ratios, relative risks and hazard ratios comparing individuals with evidence of prior infection with individuals without. A number of

studies controlled for confounding and reported adjusted figures (adjusted for variables such as age, sex, testing frequency and calendar month), while others did not. Due to heterogeneity in outcome measures and populations, meta-analysis of data was not considered appropriate.

Of the six general population studies, only one estimated the population-level risk of reinfection based on whole genome sequencing in a subset of patients with supporting evidence of reinfection.[4] The estimated risk was low (0.1% [95% CI: 0.08 to 0.11%]) in this large cohort of 43,044 anti-SARS-CoV-2 nucleocapsid antibody positive participants. Importantly, the incidence rate of reinfection by month did not show any evidence of waning of immunity over the seven months of follow-up. Compared with a cohort of 149,923 antibody-negative individuals, authors report an effectiveness of natural immunity against reinfection of 95.2% (95% CI: 94.1-96.0%) for at least seven months. However, given the observational nature of the data, any estimate of effectiveness is uncertain and subject to bias. The remaining population-based studies (conducted in Austria, Denmark, Israel and the US) also reported low absolute and relative risks of reinfection.

Only one study reported the relative risk of reinfection by age category, allowing comparisons across groups. In individuals aged 65 years or more, the aRR was 0.53 (0.37–0.75), compared with 0.17, 0.20 and 0.19 in individuals aged 0-34 years, 35-49 years and 50-64 years, respectively.[14] The lower protection in the over-65s group may be attributable to immunosenescence; however, little is known about this phenomenon in the context of COVID-19. While this study reported low rates in the 0-34 years age group, it is notable that disaggregated data specific to the paediatric population (<18 years) were not reported. Two UK studies that enrolled elderly residents of care homes reported lower relative risks of reinfection. One reported a much lower risk RR 0.038 (95% CI: 0.005 to 0.273),[10] and the only recorded reinfection occurred in a staff member and not an elderly resident of the care home. Another reported an adjusted hazard ratio of 0.15 (95% CI: 0.05 to 0.44) in residents.[11]

Only one study reported data specific to the paediatric group.[9] In this preliminary study, raw count of reinfections in individuals aged 10 to 19 years was higher than in other age categories; however, a risk or relative risk was not reported in this age category. There were a number of limitations with this study; only preliminary assessments were carried out on the study population, mainly counts and proportions; the testing indication or frequency was not reported; significance testing comparing reinfection rates in different age groups was not performed, and infection rates relative to individuals without evidence of prior infection were not estimated.

Three UK studies estimated the risk of reinfection specifically among healthcare workers (median follow-up ranged from 4.6 to 6.7 months in individuals with evidence of prior infection).[5, 6, 8] Risk was expressed as odds ratios or incidence rate ratios, comparing individuals with evidence of prior infection (antibody and or PCR positive tests) with healthcare workers with no evidence of prior infection (antibody-negative, no prior PCR positive test). The first study detected zero symptomatic infections in 1,038 healthcare workers with evidence of a prior infection, compared with 290 in 10,137 without evidence of prior infection ($p<0.0001$).[6] The second study detected two asymptomatic infections (and no symptomatic infections) out of 1,265 seropositive individuals, compared with 223 infections (100 during screening while they were asymptomatic and 123 while symptomatic) out of 11,364 seronegative individuals.[8] After adjustment for age, gender, and month of testing or calendar time, the incidence rate ratio in seropositive healthcare workers was 0.11 (95% CI: 0.03 to 0.44). The third study reported 44 reinfections in the baseline positive cohort of 6,614 individuals (15 of which were symptomatic), compared with 318 new PCR positive infections (249 of which were symptomatic) and 94 antibody seroconversions in the negative cohort of 14,173 individuals.[5] The adjusted odds ratio (aOR) was 0.17 for all reinfections (95% CI: 0.13 to 0.24), and restricting reinfections to those who were symptomatic, the aOR was 0.08 (95% CI 0.05-0.13).

Two UK studies were identified that investigated the risk of reinfection in staff and residents of care homes, a group that has been disproportionately affected by the COVID-19 pandemic, with high rates of infection and deaths among frail, elderly residents. In the first study, the relative risk of reinfection in two London care homes was very low (RR=0.038; 95% CI: 0.005 to 0.273), and the protection against reinfection after four months in seropositive group was estimated at 96.2% (95% CI: 72.7 to 99.5%).[10] This relative risk was based on a single reinfection event in a seropositive staff member, indicating the relative risk in the elderly resident cohort is even lower. In terms of whole genome sequencing, the second COVID-19 outbreaks experienced by both care homes were due to SARS-CoV-2 strains that were genetically distinct from their respective first outbreaks, and in both care homes fatal cases in residents had identical viral genomes to cases among surviving residents. The second study reported higher relative rates of reinfection[11] in a sample of staff and residents (N=2,111) across 100 LTCFs in England (median age: 85). The study, conducted between October 2020 and February 2021, coincided with a period of high community prevalence of SARS-CoV-2 in the UK, associated with the rapid emergence of the B.1.1.7 variant.[19] The estimated adjusted hazard ratio (aHR) for reinfection was 0.15 (95% CI: 0.05 to 0.44) in residents and 0.39 (95% CI: 0.19 to 0.82) in staff. The higher relative rates of infection compared with the earlier UK study raises concerns regarding the impact of new variants on the protective immunity of natural infection. Nonetheless, only four cases of possible reinfection were identified in residents, and although all cases reported symptoms, none

required hospital treatment. Taking into consideration that most residents were likely first infected during the first wave (up to six months prior), the risk of reinfection was substantially reduced in residents even in the context of high community transmission of the B.1.1.7 variant.

HIQA's earlier evidence summary (November 2020[1]) gathered information on potential individual SARS-CoV-2 reinfection cases (based on whole genome sequencing) to determine whether reinfection is possible. While the aim of the present review was to estimate the risk of reinfection and thus only considered cohort studies, a number of individual reinfection cases have been reported since the review published in November, including a number of cases involving emerging variants of concern. During the period 1 November 2020 to 22 February 2021, 28 new reinfections have been reported (Appendix 4). The sequencing results of six of these 28 reinfection cases identified variants of concern. Five reinfection cases from Brazil (three in the state of Amazonas, including one specifically from the city of Manaus,[20, 21] one in the state of Rio Grande do Norte[22] and one in the state of Bahia[23]) were attributable to the new Brazilian variant of concern (P1 lineage, which includes lineages B.1.1.28 and B.1.1.248). Additionally, one reinfection case in the UK[24] was attributable to the new UK variant of concern (B.1.1.7 lineage). The median time interval between infection events was 199 days across all cases, over six months after initial infection.

Unpublished data gathered by the Health Protection Surveillance Centre (HPSC) in Ireland support the findings of this review. The HPSC provided preliminary data relating to suspected reinfection cases during the period 2 March 2020 to 23 March 2021. Of 232,738 confirmed cases of COVID-19 notified during this time, 514 were potentially reinfections, giving a reinfection rate of approximately 0.2%. This is based on the criteria of ≥84 days interval between notification or specimen dates of PCR positives. This rate falls within the range of absolute reinfection rates identified in the present review. Further afield, the State Institute of Public Health of Czechia (SZU) have reported a reinfection rate of 0.1% (1,400 cases out of 1,225,000 infections).[25] Note that the Czech criteria for identifying cases differ from the Irish criteria – in Czechia only symptomatic reinfections are counted and the minimum interval between infection events is 60 days.

Strengths and limitations

In this review, all studies were considered large enough to adequately capture reinfection events in their respective populations. Results across studies consistently demonstrated a substantially lower risk of reinfection in previously infected individuals without a waning of the protective response over time. However, despite these strengths, there are a number of limitations associated with this review.

Firstly, as the studies are observational in nature, the prevention of reinfection cannot be causally confirmed, although longitudinal associations can be estimated. Additional concerns relating to observational studies include the greater potential for bias. Across all studies, it is possible that antibody test results affected individual behaviour. Individuals with evidence of prior infection may have believed that they possessed immunity to SARS-CoV-2, resulting in a reduction in health-seeking behaviour and testing (outcome ascertainment bias). Conversely, these individuals may have increased their engagement in social behaviour, placing them at greater risk for infection. The overall direction of bias (whether over- or under-estimating reinfection) cannot be determined.

Secondly, serological studies cannot determine whether past seroconversion, or current antibody levels, determine protection from infection. Furthermore, none could define which characteristics are associated with reinfection. The role of T-cell immunity was not assessed in any study, therefore it is not possible to determine whether protection from reinfection is conferred through the measured antibodies or T-cell immunity.

Thirdly, only two studies undertook genomic sequencing of reinfected cases. Therefore, the results of nine studies are only based on potential reinfections. The effect of this, however, is to overestimate the number of reinfections, thereby affirming the conclusion that reinfection is rare.

Fourthly, due to the nature of a number of retrospective database analyses included in this review, many studies could not correlate symptomatic infections with protection against repeat infection or evaluate disease progression comparing first and second infections. This was true for studies that accessed large databases in Austria,[13] Denmark,[14] and the US.[7]

In addition to these limitations, there are a number of issues relating to the applicability and generalisability of the presented results. Firstly, all but two studies preceded the widespread identification and spread of a number of new viral strains of international concern (for example, variant 202012/01 from the UK and 501Y.V2 from South Africa, both identified in December 2020[18]). In the first study that extended beyond December 2020, reinfection events between March 2020 and January 2021 in Israel were recorded.[9] A higher number of reinfections were recorded in January 2021 compared with previous months. However, genomic sequencing was not reported and statistical analysis of the recorded data (for example, controlling for confounders and significance testing) was not undertaken. In the second study, elderly care home staff and residents in the UK were followed between October 2020 and February 2021.[11] Sequencing data were not available for suspected reinfections, and study authors did not investigate the potential impact of new variants on the risk of reinfection. Nonetheless, the risk of reinfection

(expressed as hazard ratios in the study) was substantially reduced in elderly residents, most of whom were first infected up to six months previously. While these findings are reassuring, overall, the applicability of included studies to populations that are experiencing the emergence and spread of new variants of concern is unknown. Of note, one study included tests performed between 12 March 2020 and 7 January 2021, however no disaggregated data are presented by specific time periods.[12]

Secondly, all presented data relate to unvaccinated cohorts as they preceded vaccine roll-out in ten studies, and in the only study that was conducted during vaccine roll-out, all vaccinated individuals were excluded once 12 days had passed since their vaccination.[11] The applicability of the data to vaccinated populations is therefore unknown.

Thirdly, there is much uncertainty in relation to the risk of reinfection in younger and older age groups. Inconsistent data were identified relating to elderly populations, with one study reporting higher rates of reinfection compared with younger age groups[14] and two reporting low rates of reinfection in older groups (although these two studies did not compare risk across age groups).[10, 11] One preliminary study reported higher reinfection counts in those aged 10-19 years;[9] no other study reported disaggregated data for paediatric groups.

Future longitudinal studies should focus on the following issues that were not addressed in the aforementioned studies, including:

- the durability of immunity beyond ten months
- immune correlates of protection
- protective immunity in paediatric populations
- protective immunity in populations with comorbidities and the immunocompromised
- the impact of new variants on protective immunity.

Part 2: Scoping review of long-term humoral and cell-mediated responses

Introduction – scoping review

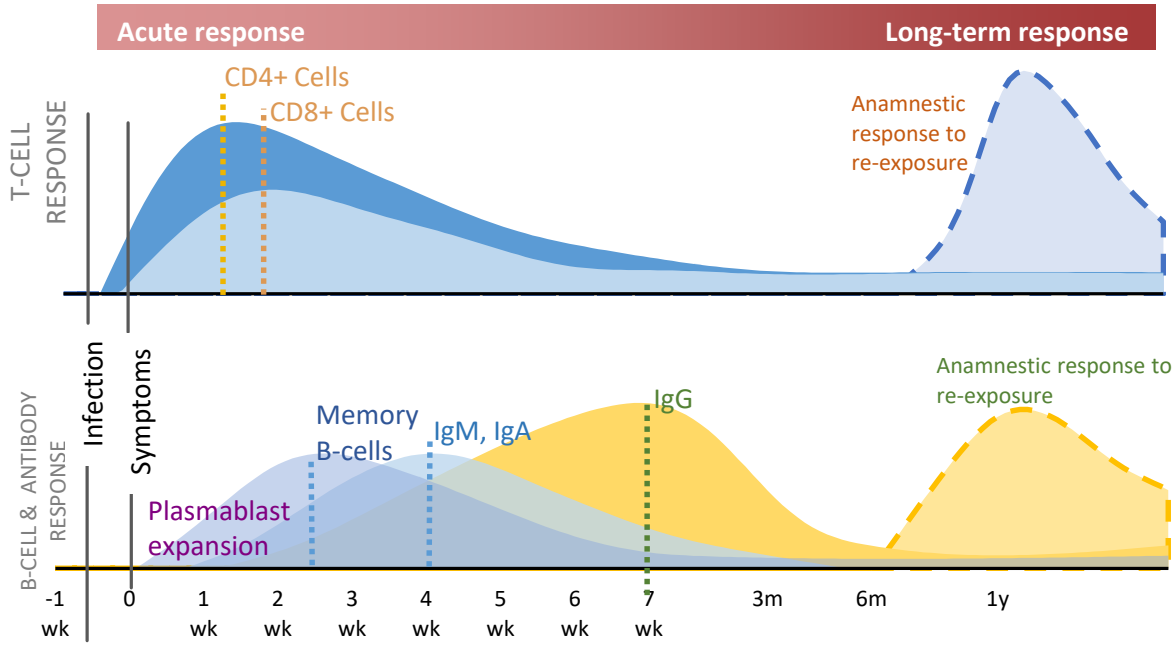
In November 2020, HIQA conducted a review of the long-term duration of antibody responses following SARS-CoV-2 infection.[1] Twenty-two studies were identified that examined the duration of immunoglobulin G (IgG) and or neutralising antibodies for longer than 60 days post-infection. This review concluded that antibody-mediated immune responses can be detected beyond two months and up to six months post-symptom onset in most individuals. In terms of antibody titres, just over half of studies (n=7/12) found that IgG titres were maintained, or increased, until the end of follow-up, while five studies reported a reduction in IgG titres over time. All but one study that reported neutralising antibody titres reported a substantial decline over time, in particular at the later stages of follow-up.

While the review focussed on the primary humoral immune response to SARS-CoV-2, (that is, the response to the first exposure to the antigen), some data were extracted that related to immune memory (secondary response). For example, one study found that cell-mediated T-cell responses were maintained in 96% (22/23) of patients three to eight months post-symptom onset. Notably, the only patient who had no T-cell response at four months had a detectable memory B-cell response.[26] Another study reported IgG specific memory B-cells increase over time,[27] and another found that virus-specific memory T and B-cells persisted and in some cases increased over three months.[28] These data were limited by the longest duration of follow-up in identified studies (across studies, mean maximum follow-up was 97 days) and the review focused mainly on the primary humoral immune response to SARS-CoV-2.

The purpose of this scoping review was to investigate longer-term duration (≥6 months) persistence of humoral *and* cell-mediated responses following SARS-CoV-2 infection.

For illustration, Figure 2 outlines the projected acute and long-term adaptive responses following SARS-CoV-2 infection (adapted from Stephens and McElrath[29]).

Figure 2. Projected acute and long-term immune responses following SARS-CoV-2 infection



Adapted from: Stephens and Mc Elrath; JAMA, 2020: Generalized model of T-cell and B-cell (plasmablast, antibody) responses to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection projected over 1 year following infection. Neutralising antibodies, memory B cells, and CD4+ and CD8+ memory T cells to SARS-CoV-2, which are generated by infection, vaccination, or after re-exposure, are key to the path to immunity. The dotted lines represent peak B-cell, T-cell, and antibody responses following infection.

Methods – scoping review

In line with HIQA standard operating procedure for the conduct of scoping reports, a search of the literature was undertaken using the PubMed Clinical Queries Tool. The results were limited to English-language studies conducted in humans and published between 9 September 2020 and 4 February 2021. The following search terms were used, in combination with the PubMed filters for identifying COVID-19 literature and transmission-related topics within COVID-19 literature: ((SARS-CoV-2 OR COVID-19) AND (antibody OR antibodies OR immunity)). This search was complemented by a desktop search (Google, Google Scholar and international public health websites).

Results – scoping review

The database search (PubMed clinical queries) resulted in the screening of 412 citations. A number of individual studies and narrative reviews of studies were identified that described the long-term duration of immune responses beyond six months post-infection, including:

- two studies that reported immune responses ≥8 months post-infection,[30, 31]
- three studies at 6-8 months post-infection,[32-34]

- one narrative review of studies reporting secondary cellular responses[29] and
- two narrative reviews of studies that report mucosal immunity.[35, 36]

Studies with ≥8 months follow-up

Two studies were identified that demonstrate SARS-CoV-2 immune responses at ≥8 months post-infection, however the durability of antibody responses differed between studies.[30, 31, 34] The first study followed a small cohort (n=25) of convalescent patients in Australia ≥8 months post-infection.[30] Serum antibodies and B-cell responses were measured between 4 and 242 days post-symptom onset, and while serum IgG to receptor binding domain (RBD) and nucleocapsid protein (NCP) was identified in all patients, antibody titres began declining at 20 days post-symptom onset. All patients demonstrated the presence of memory B cells (immune cells that "remember" viral proteins and can trigger rapid production of antibodies when re-exposed to the virus). RBD- and NCP-specific memory B cells predominantly expressed IgM+ or IgG1+ and continued to rise until 150 days. RBD-specific IgG+ memory B-cells were predominantly CD27+ and numbers significantly correlated with circulating follicular helper T cell numbers. Authors concluded that the SARS-CoV-2 antibody response contracts in convalescence, with persistence of RBD- and NCP-specific memory B cells.

The second study followed a small cohort (n=58) of COVID-19 patients in South Korea for ≥8 months post-infection.[31] The cohort consisted of seven participants with asymptomatic SARS-CoV-2 infection and 51 patients with mildly symptomatic COVID-19. Four different assays were used to detect SARS-CoV-2–specific antibodies, and to evaluate neutralising activity targeting the spike receptor–binding domain, a surrogate virus neutralisation test (sVNT) was used. Rates of antibody positivity according to three commercial kits was still high at eight months after infection (up to 91.4% positivity). Neutralising activity was detected in 53.4% of asymptomatic or mildly symptomatic participants after eight months of infection, which was considerably lower than the rate of positivity detected by binding immunoassays. The differences in antibody detection rates both within this study and compared with the first study are likely due to variations in immunoassay test characteristics and performance.

Studies with 6-8 months follow-up Three studies were identified that followed patients for 6-8 months post-infection.[32-34] The first study analysed multiple compartments of circulating immune memory to SARS-CoV-2 in 254 samples from 188 Covid-19 cases, including 43 samples at ≥6 months post-infection.[32] IgG to spike protein was relatively stable over six months or more, while CD4+ T cells and CD8+ T cells declined with a half-life of 3-5 months. However, spike-specific memory B-cells were more abundant at six months compared with one month post-symptom onset. Study authors note that it is well-recognised that the magnitude of

the antibody response against SARS-CoV-2 is highly heterogeneous between individuals. The authors observed that heterogeneous initial antibody responses did not collapse into a homogeneous circulating antibody memory; rather, heterogeneity is also a central feature of immune memory to the virus. While acknowledging that direct conclusions about protective immunity cannot be made on the basis of quantifying circulating antibodies, memory B cells, CD4+ and CD8+ T cells, immune memory in at least three immunological compartments was measurable in approximately 95% of subjects five to eight months post-symptom onset, indicating that durable immunity against secondary SARS-CoV-2 is a possibility in most individuals.

The second study investigated the durability of neutralising antibodies and T-cell responses in serum specimens collected from 17 COVID-19 patients six to seven months post-infection, comparing the results to those from cases investigated two weeks to two months post-infection.[33] All samples were positive for IgG against the S- and N-proteins of SARS-CoV-2. Notably, 14 samples available at six to seven months post-infection all showed significant neutralising activities in a pseudovirus assay, with no difference in blocking the cell-entry of the 614D and 614G variants of SARS-CoV-2. Furthermore, in ten serum samples from cases at six to seven months post-infection used for memory T-cell tests, interferon γ-producing CD4+ and CD8+ cells were increased upon SARS-CoV-2 antigen stimulation. Together, these results indicate that durable anti-SARS-CoV-2 immune responses are common in convalescent patients.

The third study sampled a small cohort (n=32) of COVID-19 patients at four longitudinal time points between 16 and 233 days post-infection.[34] Even though overall circulating anti-spike antibodies contracted over time during convalescence, RBD-specific B cells increased and persisted up to eight months post symptom onset. The total RBD-specific immunoglobulin levels, comprising of IgG, IgM, and IgA, gradually decreased between six and 31 weeks after the onset of symptoms. However, the percentage of convalescent individuals presenting detectable RBD-specific Ig levels remained stable, with a consistent seropositivity rate above 90% throughout the sampling time frame. Notably, 100% of patients still had detectable IgG at the last time point, while IgM and IgA declined more rapidly. There was also evidence of a waning neutralising response. Neutralising antibody titres were detected in 63% of the donors at six weeks post-symptom onset, however titres declined between six and 31 weeks post-symptom onset, with 77% of donors having undetectable neutralisation activity at the last time point. IgG+ RBD-specific memory B cells were detected in 100% of patients and increased up to 31 weeks.

Narrative reviews

One narrative review (Stephens and McElrath 2020) was identified that highlights the importance of ascertaining long-term B-cell and T-cell immunological memory against SARS-CoV-2 in our understanding of durable immunity.[29] Citing studies by Grifoni et al.,[37] Le Bert et al.[38] and Braun et al.,[39] they note that SARS-CoV-2 specific memory CD4+ T cells and CD8+ T cells have been identified in up to 100% and in up to 70% of patients recovering from COVID-19, respectively. Although concerns have been expressed about declining IgG neutralising antibodies to SARS-CoV-2 in convalescence, the authors describe how serological memory is maintained by smaller numbers of long-lived plasma cells. The antibody recall response comes from this pool of plasma cells and memory B-cells, which secrete antibody in the absence of antigen, including when serum antibodies are low. SARS-CoV-2-specific CD4+ and CD8+ memory T cells are also generated. While individuals with mild or asymptomatic disease are reported to exhibit robust memory T-cell responses months after infection, it is unknown whether these cells, in the absence of detectable circulating antibodies, protect against SARS-CoV-2. The authors note that 'substantial data' now demonstrate the presence of pre-existing T-cell immunity in those who have not been infected with SARS-CoV-2, which may be associated with previous infection with other coronaviruses. Cross reactive T-cells have been described in household contacts of Covid-19 cases and 'further studies may determine if cross-reactive T cells from previous coronavirus infections have been boosted with exposure to SARS-CoV-2'.

Two narrative reviews explored the mucosal immune response to SARS-CoV-2.[35, 36] Russell et al. argue that consideration of this response has been neglected in favour of studies of antibody and cell-mediated immune responses.[35] Given that the mucosal immune system is the largest component of the entire immune system, studies to determine the characteristics of IgA antibody secreting and memory B-cells should be undertaken, particularly in terms of their implications for onward transmission of disease.[35] Cervia et al. examined SARS-CoV-2–specific IgA and IgG in sera and mucosal fluids of 64 SARS-CoV-2 PCR positive patients and 109 PCR negative healthcare workers.[36] They report that systemic antibody production against SARS-CoV-2 develops mainly in patients with severe COVID-19, with very high IgA titres seen in patients with severe acute respiratory distress syndrome, whereas mild disease may be associated with transient production of SARS-CoV-2–specific antibodies, but may stimulate mucosal SARS-CoV-2–specific IgA secretion. Whether these responses confer immunity to secondary infection is not clear. The authors are following up this patient cohort longitudinally to address these uncertainties.

Discussion – scoping review

Previous reviews by HIQA concluded that most patients mount an antibody-mediated immune responses following SARS-CoV-2 infection, however some studies report a waning antibody response from two to six months post-infection.[1] This phenomenon is not unexpected and does not preclude protective immunity against subsequent infection. Subsequent encounters with the same antigen typically lead to responses called secondary immune responses that usually are more rapid, larger and better able to eliminate the antigen than primary antibody responses.[40] Therefore, studying both primary and memory immune responses (antibody, memory B cell, CD4+T cell, and CD8+T cell memory) to SARS-CoV-2 in an integrated manner is important in the understanding of the durability of protective immunity.[32] Indeed, it may be the case that evaluation of memory, diversity and durability of immune responses are more important than initial IgG responses.[41]

This scoping review identified a range of studies that demonstrate the durability of antibody- and cell-mediated immune responses beyond six months post-infection. Detection rates and titres of antibodies, and the proportion of individuals who mount memory B- and T-cell responses, differ across studies, which may be partly explained by differences in testing platforms. Reports of declining IgG and neutralising antibodies to SARS-CoV-2 in the convalescent period have raised concerns about susceptibility to reinfection,[29] however, antibody levels always decline after the acute phase of infection as most of the circulating antibody secreting cells induced during the first weeks after infection are short-lived. Following this reduction, serological memory is maintained by long-lived plasma cells that reside in the bone marrow, from which the antibody recall response comes. This review did not identify reductions in B-cell responses in the late (≥6 months) convalescent period.

While no Irish studies were identified that investigated the duration of antibody responses beyond six months post-infection, one completed study and two ongoing studies were identified that investigated the seroprevalence of SARS-CoV-2 antibodies among HCWs based in Ireland.[42-44] In the study with final results, currently published as a preprint, symptomatic and asymptomatic HCWs employed at the Rotunda Maternity Hospital, Dublin, were enrolled.[43] SARS-CoV-2 incidence was assessed using oropharyngeal or nasopharyngeal RT-PCR, accompanied by serological assessment for the presence of both the spike and nucleocapsid SARS-CoV-2 antibodies. The study enrolled 137 HCWs overall, 86 symptomatic and 51 asymptomatic at time of swab collection. SARS-CoV-2 RNA was detected in 52% (n=45/86) of symptomatic study participants with a seropositivity rate of 98% (n=44/45). Asymptomatic SARS-CoV-2 RNA infection was detected in 4% (n=2/51) of control participants with a seropositivity rate of 100% (n=2/2). Overall, 95% of

SARS-CoV-2 PCR positive participants had detectable levels of antibodies at 100 days (3.3 months) post-infection, which persisted in 91% of participants beyond 160 days (≥5.3 months).

The two ongoing Irish seroprevalence studies have published interim results. In the first study, HCWs from St. James' Hospital (SJH) in Dublin and University Hospital Galway (UHG) were enrolled in a longitudinal seroprevalence study, consisting of two sero-surveys six months apart, the first in October 2020 and the second planned for April 2021.[44] This publication is an analysis of the results of the sero-survey from 14 to 23 October 2020. All staff working in SJH and UHG (9,038 people) were invited to participate in the study. Participation rates in both a questionnaire and serology testing was 65% (3,042/4,692) in SJH and 63% (2,745/4,395) in UHG. SARS-CoV-2 antibodies were detected in 15% (464/3,042) of all participants in SJH and 4.1% (112/2,745) in UHG. In total, 95% of those who had a previously confirmed infection by RT-PCR had a detectable antibody. Thirty nine percent (226/576) of those with positive antibodies had never been diagnosed with SARS-CoV-2 infection. In the second study, 1,176 staff at Tallaght University Hospital (TUH) were enrolled in a 12-month longitudinal study.[42] Interim results after three months follow-up found that antibodies were detected in 18% of participants overall. Before this study, 12% of participants had been diagnosed with COVID-19.

On a final note, it must be acknowledged that most studies on immunity to SARS-CoV-2 have focussed on serum antibodies and cell-mediated immunity, whereas the mucosal immune system is the largest component of the immune system.[35] As SARS-CoV-2 initially infects the upper respiratory tract, its first interactions with the immune system occur in the respiratory mucosae. It is possible that the generation of memory cells at the mucosal portals could prevent viral entry.[45] Therefore, determining the characteristics of IgA and their homing potential for mucosal or systematic tissues could inform derogation policy for healthcare workers as well vaccine development and policy.[35] It is possible that analysis of cells from the peripheral blood does not represent resident SARS-CoV-2 reactive memory T- and B-cells in lymphoid tissues of the upper respiratory tract and lungs which could result in more rapid and effective immunity.[41]

Conclusion

This review consisted of a systematic search of studies that estimated the risk of SARS-CoV-2 reinfection over time, and a scoping review of the long-term duration of immune responses following SARS-CoV-2 infection.

Eleven large cohort studies were identified that estimated the risk of SARS-CoV-2 reinfection over time, including three that enrolled healthcare workers and two that enrolled elderly care home residents. All studies reported low relative SARS-CoV-2 reinfection rates in individuals with prior evidence of infection, compared with those without, for up to 10 months. The risk of reinfection across all age groups was consistently low, although very little data were retrieved on paediatric populations and there was some inconsistent evidence of a higher risk in older populations compared with younger populations. While the clinical characteristics of reinfected cases were poorly reported across studies, reinfection events were generally not associated with severe disease. There is uncertainty regarding the applicability of data to new variants of concern and to vaccinated populations. Preliminary data on the reinfection rates in Ireland are consistent with the absolute rates of reinfection reported in included studies.

A scoping review was conducted to evaluate the long-term duration of immune responses following SARS-CoV-2 infection. Five studies were identified that investigated immune responses at ≥6 months post-infection, including two studies at ≥8 months post-infection. In general, studies reported a waning of antibody responses in the late convalescent period. However, T-cell and memory B-cell responses were still present, and in many cases increased, up to eight months post-infection. The findings of low SARS-CoV-2 reinfection rates in the systematic review are supported by these observations of long-lasting secondary immune responses ≥6 months post-SARS-CoV-2 infection.

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Appendices

Appendix 1: Excluded studies with reasons

Study	Title	DOI	Exclusion reason
Abu-Raddad 2020	Two prolonged viremic SARS-CoV-2 infections with conserved viral genome for two months	10.1016/j.meegid.2020.104684	Exclusion reason: Wrong outcomes
Abu-Raddad 2020	Assessment of the risk of SARS-CoV-2 reinfection in an intense re-exposure setting	10.1093/cid/ciaa1846	Exclusion reason: Duplicate
Abu-Raddad 2021	Two prolonged viremic SARS-CoV-2 infections with conserved viral genome for two months	10.1016/j.meegid.2020.104684	Exclusion reason: Wrong outcomes
Abu-Raddad 2021	SARS-CoV-2 reinfection in a cohort of 43,000 antibody-positive individuals followed for up to 35 weeks	10.1101/2021.01.15.21249731	Exclusion reason: Duplicate
Alhusseini 2021	Persistence of SARS-CoV-2: a new paradigm of COVID-19 management	10.7416/ai.2021.2414	Exclusion reason: Wrong study design
Alturaif 2020	Recurrence of Positive SARS-CoV-2 RNA in a COVID-19 Patient: Two Case Reports from Saudi Arabia	10.21203/rs.3.rs-86920/v1	Exclusion reason: Wrong study design
Alvarez-Moreno 2020	Testing Dilemmas: Post negative, positive SARS-CoV-2 RT-PCR is it a reinfection?	10.1016/j.tmaid.2020.101743	Exclusion reason: Wrong study design
Aran 2020	Prior presumed coronavirus infection reduces COVID-19 risk: A cohort study	10.1016/j.jinf.2020.10.023	Exclusion reason: Wrong outcomes
Ariza 2021	Seroprevalence and seroconversion rates to SARS-CoV-2 in interns, residents, and medical doctors in a University Hospital in Bogota, Colombia	10.22354/IN.V25I3.938	Exclusion reason: <100 patients
Bichara 2021	Dynamics of anti-SARS-CoV-2 IgG Antibodies Post-COVID-19 in a Brazilian Amazon Population	10.21203/rs.3.rs-228739/v1	Exclusion reason: Wrong outcomes
Bilich 2021	T cell and antibody kinetics delineate SARS-CoV-2 peptides mediating long-term immune responses in COVID-19 convalescent individuals	10.1126/scitranslmed.abf7517	Exclusion reason: Wrong outcomes
Binnendijk 2021	Serological Evidence for Reinfection with SARS-CoV-2; An Observational Cohort Study	10.2139/ssrn.3800076	Exclusion reason: <100 patients

Boonyaratanakornkit 2020	Clinical, laboratory, and temporal predictors of neutralizing antibodies to SARS-CoV-2 after COVID-19	10.1101/2020.10.06.20207472	Exclusion reason: Wrong outcomes
Borena 2021	Follow-up study in the ski-resort Ischgl: Antibody and T cell responses to SARS-CoV-2 persisted for up to 8 months after infection and transmission of virus was low even during the second infection wave in Austria	10.1101/2021.02.19.21252089	Exclusion reason: Wrong study design
Brehm 2020	Seroprevalence of SARS-CoV-2 antibodies among hospital workers in a German tertiary care center: A sequential follow-up study	10.1016/j.ijheh.2020.113671	Exclusion reason: Wrong outcomes
Bruni 2020	Persistence of anti-SARS-CoV-2 antibodies in non-hospitalized COVID-19 convalescent health care workers	10.3390/jcm9103188	Exclusion reason: Wrong outcomes
Carta 2021	Prospective serological evaluation of anti SARS-CoV-2 IgG and anti S1-RBD antibodies in a community outbreak	10.1515/cclm-2021-0127	Exclusion reason: Wrong outcomes
Cassaniti 2021	Seroprevalence of SARS-CoV-2 in 1922 blood donors from the Lodi Red Zone and adjacent Lodi metropolitan and suburban area	10.1016/j.cmi.2021.01.030	Exclusion reason: Wrong outcomes
Cerutti 2020	Clinical immunity in discharged medical patients with COVID-19	Italian Journal of Medicine 2020;14(SUPPL 2):109 2020; no DOI	Exclusion reason: Follow up < 3 months (individual cases)
Cervia 2020	Systemic and mucosal antibody responses specific to SARS-CoV-2 during mild versus severe COVID-19	10.1016/j.jaci.2020.10.040	Exclusion reason: Wrong outcomes
Chen 2020	Clinical course and risk factors for recurrence of positive SARS-CoV-2 RNA: a retrospective cohort study from Wuhan, China	10.18632/aging.103795	Exclusion reason: Follow up < 3 months (individual cases)
Choi 2020	Low Seroprevalence of SARS-CoV-2 Antibodies during Systematic Antibody Screening and Serum Responses in Patients after COVID-19 in a German Transplant Center	10.3390/jcm9113401	Exclusion reason: Wrong outcomes
Choudhary 2021	SARS-CoV-2 Sequence Characteristics of COVID-19 Persistence and Reinfection	10.1101/2021.03.02.21252750	Exclusion reason: Wrong study design

Corr 2020	Seroprevalence of SARS-CoV-2 antibodies in children of United Kingdom healthcare workers: A prospective multicentre cohort study protocol	10.1136/bmjopen-2020-041661	Exclusion reason: Study protocol only;
Coutinho 2021	Model-based estimation of transmissibility and reinfection of SARS-CoV-2 P.1 variant	10.1101/2021.03.03.21252706	Exclusion reason: Wrong study design
Dan 2021	Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection	10.1126/science.abf4063	Exclusion reason: Wrong outcomes
Dao 2021	Recurrence of SARS-CoV-2 viral RNA in recovered COVID-19 patients: a narrative review	10.1007/s10096-020-04088-z	Exclusion reason: Wrong study design
denHartog 2021	Persistence of antibodies to SARS-CoV-2 in relation to symptoms in a nationwide prospective study	10.1093/cid/ciab172	Exclusion reason: Wrong outcomes
Dillner 2021	Antibodies to SARS-CoV-2 and risk of past or future sick leave	10.1038/s41598-021-84356-w	Exclusion reason: Wrong study design
Dillner 2021	High amounts of SARS-CoV-2 precede sickness among asymptomatic healthcare workers	10.1093/infdis/jiab099	Exclusion reason: Wrong outcomes
Fels 2021	Genomic surveillance of SARS-CoV-2 in the Bronx enables clinical and epidemiological inference	10.1101/2021.02.08.21250641	Exclusion reason: Wrong study design
FillMalfertheiner 2020	Immune response to SARS-CoV-2 in health care workers following a COVID-19 outbreak: A prospective longitudinal study	10.1016/j.jcv.2020.104575	Exclusion reason: Wrong outcomes
Flieder 2021	Retrospective analysis of 426 donors of a convalescent collective after mild COVID-19	10.1371/journal.pone.0247665	Exclusion reason: Wrong outcomes
Forbes 2021	Persistence of antibody response to SARS-CoV-2 in a cohort of haemodialysis patients with COVID-19	10.1093/ndt/gfab066	Exclusion reason: Wrong outcomes
Galanis 2020	Seroprevalence of SARS-CoV-2 antibodies and associated factors in health care workers: a systematic review and meta-analysis	10.1101/2020.10.23.20218289	Exclusion reason: Wrong outcomes
Gallichotte 2020	Longitudinal Surveillance for SARS-CoV-2 Among Staff in Six Colorado Long Term Care Facilities: Epidemiologic, Virologic and Sequence Analysis	10.2139/ssrn.3724248	Exclusion reason: Wrong outcomes
Ganz-Lord 2020	Title: Covid-19 symptoms, duration, and prevalence among healthcare workers in the New York metropolitan area	10.1017/ice.2020.1334	Exclusion reason: Wrong outcomes

Girardin 2021	Temporal Analysis of Serial Donations Reveals Decrease in Neutralizing Capacity and Justifies Revised Qualifying Criteria for Coronavirus Disease 2019 Convalescent Plasma	10.1093/infdis/jiaa803	Exclusion reason: Wrong outcomes
Hall 2021	Do antibody positive healthcare workers have lower SARS-CoV-2 infection rates than antibody negative healthcare workers? Large multi-centre prospective cohort study (the SIREN study), England: June to November 2020	10.1101/2021.01.13.21249642	Exclusion reason: Duplicate
Hanrath 2020	Prior SARS-CoV-2 infection is associated with protection against symptomatic reinfection	10.1016/j.jinf.2020.12.023	Exclusion reason: Duplicate
Harvey 2020	Real-world data suggest antibody positivity to SARS-CoV-2 is associated with a decreased risk of future infection	10.1101/2020.12.18.20248336	Exclusion reason: Duplicate
Haymond 2021	Viral Neutralization is Durable in Asymptomatic COVID-19 for at least 60 Days	10.1093/infdis/jiab140	Exclusion reason: Wrong outcomes
He 2021	The unexpected dynamics of COVID-19 in Manaus, Brazil: Herd immunity versus interventions	10.1101/2021.02.18.21251809	Exclusion reason: Wrong study design
Higgins 2021	Longitudinal SARS-CoV-2 antibody study using the Easy Check COVID-19 IgM/IgG lateral flow assay	10.1371/journal.pone.0247797	Exclusion reason: Wrong outcomes
Jin 2020	Correlation between viral RNA shedding and serum antibodies in individuals with coronavirus disease 2019	10.1016/j.cmi.2020.05.022	Exclusion reason: Wrong outcomes
Karbiener 2021	Longitudinal analysis of SARS-CoV-2 antibodies in 8000 U.S. first-time convalescent plasma donations	10.1111/trf.16291	Exclusion reason: Wrong outcomes
Lai 2020	Population-based seroprevalence surveys of anti-SARS-CoV-2 antibody: An up-to-date review	10.1016/j.ijid.2020.10.011	Exclusion reason: Wrong study design
Lampasona 2020	Antibody response to multiple antigens of SARS-CoV-2 in patients with diabetes: an observational cohort study	10.1007/s00125-020-05284-4	Exclusion reason: Wrong outcomes
Laursen 2021	Prevalence of SARS-CoV-2 igg/igm antibodies among danish and swedish falck emergency and non-emergency healthcare workers	10.3390/ijerph18030923	Exclusion reason: Wrong outcomes

Li 2020	Molecular and serological characterization of SARS-CoV-2 infection among COVID-19 patients	10.1016/j.virol.2020.09.008	Exclusion reason: Wrong outcomes
Ling 2020	Persistence and clearance of viral RNA in 2019 novel coronavirus disease rehabilitation patients	10.1097/cm9.0000000000000774	Exclusion reason: Wrong outcomes
Liu 2021	Clinical characteristics and follow-up analysis of 324 discharged covid-19 patients in shenzhen during the recovery period	10.7150/ijms.50873	Exclusion reason: Follow up < 3 months (individual cases)
Lumley 2020	Antibody Status and Incidence of SARS-CoV-2 Infection in Health Care Workers	10.1056/NEJMoa2034545	Exclusion reason: Duplicate
Lumley 2020	Antibodies to SARS-CoV-2 are associated with protection against reinfection	10.1101/2020.11.18.20234369	Exclusion reason: Duplicate
Luo 2020	Clinical Characteristics, Risk Factor and Transmission of the COVID-19 Discharged Cases with Positive Retest in Guangzhou, China: A Retrospective Cohort Study	10.2139/ssrn.3732143	Exclusion reason: Follow up < 3 months (individual cases)
Mack 2021	Prevalence of SARS-CoV-2 IgG antibodies in a large prospective cohort study of elite football players in Germany (May-June 2020): implications for a testing protocol in asymptomatic individuals and estimation of the rate of undetected cases	10.1016/j.cmi.2020.11.033	Exclusion reason: Wrong outcomes
Mattiuzzi 2020	Sars-cov-2 recurrent rna positivity after recovering from coronavirus disease 2019 (COVID-19): A meta-analysis	10.23750/abm.v91i3.10303	Exclusion reason: Wrong study design
Muecksch 2021	Longitudinal Serological Analysis and Neutralizing Antibody Levels in Coronavirus Disease 2019 Convalescent Patients	10.1093/infdis/jiaa659	Exclusion reason: Wrong outcomes
Mumoli 2020	Clinical immunity in discharged medical patients with COVID-19	10.1016/j.ijid.2020.07.065	Exclusion reason: Follow up < 3 months (individual cases)
Murillo-Zamora 2020	Predictors of severe symptomatic laboratory-confirmed SARS-COV-2 reinfection	10.1101/2020.10.14.20212720	Exclusion reason: Follow up < 3 months (individual cases)
Nag 2020	A Prospective Study on Rapidly Declining SARS-CoV-2 IgG Antibodies Within One to Three Months of Testing IgG Positive: Can It Lead to Potential Reinfections?	10.7759/cureus.11845	Exclusion reason: Follow up < 3 months (individual cases)
Nielsen 2020	SARS-CoV-2 elicits robust adaptive immune responses regardless of disease severity	10.1101/2020.10.08.331645	Exclusion reason: Wrong outcomes

Noh 2021	Longitudinal assessment of anti-SARS-CoV-2 immune responses for six months based on the clinical severity of COVID-19	10.1093/infdis/jiab124	Exclusion reason: Wrong study design
Ortega 2021	Seven-month kinetics of SARS-CoV-2 antibodies and protective role of pre-existing antibodies to seasonal human coronaviruses on COVID-19	10.1101/2021.02.22.21252150	Exclusion reason: Wrong study design
Osman 2020	Re-positive coronavirus disease 2019 PCR test: could it be a reinfection?	10.1016/j.nmni.2020.100748	Exclusion reason: Wrong study design
Patwardhan 2020	Sustained Positivity and Reinfection With SARS-CoV-2 in Children: Does Quarantine/Isolation Period Need Reconsideration in a Pediatric Population?	10.7759/cureus.12012	Exclusion reason: Follow up < 3 months (individual cases)
Peluso 2021	Long-Term SARS-CoV-2-Specific Immune and Inflammatory Responses Across a Clinically Diverse Cohort of Individuals Recovering from COVID-19	10.1101/2021.02.26.21252308	Exclusion reason: Wrong outcomes
Peluso 2021	SARS-CoV-2 antibody magnitude and detectability are driven by disease severity, timing, and assay	10.1101/2021.03.03.21251639	Exclusion reason: Wrong outcomes
Piri 2021	A systematic review on the recurrence of SARS-CoV-2 virus: frequency, risk factors, and possible explanations	10.1080/23744235.2020.1871066	Exclusion reason: Wrong study design
Pradenas 2021	Stable neutralizing antibody levels 6 months after mild and severe COVID-19 episodes	10.1016/j.medj.2021.01.005	Exclusion reason: Wrong outcomes
Qin 2021	The seroprevalence and kinetics of IgM and IgG in the progression of COVID-19	10.1186/s12865-021-00404-0	Exclusion reason: Wrong outcomes
Ravichandran 2021	Longitudinal antibody repertoire in "mild" versus "severe" COVID-19 patients reveals immune markers associated with disease severity and resolution	10.1126/sciadv.abf2467	Exclusion reason: Wrong outcomes
Sadr 2021	SARS-CoV-2 Reinfection within the first 3 months of COVID-19 Recovery in A Referral Hospital, Tehran, Iran	10.21203/rs.3.rs-271345/v1	Exclusion reason: Follow up < 3 months (individual cases)
Sakharkar 2021	Prolonged evolution of the human B cell response to SARS-CoV-2 infection	10.1126/sciimmunol.abg6916	Exclusion reason: Wrong outcomes

Salehi 2021	COVID-19 Re-infection or Relapse? A Retrospective Multi Center Cohort Study From Iran	10.21203/rs.3.rs-262191/v1	Exclusion reason: Wrong study design
Sandberg 2021	Longitudinal characterization of humoral and cellular immunity in hospitalized COVID-19 patients reveal immune persistence up to 9 months after infection	10.1101/2021.03.17.435581	Exclusion reason: Wrong study design
Sarapultseva 2021	SARS-CoV-2 Seropositivity among Dental Staff and the Role of Aspirating Systems	10.1177/2380084421993099	Exclusion reason: Wrong outcomes
Self 2020	Decline in SARS-CoV-2 Antibodies After Mild Infection Among Frontline Health Care Personnel in a Multistate Hospital Network - 12 States, April-August 2020	10.15585/mmwr.mm6947a2	Exclusion reason: Wrong outcomes
Shah 2020	Immunity status of Health Care Workers post recovery from COVID-19: An online longitudinal panel survey	10.1101/2020.11.27.20239426	Exclusion reason: Wrong outcomes
Sokal 2021	Maturation and persistence of the anti-SARS-CoV-2 memory B cell response	10.1016/j.cell.2021.01.050	Exclusion reason: Wrong outcomes
Song 2021	Dynamics of viral load and anti-SARS-CoV-2 antibodies in patients with positive RT-PCR results after recovery from COVID-19	10.3904/kjim.2020.325	Exclusion reason: <100 patients
Talbot 2021	Prevalence of IgM and IgG antibodies to SARS-CoV-2 in health care workers at a tertiary care New York hospital during the Spring COVID-19 surge	10.1186/s13741-021-00177-5	Exclusion reason: Wrong outcomes
Trieu 2021	SARS-CoV-2-Specific Neutralizing Antibody Responses in Norwegian Health Care Workers After the First Wave of COVID-19 Pandemic: A Prospective Cohort Study	10.1093/infdis/jiaa737	Exclusion reason: Wrong outcomes
Tuells 2021	Seroprevalence Study and Cross-Sectional Survey on COVID-19 for a Plan to Reopen the University of Alicante (Spain)	10.3390/ijerph18041908	Exclusion reason: Wrong study design
VanElslande 2021	Longitudinal follow-up of IgG anti-nucleocapsid antibodies in SARS-CoV-2 infected patients up to eight months after infection	10.1016/j.jcv.2021.104765	Exclusion reason: Wrong outcomes

Vibholm 2021	SARS-CoV-2 persistence is associated with antigen-specific CD8 T-cell responses	10.1016/j.ebiom.2021.103230	Exclusion reason: Wrong outcomes
WÃing 2020	Ct suggests discharged covid-19 patients who were retested rt-pcr positive again for sars-cov-2 more likely had false negative rt-pcr tests before discharging	10.21037/QIMS-2020-19	Exclusion reason: Wrong study design
Wallace 2020	SIREN protocol: Impact of detectable anti-SARS-CoV-2 on the subsequent incidence of COVID-19 in 100,000 healthcare workers: do antibody positive healthcare workers have less reinfection than antibody negative healthcare workers?	10.1101/2020.12.15.20247981	Exclusion reason: Study protocol only
Wheatley 2021	Evolution of immune responses to SARS-CoV-2 in mild-moderate COVID-19	10.1038/s41467-021-21444-5	Exclusion reason: Wrong outcomes
Wu 2020	A follow-up study shows no new infections caused by patients with repeat positive of COVID-19 in Wuhan	10.1101/2020.11.18.20232892	Exclusion reason: Follow up < 3 months (individual cases)
Wu 2021	A follow-up study shows that recovered patients with re-positive PCR test in Wuhan may not be infectious	10.1186/s12916-021-01954-1	Exclusion reason: Wrong outcomes
Yuan 2020	Recurrence of positive SARS-CoV-2 viral RNA in recovered COVID-19 patients during medical isolation observation	10.1038/s41598-020-68782-w	Exclusion reason: Follow up < 3 months (individual cases)
Zheng 2020	Incidence, clinical course and risk factor for recurrent PCR positivity in discharged COVID-19 patients in Guangzhou, China: A prospective cohort study	10.1371/journal.pntd.0008648	Exclusion reason: Follow up < 3 months (individual cases)
Zheng 2021	Sustainability of SARS-CoV-2 Induced Humoral Immune Responses in COVID-19 Patients from Hospitalization to Convalescence Over Six Months	10.1007/s12250-021-00360-4	Exclusion reason: Wrong outcomes

Appendix 2: Data extraction

Author DOI Title Country Study design Publication status	Population (number of participants, follow-up duration) Patient demographics	Primary endpoints Test parameters: Serial testing intervals SARS-CoV-2 confirmation Serological confirmation Clinical description	Relative risk of reinfection (or Odds Ratio) Adjusted estimates (for covariates) Absolute (/crude) reinfection events Conclusion/relevance
Abu-Raddad 2021 10.1101/2021.01.15.21249731 SARS-CoV-2 reinfection in a cohort of 43,000 antibody positive individuals followed for up to 35 weeks Qatar Retrospective cohort study Preprint	N=43,044 anti-SARS-CoV-2 antibody positive persons Median follow-up: 16.3 weeks Maximum duration of follow-up: 34.6 weeks Criteria for cases: ▪ Suspected reinfection: All SARS-CoV-2 antibody-positive persons in Qatar with at least one PCR-positive swab that occurred ≥14 days after the first-positive antibody test. ▪ Good evidence for reinfection: Suspected reinfection cases with a PCR Ct ≤30 for the reinfection swab (suggestive of a recent active infection) and who had not had a PCR-positive swab for 45 days preceding the reinfection swab (to rule out persisting PCR positivity due	**Primary endpoint:** Risk of reinfection and efficacy of natural immunity **Risk calculations**: ▪ Risk of reinfection: proportion of cases with good or some evidence for reinfection among all eligible anti-SARS-CoV-2 +ve cases (with an antibody-positive test ≥14 days from end-of-study censoring). ▪ Incidence rate of reinfection: number of cases with good or some evidence for reinfection divided by the number of person-weeks contributed by all anti-SARS-CoV-2 positive cases. ▪ Follow-up person-time: starting 14 days after the first positive antibody test until the reinfection swab, all-cause death, or end-of-study censoring (set on December 31, 2020). ▪ Adjusted estimates for the risk of reinfection and the incidence rate of reinfection derived by applying the confirmation rate obtained from viral genome sequencing analysis.	314 individuals (0.7%) had at least one PCR positive swab ≥14 days after the first-positive antibody test. Of these 314 individuals, 129 (41.1%) had supporting epidemiological (with good or some) evidence for reinfection. ▪ Applying the viral-genome-sequencing confirmation rate, the risk of reinfection was estimated at 0.10% (95% CI: 0.08-0.11%). ▪ Incidence rate of reinfection: 0.66 per 10,000 person-weeks (95% CI: 0.56-0.78). ▪ Risk over time: Incidence rate of reinfection by month of follow-up did not show any evidence of waning of immunity for over 7 months of follow-up. Seronegative comparison: N=149,923 antibody-negative persons followed for a median of 17.0 weeks (range: 0-45.6), risk of infection was estimated at 2.15% (95% CI: 2.08-2.22%) and incidence rate of infection was

	to non-viable virus fragments). ▪ Some evidence for reinfection: Suspected reinfection cases who had not had a PCR-positive swab for 45 days preceding the reinfection swab, but whose Ct value for the reinfection swab was >30. ▪ Weak evidence for reinfection: Suspected reinfection cases who had a PCR-positive swab within the 45 days preceding the reinfection swab. Demographics: The cohort included 8,953 (20.8%) women and 34,091 men (79.2%) of 158 nationalities. Median age was 35 years for women (interquartile range (IQR): 28-45 years) and 38 years for men (IQR: 31-47 years)	**Efficacy (of natural infection against reinfection):** ▪ SARS-CoV-2 incidence was also assessed in a complement cohort including all those testing SARS-CoV-2 antibody-negative in Qatar, to provide an antibody-negative comparator group and to assess the efficacy of natural infection against reinfection. ▪ Efficacy=1-(Risk in exposed)/(Risk in unexposed) **Test parameters** RT-qPCR: TaqPath™ COVID-19 Combo Kits (Thermo Fisher Scientific, USA) on ABI 7500 FAST (Thermo Fisher, USA) Serology: Roche Elecsys® Anti-SARS-CoV-2 assay (Roche, Switzerland) [ECLIA] **Viral genome sequencing:** For a subset of investigated reinfection cases with good or some evidence for reinfection (where it was possible to retrieve the first infection PCR+ve swab and the reinfection swab), sequencing was conducted to confirm reinfection	estimated at 13.69 per 10,000 person-weeks (95% CI: 13.22-14.14). **Efficacy of natural infection against reinfection:** 95.2% (95% CI: 94.1-96.0%). **Severity:** Of the 8 reinfection cases that received severity classification, only 1 reinfection was severe, 2 were moderate, and 0 were critical or fatal. **Symptomatic/serial testing:** Most reinfections (N=86/129, 66.7%) were diagnosed incidentally through random or routine testing, or through contact tracing. **Whole genome sequencing:** ▪ Of the 16 cases where viral genome sequencing evidence was available, 5 cases were confirmed as reinfections, a confirmation rate of 31.3%. ▪ For 1 pair, there were few changes of allele frequency offering supporting evidence for reinfection. For 4 other pairs, there were multiple clear changes of allele frequency indicating strong evidence for reinfection. 1 of the latter pairs also documented the presence of the D614G mutation (23403bp A>G) at the reinfection swab—a variant that has progressively replaced the original D614 form.
Hanrath 2020 10.1016/j.jinf.2020.12.023 Prior SARS-CoV-2 infection is associated	**Analysis period and time interval:** ▪ Two periods for analysis: 1st wave: 10 March - 6 July 2020; 2nd wave: 7 July - 20 November.	**Primary endpoint:** symptomatic SARS-CoV-2 infection. **Time interval:** In those previously infected, there was a median of 173 (IQR: 162–229) days from the	**Risk difference:** ▪ During 2nd time period, 2,243 HCWs underwent PCR testing for symptoms. 128 had previous confirmed SARS-CoV-2 infection, while 2,115 had not.

with protection against symptomatic reinfection UK Retrospective cohort study Published (Journal of Infection)	▪ Follow-up: median 5.8 months (173 days, IQR: 162–229 days, between first positive test and end of follow-up period). **Number of participants:** ▪ 1st wave: N=1,038 HCWs with prior SARS-CoV-2 infection (PCR and or antibody testing) and N=10,137 HCWs without prior exposure. ▪ Of those with prior exposure: 481/3,338 symptomatic HCWs tested positive for SARS-CoV-2 by PCR, while SARS-CoV-2 IgG was detected in 937/11,103. **Demographics:** Median age: 39.5 (prior infection), 40 (no infection) Female: 82.5% (prior infection), 80.5% (no infection)	date of first positive PCR/antibody result to the end of the analysis period. **Test parameters:** ▪ Public Health England (PHE) approved RT-PCR assays containing two SARS-CoV-2 gene targets. ▪ SARS-CoV-2 nucleocapsid IgG antibody testing using the Roche Anti-SARS-CoV-2 IgG assay	▪ A positive PCR test was returned in 0/1,038 (0% [95% CI: 0–0.4) of those with previous infection, compared to 290/10,137 (2.9% [95% CI: 2.6–3.2) of those without (*P*<0.0001 χ2 test). **Symptomatic testing:** ▪ Fewer HCWs in the previous infection group presented for symptomatic testing. 128/1,038 (12.3% [95% CI: 10.5–14.5]) of those with evidence of prior infection had a test due to symptoms in the second period compared to 2115/10,137 (20.8% [95% CI: 20.1–21.6]) in the group without previous infection (P<0.0001 χ2 test). **Asymptomatic screening:** Asymptomatic PCR screening was undertaken on a pilot basis in an additional 481 HCWs, 106 with past infection and 375 without. There were similarly no positive results in the group with previous infection 0/106 (0% [95% CI: 0–3.5]), compared to 22/375 (5.9% [95% CI: 3.9–8.7], P = 0.011) positive PCR results in the group without previous infection. **Author conclusions:** ▪ There were no symptomatic reinfections in a cohort of healthcare workers
Harvey 2020 10.1101/2020.12.18.20248336 Real-world data suggest antibody	N=3,257,478 (national sample from EHRs) with an index antibody test. 88.3% (n= 2,876,773) had negative index test; 11.6% (n=378,606) positive and 0.1% (n=2,099) inconclusive	**Primary endpoints:** index antibody test results and post-index diagnostic NAAT* results, with infection defined as a positive diagnostic test post-index, as measured in 30-day intervals (0-30, 31-60, 61-90, >90 days).	**Duration of seropositivity in the index positive cohort:** 2.6% (n=9,895) of those with a positive antibody test at index had at least one subsequent antibody test during follow-up. Of these:

positivity to SARS-CoV-2 is associated with a decreased risk of future infection USA Retrospective cohort study Pre-print	(the latter excluded from follow-up) **Demographics:** (negative index test group/positive index test group) Mean age =47.66/44.34 years; Female 56.7%/54.1%	**Test:** Antibody test and/or diagnostic nucleic acid amplification test (NAAT). NAAT is considered a proxy representing a new infection or may represent continued viral shedding depending on the context and timing **Cycle threshold:** N/R **Median follow-up:** ▪ 47 days for the seronegative group (IQR 8 to 88 days) ▪ 54 days for the seropositive group (IQR: 17 to 92 days). 11.0% seropositives and 9.5% seronegatives had >1NAAT during follow-up, (mean of 3.3 NAAT for seropositives and 2.3 seronegatives over the follow-up period) 2.6% of those with a positive antibody test at index had at least one subsequent antibody test during follow-up **Serology:** The commercial laboratories antibody testing included a limited set of high throughput antibody tests with validation against a known standard providing between 98% to 100% agreement with both known antibody-positive and antibody-negative specimens, with a 95% confidence interval of 99-100% agreement. The majority of tests performed during the study period were IgG (>91%). Most COVID-19 signs and symptoms were similar between the seropositive and seronegative groups.	▪ 12.4% (n=1,227) tested negative when retested within 0-30 days ▪ 18.4% (n=unclear) testing seronegative when the subsequent antibody test occurred >90 days Ratio (CI) of positive NAAT results in those with positive antibody test at index versus those with negative: ▪ 2.85 (2.73 - 2.97) at 0-30 days ▪ 0.67 (0.6 - 0.74) at 31-60 days ▪ 0.29 (0.24 - 0.35) at 61-90 days) ▪ 0.10 (0.05 - 0.19) at >90 days. **Duration of NAAT positivity:** Those seropositive at baseline: ▪ 11.3% (n=3,226) had a positive NAAT 0 to 30 days ▪ 2.7% (n=771) from 31-60 days* ▪ 1.1% (n=314) from 61-90 days* ▪ 0.3% (n=86) at >90 days* *Based on calculation Those seronegative at baseline: ▪ 3.9% (n=5,638) had positive NAAT result 0 to 30 days ▪ ~3.0% had positive NAAT over all subsequent periods of observation, including at >90 days
Hall 2020 10.1101/2021.01.13.21249642	N=20,787; Study period: 18 June to 09 November 2020 **Baseline:**	**Primary endpoint:** reinfection and incidence rates in those that had evidence of prior infection compared with those that without evidence of a prior infection. Study definitions of reinfection available ranging from	**Rate of reinfection:** ▪ 44 reinfections (2 probable, 42 possible) in positive cohort (1,339,078 days of follow-up)

Do antibody positive healthcare workers have lower SARS-CoV-2 infection rates than antibody negative healthcare workers? Large multi-centre prospective cohort study (the SIREN study), England: June to November 2020 UK Prospective cohort study Pre-print	▪ 32% (n=6,614) positive cohort (antibody positive or prior PCR/antibody test positive ▪ 68% (n=14,173) negative cohort (antibody negative, not previously known to be PCR/antibody positive). **Demographics:** 84% female; 88% white; median age 45.9 years. **Study duration: Enrolment began** 1 February 2020; data censored on 24 November 2020. : Between 18 June and 09 November 2020, 1,339,078 days of follow-up data was analysed from the baseline positive cohort of 6,614 participants.	confirmed to possible dependent on the strength of serological, genetic and virological evidence **Test:** SARS-CoV-2 antibody (Roche cobas® or Abbott immunoassay®) and Nucleic Acid Amplification Testing (NAAT) **Cycle threshold:** 'Probable' Ct=21 to 24; 'symptomatic possible' Ct=13 to 37; 'all probable/possible' Ct=13 to 45 **Frequency of testing:** Questionnaires and PCR every two weeks, antibody every four weeks Median no. post-enrolment PCR and antibody tests=5 (IQR 3-7) and 3 (IQR 2-5), June to November 2020. **Clinical description:** Of 44 possible and probable reinfections, 15 (34%) symptomatic; 2 'probable' were symptomatic; of 42 possible; 13 symptomatic, two (23%) of whom reported typical COVID-19 symptoms.	– event rate of 0.67% (44/6614) [CI: 0.48-0.86%] ▪ Cumulative incidence of 6.7 per 1,000 ▪ Risk of infection OR: 0.17 (0.13-0.24), aOR: 0.17 (0.12-0.23) ▪ 318 new PCR positive infections and 94 antibody seroconversions (not included) in the negative cohort (1,868,646 days of follow-up) – event rate of 2.24% (318/14,173) [2.00-0.49%] ▪ Cumulative incidence of 22.4 per 1,000 **Incidence density per 100,000 person days**: ▪ 3.3 reinfections in the positive cohort ▪ 22.4 new PCR confirmed infections in the negative cohort. **Odds ratio:** ▪ Using a symptomatic case definition aligned with positive PCR results, previous infection reduced the odds of infection by at least 90% - adjusted OR 0.06 (95%CI of 0.03 to 0.09) ▪ When all possible and probable reinfections were included previous infection reduced the odds of reinfection by at least 75% aOR was 0.17 for all reinfections (95% CI 0.13-0.24) compared to PCR confirmed primary infections. **Median interval between primary infection and reinfection**: The median interval between primary infection and reinfection beyond 90 days was 172 days (90-227) and for the 21 reinfections with a historic PCR positive test before enrolment, the median interval between the historic PCR

			positive date and the reinfection PCR positive date was 162 days (95-223). **Conclusions/relevance:** A prior history of SARS-CoV-2 infection was associated with an 83% lower risk of infection, with median protective effect observed 5 months following primary infection. This is the minimum likely effect as seroconversions were not included.
Hansen 2021 doi.org/10.1016/S0140-6736(21)00575-4 Assessment of protection against reinfection with SARS-CoV-2 among 4 million PCR-tested individuals in Denmark in 2020: a population-level observational study Denmark Retrospective cohort study Published in Lancet	N=11,068 PCR positive at baseline were analysed in the main analysis. Two 'surges' were defined (in this report 'wave' is used). During the first wave (before June, 2020), N=533,381 people were tested, of whom 11,727 (2.20%) were PCR positive. N=525,339 were eligible for follow-up in the second wave (1 Sept 31 Dec 2020), of whom 11,068 (2.11%) had tested positive during the first wave. Alternative cohort analysis: 2,432,509 individuals were included in the alternative cohort analysis, with 28,875 (1.19%) individuals contributing exposed time periods and 2,405,683 (98.90%) contributing unexposed time periods, with 2,049 contributing to both unexposed and exposed time periods.	**Primary endpoint:** Main analysis: Rate of infection: the number of individuals with positive PCR tests during the second wave divided by the cumulative number of person-days at risk. The number of days at risk for each individual in the sample was the number of days from Sept 1, 2020, until the first positive test, or Dec 31, 2020, whichever came first. Follow-up time was censored in the event of death. Adjusted rate ratio (RR) and accompanying 95% CI was obtained using Poisson regression, adjusted for sex, age group (0–5, 6–14, 15–24, 25–34, 35–44, 45–54, 55–64, 65–74, 75–84, and ≥85 years), and test frequency (number of PCR tests done on each person in 2020 categorised as 1–2, 3–5, 6–10, and ≥11 tests) to control for potential confounding. Additional cohort analysis: All available data was used to investigate rates of reinfection throughout the epidemic, not just during the second wave. Each individual with a PCR test result was followed up from the time of their first test, irrespective of the date and whether they had a positive or negative result, until Dec 31, 2020, or a new positive test at least 90 days later. If the initial test was negative, a subsequent positive test within	Max follow-up was 295 days (9.8 months). **Main analysis:** 72 confirmed new infections during follow-up out of 1,346,920 person-days in those positive in first wave, compared with 16,819 new infections out of 62,151,056 person-days in those negative in first wave. Adjusted rate ratio (aRR) of reinfection=0.195 (0.155–0.246) **Additional cohort analysis:** aRR**=**0.212 (0.179–0.251) By age group: 0-34 years: aRR=0.173 (0.131–0.229) 35–49 years: aRR=0.199 (0.141–0.282) 50–64 years: aRR=0.187 (0.127–0.274) ≥65: years: aRR=0.529 (0.372–0.753)

	Mean follow-up: In primary analysis, 1,346,920 person-days follow-up in positive cohort of 11,068 individuals (approx 4 months) and 62,151,056 person-days of follow-up in negative cohort of 514,271 individuals (approx 4 months). **Duration of study:** Data between 26 Feb and 31 Dec 2020 were included in analyses. For the analysis of reinfection rate over time, reinfection at 3-6 months follow-up was compared to ≥7 months. **Demographics:** Of those PCR positive in first wave (N=72/11,068): Sex: N=46 women, N26 men Age: N=4 aged 0-19 years, N=15 aged 20-34years, N=20 aged 35-50 years, N=16 aged 50-64 years, N=8 aged 65-79 years, N=9 aged 80+.	the 90 days changed an individual's status from uninfected to previously infected. Additional cohort anlaysis was then expanded the to include interaction terms with sex and age group (restricted to four age groups [0–34, 35–49, 50–64, ≥65 years] to avoid strata with few events). **Test:** The clinical microbiology laboratories applied a range of CE-marked commercial platforms or in-house assays that were all quality controlled according to clinical microbiology diagnostic standards. The TestCenter Denmark laboratory applied an RT-PCR assay with the E gene on SARS-CoV-2 as the target. Rapid antigen test results were excluded from analysis. **Intervals:** No specific time interval – all PCR tests were analysed. **Cycle threshold:** N/R **Whole Genome Sequencing:** Not performed	
Jeffery-Smith 2021 10.2807/1560-7917.ES.2021.26.5.2100092 Antibodies to SARS-CoV-2 protect against re-infection during	N=88 with evidence of prior infection (antibody positive N=87; RT-PCR positive N=1) Outbreak in Sept/Oct 2020 was compared to serological evidence of prior infection in May/June	**RT-PCR testing** Nasal swabs were subjected to SARS-CoV-2 RT-PCR at the Public Health England (PHE) national reference Laboratory. **Antibody testing**	Reinfection rate: N=1/88 (1.1%) Infection rate in seronegative cohort: 30.1% (N=22/73, includes 4 people diagnosed by seroconversion) RR=0.038 (95% CI: 0.005-0.273; p < 0.0001)

outbreaks in care homes, September and October 2020 UK Retrospective cohort Published Eurosurveillance	2020. Follow-up was approx 4 months. Two sites: <u>Care home A</u> N=52 residents (median age 84 years; IQR: 76-89). Serological investigations in June 2020 found 33/66 (50.0%) had SARS-CoV-2 antibodies after the first outbreak (18/32 residents; 15/34 staff). <u>Care home L</u> N=64 residents (median age 85 years; IQR: 78-89). Serological investigation in May 2020 identified 59/117 (50.4%) as seropositive (26/52 residents; 33/65 staff). **Case definitions:** A COVID-19 case was defined as any individual testing positive by RT-PCR for SARS-CoV-2, whether tested as a result of symptoms or through routine care home Screening. A re-infection was defined as an individual testing SARS-CoV-2 RT-PCR positive while having evidence of previous seropositivity by any assay, or a previous RT-PCR-positive result more than 90 days earlier in an individual without serological analysis	Serological testing was conducted using in-house native virus lysate (PHE, UK) and receptor binding domain (RBD) EIA assays (PHE, UK), and a commercial nucleocapsid (N) assay (Abbott, Illinois, United States) Seropositivity was determined by reactivity in any assay; > 80% of samples were positive in ≥ 2 assays. Neutralising antibody titres were determined by live virus neutralisation **Whole Genome Sequencing** WGS was attempted on all RT-PCR-positive samples tested at the PHE reference laboratory; completed viral genomes were deposited in GISAID.	Effectiveness: protection against reinfection after 4 months estimated at 96.2% (95% CI: 72.7–99.5%) **Whole Genome Sequencing:** • The second COVID-19 outbreaks experienced by both care homes were due to SARS-CoV-2 strains that were genetically distinct from their respective first outbreaks. • In both care homes, fatal cases in residents had identical viral genomes to surviving residents. Care home A: ▪ Virus strains from the earlier outbreak had S gene 614D, whereas the strains in the later outbreak were 24–27 single nucleotide polymorphisms (SNPs) different and contained S gene 614G. In the second outbreak, 9 individuals were infected by an identical strain, which differed by 1–2 SNPs from 3 other COVID-19 cases. ▪ The individual with a probable re-infection (S#) shared a virus sequence from B1.36 lineage and the same UK1350_1.2.1.1 phylotype as the other residents and staff, with 6 SNPs differences from the main cluster, including 3 mixed bases which were all outside the S protein RBD coding region. Care home L: ▪ Virus strains from the earlier outbreak arose from several introductions and contained a mixture of 614D and 614G strains, whereas the second outbreak

	(assumed to have seroconverted).		strains were all S gene 614G and differed by 11–18 SNPs from earlier strains. ▪ In both care homes, fatal cases in residents had identical viral genomes to surviving residents.
Krutikov 2020 10.1101/2021.03.08.21253110 Incidence of SARS-CoV-2 infection according to baseline antibody status in staff and residents of 100 Long Term Care Facilities (VIVALDI study) UK Prospective cohort study Pre-print	N=634 seropositive at baseline. N=2,111 participants enrolled in total, comprising 682 residents and 1429 staff. Baseline antibodies to nucleocapsid were detected in 226 residents (33%) and 408 staff (29%) **Setting** Study followed residents and staff at 100 Long Term Care Facilities (LTCFs) **Duration of study** ▪ Blood samples were collected at baseline (June 2020). Blood sampling was ▪ offered to all participants at 3 time points separated by 6-8 week intervals in June, ▪ Aug and Oct 2020. ▪ PCR testing for SARS-CoV-2 was undertaken weekly in staff and monthly in residents. ▪ Patients were followed between Oct 2020 and	**Primary outcome:** All positive PCR tests after entry time were considered to indicate infection or reinfection. Cox regression was used to estimate hazard ratios (HRs) for baseline antibody positivity. The baseline hazard was defined over calendar time, with participants entering the 'risk set' on their entry date (in most cases 1st October 2020) **Antibody testing** All participants were classified into 2 cohorts (positive and negative) according to their first (baseline) antibody test. Exposure status was based on IgG antibodies to nucleocapsid (Abbott) because this test was available for all participants. Subsequent seroconversion was not considered in our primary analysis due to small numbers of participants in which this occurred **Titres** Quantitative antibody data were available for 11/14 reinfection cases, and 42 control participants who were antibody positive at baseline and remained PCR negative throughout follow-up. There was no statistically significant difference in antibody titres to spike and nucleocapsid in individuals who were re-infected and those who remained PCR-negative during follow-up, when considering antibodies at the first testing round (baseline), and at the last antibody	**Infection events by group and antibody status:** Residents: 93 infections out of 456 antibody negative residents, compared with 4 reinfections out of 226 antibody positive residents Rate of PCR positive infection per month at risk: 0.054 seronegative versus 0.007 seropositive Staff: 111 infections out of 1,021 antibody negative residents, compared with 10 reinfections out of 408 antibody positive residents Rate of PCR positive infection per month at risk: 0.042 seronegative versus 0.009 seropositive **RR** Relative adjusted hazard ratios for PCR positive infection comparing seropositive versus seronegative: Residents aHR: 0.15 (0.05-0.44)* Staff aHR: 0.39 (0.19-0.82)* *Multivariate analysis of risk of PCR positive infection by baseline antibody status, stratified by LTCF and adjusted for sex and age **Symptoms:**

	Feb 2021 for evidence of infection ▪ Staff and residents contributed 3,749 and 1,809 months of follow-up time respectively (mean 2.6 months per participant) ▪ Maximum f/u: 300 days (10 months), based on an assumption as to when the earliest infections took place. **Demographics** The median age of residents was 86 years (IQR: 79-91) and 47 years in staff (IQR: 34-56).	testing round stratified by the time gap between the antibody test and the PCR test **Cycle threshold:** Ct values were retrieved for 13/14 reinfection samples. The median Ct value for reinfection cases was 36 (30.1-37.0). 6/7 samples that were analysed using the same PCR assay, and 9/14 samples that were tested using assays that targeted the ORF1ab had Ct values >30	Of 12 reinfected participants with data on symptoms, 11 were symptomatic. **Titres:** Antibody titres to spike and nucleocapsid were comparable in PCRpositive and PCR-negative cases.
Lumley 2020 10.1056/NEJMoa2034545 Antibody status and incidence of SARS-CoV-2 infection in health care workers UK Prospective longitudinal cohort study Published NEJM	N=12,541 HCWs: 90.6% (N=11,364) seronegative, 9.4% (N=1,265) seropositive at baseline (anti-spike IgG assay) including 88 who seroconverted during the study **Median follow-up**: 200 days (IQR 180 to 207) after a seronegative test and 139 days (IQR 117 to 147) after a seropositive test. **Duration of study:** participants were followed up to 31 weeks **Demographics (seronegative/seropositive):**	**Primary endpoint:** Relative incidence of subsequent positive SARS-CoV-2 PCR tests and symptomatic infections in HCWs (seropositive *or* seronegative for SARS-CoV-2 antibodies at baseline) Those seropositive considered at risk for infection/reinfection from 60 days of first PCR positive test **Test:** Anti-trimeric spike IgG enzyme-linked immunosorbent assay (ELISA) and anti-nucleocapsid IgG assay (Abbott) **Intervals:** PCR testing every 2 weeks; serological testing every 2 months from April 2020 to November 2020	Participants were followed for up to 31 weeks. During this time no symptomatic infections and only 2 PCR-positive results in asymptomatic HCWs were seen out of 1,026 HCWs with anti-spike antibodies, compared with 223 PCR-positive results out of 11,364 seronegative HCWs (adjusted IRR: 0.11). This suggests that previous infection resulting in antibodies to SARS-CoV-2 is associated with protection from reinfection for most people for at least 6 months **Relative risk of reinfection** 3 of those seropositive at baseline subsequently had PCR-positive tests i.e., possible SARS-CoV-2 reinfection (1 anti-spike IgG only, 1 antinucleocapsid IgG only, 1 with both) as follows:

	Mean age 38/41; Female 74.1%/77%; White 73.1%/66%.	**In those seropositive at baseline, days between episodes:** ▪ Worker 1 (anti-nucleocapsid positive at baseline) 160 days ▪ Worker 2 (both anti-spike and anti-nucleotide positive) 190 days ▪ Worker 3 (anti-spike positive at baseline) 199 days **Whole Genome Sequencing:** Not performed **Cycle threshold**: N/R overall**-**only reported for the HCW positive for both antibodies and who tested PCR positive: Ct=31 (CN=21)	**1. In those who were baseline seropositive for anti-spike** n=2/1,265 seropositive (both mild symptomatic on 1st and asymptomatic on 2nd episode) and n=223/11,364 seronegative had positive PCR; IRR in seropositive: 0.12 (95% CI, 0.03 to 0.47; P = 0.002) After adjustment for age, gender, and month of testing or calendar time as a continuous variable, the IRR in seropositive: 0.11 (95% CI, 0.03 to 0.44; p=0.002). **2. In those who were baseline seropositive for anti-nucleotide** n=2/1,172 seropositive(1 asymptomatic 1st episode and mild symptomatic 2nd; 1 mild symptomatic 1st and asymptomatic 2nd episode) and n=226/11,543 had positive PCR; IRR 0.11 (95% CI, 0.03 to 0.45; P = 0.002) **3. In those who were baseline positive for both anti-spike and anti-nucleocapsid** n=1/1021 seropositive (mild symptomatic 1st episode and asymptomatic 2nd) and n=218/11,182 seronegative; IRR, 0.06; (95% CI, 0.01 to 0.46). Note: This is the only HCW with a history of PCR-confirmed symptomatic infection. After 5 negative tests, 1 positive with low viral load (Ct=31) If this result was a false positive, the IRR for PCR positivity: ▪ if anti-spike IgG–seropositive would fall to 0.05 (95% CI, 0.01 to 0.39) ▪ if anti-nucleocapsid IgG–seropositive would fall to 0.06 (95% CI, 0.01 to 0.40).

			4. In those with baseline mixed seropositivity n=2/344 workers with mixed antibody assay results had subsequent PCR-positive tests; IRR 0.42; 95% CI, 0.10 to 1.69)
Perez 2021 DOI: 10.1101/2021.03.06.21253051 A 1 to 1000 SARS-CoV-2 reinfection proportion in members of a large healthcare provider in Israel: a preliminary report Retrospective cohort study Pre-print	N=149,735 with history of prior infection Database covered all members in a healthcare provider (Maccabi Healthcare Services) with 2.5 million members (25% of population) Individuals were evaluated for reinfection if they had 2 positive PCR tests at least 100 days apart from 16 Mar 2020 to 27 Jan 2021. **Median f/u:** 165 days (5.5 months) **Maximum f/u:** Approx. 325 days (10.8 months)	The primary outcome was the rate of reinfection (2 positive PCR tests at least 100 days apart) Mean age (SD): 31.5 (19.5); male: 94 (61%) Mean interval between infection events: 165.7 days (SD: 57.6); Range between first and second positive PCR: 100 to >300 days. 11 (7.1%) hospitalised on 1st infection, 4 (2.6%) on 2nd; death 1 (0.6%) on 2nd The age distribution suggests higher count of reinfection among younger individuals. Of 154 with a second PCR positive test, 73 reported symptoms (47.4%) at both tests. **Cycle threshold:** N/R **Whole Genome Sequencing:** Not performed	Of 149,735 individuals with a record of positive PCR test (Mar 2020 to Jan 2021), 154 had 2 positive tests at least 100 days apart (0.1% proportion of reinfection). The reinfection counts were numerically higher in Jan 2021 compared with previous months. The reinfection counts were numerically higher in the 10-19 years age group compared with other age groups.
Pilz 2021 DOI: 10.1111/eci.13520 SARS-CoV-2 re-infection risk in Austria Austria Retrospective observational study	N=14,840 with history of prior infection at baseline These 14,840 represent recovered patients from the first wave and were compared with 8,885,640 of all the remaining general population from Austrian Epidemiological Reporting System. Of those with tentative reinfections, 62.5% were women;	Primary outcome was the odds of SARS-CoV-2 reinfections of COVID-19 survivors of the first wave (Feb to Apr 30 2020) versus odds of first infections during the second wave (Sept 1 to Nov 30 2020). Mean (SD) time from first to tentative reinfection was 212±25days (4, 12 and 24 reinfections documented in Sept, Oct and Nov, respectively) Range 148 to 251 days One 72-year old woman died following tentative reinfection – she was not hospitalised and cause of death was not causally attributed to COVID-19.	40 possible reinfections were recorded in 14,840 individuals with history of prior infection from the first wave (0.27%), compared with 253,581 infections in 8,885,640 (2.85%) in the remaining general population. OR was estimated at 0.09 (95% CI: 0.07 to 0.13)

Published in Eur J Clin Invest	median age (IQR) = 39.8 (25.9 to 54.5). **Median f/u:** 210 days (7 months) **Maximum f/u:** 300 days (10 months)	Hospitalisation status was coded yes (n=8), no (n=31), unknown (n=1) for first infection and yes (n=5), no (n=27), unknown (n=8) for reinfection (4 were hospitalised during first infections and reinfection) **Cycle threshold:** N/R **Whole Genome Sequencing:** Not performed	
Sheehan 2021 DOI: https://doi.org/10.1101/2021.02.14.21251715 Reinfection rates among patients who previously tested positive for COVID-19; a retrospective cohort study US Retrospective cohort study Pre-print	N=8,845 with history of prior infection at baseline All 150,325 patients who were tested for COVID-19 via PCR from Mar 12 2020 to Aug 30 2020 from one multi-hospital healthcare system were included. Of these, 8,845 (5.9%) tested positive and of these, 974 were re-tested after 90 days. These were compared with N=32,308 with no prior evidence of reinfection who were re-tested after 90 days. **Median f/u:** 131 days (4.4 months) **Maximum f/u:** 269 days (9 months)	Main outcome was risk of reinfection, defined as a positive PCR test ≥90 days after initial testing. Secondary outcomes were symptomatic infection and protective effectiveness of prior infection. Patients with a negative status who tested positive within 90 days of their initial test were excluded. Infection rates were determined for distinct periods following initial test: 4-5 months; 6-7 months and ≥8 months. Of 56 possible reinfections, 26 were symptomatic (shortness of breath being the most common symptom; no patient lost the sense of smell). 17 were hospitalised within 30 days of the positive test, 5 with symptoms considered related to COVID-19. Of those 5, none required ICU or mechanical ventilation. **Cycle threshold:** N/R **Whole Genome Sequencing:** Not performed	**Risk of reinfection** N=974 (11%) of the positive patients were retested after 90 days and 56 had possible reinfections. Of those, N=26 (46.6%) were symptomatic. Of those with negative initial tests, 22.8% (32,208/141,480) were retested and 4,163 (12.9%) were positive **Protective effectiveness** Protective effectiveness of prior infection was 78.5% (95%CI 72.0% to 83.5%)* and against symptomatic infection was 83.1% (95%CI 75.1% to 88.5%). *Effectiveness = 1-((56/8845)/(4163/141480)) **Risk of reinfection over time** ▪ Risk of reinfection was greatest just after 90 days and declined thereafter. ▪ Consequently, effectiveness was lowest in months 4-5 and increased for up to 8 months after infection.

			Many reinfections occurred close to 90 days after initial infection and average time to reinfection was 131.4±40.4days (range 90.2 to 269.0days) Protective effectiveness was lowest in months 4-5 and increased for up to 8 months after infection.

Key: aHR – adjusted hazard ratio; aOR – adjusted odds ratio (adjusted for week group); CI – confidence interval; Ct – cycle threshold value; f/u – follow-up; NAAT – nucleic acid amplification test; RT-qPCR – real time reverse transcription polymerase chain reaction; WGS – whole genome sequencing

Appendix 3: Quality Appraisal (NIH assessment tool)

Tool: The National Institutes of Health (NIH) quality assessment tool for observational cohort and cross-sectional studies, available at: https://www.nhlbi.nih.gov/health-topics/study-quality-assessment-tools

Quality appraisal question	Response					
	Abu-Raddad 2021 [assessment: 'fair']	Hall 2020 [assessment: 'good']	Hanrath 2021 [assessment: 'fair']	Hansen 2021 [assessment: 'good']	Harvey 2020 [assessment: 'poor']	Jefferey-Smith 2021 [assessment: 'fair']
1. Was the research question or objective in this paper clearly stated?	Yes	Yes	Yes	Yes	Yes	Yes
2. Was the study population clearly specified and defined?	Yes	Yes	Yes	Yes	Yes	Yes
3. Was the participation rate of eligible persons at least 50%?	Yes	Yes	Yes	Yes	Yes	Yes
4. Were all the subjects selected or recruited from the same or similar populations (including the same time period)? Were inclusion and exclusion criteria for being in the study prespecified and applied uniformly to all participants?	Yes	Yes	Yes	Yes	Yes	Yes
5. Was a sample size justification, power description, or variance and effect estimates provided?	N/A	N/A	N/A	N/A	N/A	N/A
6. For the analyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured?	Yes	Yes	Yes	Yes	Yes	Yes
7. Was the timeframe sufficient so that one could reasonably expect to see an association	Yes	Yes	Yes	Yes	Unclear	Yes

between exposure and outcome if it existed?						
8. For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g., categories of exposure, or exposure measured as continuous variable)?	N/A	N/A	N/A	N/A	N/A	N/A
9. Were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	Yes	Yes	Yes	Yes	No – All had an antibody test in the database, but type of antibody test and validity cannot be determined	Yes
10. Was the exposure(s) assessed more than once over time?	Yes	Yes	Yes	Yes	No	Yes
11. Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	Yes	Yes	Yes	Yes	No – All had NAAT, but type of NAAT cannot be determined	Yes
12. Were the outcome assessors blinded to the exposure status of participants?	No; Retrospective study	Unclear; Prospective study	No; Retrospective study	No; Retrospective study	No; Retrospective study	No; Retrospective study
13. Was loss to follow-up after baseline 20% or less?	Yes	Yes	Yes	Yes	Not Reported	Yes
14. Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)?	Database analysis; adjustment for WGS (removing viral shedding); unclear if all confounders measured	Yes	No	Yes	Statistical analysis and adjustment for confounders not reported	No

Quality appraisal question	Response				
	Krutikov 2021 [assessment: 'good']	Lumley 2020 [assessment: 'good']	Perez 2021 [assessment: 'fair']	Pilz 2021 [assessment: 'fair']	Sheehan 2021 [assessment: 'fair']
1. Was the research question or objective in this paper clearly stated?	Yes	Yes	Yes	Yes	Yes
2. Was the study population clearly specified and defined?	Yes	Yes	Yes	Yes	Yes
3. Was the participation rate of eligible persons at least 50%?	Yes	Yes	Yes	Yes	Yes
4. Were all the subjects selected or recruited from the same or similar populations (including the same time period)? Were inclusion and exclusion criteria for being in the study prespecified and applied uniformly to all participants?	Yes	Yes	Yes	Yes	Yes
5. Was a sample size justification, power description, or variance and effect estimates provided?	N/A	N/A	N/A	N/A	N/A
6. For the analyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured?	Yes	Yes	Yes	Yes	Yes
7. Was the timeframe sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed?	Yes	Yes	Yes	Yes	Yes
8. For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g.,	N/A	N/A	N/A	N/A	N/A

categories of exposure, or exposure measured as continuous variable)?					
9. Were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	Yes	Yes	Yes	Yes	Yes
10. Was the exposure(s) assessed more than once over time?	Yes	Yes	Yes	Yes	Yes
11. Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	Yes	Yes	Yes	Yes	Yes
12. Were the outcome assessors blinded to the exposure status of participants?	Unclear; Prospective study	Unclear; Prospective study	No; Retrospective study	No; Retrospective study	No; Retrospective study
13. Was loss to follow-up after baseline 20% or less?	Yes	Yes	Yes	Yes	Yes
14. Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)?	Yes	Yes	No	No	No

Appendix 4 Reinfection cases (1 November 2020 to 22 February 2021)

Source/First Author Date of reinfection	Location	Patient details	Interval (days)	Symptoms (initial infection)	Symptoms (reinfection) z	Whole genome sequencing & details of variants‡
Case 1 Media report **20/2/2021**	Panama	Not Reported	180 (approx.)	Not Reported	Not Reported	Not Reported
Case 2 Fels 2021 **18/2/2021**	United States (Bronx, NY)	10-15/F	142	Mild	Mild	The first and second samples fall in different local phylogenetic clades in the Bronx phylogenetic tree
Case 3 Media report **18/2/2021**	Paraguay	M	120 (approx.)	Not Reported	Not Reported	Not Reported
Case 4 Government press release **9/2/2021**	Brazil (Amazonas)	50/F	92	Not Reported	Not Reported	**Reinfection: P1 variant** (initial infection not reported)
Case 5 Government press release **9/2/2021**	Brazil (Amazonas)	40/F	282	Not Reported	Not Reported	**Reinfection: P1 variant** (initial infection not reported)
Case 6 Personal communication - Corey Egel **27/1/2021**	United States	Not Reported	≥120	Symptomatic	Symptomatic	Both were different strains (strain not reported)
Case 7 Personal communication - Corey Egel **27/1/2021**	United States	Not Reported	≥120	Symptomatic	Symptomatic	Both were different strains (strain not reported)
Cases 8-13	China	F	34	Serious	Mild	

Zhang 2021 **26/1/2021**	China	Not Reported	19	Moderate	Mild	D614G haplotype present on reinfection event in five out of six reinfection cases
	China	Not Reported	57	Moderate	Mild	
	China	Not Reported	37	Moderate	Not Reported	
	China	Not Reported	24	Moderate	Not Reported	
	China	Not Reported	24	Serious	Not Reported	
Cases 14-18 **Abu-Raddad 2021** **16/1/2021**	Qatar	40-44/F	84	Not Reported	Moderate	Included in our review
	Qatar	35-39/F	110	None	None	
	Qatar	35-39/M	59	Not Reported	None	
	Qatar	30-34/M	81	Serious	Serious	
	Qatar	35-39/M	84	Not Reported	None	
Case 19 **Naveca 2021** **13/1/2021**	Brazil (Manaus, Amazonas)	29/F	281	Mild	Mild	Initial infection: B.1 lineage **Reinfection: P.1 lineage (alias of B.1.1.28.1)**
Case 20 **Harrington 2021** **10/1/2021**	UK	78/M	250	Mild	Serious	Initial infection: B.2 lineage, with no mutations observed in the S region. **Reinfection: B.1.1.7 lineage, and accumulated 18 amino-acid replacements across the genome**
Case 21 **Government press release** **8/1/2021**	Brazil (Bahia)	45/F	147	Mild	Mild (more intense)	Initial infection: B.1.1.33 lineage **Reinfection: B.1.1.248 lineage with mutation found in the new South African variant in the protein Spike located in the RDB (E484K)**
Case 22 **News report** **22/12/2020**	Israel	74/M	90 (approx.)	Symptomatic	Symptomatic	Not reported
Case 23 **Facebook post** **17/12/2020**	Mexico	M	64 (approx.)	Symptomatic	Symptomatic	
Case 24	Brazil	41/F	145	Symptomatic	Symptomatic	Following details on sequencing data: "One of them [strains] was found exclusively in Brazil, and the other has already

Government press release 16/12/2020						been identified both in Brazil and in the United States, United Kingdom, Australia and Chile"
Case 25 Facebook post 11/12/2020	Peru	6/F	97	Mild	Mild	
Case 26 Resende 2020 9/12/2020	Brazil (Rio Grande do Norte)	37/F	116	Mild	Mild	Initial infection: B.1.1.33 lineage **Reinfection: B.1.1.28 lineage. "Notably, the B.1.1.28 virus detected at reinfection corresponds to a new emergent Brazilian viral lineage, initially detected in the Rio de Janeiro state, containing the mutation E484K in the Spike protein"**
Case 27 Lee 2020 21/11/2020	South Korea	21/F	10	Mild	Mild	Initial infection: V clade (nsp6 L37F and ORF3a G251V) Reinfection: G clade, with the spike protein D614G substitution
Case 28 Selhorst 2020 10/11/2020	Belgium	39/F	185	Mild (long)	Mild (milder)	Initial infection: V clade Reinfection: G clade Transmissibility: Although contact tracing and viral culture remained inconclusive, the healthcare worker formed a transmission cluster with 3 patients and showed evidence of virus replication but not of neutralising antibodies in her nasopharyngeal swabs

Source: Covid-19 reinfection tracker (https://bnonews.com/index.php/2020/08/covid-19-reinfection-tracker/)

‡ Newly identified variants of concern (VoC) are identified in bold text. These comprise: **P.1 lineage (alias of B.1.1.28.1), B.1.1.248, B.1.1.28 lineage**

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