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

Adaptive immunity	Adaptive immunity, also known as acquired immunity, is a type of immunity that occurs after exposure to an antigen either from a pathogen or a vaccination. An adaptive immune response relies on lymphocytes (B and T cells) and the products of these cells to respond to threats.
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 to not inhibit the multiplication of viruses within cells.
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 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.They are important in relation to T cells' interaction with and stimulation of lymphocytes and other cells, but do not recognise the antigen or components of the antigen.
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 serum	Convalescent serum is serum collected during convalescence, the clinical period during which the acute phase of illness has passed, but the person has not recovered full function.
Cycle threshold (Ct)	In reverse transcriptase 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.
Epitope	The portion of an antigen to which an antibody binds
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 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.
Innate immunity	The first line of defence against microbes; provided by the skin, mucosal tissues, and non-specific immune cells, plasma proteins. Also, initiates adaptive immunity
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)	Neutralising antibodies are antibodies that are capable of preventing viruses from infecting cells. Neutralising antibodies usually bind the pathogen protein, which binds the receptor.
Pathogen	Pathogens are microorganisms that can cause disease when they infect a host.
Plasma cells	Plasma cells are specialised cells derived from B cells after B cells are stimulated by an antigen. They make antibodies against the stimulating antigen.
Primary immune response	The immune response initiated by lymphocytes called naïve lymphocytes that are encountering an antigen for the first time.
Receptor-binding domain (RBD)	In the context of SARS-CoV-2, RBD refers to a specific section of the spike protein that binds to a molecule (ACE2 receptor) on the surface of human cells that allows the virus to enter the cell.
Reverse transcriptase–	The reverse transcriptase–polymerase chain reaction (RT-PCR) is used to amplify RNA sequences. The enzyme reverse transcriptase is

polymerase chain reaction	used to convert an RNA sequence into a cDNA sequence, which is then amplified by PCR.
Secondary (memory) immune response	Following the primary response and once the pathogen has been subdued, most pathogen-specific B- and T-cells die, but some of them persist in specialised, long-lived immune memory cells. On reinfection memory B-cells, combined with reactivated memory T-cells will add large numbers of high-affinity antibodies to those already present in the serum, blocking the attack.
Seroconversion	Seroconversion timing refers to the first time an individual tests positive for antibodies (based on serial serological samples).
Seropositive	When someone has detectable antibodies against a specific antigen
Seronegative	When someone does not have detectable antibodies against a specific antigen
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 RNA 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 an organisml 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	14 April 2021	Updated search with 6 new reinfection studies
V7.0	3 June 2021	Updated search with 11 new reinfection studies and systematic search of immune memory responses following SARS-CoV-2 infection
V7.1	22 June 2021	Minor wording change to final paragraph on page 53, summarising the Bernal et al study published as a preprint on 24 May 2021.

Acknowledgements

We would like to thank HSE librarians for their assistance in designing database search and conducting searches.

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

Key points

- This evidence synthesis, which informed HIQA's advice, consisted of two systematic reviews. The first identified studies that investigated the risk of SARS-CoV-2 reinfection over time, with the second identifying studies that investigated immune memory responses at least six (≥6) months post-infection.

Part 1 – risk of reinfection

- Nineteen observational cohort studies, that investigated the risk of SARS-CoV-2 reinfection over time, were identified that met the inclusion criteria. Five studies exclusively enrolled healthcare workers and two studies enrolled both staff and residents of elderly care homes; six of these seven studies were conducted in the UK. The remaining twelve studies were in the general population, conducted in ten different countries.
- Across studies, the total number of PCR- or antibody-positive participants at baseline was 641,911 (median: 1,899; range: 88 to 378,606).
- The median follow-up of individuals within studies was 135 days (4.5 months) (range of medians: 54-249 days), with a maximum follow-up of ≥300 days (ten months) in six studies.
- Reinfection was a rare event: the median PCR-confirmed reinfection rate was 0.6% across studies, ranging from 0% (zero reinfections in three studies) to 2.8% (which was observed among dental practitioners in the UK).
- All studies reported low relative rates of reinfection comparing prior positive (PCR and or antibody positive) and prior negative groups (no PCR positive and or antibody negative). However, between-study estimates were not directly comparable due to varying definitions for reinfection and different outcome measures. No study reported an increased relative risk of reinfection over time. All studies, that separately reported symptomatic and 'all' reinfection events, reported lower relative rates of symptomatic reinfections. For example, in a large sample of UK health care workers, the relative risk for 'any reinfection' was 0.159 (95% CI: 0.13–0.19), falling to 0.074 (95% CI: 0.06–0.10) for reinfections with COVID-19 symptoms.
- Of the 11 general population studies, only one study estimated the population-level risk of reinfection based on whole genome sequencing on a representative sample. 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.

- Only one study reported the relative risk of reinfection by age group, noting higher rates in older individuals. In individuals aged 65 years or older, 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. One other study reported risk of reinfection in an older age group. This UK study reported an adjusted hazard ration of 0.15 in elderly residents of care homes (median age ≥84 years).
- One study assessed the protective effectiveness of natural infection against reinfection in both vaccinated and unvaccinated healthcare workers in the UK, and coincided with widespread transmission of the B.1.1.7 variant. This study found:
    - Compared to unvaccinated seronegative HCWs, natural immunity and two vaccination doses provided similar protection against symptomatic infection: no HCW vaccinated twice had symptomatic infection, and incidence was 98% lower in seropositive HCWs (adjusted incidence rate ratio 0.02 [95%CI <0.01-0.18]).
    - Two vaccine doses or seropositivity reduced the incidence of any PCR-positive result (with or without symptoms) by 90% (0.10 [0.02-0.38]) and 85% (0.15 [0.08-0.26]), respectively.
    - Single-dose vaccination reduced the incidence of symptomatic infection by 67% (0.33 [0.21-0.52]) and any PCR-positive result by 64% (0.36 [0.26-0.50]).
    - There was no evidence of differences in immunity induced by natural infection and vaccination for infections with B.1.1.7 and a proxy for B.1.1.7 (S-gene target failure.
- One study directly assessed the relationship between serological antibody levels and reinfection risk among a cohort of dental practitioners in the UK. In this study, the risk of infection was 9.6% in participants who were seronegative at baseline compared to 2.8% in individuals who were seropositive (p=0.001). However, there were no PCR-proven infections among 64 individuals with a baseline anti-SARS-CoV-2 IgG level greater than 147.6 IU/ml (with respect to the WHO international standard NIBSC 20/136).
- Only four of the included studies were considered of high methodological quality, with a number of issues identified across studies. Apart from the

inherent biases associated with observational study designs, many studies were downgraded due to poor quality of reporting and for inadequate control of confounders. A recognised limitation of a number of studies was the risk of outcome ascertainment bias. In addition, 10 of the 19 studies are currently published as preprints.

- There are also limitations relating to the applicability and generalisability of identified studies. There is uncertainty in relation to:
    - paediatric populations
    - those with comorbidities and those who are immunocompromised
    - vaccinated populations
    - new variants.

Part 2 – immune memory

- Thirteen studies were identified that investigated immune memory responses at ≥6 months post-infection, including one study at ≥9 months post-infection. Study numbers were small, ranging from 15 to 188 participants.
- In 11 studies that considered memory B-cells, with the exception of a decline in IgM+ memory B-cells reported in two studies, memory B-cell response was found to be maintained for the duration of follow-up, which extended to nine months post infection in one study.
- In six studies that considered memory T-cells, all reported persistence over periods of six to nine months, however a number reported declining frequency over time.
- Eight studies, reporting the proportion with a response, identified that most or all of those tested developed either memory B- or memory T-cell responses.
- Two studies examined the development of neutralising antibodies from memory B-cells, and both demonstrated that memory B-cells generated neutralising antibodies. One of these studies found that, over a six month period, these antibodies increased in potency and breadth.
- The studies identified suggest that immune memory develops in most or all of those who have been infected with SARS-CoV-2 and lasts for up to nine months. There is substantial uncertainty in relation to the immune response to SARS-CoV-2 given the small study sizes and lack of clarity in relation to potential confounders.
- No studies were identified that examined mucosal immune memory or immune memory in tissues. These are likely to be key factors in preventing onward transmission of disease.

- In conclusion:
    - A large volume of data supports the likelihood that the risk and relative risk of SARS-CoV-2 reinfection is low for over ten months post-infection. While limited evidence from one study supports the hypothesis that natural infection and vaccination both result in robust immune responses, including against the variant B.1.1.7, the emerging evidence relating to new variants and vaccinated populations should be kept under review.
    - While more limited data were identified in relation to the immune memory response to SARS-CoV-2 infection, studies generally found that immune memory lasts for up to nine months post-infection and support the findings of the reinfection review.

Duration of protective immunity 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 questions were developed and will form the basis of this evidence summary:

Research question 1: How long does protective immunity (that is, prevention of RT-PCR confirmed reinfection) last in individuals who were previously infected with SARS-CoV-2 and subsequently recovered?

Research question 2: What is the duration of immune memory responses (T-cell and B-cell memory and or their components' responses) following SARS-CoV-2 infection?

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:

- How long can asymptomatic individuals 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?
- How long can asymptomatic health care workers who have recovered from a prior SARS-CoV-2 infection be exempted from exclusion from work policies if they become a close contact of a confirmed COVID-19 case?
- 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)?
- How long can asymptomatic patients who have recovered from a prior SARS-CoV-2 infection be exempted from testing prior to scheduled admission to hospital or inter institutional transfer?

- How long can asymptomatic individuals who have recovered from a prior SARS-CoV-2 infection meet indoors without wearing face coverings or staying two metres apart:
    - with other asymptomatic individuals who have recovered from a prior SARS-CoV-2 infection or with vaccinated individuals from up to two other households
    - with people from one other household who are not vaccinated as long as no more than three other households are there

Prior to this review, six evidence summaries relating to immunity following SARS-CoV-2 infection were published by HIQA (13 May 2020, 9 June 2020, 6 August 2020, 11 November 2020, 5 March 2021 and 14 April 2021). In the 14 April 2021 review, HIQA concluded that SARS-CoV-2 reinfection rates remain low for 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.

Due to the rapidly evolving evidence base relating to SARS-CoV-2 immunity, this review updates the evidence base relating to protective immunity following SARS-CoV-2 infection. Part 1 relates to the systematic review of the risk of SARS-CoV-2 reinfection over time, and directly updates the systematic search employed in the 14 April 2021 review. Part 2 relates to a de novo systematic review relating to the long-term immune memory responses following SARS-CoV-2 infection.

Methods

Part 1 – risk of reinfection

A standardised protocol was adhered to and is available on the HIQA website. Databases (PubMed, Embase and EuropePMC) were searched on 4 May 2021.

Table 1 outlines the Population Outcome Study design (POS) criteria for study selection relating to the systematic search for observational cohort studies that report the risk of reinfection over time.

Table 1. Population Outcome Study design (POS) criteria – reinfection review

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) ▪ Case studies ▪ 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).[1] **Definitions used by HSE[2]

Part 2 – immune memory

HIQA's previous review on immune response to SARS-CoV-2 included a scoping review of immune memory. In this updated review, HIQA undertook a systematic search for relevant studies on immune memory. Similar to Part 1, a standardised protocol was adhered to and is available on the HIQA website.

The purpose of this component of the review is to investigate longer-term duration (≥6 months) persistence of B- and T-cell immune responses following the immune system's primary response to SARS-CoV-2.

Primary immune response and immune memory

Following infection with SARS-CoV-2, the first line of defense is provided by the non-specific *innate* immune system. Epithelial barriers of the skin and mucosal membranes, cells and natural antimicrobial subtances in the epithelia all block the entry of microbes. The innate immune system does not have a memory function, and therefore does not contribute to defense against reinfection after infection, or the protective response to vaccination.

In addition, the innate immune response initiates *adaptive* immune responses to the infectious agent.[3, p.3] There are two types of adaptive immunity; *humoral* and *cell-mediated*. Humoral immunity is mediated by antibodies produced by B lymphocytes (B-cells) and plasma cells. These antibodies enter the circulation, extracellular tissue fluids and lumens of the mucosal organs and defend against microbes by preventing them from invading tissue cells and by neutralising them.[3, pp 5-6] Humoral immunity is not generally effective after pathogens have entered cells; however, *cell-mediated* immunity which is mediated by T lymphocytes is important.

This *primary* immune response is initiated when lymphocytes (called naïve lymphocytes) encounter a specific antigen that binds to their specific cell surface antibody and stimulates them to respond. However, subsequent encounters with the same, or similar antigens, lead to secondary immune responses, that are usually more rapid, larger and better able to eliminate the antigen than primary responses. Secondary responses result from the activation of *memory* lymphocytes; long-lived cells that are induced during the primary immune response.[3, p.8] [4]

Following the primary response and once the pathogen has been controlled, most pathogen-specific B- and T-cells die, but some of them (B-cells, T-cells and antibody secreting plasma cells), persist as specialised, long-lived immune memory cells. [5] Plasma cells do not express surface-bound antigen receptor and cannot sense antigens. Rather, they are 'antibody factories' that release their products at a constant rate.[6] On reinfection, antibodies produced by plasma cells act as an

immediate line of defense. In contrast, antigen must be present to trigger memory B-cells recall response.[6] During reinfection these memory B-cells will add large numbers of high-affinity antibodies to those already present in the serum, enhancing the humoral immune defense.[6]

Thus, although concerns have been expressed about declining IgG neutralising antibodies to viruses such as SARS-CoV-2 in convalescence, immunological memory is usually maintained.[7] This enables a quicker and stronger response on subsequent encounter with the virus (or a closely related virus), often before symptoms develop, and may offer long-lasting protective immunity.[8]

Type of immune memory cells

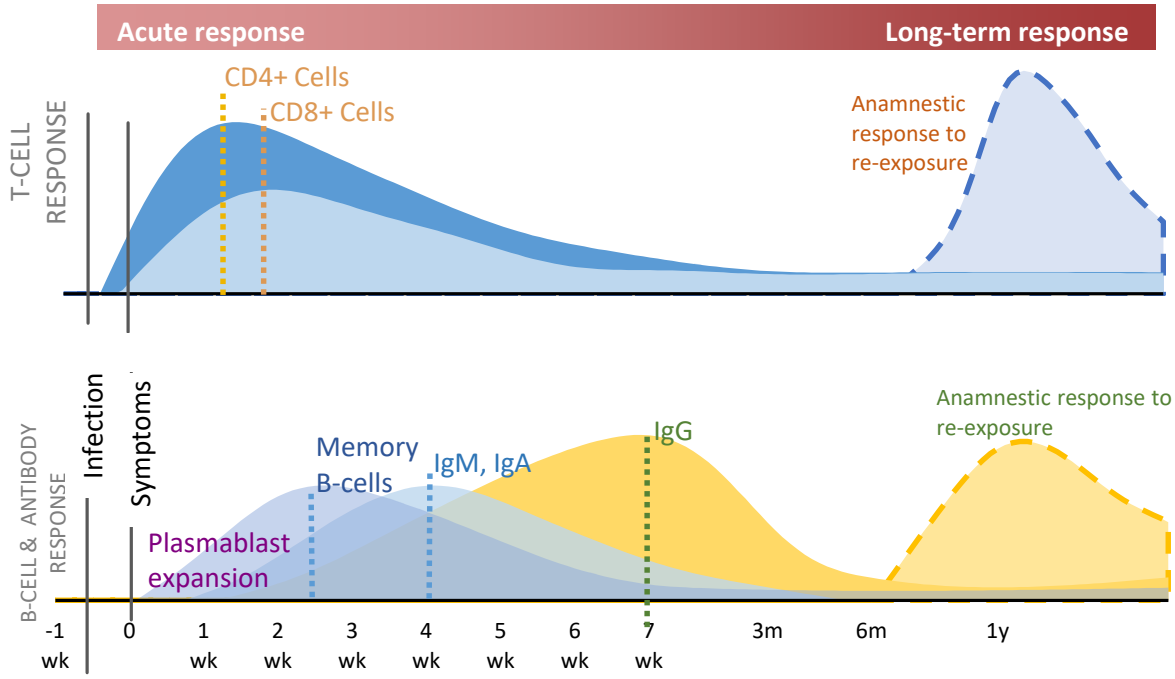
There are three main components of immune memory: memory B-cells, memory CD4+ T-cells and memory CD8+ T-cells, as well as subtypes of each and local tissue immune memory.[9]

Epitopes- where immune cells attach to antigens

An epitope is the specific portion of an antigen to which an antibody receptor binds.[3, p.268, 5] For example, the ability of an antibody to neutralise a virus (prevent the virus from entering into the cell) may focus on one specific epitope on the virus. If a mutation occurs that changes that specific epitope, the variant may escape recognition by the immune system and cause infection in individuals who have adaptive immunity to previous variants.

For illustration, Figure 1 outlines the projected acute and long-term adaptive responses following SARS-CoV-2 infection (adapted from Stephens and McElrath[7]).

Figure 1. Projected acute and long-term immune responses following SARS-CoV-2 infection



Adapted from: Stephens and Mc Elrath; JAMA, 2020[7] 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.

Why does this matter?

As severe COVID-19 in humans is relatively slow to progress (median 19 days post-symptom onset for fatal cases), protective immunity to SARS-CoV-2 reinfection may involve immune memory compartments which can take several days to reactivate and generate recall T-cell and/or B-cell responses.[10] Evaluation of the various components of immune memory (memory B-cells, CD4+ T-cells, CD8+ T-cells) is required as these different cell types have immune memory kinetics relatively independent of each other. Understanding their complexities will help gain insights into the likelihood of durability of protective immunity against reinfection.[10]

Immune memory and other viral infections

Data on immunological memory is constantly evolving. However, knowledge of how immune memory components relate to other infectious diseases can inform current knowledge regarding SARS-CoV-2. Studies have shown that:

- B-cell memory can be long-lived, including 60+ years after smallpox vaccination, or 90+ years after influenza[11]

- Durability of CD4+ T-cells to smallpox were estimated to have a half-life ($t_{1/2}$) of ~10 years[10]
- SARS-CoV memory T-cells were detected 17 years after infection[10, 12, 13]
- SARS-CoV memory B-cells were reportedly lost within six years of infection [13, 14]
- SARS-CoV neutralising antibodies were detected 17 years after infection[13, 15]
- MERS memory T-cells persisted for two years [13, 16]
- T-cell memory in tissues may be key players in upper respiratory tract infections, although this has not been studied in humans[13, 17, 18]
- T-cell memory has a role in protection from influenza disease severity in humans[13, 19-21]

Immune memory in the absence of detectable SARS-CoV-2

SARS-CoV-2–specific memory T-cell responses have been reported in close contacts of people infected with SARS-CoV-2 despite lacking a detectable infection. An analysis of 69 close contacts from 45 family clusters found that 58% and 14.5% of close contacts' samples contained virus-specific CD4+ and CD8+ T-cells, respectively.[22] In addition, multiple studies confirm the cross-protective nature of memory immune cells from other viruses towards SARS-CoV-2, probably based on exposure to common cold coronaviruses.[12, 23-28]

Immune memory and the common cold

There has been a concern that infection with the common cold human coronaviruses (HCoVs) fails to induce durable protective immunity.[13] This thinking derives from one interpretation of a study from a seminal rechallenge study in 1990 by Callow et al.[29] However, alternative interpretations of the study results can be made, including that the study demonstrated that immune memory provided 100% protective immunity from symptomatic disease, as 'reinfection' was defined as viral shedding for at least one day or change in antibody titre.[9]

Databases (PubMed and Embase) were searched from 1 January 2020 to 12 May 2021.

Table 2 outlines the Population Outcome Study design (POS) criteria for study selection relating to the systematic search for observational cohort studies that report on the duration of immune memory responses (T-cell and B-cell memory and or their components' responses) following SARS-CoV-2 infection.

Table 2. Population Outcome Study design – immune memory

Population	▪ Individuals (of any age) with evidence of prior SARS-CoV-2 infection; 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)
Outcomes	▪ Development of immune memory B and or T cells or their components.
Types of studies	**Include:** ▪ Observational studies (prospective or retrospective) with follow-up ≥6 months post-infection or post-symptom onset. **Exclude:** ▪ modelling studies ▪ studies of cross-protection from immune memory development from a prior infection with a virus other than SARS-CoV-2 ▪ systematic or narrative reviews ▪ case series ▪ case reports ▪ studies that describe precursors to immune memory cells ▪ studies of deceased patients ▪ studies in animals ▪ studies of populations vaccinated against SARS-CoV-2.

Analyses of populations or blood samples conducted six months or greater post-infection (or post-symptom onset) were included in this review.

Results

Part 1 – risk of reinfection

The collective database search resulted in 2,455 citations, with eight citations retrieved from other sources (grey literature search). Following removal of duplicates, 2,315 citations were screened for relevance. This resulted in 133 studies eligible for full text review (Figure 2), where a further 114 studies were excluded (Appendix 1.1).

Nineteen studies were identified that met the inclusion criteria.[30-48] Five studies exclusively enrolled healthcare workers[31, 32, 42, 47, 49] and two studies enrolled both staff and older residents of care homes;[35, 36] six of the seven were conducted in the UK with one conducted in the US.[47] The remaining twelve studies were all general populations, conducted in Austria,[38] Denmark,[39] Iran,[40] Israel,[34] Italy,[46] Qatar[30] Spain,[41] Switzerland,[45] the UK[43] and the US.[33, 37, 47] Ten studies are currently published as preprints.[34, 36, 37, 40, 42, 44-46, 48, 50] Across studies, the total number of PCR- or antibody-positive participants at baseline was 641,911 (median: 1,899; range: 88 to 378,606). 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 135 days (4.5 months) (range of medians: 54-249 days), with a maximum follow-up of ≥300 days (ten months) in six studies.[34, 36, 38, 43, 45, 51] Studies reported a range of primary endpoints (Table 3 and Appendix 2.1).

Figure 2. PRISMA diagram of study selection (Part 1 – reinfection review)

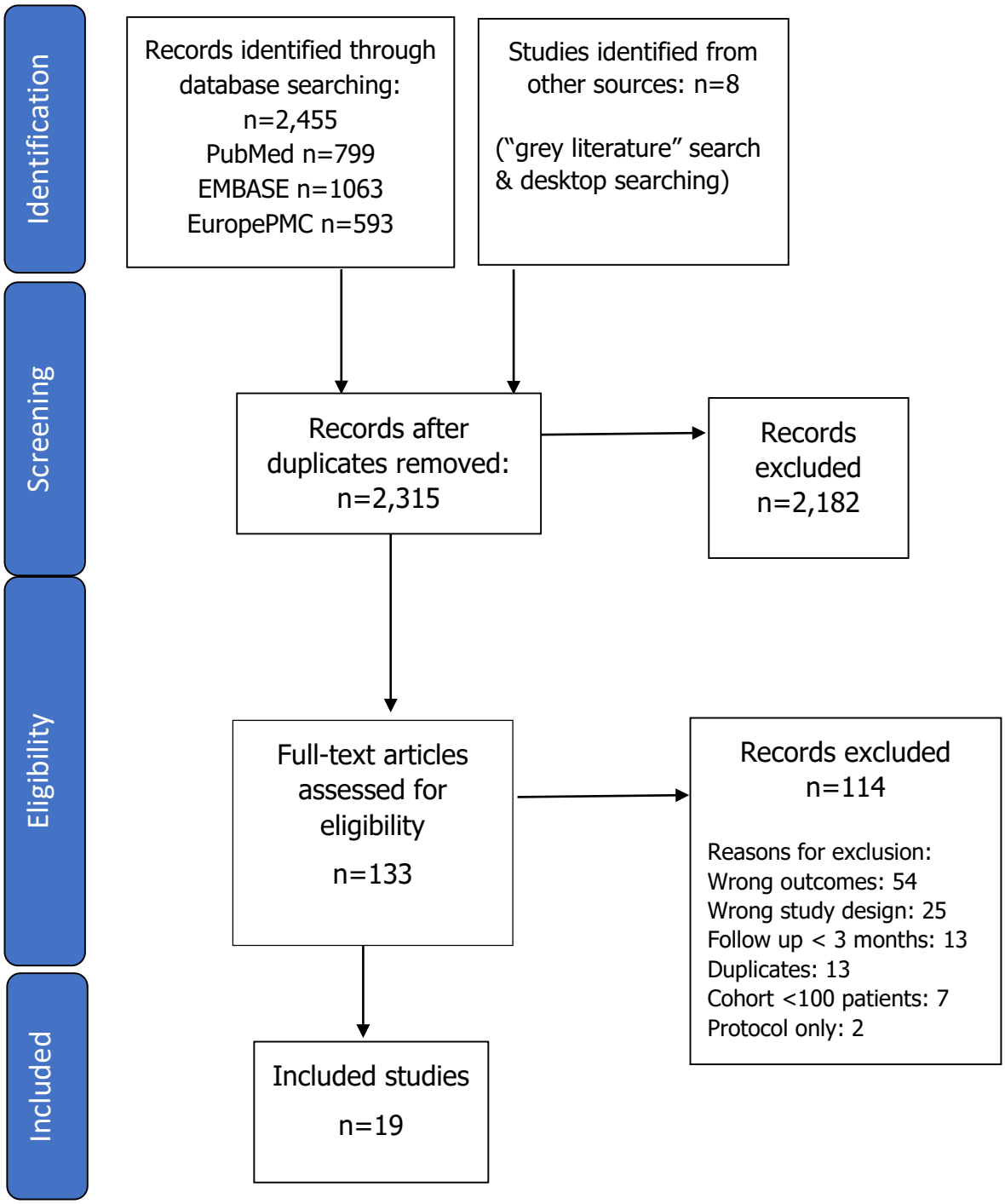


Table 3 Summary of included studies and primary outcome results

First author Country	Participants[a] Follow-up	Author reported primary outcomes	Quality appraisal[l]
General population			
Abu-Raddad 2021[30] **Qatar**	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
Breathnach 2021[43] **UK**	N=10,727 **Median f/u:** N/R **Maximum f/u:** Approx. 11 months (February to December 2020)	**Risk of reinfection:** 0.07% (with ≥90 days between infection events) Of note, there were no reinfections in the first seven months after the peak of the first wave; all eight patients with likely reinfections were diagnosed in December, the last month of the study period; reinfections accounted for 1.69% of all infections in that month[m] **Relative risk of reinfection[c]**= 0.058 (95% CI: 0.029 to 0.116)	'Fair' quality
Hansen 2021[39] **Denmark**	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[33] **USA**	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
Leidi 2021[45] **Switzerland**	N=498 **Mean f/u:** 249 days (8.3 months) **Maximum f/u:** Approx. 10 months	Seropositive group: 5/498 reinfections; incidence: 0.3 per 1,000 person-weeks (considered 'likely' reinfections)[e] Seronegative group: 154/996 infections; incidence: 4.8 per 1,000 person-weeks **Hazard ratio for reinfection**: 0.06, 95% CI 0.02 to 0.14, p<0.001 (with propensity matching)	'Good' quality
Manica 2021[46] **Italy**	N=1,402 **Maximum f/u:** 8 months	Cumulative incidence of symptomatic infections in seropositive group: 0.14% (95%CI: 0.04% to 0.58%) Cumulative incidence of symptomatic infections in seronegative group: 2.67% (95% CI: 2.12% to 3.37%) **Adjusted odds ratio** of developing symptomatic infection: 0.05 (95% CI: 0.01 to 0.17) Note: Investigators used RT-PCR *or* rapid antigen testing to identify reinfection cases.	'Good' quality

Masia 2021[41] **Spain**	N=146 **Maximum f/u:** 6 months	**Reinfection rate based on whole genome sequencing:** 1 confirmed reinfection out of 146 primary infections (0.68%)	'Good' quality
Mohamadreza 2021[40] **Iran**	N=1,899 **Maximum f/u:** 6 months	Symptomatic reinfection rate: 1.9% (37/1,899)	'Poor' quality
Perez 2021[34] **Israel**	N=149,735 **Median f/u:** 165 days (5.5 months) **Maximum f/u:** Approx. 325 days[f] (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[38] **Austria**	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
Qureshi 2021[44] **USA**	N=9,119 **Mean** interval between positive tests: 116 days (3.9 months) **Maximum f/u:** N/R; time period applied to dataset: 1 December 2019 to 13 November 2020.	Reinfection rate: 0.7% (95% CI: 0.5%-0.9%), 63/9,119 individuals	'Fair' quality
Sheehan 2021[37] **USA**	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%)[g] Protective effectiveness against symptomatic infection: 83.1% (95% CI: 75.1% to 88.5%)	'Fair' quality
Health care workers			
Hall 2021[51] **UK**	N=8,278 **Median f/u:** 275 days (9.1 months) (IQR 218–291 days) for the positive cohort and 195 days (6.5 months) (IQR 131–214 days) for the negative cohort. **Maximum f/u:** >11 months	**Incidence density:** 7.6 reinfections per 100,000 person-days in the previous positive cohort compared with 57.3 primary infections per 100,000 person-days in the previous negative cohort **Adjusted incidence rate ratio of reinfection comparing antibody or PCR-positive group with negative group:[h]** ▪ All events (possible and probable reinfections): 0.16 (95% CI: 0.13–0.19) ▪ Symptomatic reinfections only (with COVID-19 symptoms): 0.07 (95% CI: 0.06–0.10) ▪ Asymptomatic reinfections only: 0.48 (95% CI: 0.37–0.63) ▪ Probable reinfections only: 0.002 (95% CI: 0.00–0.01)	'Good' quality
Hanrath 2020[32] **UK**	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 χ^2 test).	'Fair' quality

Lumley 2021[48] UK	N=1,273 **F/u:** 216 days (7.2 months) (13,109 individuals contributed 2,835,260 person-days follow-up)	▪ Compared to unvaccinated seronegative HCWs, natural immunity provided similar protection against symptomatic infection as two vaccination doses: no HCW who received two vaccine doses had symptomatic infection, and incidence was 98% lower in seropositive HCWs (adjusted incidence rate ratio 0.02 [95%CI <0.01-0.18][j]). ▪ Two vaccine doses or seropositivity reduced the incidence of any PCR-positive result with or without symptoms by 90% (0.10 [0.02-0.38]) and 85% (0.15 [0.08-0.26]), respectively. ▪ Single-dose vaccination reduced the incidence of symptomatic infection by 67% (0.33 [0.21-0.52]) and any PCR-positive result by 64% (0.36 [0.26-0.50]). ▪ There was no evidence of differences in immunity induced by natural infection and vaccination for infections with S-gene target failure and B.1.1.7.	'Good' quality
Papasavas 2021[47] USA	N=433 **Median f/u:** 5.5 months **Maximum f/u:** 196 days (6.5 months)	0/35 seropositive participants had a subsequent PCR test at least 30 days following the positive antibody test had a positive test 1.3% (29/2173) of seronegative participants had a subsequent positive PCR test	'Fair' quality
Shields 2021[42] UK	N=246 (dental practitioners) Maximum f/u: 6 months	**Adjusted risk ratio** for reinfection: 0.26 (95% CI 0.11 to 0.63) The risk of infection was 9.6% in participants who were seronegative at baseline, compared to 2.8% in individuals who were seropositive (p=0.001) Serological analysis: there were no PCR-proven infections in 64 individuals with a baseline anti-SARS-CoV-2 IgG level greater than 147.6 IU/ml (with respect to the WHO international standard NIBSC 20/136).	'Good' quality
Staff and residents of care homes for older people			
Jeffery-Smith 2021[35] UK	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[36] UK	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)[i] Staff of care home: aHR=0.39 (0.19-0.82)[i]	'Good' 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; PM – propensity matching; 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]This is the relative risk during second wave (August-December 2020) comparing those previously PCR/antibody positive after first wave (February-July 2020) with PCR/antibody negative after first wave.

[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] Three adjudicators assessed the likelihood of reinfection based on timing, clinical characteristics and Ct values ('likely')

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

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

[h]'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

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

[j]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

[k]After adjustment for age, gender, and month of testing or calendar time as a continuous variable.
[l]Based on National Institutes of Health (NIH) quality appraisal criteria
[m] This month (December 2020) coincided with the identification and widespread transmission of variant B.1.1.7 in the UK

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

Twelve studies were identified that investigated reinfection in the general population. Three studies were conducted in the US,[33, 37, 44] and one each was conducted in Austria,[38] Denmark,[39] Iran,[40] Israel,[34] Italy,[46] Qatar,[30] Spain,[41] Switzerland,[45] and the UK.[43]

Austria

In the study by Pilz et al.,[38] 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.,[39] individual-level data were collected on patients who had been tested 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 95% CI: 0.13–0.23
- 35–49 years: aRR=0.20 95% CI: 0.14–0.28
- 50–64 years: aRR=0.19 95% CI: 0.13–0.27
- ≥65: years: aRR=0.53 95% CI: 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.

Iran

In the study by Mohamadreza et al.,[40] symptomatic reinfection rates were retrospectively investigated in the three referral hospitals in Iran, six months after the pandemic onset. A total of 32,567 tests were performed involving 1,899 patients. Of these, 37 cases were considered reinfections based on prespecified criteria (two positive RT-PCR tests at least three months apart, with a negative RT-PCR test

between the two positive tests). The mean duration between the discharge and second presentation was 117±61.42 days. The proportions of patients with mild, moderate or severe disease was not significantly different comparing primary and secondary infections. Seven (18.9%) patients were hospitalised during the secondary infection compared with two (5.4%) patients during the primary infection. The clinical, radiological, and laboratory characteristics were not significantly different between the two episodes.

Israel

In the preliminary preprint report by Perez et al.,[34] 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.

Italy

In the study by Manica et al.,[46] IgG serological screening of individuals in five Italian municipalities within the Province of Trento, Italy, was conducted in May 2020. These municipalities were selected as those showing the highest cumulative case incidence in the province during the first COVID-19 wave (ranging between 18.7 and 27.6 per 1,000 individuals).

The serological screening involved 6,074 individuals (median age 50; IQR: 32-63), representing 77.1% of the resident population. Of these, 1,402 (23.1%) were seropositive for IgG. Between 1 June 2020 and 31 January 2021, regular surveillance activities identified 221 new positive SARS-CoV-2 infections (124 symptomatic) among study participants (RT-PCR or rapid antigen positive). The cumulative incidence of identified symptomatic infections over the observation period was

2.67% (95% CI: 2.12% to 3.37%) in the seronegative group and 0.14% (95% CI: 0.04% to 0.58%) in the seropositive group. The odds ratio of being confirmed as a symptomatic SARS-CoV-2 infection in IgG positive relative to IgG negative participants was 0.054 (95% CI: 0.009 to 0.169), adjusted for age and geographical municipality.

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.[30] 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 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). However, these declining rates may be suggestive of persistent shedding of viral RNA early in the convalescent period, rather than true reinfections. 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%).

Spain

In the study by Masia et al.,[41] 146 patients admitted to hospital due to COVID-19 were followed-up at 1, 2 and 6 months for evidence of reinfection. Suspected reinfection cases, based on a minimum interval of 90 days between positive RT-PCR tests, were confirmed using whole genome sequencing.

There were five suspected reinfection cases in total. Median time between infection events was 183 days (range: 167–204). Age ranged from 44 to 73 years. Two patients were symptomatic and readmitted on suspected reinfection, and three

patients remained asymptomatic. One patient had a Ct<33, in the other four patients the Cts ranged from 33 to 38.

Genomic sequencing was performed in four individuals with available paired samples. In the three patients with Ct≥33, all were asymptomatic and the same clade 20B was detected. In two of these cases, the clade showed the same hallmark single nucleotide variants. In the third patient, the follow-up sample showed two new mutations, a K374R substitution in the N gene and an A222V substitution in the S gene, probably reflecting adaptive viral changes associated to persistent infection.

Genomic sequencing of the symptomatic patient with a Ct of 18 showed phylogenetically distinct genomic sequences; the first sample was member of the clade 20A, and the most recent sample was member of the clade 20B. Assuming that this is the only confirmed case of reinfection, the reinfection rate was 0.068% (1/146) in this cohort.

In terms of antibody levels, the three patients with asymptomatic recurrence and the symptomatic patient with no sequencing data available showed detectable antibody levels at the time of RT-PCR testing. The patient with symptomatic reinfection had no detectable antibody levels at the time of RT-PCR testing.

Switzerland

In the study by Leidi et al., a seroprevalence survey was conducted based on a representative sample of individuals aged 12 years and older in the canton of Geneva between April and June 2020, immediately after the first pandemic wave.[45] Individuals who developed anti-spike IgG antibodies were matched one-to-two to seronegative controls, using a propensity-score including age, gender, immunodeficiency, body mass index, smoking status and education level.

Among 8,344 seroprevalence survey participants, 498 seropositive individuals were selected and matched with 996 seronegative controls. After a mean follow-up of 35.6 (standard deviation [SD]: 3.2) weeks, 7 out of 498 (1.4%) seropositive participants had a positive SARS-CoV-2 test, of which 5 (1.0%) were classified as likely and two as unlikely reinfections (three adjudicators assessed the likelihood of reinfection based on timing, clinical characteristics and Ct values). This corresponded to an incidence of 0.3 (95% CI 0.1 to 0.7) per 1,000 person-weeks. By contrast, the rate of confirmed SARS-CoV-2 infections was significantly higher in seronegative individuals (15.5%, 154/996) corresponding to an incidence rate of 4.8 (95% CI 4.6 to 6.2) per 1,000 person-weeks, during a similar mean follow-up of 34.7 (SD 3.2) weeks.

Over the study follow-up, seropositive individuals were 94% less likely to have a virologically confirmed SARS-CoV-2 infection, when compared to individuals with no detectable anti-SARS-CoV-2 antibodies at study inclusion (hazard ratio of 0.06, 95% CI 0.02 to 0.14, p<0.001).

UK

In the study by Breathnach et al.,[43] reinfection rates recorded at one London laboratory are reported. This laboratory serves four hospitals and a population of 1.3 million. Individuals who had PCR- or antibody-confirmed SARS-CoV-2 infection during the first wave in the UK (February to July 2020, with a peak in early April) were identified, and their risk of having a positive SARS-CoV-2 RT-PCR assay in the first five months of the second wave (August to December 2020) was determined. These rates were compared with patients who had a previous negative PCR or antibody test. Cases where the second positive result was ≤90 days after the first were excluded. The samples included a significant proportion from healthcare workers, who were offered testing for SARS-CoV-2 antibodies in June 2020.

In total, 66,001 patients had a PCR and or serological SARS-CoV-2 assay before the end of July, of whom 10,727 tested positive (PCR and or antibody positive). Of these, eight had a positive PCR assay between 1 August and 30 December 2020, resulting in an absolute reinfection rate of 0.07%. Of 55,274 patients with no laboratory evidence of COVID-19 in the first wave, 713 subsequently had SARS-CoV-2 detected in the second wave (1.29%). The relative risk of SARS-CoV-2 reinfection was reported as 0.06 (95% CI: 0.03 to 0.12). The risk or relative risk over time was not reported.

It is notable that there were no reinfections in this dataset in the first seven months after the peak of the first wave; all eight patients with likely reinfections were diagnosed in December, the last month of the study period, which also coincided with the identification and widespread transmission of variant B.1.1.7 in the UK. That month, reinfections accounted for 1.69% of all infections.

USA

Three 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.[33]). 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. These findings likely indicate persistent viral RNA shedding from the primary infection in the early stages post-infection. While detection of viral RNA at >90 days may reflect prolonged viral shedding, these may constitute reinfection cases.

In the second study (Sheehan et al.[37]), 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.

In the third study by Qureshi et al.,[44] 9,119 patients with SARS-CoV-2 infection who received serial tests across 62 healthcare facilities in the US were followed between 1 December 2019 and 13 November 2020 for evidence of reinfection. Reinfection was defined as two positive RT-PCR tests separated by an interval of ≥90 days after resolution of first infection (confirmed by two or more consecutive negative RT-PCR tests).

Reinfection was identified in 63 patients (0.7%, 95% CI: 0.5%-0.9%). The mean interval between infections was 116 days (SD: 21). There were two deaths (3%) associated with reinfection. Intubation/mechanical ventilation was required in two patients (3%) during primary infection, but in none during reinfection. There was a significantly lower rate of pneumonia, heart failure, and acute kidney injury observed with reinfection compared with primary infection among the 63 patients with reinfection.

Health care workers

Five studies were identified that exclusively enrolled healthcare workers, including four conducted in the UK[32, 42, 48, 51] and one in the US.[47] Additionally, a further two studies were identified that enrolled both staff and residents of care homes for older people (see next section).

The study by Hall et al.[51] reports interim results after seven months of follow-up from Public Health England's 'SIREN' study. In total, 30,625 hospital staff (including healthcare workers, support staff and administrative staff of NHS hospitals across the UK) were enrolled into the study from 18 June 2020 to 31 December 2020, of which 25,661 participants with linked data on antibody and PCR testing were included in the analysis. Data were extracted from all sources on 5 February 2021, and included data up to 11 January 2021. These results update previously published interim results,[31] which related to 20,787 hospital staff, followed between 18 June and 9 November 2020.

Overall, 8,278 participants were assigned to the PCR/antibody-positive cohort and 17,383 to the negative cohort. Of the 8,278 participants in the positive cohort,

91.2% were antibody positive at enrolment, 7.0% were antibody negative at enrolment, but had a previous antibody positive result or positive PCR result and 1.8% had a previous PCR positive result, but no linked antibody data. The total follow-up time up to 11 January 2021 was 2,047,113 person-days for the positive cohort and 2,971,436 person-days for the negative cohort. The median length of follow-up per participant was 275 days, IQR 218–291 (9.2 months, IQR 7.3-9.7) for the positive cohort and 195 days, IQR 131–214 (6.5 months, IQR 4.4-7.1) for the negative cohort.

A median of eight post-enrolment PCR tests (IQR 6–11) and five post-enrolment antibody tests (IQR 3–7) were done. The PCR test density during follow-up was 64 per 1,000 days of participant follow-up in the positive cohort and 70 per 1,000 days of participant follow-up in the negative cohort. During the follow-up period (between 8 December 2020 and 11 January 2021), 13,401 (52.2%) participants were vaccinated, 9,468 in the negative cohort and 3,933 in the positive cohort. Vaccine roll-out accelerated in January 2021. The number of participants who contributed follow-up time to this analysis who had been vaccinated for 21 days or more (the period at which a protective effect from vaccination would be expected) was 833 from the positive cohort, contributing 4,941 days of follow-up, and 2,279 from the negative cohort, contributing 12,839 days of follow-up. In total, 0.4% of the study's person-time of follow-up included participants 21 days or more following vaccination.

PCR positivity for primary infections in the positive cohort peaked in the first week of April, in the negative cohort PCR positivity peaked in the last week of December 2020. By 11 January 2021, 1,859 new infections were detected in the study population: 1,704 primary infections in the negative cohort and 155 reinfections in the positive cohort. Of the primary infections, 1,369 (80.3%) of these cases were symptomatic at infection, 1,126 (66.1%) with typical COVID-19 symptoms, and 243 (14.3%) with other symptoms; 293 (17.2%) were asymptomatic; and 42 (2.5%) did not complete a questionnaire at the time of their symptoms. There were 864 seroconversions in participants without a positive PCR test; these were not included as primary infections in this interim analysis.

There were 155 reinfections identified in the positive cohort, two of which were categorised as probable and 153 as possible. A probable case additionally required "supportive quantitative serological data or supportive viral genomic data from samples available". Of these 155 cases, 78 (50.3%) were symptomatic, 50 (32.3%) with typical COVID-19 symptoms, including both probable cases. At baseline antibody testing, 127 of the reinfection cases were antibody positive, 18 were antibody negative, but had a previous antibody positive or positive PCR test result, seven had no history of an antibody positive result, but had a previous positive PCR result. There were also three participants who were antibody negative at baseline

but due to having had both a primary infection and reinfection during follow-up moved cohort.

The median interval between the primary infection and reinfection episode for the 47 cases with a positive PCR test from their primary episode was 201 days (range 95–297). For the 99 cases who provided a history of COVID-19 symptoms, used as a proxy to estimate the date of their primary infection, the median interval between primary infection and reinfection was 241 days (range 90–345).

The incidence of COVID-19 symptomatic infections was 64.8 cases per 1,000 participants; other symptomatic infections was 14.0 cases per 1,000; asymptomatic cases was 16.9 cases per 1,000, and all new PCR positive infections was 98.0 cases per 1,000 in the negative cohort. The incidence density between June 2020 and January 2021 was 7.6 reinfections per 100,000 person-days of follow-up in the positive cohort and 57.3 new PCR positive infections per 100,000 person-days of follow-up in the negative cohort.

A proportional hazards frailty model using a Poisson distribution was used to estimate incidence rate ratios (IRRs) to compare the incidence rates in the positive and negative cohorts to provide a relative estimate of the protective effect of a previous SARS-CoV-2 infection. The fixed covariates included in the model were age, gender, ethnicity, region, staff group, and index of multiple deprivation. Time varying covariates included in the model were 21 days after COVID-19 vaccination and regional prevalence of the B.1.1.7 variant.

Restricting reinfections to probable reinfections only, the adjusted IRR (aIRR) was 0.002 (95% CI 0.00–0.01), after controlling for other risk factors and for a given site. Therefore, participants in the positive cohort had 99.8% lower risk of new infection than did participants in the negative cohort. Restricting infections to those who had COVID-19 symptoms on reinfection, the aIRR was 0.074 (95% CI 0.06–0.10) (93% lower incidence of new infection than did participants in the negative cohort). Using the broadest definition of reinfections, including all those who were possible or probable, the aIRR was 0.159 (95% CI 0.13–0.19). Although the results showed that previous infection offered protection against all five categories of reinfection, the lowest protection was provided against asymptomatic reinfection (aIRR 0.48 95% CI 0.37–0.63).

Study authors did not find any evidence that increased prevalence of the B.1.1.7 variant adversely affected reinfection rates in the cohort during this follow-up period. Models suggested that the protective effect of previous infection increased when the B.1.1.7 variant was dominant (IRR 0.18, 95% CI 0.15–0.23) compared with IRR 0.13 (0.10–0.17).

In the study by Hanrath et al.,[32] 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 study by Lumley et al., reinfection rates among health care workers were reported according to vaccination status and in relation to the B.1.1.7 variant.[48]

This study updates the 2020 study by the same authors[49] and presents data up to 28 February 2021. In this longitudinal cohort study in Oxfordshire, UK, protection from symptomatic and asymptomatic PCR-confirmed SARS-CoV-2 infection conferred by vaccination (Pfizer-BioNTech BNT162b2 or Oxford-AstraZeneca ChAdOx1 nCOV-19) and prior infection (determined using anti-spike antibody status), was assessed using Poisson regression adjusted for age, sex, temporal changes in incidence and role. Staff members were classified into five groups: a) unvaccinated and consistently seronegative during follow-up; b) unvaccinated and ever seropositive; c) one vaccine dose, always seronegative prior to vaccination; d) two vaccine doses, always seronegative prior to first vaccine dose; e) vaccinated (one or two doses) and ever seropositive prior to first vaccination. Vaccinated groups were considered at-risk of infection >14 days after each vaccine dose. The staff vaccination programme began on 8 December 2020, starting with the Pfizer-BioNTech BNT162b2 vaccine, with the addition of the Oxford-AstraZeneca ChAdOx1 nCoV-19 vaccine from 4 January 2021. Some staff members received the ChAdOx1 nCoV-19 vaccine in clinical trials beginning 23 April 2020 and were included following unblinding.

In total, 13,109 individuals participated; 8,285 received the Pfizer-BioNTech vaccine (1,407 two doses) and 2,738 the Oxford-AstraZeneca vaccine (49 received two doses). Compared to unvaccinated seronegative workers, natural immunity (that is, seropositivity due to prior infection) provided similar protection to two vaccine doses against symptomatic infection: no HCW with two vaccine doses had symptomatic infection, and incidence was 98% lower in seropositive HCWs (adjusted incidence rate ratio 0.02 [95%CI <0.01-0.18]). Two vaccine doses or seropositivity reduced the incidence of any PCR-positive result with or without symptoms by 90% (0.10 [0.02-0.38]) and 85% (0.15 [0.08-0.26]), respectively. Single-dose vaccination reduced the incidence of symptomatic infection by 67% (0.33 [0.21-0.52]) and any PCR-positive result by 64% (0.36 [0.26-0.50]).

Viral whole genome sequencing was undertaken to determine infecting lineages from 1 December 2020 onwards. Of these, 343/463 (74%) were successfully sequenced; 193/343 (56%) were B.1.1.7, and an additional 19/463 (4%) were not sequenced, but S-gene positive (i.e., unlikely to be B.1.1.7). There was no evidence that B.1.1.7 changed the extent of protection from any-PCR positive infection in those who were seropositive (aIRR vs non-B.1.1.7=0.40 [95%CI 0.10-1.64; p=0.20]) or following a first vaccine dose (aIRR=1.84 [0.75-4.49; p=0.18). Additionally, 17% of S-gene target failure (SGTF) was due to a lineage other than B.1.1.7. No other variants of concern (B.1.1.7 with E484K, B.1.351 or P.1) were identified in participants, in an at-risk period. There was no evidence of differences in immunity induced by natural infection and vaccination for infections with S-gene target failure and B.1.1.7.

Study authors concluded that natural infection resulting in detectable anti-spike antibodies and two vaccine doses both provide robust protection against SARS-CoV-2 infection, including against the B.1.1.7 variant.

In the study by Shields et al.,[42] 1,507 dental care professionals in the UK were recruited in June 2020 and followed longitudinally for six months, which included commencement of vaccination. Baseline seroprevalence of antibodies against SARS-CoV-2 spike glycoprotein was 16.3% in this cohort, compared to estimates in the general population of 6-7%. At six months, 61.4% (n=926/1,507) of the cohort returned questionnaires regarding SARS-CoV-2 infections and blood samples were retrieved from 59.2% (n=873/1,507). Overall, 77 PCR-positive SARS-CoV-2 infections were reported by study participants, representing an overall infection risk of 8.3%. The risk of infection was 9.6% in participants who were seronegative at baseline compared with 2.8% in individuals who were seropositive (p=0.001). The emergence of antibodies following natural infection was associated with a 74% risk reduction for reinfection, with an adjusted risk ratio of 0.26 (95% CI: 0.11 to 0.63, adjusted for age, sex, ethnicity and smoking).

In reference to the first WHO standard for SARS-CoV-2 immunoglobulin (NIBSC 20/136), study authors estimated that the minimum level of anti-SARS-CoV-2 spike glycoprotein IgG antibodies necessary to confer six months protection from infection was 147.6 IU/ml. Using the NIBSC standard 20/162 generated a similar estimate of 195.2 U/ml.

It is notable that this study coincided with vaccine roll-out. However, as the seropositive cohort was based on samples from June 2020, the relative reinfection rates relate to the effectiveness of natural immunity to prevent reinfection. Vaccine effectiveness rates were not reported. However, the serological responses of individuals receiving a single dose of the Pfizer-BioNTech SARS-CoV-2 were analysed based on prior exposure to the virus, defined by either positive baseline serology, or PCR-confirmed infection during the follow up period. Vaccination on the background of prior exposure to the virus was associated with a more rapid and quantitatively greater total antibody response against the SARS-CoV-2 spike glycoprotein, consistent with the boosting of immunological memory.

In the study by Papasavas et al.,[47] a longitudinal evaluation of the seroprevalence and epidemiology of SARS-CoV-2 specific antibodies on US health care workers was performed, which included RT-PCR testing at follow-up, over a period of approximately six months. The baseline prevalence of SARS-CoV-2 antibody among 6,863 HCWs was 6.3%. The incidence of reinfection in the seropositive group was zero: 0/35 seropositive participants who had a subsequent PCR test at least 30 days

following the positive antibody test had a positive test, compared with 1.3% (29/2,173) seronegative participants had a subsequent positive PCR test.

Residents and staff of care homes for older poeple

Two studies were identified that enrolled both residents and staff at UK care homes.[35, 36]

In the first study (Jeffery-Smith et al.[35]), the risk of reinfection according to antibody seropositivity was investigated following outbreaks in two London care homes[35, 52] 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 2.1), and fatal cases in residents had identical viral genomes to surviving residents. Ct values were not reported.

In the second study by Krutikov et al.[36], staff and residents in 100 long term care facilities (LTCFs) in England were followed between October 2020 and February 2021. 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 tool was used for appraisal of observational cohort studies.[53] Seventeen studies were considered of 'good' or 'fair' methodological quality (Appendix 3.1), Four studies were deemed of 'good' methodological quality,[31, 36, 39, 48] 11 studies were deemed 'fair' and two studies[33, 40] were considered of poor methodological quality.

In the first study of poor methodological quality, currently published as a preprint, details of the testing methodology employed was not provided by study authors.[40] In the second study of poor methodological quality, a proxy measure for outcomes (NAAT positivity) was used.[54] 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).

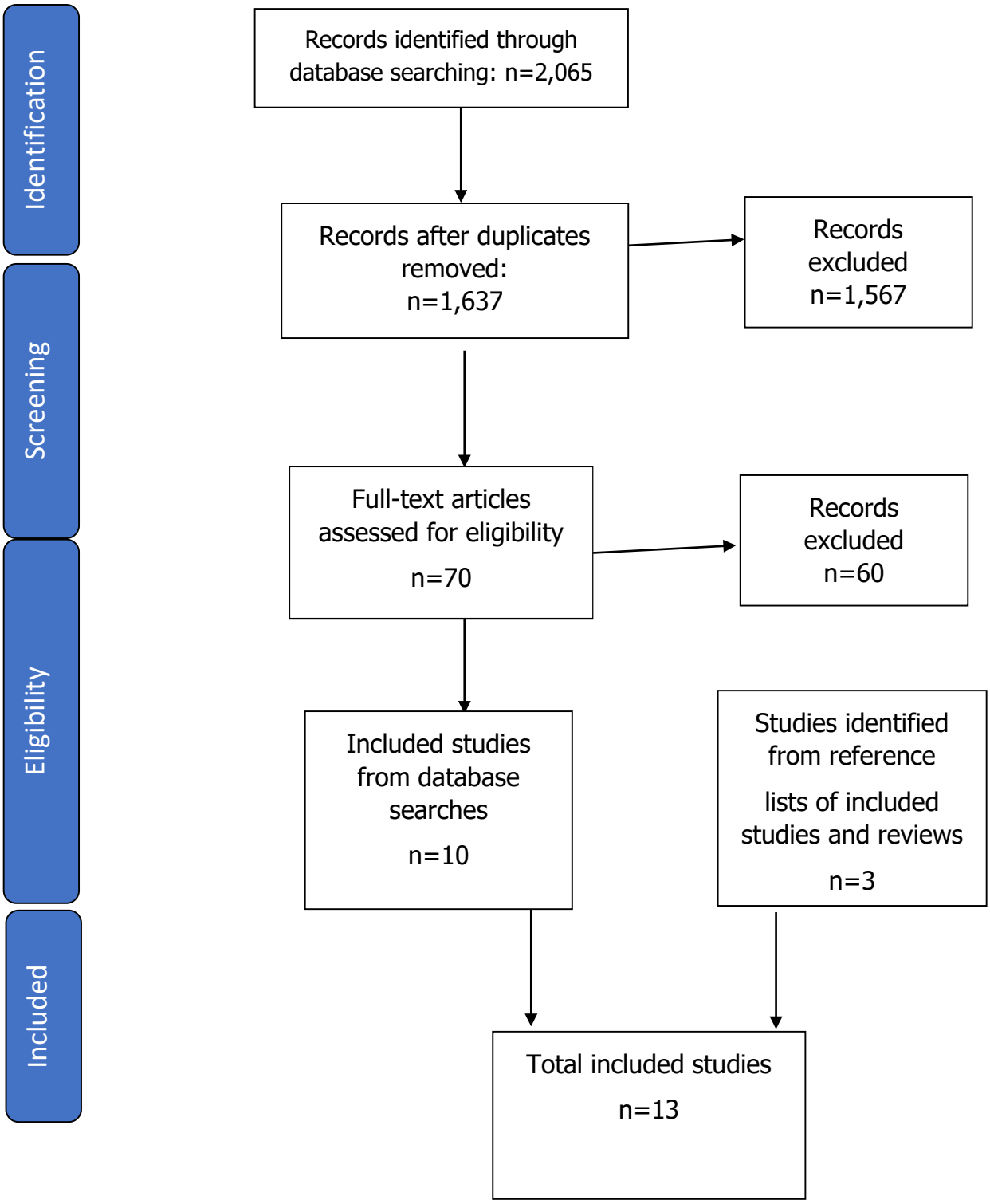
The studies deemed of 'fair' methodological quality were downgraded for a number of reasons, the most common reason being a lack of controlling for confounders (seven studies did not adequately control for confounding, and controlling for confounding was unclear in a further eight studies). In these studies, potential confounding variables were either not assessed or not measured appropriately, or the statistical analysis was not adequately described (Appendix 3.1). Additionally, 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 (11 of 19) were retrospective in nature.

Ten studies are currently published as preprints,[34, 36, 37, 40, 42, 44-46, 48, 50] 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.

Results (Part 2 – immune memory)

The collective database search resulted in 2,065 citations. Following removal of duplicates, 1,637 citations were screened for relevance. This resulted in 70 studies being eligible for full text review (Figure 3), where a further 60 studies were excluded (Appendix 1.2), leaving ten studies for inclusion. Three additional studies were retrieved from the reference lists of included studies and published reviews of the topic.

Figure 3 PRISMA flow diagram – immune memory review



Thirteen studies were identified that met the inclusion criteria. Four of these were conducted in the US,[55-58] two in China,[15, 59] two in Australia,[4, 60] and one each in Sweden,[61] Canada,[62] South Korea,[63] France[64] and Sweden/India.[65] Two of these studies are currently published only as preprints. [56, 61]

In twelve of the included studies, participants (or samples thereof) had infection confirmed by PCR; two of which[55, 57] included some close contacts of PCR-confirmed cases, all of whom had seroconverted. In Dan et al.[11], however, only 77% of participants were PCR-confirmed cases. Infection was confirmed by nucleic acid amplification test in one study.[4]

Six studies examined memory B-cell response only,[4, 57, 60, 62, 64, 65] three examined T-cell responses only,[15, 55, 63] and four examined both B- and T-cell responses.[56, 58, 59, 61] No studies were identified that considered upper respiratory tract mucosal immune memory.

Nine studies used blood samples from healthy donors as controls,[4, 55, 56, 59, 62-66] whereas four analysed only those who had been infected with SARS-CoV-2.[15, 57, 58, 61]

Memory B-cell responses

The studies included in this review varied in terms of length of follow-up. All studies reported on participants or blood samples that were greater than six months post-infection; the maximum follow-up was nine months. In general, studies reported that the frequency of memory B-cells either increased (up to six months,[4, 57] or was maintained up to the end of the study period (six months,[64] eight months;[62] or nine months [65]). Dan et al.[11] noted that the frequency of memory B-cells increased to 110 days and then plateaued. (See Table 4)

Hartley et al.[60] reported that the frequency of receptor-binding-domain (RBD)- and nucleocapsid (NCP)-specific memory B-cells rose up to 150 days post infection, plateauing thereafter. Cohen reported that the frequency of RBD- and NCP-specific memory B-cells rose to 150 days, with a decline in RBD-specific memory B-cells thereafter while NCP-specific memory B-cells were maintained for the duration of the study follow-up.[56] Similarly, Hartley et al.[60] found that total and IgM+-specific memory B-cell frequencies were lower after 200 days than in the earlier periods (21 to 106 days), whereas IgG+ memory B-cell frequencies remained stable.

Sandberg et al.[61] reported that while S-specific IgG+ was detectable in all patients at both time points in their study (5 months and 9 months) and N-specific IgG+ was detectable in all but one patient at the later time point, few patients had detectable S-specific or N-specific IgA+ memory antibody-secreting-cells at nine months.

Similarly, Hartley, Edwards (60) found that while total and IgM+ memory B-cell frequency tested at >200 days were lower than in the first sample (21 to 106 days), IgG+ memory B-cell frequency remained high. Sandberg et al.[61] noted that there was a high variation in frequencies of S1- or N-specific cells among patients. The decline in IgA and IgM levels is typical of viral infection.

Abayasingam et al.[4] found that RBD-specific memory B-cells generated monoclonal antibodies with SARS-CoV-2 neutralising capacity, while Gaebler et al.[57] found that over a six month period memory B-cells expressed antibodies with increasing neutralising potency and breadth.

Memory T-cell responses

Cohen et al.[56] who followed patients for the longer period of eight months found that frequency of SARS-CoV-2 T-cells peaked within the first month and then declined slowly over the next six to seven months. Similarly, Kang et al.[63] found that while frequencies of SARS-CoV-2-specific memory CD4+ and CD8+ T-cells were higher in patients than controls, the frequency of these cells tended to decline over the eight-month study period.

Dan et al.[11] reported that while 70% of patients had detectable SARS-CoV-2-specific CD8+ T cells at one month this declined to 50% of patients at ≥six month follow-up. In contrast, 93% and 92% of patients had detectable SARS-CoV-2-specific memory CD4+ T-cells at one and ≥six months, respectively. Tan et al.[15] reported that SARS-CoV-2–specific memory T-cells persisted for up to seven months and Sandberg et al.[61] found that they persisted up to nine months.

Immune memory by severity, age and sex

A US study (n=43) with eight month follow-up reported differences in immune response in previously hospitalised (n=13) compared with never hospitalised cases (n=30). Compared with non-hospitalised cases, the hospitalised cases had higher frequency of spike and RBD-specific memory B-cells, lower frequency of memory CD4+ T-cell titres, and comparable frequency of memory CD8+ T-cell. However, the authors noted that the conclusions are limited by the small number of hospitalised cases. Abayasingam et al.[4] reported the CD27+RBD+IgD-IgG+ memory B-cell response in 15 post-infection patients compared with six healthy controls. At 110 and 181 days post-symptom onset, three out of the fifteen patients did not have a response greater than controls. Two of these were from the low end point titre group, and had experienced mild or moderate clinical disease; the third was from the high end point titre group and had experienced severe disease.

In a sample of 24 participants, Kang et al.[63] found that while memory CD4+ T-cell frequencies tended to be higher at two months in patients who had experienced severe illness compared with those with mild or asymptomatic disease, the difference between the groups decreased over time with frequency declining in all patients.

Long et al.[59] compared individuals six months post recovery from either asymptomatic infection or from symptomatic infection. A greater proportion of individuals who previously had asymptomatic disease tested positively in memory B-cell functional tests compared with those who had had asymptomatic infections (50% vs. 15%)No difference was observed in memory CD4+ or CD8+ T-cell frequencies.

Sandberg et al.[61] found no difference in the magnitude of the B- and T-cell response between patients who experienced moderate versus severe disease at five (n=17) or nine months (n=13) follow-up. Sherina et al.[65] found that SARS-CoV-2-specific memory B-Cells were detected and remained detectable in almost all patients (n=32) followed-up for at least six to eight months, regardless of disease severity.

Therefore, of five studies that reported on memory immune response by disease severity, three reported no difference in either the CD4+ or CD8+ T-cell response.[59, 61, 65] Two studies reported no difference in the CD8+ T-cell response, but reported an increase in CD4+ T-cell frequency with greater disease severity;[58, 63] one study of these reported that the difference in CD4+ T-cell frequency declined over time. Two studies reported that memory B-Cells frequency or their functionality was increased in patients who experienced a more severe disease,[58, 59] although this finding was inconsistent between studies.[4]

Cohen et al.[56] reported that increased age positively correlated with increased frequencies of spike (n=99) and RBD-specific (n=135) IgG+memory B-cells, with 1.19 to 1.24-fold higher responses per decade of age (controlling for disease severity).

Dan et al.[11] reported on immune memory by sex. No difference was found in SARS-CoV-2 memory B-cells, SARS-CoV-2 memory CD8+ T-cell or CD4+ T-cell frequencies between males and females at ≥6 months follow-up.

Quality of included studies

The Joanna Briggs Institute checklist was used to appraise the quality of included studies (See Appendix 3.2). However, the applicability of this tool was limited as most of the studies concerned blood samples of cohorts of convalescent participants.

The included studies were laboratory-based and of experimental design. Appropriate experimental design and the inclusion of appropriate controls are fundamental to a high quality study.

The main limitation of the individual studies identified is that it is unclear whether the blood samples used in the included studies are representative of the general population. Many studies examined immune memory in a small subset of patients and controls, and most did not describe whether this subset was representative of the larger cohort. Related to this issue is the lack of information on potential confounders, which could potentially bias the findings. In addition, the small study numbers (ranging from 15 to 188 participants) does not allow identification of patient characteristics that would predict maintenance of immune memory.

Finally, the included studies are all based on lymphocytes derived from blood samples, which is usually the only accessible sample type. However, only 1-2% of total body lymphocytes are present in peripheral blood, with most lymphocytes distributed in secondary lymphoid organs and other tissues. While finding evidence of immunological memory in lymphocytes from the blood is of interest, it may not be representative of tissue-resident memory cells. Absence of evidence of immunity in blood samples does not preclude tissue resident memory cells which may persist and contribute to protection from infection.

Table 4. Immune memory response: table of study characteristics and primary outcome data*

Author (Country)	Components analyses	Samples analysed	Max. follow-up	Results relevant to this review*	Authors' conclusions	Published
Abaysingham (Australia)	Memory B-cells and their neutralising capacity	n=15 post-infection patients ; n=6 healthy controls at TP1 n=5 post-infection patients at TP2 181 days (median 132 days) post infection	~6 months	12/15 post-infection patients had detectable RBD-specific memory B-cells and these generally are increasing out to 6 months. MABs with SARS-CoV-2 neutralising capacity were generated from these memory B-cells.	Our study suggests that the loss of NAs in plasma may be countered by the maintenance of neutralising capacity in the memory B-cell repertoire.	Yes
Anand (Canada)	Memory B-cells	Post-infection patients n=32 TP1; n=28 TP2; n=28 TP3; n=13 TP4 TP1= 16-95 days (median 43 days); TP2= 48-127 days (median 77 days;TP3= 116-171 days (median 145 days); TP4=201-233 days (median 218 days) post infection	~8 months	Total RBD-specific memory B-cells were detected in 100% of post-infection patients and the mean frequency remained stable between 6 and 31 weeks post-symptom onset.	(w)e show that COVID-19 patients generate RBD-specific memory B-cells that persist for over 8 months … the decline of AB levels does not negate the protective potential.	Yes
Breton (USA)	Circulating memory T-cells phenotypes	n=41 post-infection patients and close contacts; n=20 healthy controls TP1 = 1.3 months; TP2= 6.1 months post infection	~6.1 months	There are significant shifts in circulating CD4+ and CD8+ memory T-cell compartments that persist for 6 months after SARS-CoV-2 infection. (The relative distribution of all the clusters** remained abnormal at 6.1 months.) Recovered individuals show persistent polyfunctional SARS-CoV-2 antigen-specific memory that could contribute to rapid recall responses T-cell central memory decreased and this defect persisted throughout the observation period.	The data indicate that recovered individuals show persistent polyfunctional SARS-CoV-2 antigen-specific memory that could contribute to rapid recall responses.	Yes
Cohen (USA)	Memory B- and T-cells	n=111 post-infection patients; n=29 healthy controls TP1= 30 days; TP2=3 months; TP3=6 months; TP4 = 9 months	8 months	The spike IgG+ MBCs were significantly increased in post-infection patients compared with healthy controls. After a steep early expansion over the first 2-3 months, the spike IgG+ MBCs persisted in post-infection patients with no decline out to 250 days post-symptom onset.	This in-depth longitudinal study demonstrates that durable immune memory persists in most COVID-19 patients, including those with mild disease.	Preprint

				The spike IgM+ MBC appeared within the first two weeks post-symptom onset and quickly declined. 89% (102/113) of post-infection patients mounted CD4+ T-cells response and 69% of post-infection patients generated CD8+ T-cells in contrast to infrequent to rare responses in the healthy controls SARS-CoV-2 specific T-cells peak early, within the first month, and then slowly decline over the next 6-7 months.		
Dan (USA)	Memory B- and T-cells	n=43 sampled at 6-8 months TP1=36 to 136 days; TP2 =11-249 days post infection	~8months	Frequencies of SARS-CoV-2-specific MBC increased over the first 110 days and then plateaued. 70% of post-infection patients had detectable SARS-CoV-2 memory CD8+T-cells at 1 month post-symptom onset and 50% at ≥6 months. 93% of post-infection patients had detectable SARS-CoV-2 memory CD4+T-cells at 1 month post-symptom onset and 92% at ≥6 months	Our data show immune memory in at least three immunological compartments was measurable in 95% of subjects 5 to 8 months post-symptom onset, indicating that durable immunity against secondary COVID-19 disease is a possibility in most individuals.	Yes
Gaebler (USA)	Memory B-cells Antibodies produced by memory B-cells	n=21 for MBCs; n=6 for Abs TP1=1.3 months; TP2= 6 months post infection	6 months	MBC response evolved between 1.3 and 6 months post-symptom onset and these expressed ABs with increasing neutralising potency and breadth.	We conclude that, although the magnitude of the RBD-specific memory B cell compartment is conserved between 1.3 and 6.2 months after infection with SARS-CoV-2, there is extensive clonal turnover and antibody sequence evolution that is consistent with prolonged germinal centre reactions.	Yes
Hartley (Australia)	Memory B-cells	n=11 post-infection patients with paired samples;	~8 months	RBD and NCP MBCs continued to rise to 150 days. RBD-specific MBCS were highest between 100-150 days post-symptom onset. NCP-specific MBCs did not decline between 150 and 240 days	The SARS-CoV-2 AB response contracts in convalescence with persistence of RBD and NCP-specific MBCs.	Yes

		TP1= 21-106 days; TP2 = 116-242 days post infection		Total and IgM+ MBCs taken >200 days were lower than in the corresponding first samples, whereas IgG+ MBCs remained stable.		
Kang (South Korea)	Memory T-cells	n=24 post-infection patients; n=6 healthy controls; n= 7 MERS controls*** TP1= 2months; TP2= 5 months;TP3= 8 months post infection	8 months	SARS-CoV-2-specific memory CD4+ and CD8+T-cells were higher in post-infection patients than healthy controls at 8 months post-symptom onset. SARS-CoV-2-specific CD4+ T cells and CD8+ T cells persisted at 8 months post-symptom onset Also, antigen-specific cytokine-producing or polyfunctional CD4+ T cells were maintained for up to 8 months post-symptom onset Memory CD4+ T-cell responses tended to be greater in patients who had severe illness than in those with mild or asymptomatic disease The frequency of SARS-CoV-2-specific memory CD4+ and CD8+ T-cells tended to decline over time	Memory response to SARS-CoV-2 based on the frequency and functionality persists for 8 months post-symptom onset. Further investigations involving its longevity and protective effect from reinfection are warranted.	Accepted manuscript
Long (China)	Memory B- and T-cells	n=20 recovered symptomatic patients; n=13 recovered asymptomatic patients; n=10 healthy controls Tested once mean TP=169 days (IQR 164-174 days) post infection	~6months	SARS-CoV-2 RBD-specific memory B cell response was significantly lower in asymptomatic versus symptomatic patients (2/13 versus 10/20) The proportion of virus-specific MBCs in recovered symptomatic patients and RAs was higher than healthy controls but no significant difference between recovered symptomatic patients and RAs The S1 memory T-cell peptide pool had higher reactivity in patients than healthy controls	NA	Yes
Sandberg (Sweden)	Memory B- and T-cells Antibodies produced by memory B-cells	n=8 moderate post-infection patients; n=5 severe post-infection patients at last TP TP1=5 months;TP2= 9 months post infection	9 months	S-specific IgG mASCS were detected in all patients at both TPs Very few post-infection patients had detectable S1 or N-specific IgA mASCS There was a high variation in frequencies of S1- or N-specific cells between post-infection patients (range 0.2% to 20%) Polyfunctional SARS-CoV-2 –specific T-cell memory persists up to 9 months	S1- and N-specific IgG MBCs are readily detectable in circulation at both 5 and 9 months post-symptom onset in all patients in this cohort, although the magnitude of these responses is highly variable.	Preprint

				Robust specific memory B cell responses and polyfunctional T cell responses at five- and nine-months after symptom onset in both moderate and severe COVID-19 patients		
Sherina (Italy and Sweden)	Memory B-cells	n=11 at last TP; n=4 HCs TP1=2-4 weeks;TP2=3-6 months; TP3=6-8 months post infection	8 months	SARS-CoV-2-specific memory B-cells and T-cells developed and remained present in 95% of post-infection patients followed-up to latest date of the study. One post-infection patient who had no detectable T-cell response at 4 months had detectable B-cell response. T-cell response was detectable in all post-infection patients analysed at 6-8 months A clear shift from the production of specific ABs at the early TP to the generation of MBCs and memory T-cells at later TPs was observed.	SARS-CoV-2-specific memory B- and T-cell responses developed with time and were persistent in all patients followed up for 6 to 8 months.	Yes
Sokal (France)	Memory B-cells	n=21 severe post-infection patients; n=18 mild post-infection patients; n= 6 healthy controls TP1=median 18.8 days (SD±, 8.8 days); TP2= 35.5 days (SD±12.8 days). Two additional samples collected a 3 months and 6 months post infection	6 months	At 6 months severe post-infection patients showed significantly higher frequencies of S-specific MBCs and most mild post-infection patients harboured a sizeable population of S-specific MBCs. Only 1 mild post-infection patient showed a frequency of S-specific CD27+ MBCs below prepandemic healthy controls. Both mild post-infection patients whose serum levels of S-specific IgG had dropped below detectable levels at 6 months still harboured a clear population of S-specific MBCs	These findings demonstrate that an antigen-driven activation persisted and matured up to 6 months after SARS-CoV-2 infection and may provide long-term protection.	Yes
Tan (China)	Memory T-cells	n=10 Tested once at 6-7 months post infection	6-7 months	IFN-ɣ CD4+ and CD8+ memory T-cells were increased upon SARS-CoV-2 antigen stimulation compared with non-stimulated samples	These observations indicate that memory T-cells for SARS-CoV-2 can persist for up to 6-7 months post-infection, in agreement with the status of humoral immunity.	Yes

A=analysis; AB=antibody; ABS=antibody-secreting; CD4+ and CD8+ are types of T-cells; IFN-ɣ= interferon gamma-producing; MAB=monoclonal antibody; mASCS= memory B-cell derived antibody-secreting cells; MBC=memory B-cell; NA=neutralising antibody; NCP=nucleocapsid; RBD=receptor binding domain; S=spike; TP=time points of tests;
*More detailed results are reported in Appendix 2.2.
** 'Clusters' refers to classification of immune cells based on which molecules are present on their surface
*** Serum samples from those who had been infected with middle-east respiratory syndrome (MERS-CoV) 5 years previously

Discussion

Part 1 – risk of reinfection

Summary of findings

This review identified nineteen observational cohort studies that assessed the risk and or relative risk of SARS-CoV-2 reinfection over time, comparing individuals with evidence of prior infection (prior SARS-CoV-2 diagnosis or antibody positivity) with those without. Five studies exclusively enrolled healthcare workers and two studies enrolled both staff and residents of care homes for older people; six of these seven studies were conducted in the UK. The remaining twelve studies were conducted in general populations in ten different countries. Across studies, the total number of PCR- or antibody-positive participants at baseline was 641,911 (median: 1,899; range: 88 to 378,606). The median follow-up of individuals within studies was 135 days (4.5 months) (range of medians: 54-249 days), with a maximum follow-up of ≥300 days (ten months) in six studies.

Reinfection was a rare event: the median PCR-confirmed reinfection rate was 0.6% across studies, ranging from 0% (zero reinfections in three studies) to 2.8% (which was observed among dental practitioners in the UK[42]).

Apart from the crude 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 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. However despite the inability to pool data, all studies consistently reported low relative rates of reinfection comparing seropositive and seronegative groups, which remained low for the duration of the studies. In addition, all studies that separately reported symptomatic and 'all' reinfection events consistently reported lower relative rates of symptomatic reinfections. For example, in one large sample of UK health care workers, the relative risk for 'any reinfection' was 0.159 (95% CI: 0.13–0.19), falling to 0.074 (95% CI: 0.06–0.10) for reinfections with COVID-19 symptoms.[51]

Impact of vaccination and new variants

While the objective of this review was to investigate immune responses following natural infection, a number of studies coincided with vaccine-roll out. The comparative effectiveness of natural versus vaccine-mediated immunity is of

considerable interest and likely to impact policy going forward. Additionally, recent studies have coinicided with widespread transmission of new variants, namely variant B.1.1.7 in the UK.

One study simultaneously assessed the protective effectiveness of natural infection in both vaccinated and unvaccinated individuals, and in the context of widespread community transmission of a new variant (B.1.1.7 in the UK).[48] Robust immune responses following both natural infection and vaccination were reported, including against variant B.1.1.7. Study authors reported that, compared to unvaccinated seronegative HCWs, natural immunity and two vaccine doses provided similar protection against symptomatic infection: no HCW who received two vaccine doses had symptomatic infection, and incidence was 98% lower in seropositive HCWs (adjusted incidence rate ratio 0.02 [95% CI: <0.01-0.18]). Two vaccine doses or seropositivity reduced the incidence of any PCR-positive result with or without symptoms by 90% (0.10, 95% CI [0.02-0.38]) and 85% (0.15, 95% CI [0.08-0.26]), respectively. One vaccine dose reduced the incidence of symptomatic infection by 67% (0.33, 95% CI [0.21-0.52]) and any PCR-positive result by 64% (0.36, 95% CI [0.26-0.50]). There was no evidence of differences in immunity induced by natural infection and vaccination for infections with S-gene target failure and B.1.1.7.

Authors of the SIREN study (Hall et al.[51]), included in this review, published updated results on 23 May 2021.[67] SIREN initially investigated the effect of previous infection on protection against reinfection and this was amended to investigate COVID-19 vaccine effectiveness in January 2021. The factors associated with both BNT162b2 and ChAdOx1 nCoV-19 vaccine coverage and early vaccine effectiveness of the BNT162b2 vaccine against all (asymptomatic and symptomatic) infections was assessed. A single dose of BNT162b2 vaccine showed vaccine effectiveness of 70% (95% CI: 55–85) 21 days after first dose and 85% (95% CI: 74–96) 7 days after two doses in the study population. Given the dominance of the B.1.1.7 variant in England during the study period, findings suggested that the BNT162b2 vaccine is effective against variant B.1.1.7.

In another study by Public Health England, published as a preprint on 24 May 2021, the effectiveness of vaccines against variant B.1.617.2 (Indian variant) was assessed.[68] This was a test negative case control design that estimated the effectiveness of vaccination against symptomatic disease over the period that B.1.617.2 began circulating, with cases identified based on sequencing and S-gene target status. After two doses of either BNT162b2 or ChAdOx1 COVID-19 vaccine, the authors reported only modest differences in vaccine effectiveness for each of the two vaccines against the B.1.617.2 variant compared with the dominant B.1.1.7 strain. Overall 2-dose vaccine effectiveness was lower for ChAdOx1 than with

BNT162b2 (66.1% vs. 93.4% and 59.8% vs 87.9% for B.1.1.7 and B.1.617.2, respectively). Pooled estimates highlight that effectiveness was notably lower after one dose of either vaccine for the B.1.617.2 variant (33.5%; 95% CI: 20.6 to 44.3) compared with the B.1.1.7 variant (51.1%; 95% CI: 47.3 to 54.7), with similar results for both vaccines.

Sequencing-confirmed reinfection rates

While a number of studies undertook whole genome sequencing on a small sample of individuals, only one study estimated the population-level risk through genomic sequencing of a representative sample.[50] This sample represented 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%]). Importantly, the incidence rate of reinfection by month did not show any evidence of waning 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.

Infection by age group

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.[39] 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),[35] 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.[36] Only one study reported data specific to the paediatric group.[34] 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.

Reinfection risk by serological antibody levels

One study directly assessed the relationship between serological antibody levels and reinfection risk. In this study, conducted among UK dental practitioners, the risk of infection was 9.6% in participants who were seronegative at baseline, compared to 2.8% in individuals who were seropositive (p=0.001). However, there were no PCR-proven infections among 64 individuals with a baseline anti-SARS-CoV-2 IgG level greater than 147.6 IU/ml (with respect to the WHO international standard NIBSC 20/136). Further research is needed on this subject, and while serological levels that are protective against PCR-confirmed infection may be found, the serological response that prevents transmission is unknown.

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.

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.

Included studies could not determine whether past seroconversion, or current antibody levels, determine protection from infection, although one study did consider the IgG level at which no reinfections occurred.[42] 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.

Only four studies undertook genomic sequencing of reinfected cases.[30, 35, 41, 48] The effect of not undertaking genomic sequencing, however, is to overestimate the number of reinfections, thereby affirming the conclusion that reinfection is rare. 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.

One study determined reinfection cases by either RT-PCR or rapid antigen test, despite antigen testing not being considered the optimal testing methodology.[46] In this study, authors report a sensitivity of >90% and specificity >97% for their rapid antigen test. The results of this study, however, were consistent with other studies that exclusively used RT-PCR to diagnose reinfections (aOR of 0.05 [95% CI: 0.01 to 0.17] comparing seropositive and seronegative groups). Another study also used antigen testing in a proportion of cases,[51] however these were subsequently confirmed with RT-PCR.

A number of studies employed definitions of reinfection that may have identified a significant number of cases of prolonged shedding of dead viral remnants following the primary infection rather than true reinfection cases. For example, one study used a proxy measure for reinfection (NAAT positivity).[54] Additionally, a number of other studies used time intervals between infection events that are unlikely to rule out persistent shedding, the shortest interval being 45 days in one study.[50] Studies that required additional supporting evidence, such as additional epidemiological or laboratory evidence (Ct values, serological status) were more likely to rule out persistent shedding. Only studies that employed whole genome sequencing could provide confirmation of true reinfection events.

Outcome ascertainment bias may have been an issue in a number of studies, as antibody test results, or knowledge of prior PCR-positive infection, may have affected individual behaviour. For instance, 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. 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. In addition, studies with low uptake rates or high attrition may have introduced selection bias.

A final limitation is that only four studies were considered of 'good' methodological quality,[31, 36, 39, 48] and over half (n=10) of studies are currently published as preprints.[34, 36, 37, 40, 42, 44-46, 48, 50]

Research in context

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.

In addition, O'Donnell et al.[69] have recently reported the case of a HCW in Ireland who presented with mild symptoms of COVID-19 seven months after primary infection. Both episodes were confirmed by RT-PCR and WGS, which established the latter infection as phylogenetically distinct from the first.

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).[70] 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.

Part 2 – immune memory

Reports of declining IgG and neutralising antibody titres to SARS-CoV-2 in the convalescent period have raised concerns about susceptibility to reinfection.[7] However, this picture is consistent with typical post-viral infection whereby a decline in antibody levels after the acute phase of an infection is observed as most of the circulating antibody secreting cells induced during the first weeks after infection are short-lived. Therefore, studying both primary and memory immune responses to SARS-CoV-2 in an integrated manner is important to understand the durability of protective immunity.[10] Indeed, it may be the case that immune memory responses are more important than initial IgG responses.[71]

Previous studies have demonstrated the presence of SARS-CoV-2-specific memory B-cells for at least 30 to 90 days,[72-75] and memory T-cells around 90 days post-infection.[74] However, data from at least six months post-infection,[10, 13] if not several years, are needed to define the duration of immune memory to SARS-CoV-2. In addition, the included studies are based on serum samples, which may not be representative of tissue resident memory cells, and differences should be interpreted with caution. When evidence of immunity in blood samples is absent, protection from infection may still be present, as tissue resident memory cells may persist.

Summary of findings

The studies included in this review reported long-lasting memory B- and T-cell responses to SARS-CoV-2 over a six to nine month period, the maintenance of which

will inform future health policy. However, primary and memory immunological responses are only part of the full picture of immunity to SARS-CoV-2.

The second issue may be the comparative development of mucosal immunity. Previous vaccines for other coronaviruses induced high systemic levels of serum neutralising antibodies and conferred protection against disease. However, they had lower efficacy against mucosal coronavirus infections and did not prevent viral shedding in vaccinated people. A high level of seroprevalence of these endemic human coronaviruses is dynamically maintained by intermittent reinfection and this affords protection from severe infection in the vulnerable.[76] Similarly, it has been argued that post-pandemic population immunity will depend on the endemic presence of SARS-CoV-2 in conjunction with vaccination.[76]

As SARS-CoV-2 is an infection that firstly involves the upper respiratory tract, the immune memory that develops in this area is important to understand, particularly when considering the impact of immunity from infection or from vaccination on onward transmission.[77, 78] While systemic and mucosal antibodies have been identified in patients diagnosed with COVID-19 for at least three months post infection,[79] little is known about the duration of mucosal immunity or development of mucosal immune memory. 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, which in conjunction with epidemiological studies will further inform current understanding of onward transmission of disease.[77] However, as noted, this review did not identify any studies that reported on mucosal immune memory.

Many unexposed individuals have pre-existing immune responses that react with SARS-CoV-2 antigens (cross-reacting responses), probably based on exposure to endemic (common cold) coronaviruses.[12, 23-26]

There has been much debate on how effective immunity from SARS-CoV-2 infection or vaccination is at preventing infection in the context of variants of interest or variants of concern. Fortunately, a very broad array of epitopes are recognised in humans with COVID-19[13, 28] consisting of T-cell responses of up to >10 epitopes distributed throughout the SARS-CoV-2 genome. This may indicate that SARS-CoV-2 is a relatively easy target and that it elicits a diverse array of antibodies in each person. Given this, it has been suggested that it is unlikely that the SARS-COV-2 escape variants will emerge that avoid the majority of humoral and cellular immune memory in COVID-19 cases.[9] As vaccine-induced immunity is based only on spike protein, this broad immune response may not be applicable. However, robust immune responses following both natural infection and vaccination against variant

B.1.1.7 as described by Lumley et al.[48] in section 1 of this review further supports this suggestion.

Limitations

Although preprints were not excluded from the review, databases that include preprint studies were not searched for the immune memory section of this review. Thus, there may be additional relevant preprint papers that have not been included.

Some of the studies included in this review also reported outcomes such as the comparison of primary immune response with memory immune response and the cross-protection afforded from immune memory developed by exposure to viruses other than SARS-CoV-2. However these outcomes are not reported in this review. No included studies compared immune memory responses in vaccinated people with immune memory responses in those with natural immunity.

Importantly, the included studies were based on analyses of blood samples and cannot verify the presence of immune memory resident in tissues, which form the larger part of immune memory. Therefore, the absence of immune memory in the blood may not be indicative of an absence of immune memory overall.

Finally, as noted, while most studies on immunity to SARS-CoV-2 have focused on serum antibodies and cell-mediated immunity, the mucosal immune system is the largest component of the immune system.[77] 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.[80] However, no studies were identified in this review that could inform this topic.

Additional studies post systematic search

Since the formal systematic search on 12 May 2021, two further relevant studies have been identified.

The first, by Turner et al.[81] reported a longitudinal analysis of circulating anti-SARS-CoV-2 serum antibodies in 77 SARS-CoV-2 convalescent patients, the majority of whom had experienced mild disease. Consistent with some of the findings in our review, most convalescent patients had detectable anti-SARS-CoV-2 antibodies one month post-symptom onset (PSO). Following an initial rapid decline in titres in the first four months, the rate of decline slowed, with antibodies remaining detectable at least 11 months PSO. The authors also reported on the ability of SARS-COV-2 infection to induce a durable humoral immune response through detection of antigen-specific bone marrow plasma cells (BMPC) and memory B cells (MBC). SARS-

CoV-2 IgG and IgA S-specific BMPC were detected in 15 of 19 convalescent patients seven months PSO and in none of the 11 control participants. When retested at 11 months PSO, frequencies of anti-S IgG BMPC and anti-S IgA BMPC were stable in five of five and four of five convalescent patients. S-binding MBC were detectable at one month PSO and were maintained for at least seven months at significantly higher frequencies than in healthy controls. This detection of long-lived BMPC and MBC support the hypothesis of a durable humoral immune response to SARS-COV-2 infection.

The second study, a pre-print by Wang et al.[82] reports 12-month post-infection follow-up data for 63 participants from the study by Gaebler et al.[57] included in this review. Twenty six of the 63 (41%) had received at least one dose of a mRNA vaccine. While vaccination increased all components of the humoral response, antibody reactivity to the receptor binding domain (RBD) of SARS-CoV-2, neutralising activity and the number of RBD-specific memory B cells remained relatively stable from six to 12 months in all participants. Monoclonal antibodies titres were lower at 12 months than at six months PSO irrespective of vaccination status, but the neutralising activity of these antibodies increased in potency over time. Serum neutralising activities against the variants of concern B.1.351, B1.1.7 and B.1.526 were reported in both vaccinated and unvaccinated participants and were increased following vaccination. The authors also reported ongoing clonal evolution of memory B-cells with those vaccinated having a greater absolute number of B-cells representing persistent clones. Somatic hypermutation (which results in greater affinity of antibodies) of antibody genes also continued over 6 to 12 months with slightly higher levels of mutations found in non-vaccinated individuals.

While the studies by Turner et al. and Wang et al. have not formally been quality-appraised, they appear to support the findings of this review, that is, that immunity to SARS-CoV-2 appears to be long-lasting and involves the evolution of a robust immune memory. The findings of Wang et al. further support the likelihood that both natural and vaccine-induced immunity will protect against at least some of the known variants of concern.

Conclusion

A large volume of data supports the likelihood that the risk of SARS-CoV-2 reinfection, and relative risk compared with individuals without prior evidence of SARS-CoV-2 infection, is low for over ten months post-infection. While limited evidence from one study supports the hypothesis that natural infection and vaccination result in equally robust immune responses, including against new variants such as B.1.1.7, more studies are necessary to confirm this finding.

More limited data were identified in relation to the immune response to SARS-CoV-2 infection. The studies identified suggest that immune memory develops in most or all of those who have been infected with SARS-CoV-2 and lasts for up to nine months. While two studies reported the neutralising capacity of this immune memory, further studies would be required to define the relevant level of neutralisation. In addition, all studies were based on blood samples and cannot verify the presence of immune memory in the tissues, which forms the larger part of immune memory. There is substantial uncertainty in relation to the immune response to SARS-CoV-2 given the small study sizes and lack of clarity in relation to potential confounders.

There is still uncertainty on the following issues that have not yet been addressed within the literature:

- the durability of immunity beyond one year and longevity of immune memory to SARS-CoV-2
- the presence of immune memory resident in tissue
- protective immunity in paediatric populations
- protective immunity in populations with comorbidities and immunocompromised individuals
- the impact of new variants on protective immunity.

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Appendices

Appendix 1: Excluded studies with reasons

Appendix 1.1: Excluded studies from reinfection systematic search

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
Abu-Raddad 2021	SARS-CoV-2 antibody-positivity protects against reinfection for at least seven months with 95% efficacy	10.1016/j.eclinm.2021.100861	Exclusion reason: Already included in prior review
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
Asakura 2021	One Possible Reinfection with SARS-CoV-2 Validated by 205-days Interval of Re-detection in Sapporo City, Japan	10.20944/preprints202104.0439.v1	Exclusion reason: Cohort <100 people
Babiker 2021	The Importance and Challenges of Identifying SARS-CoV-2 Reinfections	10.1128/jcm.02769-20	Exclusion reason: Wrong study design
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
Binnendijk 2021	Serological Evidence for Reinfection with SARS-CoV-2; An Observational Cohort Study	10.2139/ssrn.3800076	Exclusion reason: Cohort <100 people
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
Deisenhammer 2021	6-month SARS-CoV-2 antibody persistency in a Tyrolian COVID-19 cohort	10.1007/s00508-020-01795-7	Exclusion reason: Wrong outcomes
Deng 2021	Transmission, infectivity, and antibody neutralization of an emerging SARS-CoV-2 variant in California carrying a L452R spike protein mutation	10.1101/2021.03.07.21252647	Exclusion reason: Wrong outcomes
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
Galiana 2021	Late Reinfection With a Different SARS-CoV-2Â Clade in a Patient With Refractory Arterial Hypertension: a Case Report	10.21203/rs.3.rs-392287/v1	Exclusion reason: Cohort <100 people
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	10.1093/infdis/jiaa803	Exclusion reason: Wrong outcomes

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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
Hanrath 2021	Prior SARS-CoV-2 infection is associated with protection against symptomatic reinfection	10.1016/j.jinf.2020.12.023	Exclusion reason: Already included in prior review
Hansen 2021	Assessment of protection against reinfection with SARS-CoV-2 among 4 million PCR-tested individuals in Denmark in 2020: a population-level observational study	10.1016/s0140-6736(21)00575-4	Exclusion reason: Already included in prior review
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
Hollinghurst 2021	COVID-19 Infection Risk amongst 14,104 Vaccinated Care Home Residents: A national observational longitudinal cohort study in Wales, United Kingdom, December 2020 to March 2021	10.1101/2021.03.19.21253940	Exclusion reason: Wrong study design
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
Kang 2021	Longitudinal Analysis of Human Memory T-Cell Response according to the Severity of Illness up to 8 Months after SARS-CoV-2 Infection	10.1093/infdis/jiab159	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
Klein 2021	Case Study: Longitudinal immune profiling of a SARS-CoV-2 reinfection in a solid organ transplant recipient	10.1101/2021.03.24.21253992	Exclusion reason: Wrong study design
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
Letizia 2021	SARS-CoV-2 Seropositivity and Subsequent Infection Risk in Healthy Young Adults: A Prospective Cohort Study	10.2139/ssrn.3779907	Exclusion reason: Follow-up <3 months
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
Perez 2021	A 1 to 1000 SARS-CoV-2 reinfection proportion in members of a large healthcare provider in Israel: a preliminary report	10.1101/2021.03.06.21253051	Exclusion reason: Already included in prior review
Pilz 2021	SARS-CoV-2 re-infection risk in Austria	10.1111/eci.13520	Exclusion reason: Already included in prior review
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
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
Salvato 2021	Epidemiological investigation reveals local transmission of SARS-CoV-2 lineage P.1 in Southern Brazil	10.21203/rs.3.rs-280297/v1	Exclusion reason: Wrong outcomes
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
Sheehan 2021	Reinfection Rates among Patients who Previously Tested Positive for COVID-19: a Retrospective Cohort Study	10.1101/2021.02.14.21251715	Exclusion reason: Already included in prior review
Silva 2021	Early detection of SARS-CoV-2 P.1 variant in Southern Brazil and reinfection of the same patient by P.2	10.21203/rs.3.rs-435535/v2	Exclusion reason: Cohort <100 people
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
Wang 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
Wang 2021	COVID-19 reinfection: A Rapid Systematic Review of Case Reports and Case Series	10.1101/2021.03.22.21254081	Exclusion reason: Wrong study design
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 1.2: Excluded studies from immune memory systematic search

Study	Title	DOI	Exclusion reason
Alrubayyi 2020	Coordinated and sustained immune memory responses after mild COVID-19	10.1038/s41577-020-00450-6	Exclusion reason: Duplicate
Alrubayyi 2021	B cell persistence and evolution to SARS-CoV-2	10.1101/2020.1103.367391	Exclusion reason: Brief report of excluded study
Ansari 2021	Immune memory in mild COVID-19 patients and unexposed donors from India reveals persistent T cell responses after SARS-CoV-2 infection	10.1101/2020.11.16.20232967	Exclusion reason: Wrong follow-up period
Ansari 2021	Immune memory in mild COVID-19 patients and unexposed donors from India reveals persistent T cell responses after SARS-CoV-2 infection	10.1101/2020.11.16.20232967	Exclusion reason: Wrong follow-up period
Bacher 2020	Low-Avidity CD4 + T Cell Responses to SARS-CoV-2 in Unexposed Individuals and Humans with Severe COVID-19	10.1016/j.immuni.2020.11.016	Exclusion reason: Duplicate
Bacher 2020	Low-Avidity CD4⁺ T Cell Responses to SARS-CoV-2 in Unexposed Individuals and Humans with Severe COVID-19	10.1016/j.immuni.2020.11.016	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: Examines T cells that may correlate with memory
Breathnach 2021	Prior COVID-19 significantly reduces the risk of subsequent infection, but reinfections are seen after eight months	10.1016/j.jinf.2021.01.005	Exclusion reason: Wrong outcome
Breton 2021	Persistent cellular immunity to SARS-CoV-2 infection	10.1084/jem.20202515	Exclusion reason: Duplicate
Byazrova 2021	Pattern of circulating SARS-CoV-2-specific antibody-secreting and memory B-cell generation in patients with acute COVID-19	10.1002/cti2.1245	Exclusion reason: Wrong follow-up period
Carsetti 2020	Different Innate and Adaptive Immune Responses to SARS-CoV-2 Infection of Asymptomatic, Mild, and Severe Cases	10.3389/fimmu.2020.610300	Exclusion reason: Wrong study design
CimenBozkus 2020	Long-lasting SARS-CoV-2-specific T cell memories	10.1101/2020.08.13.249433	Exclusion reason: Wrong follow-up period
Cohen 2021	Longitudinal analysis shows durable and broad immune memory after SARS-CoV-2 infection with persisting antibody responses and memory B and T cells	10.1101/2021.04.19.21255739	Exclusion reason: Wrong study design

Cohen 2021	Longitudinal analysis shows durable and broad immune memory after SARS-CoV-2 infection with persisting antibody responses and memory B and T cells	10.1101/2021.04.19.21255739	Exclusion reason: Wrong study design
Compeer 2020	Antibody response to SARS-CoV-2 - sustained after all?	10.1101/2020.07.21.20159178	Exclusion reason: Wrong outcomes
Dan 2021	Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection	10.1126/science.abf4063	Exclusion reason: Duplicate
DeBiasi 2020	Expansion of plasmablasts and loss of memory B cells in peripheral blood from COVID-19 patients with pneumonia	10.1002/eji.202048838	Exclusion reason: Wrong follow-up period
DiMuzio 2021	Unbiased interrogation of memory B cells from convalescent COVID-19 patients reveals a broad antiviral humoral response targeting SARS-CoV-2 antigens beyond the spike protein	10.1016/j.jvacx.2021.100098	Exclusion reason: Wrong follow-up period
Ferreras 2021	SARS-CoV-2-Specific Memory T Lymphocytes From COVID-19 Convalescent Donors: Identification, Biobanking, and Large-Scale Production for Adoptive Cell Therapy	10.3389/fcell.2021.620730	Exclusion reason: Wrong follow-up period
Ferretti 2020	Unbiased Screens Show CD8⁺ T Cells of COVID-19 Patients Recognize Shared Epitopes in SARS-CoV-2 that Largely Reside outside the Spike Protein	10.1016/j.immuni.2020.10.006	Exclusion reason: Wrong outcomes
Gaebler 2020	Evolution of Antibody Immunity to SARS-CoV-2	10.1101/2020.11.03.367391	Exclusion reason: Duplicate;
Grifoni 2020	Targets of T Cell Responses to SARS-CoV-2 Coronavirus in Humans with COVID-19 Disease and Unexposed Individuals	10.1016/j.cell.2020.05.015	Exclusion reason: Wrong outcomes
Guthmiller 2020	SARS-CoV-2 infection severity is linked to superior humoral immunity against the spike	10.1101/2020.09.12.294066	Exclusion reason: Wrong follow-up period
Habel 2020	Suboptimal SARS-CoV-2-specific CD8 + T cell response associated with the prominent HLA-A*02:01 phenotype	10.1073/pnas.2015486117	Exclusion reason: Wrong follow-up period
Hartzell 2020	Evidence of potent humoral immune activity in COVID-19-infected kidney transplant recipients	10.1111/ajt.16261	Exclusion reason: Wrong follow-up period
Juno 2020	Humoral and circulating follicular helper T cell responses in recovered patients with COVID-19	10.1038/s41591-020-0995-0	Exclusion reason: Wrong follow-up period
Kared 2020	Broad and prevalent SARS-CoV-2 CD8+ T cell response in recovered COVID-19 individuals demonstrates kinetics of early differentiation	10.1172/JCI145476	Exclusion reason: Wrong study design

Leslie 2020	T cells found in coronavirus patients 'bode well' for long-term immunity	10.1126/science.368.6493.809	Exclusion reason: Wrong study design
Lineburg 2020	Rapid detection of SARS-CoV-2-specific memory T-cell immunity in recovered COVID-19 cases	10.1002/cti2.1219	Exclusion reason: Wrong follow-up period
Liu 2020	Severe COVID-19 cases with a history of active or latent tuberculosis	10.5588/ijtld.20.0163	Exclusion reason: Wrong outcomes
Ma 2021	Protracted yet coordinated differentiation of long-lived SARS-CoV-2-specific CD8+ T cells during COVID-19 convalescence	10.1101/2021.04.28.441880	Exclusion reason: Wrong study design
Mansi 2021	Study of the SARS-CoV-2-specific immune T-cell responses in COVID-19-positive cancer patients	10.1016/j.ejca.2021.03.033	Exclusion reason: Wrong follow-up period
Mansi 2021	Study of the SARS-CoV-2-specific immune T-cell responses in COVID-19-positive cancer patients	10.1016/j.ejca.2021.03.033	Exclusion reason: Wrong follow-up period
Mazet 2020	CD8+ T cells remember same bits of SARS-CoV-2	NA	Exclusion reason: Brief report of excluded study
Nayak 2021	Characterization of neutralizing versus binding antibodies and memory B cells in COVID-19 recovered individuals from India	10.1016/j.virol.2021.02.002	Exclusion reason: Wrong follow-up period
Newell 2021	Switched and unswitched memory B cells detected during SARS-CoV-2 convalescence correlate with limited symptom duration	10.1371/journal.pone.0244855	Exclusion reason: Wrong follow-up period
Nguyen-Contant 2020	S Protein-Reactive IgG and Memory B Cell Production after Human SARS-CoV-2 Infection Includes Broad Reactivity to the S2 Subunit	10.1128/mBio.01991-20	Exclusion reason: Wrong follow-up period
Noh 2021	Longitudinal assessment of anti-SARS-CoV-2 immune responses for six months based on the clinical severity of COVID-19	10.2139/ssrn.3719075	Exclusion reason: Wrong outcomes
Ogega 2021	Durable SARS-CoV-2 B cell immunity after mild or severe disease	10.1172/JCI145516	Exclusion reason: Wrong follow-up period
Oliviero 2020	Expansion of atypical memory B cells is a prominent feature of COVID-19	10.1038/s41423-020-00542-2	Exclusion reason: Wrong follow-up period
Peng 2006	Long-lived memory T lymphocyte responses against SARS coronavirus nucleocapsid protein in SARS-recovered patients	10.1016/j.virol.2006.03.036	Exclusion reason: Wrong follow-up period
Poon 2021	Lasting memories of SARS-CoV-2 infection	10.1084/jem.20210210	Exclusion reason: Wrong study design
Rodda 2020	Functional SARS-CoV-2-specific immune memory persists after mild COVID-19	10.21203/rs.3.rs-57112/v1	Exclusion reason: Review
Rodda 2021	Functional SARS-CoV-2-Specific Immune Memory Persists after Mild COVID-19	10.1016/j.cell.2020.11.029	Exclusion reason: Review

Sekine 2020	Robust T Cell Immunity in Convalescent Individuals with Asymptomatic or Mild COVID-19	10.1016/j.cell.2020.08.017	Exclusion reason: Wrong follow-up period
Sette 2020	Pre-existing immunity to SARS-CoV-2: the knowns and unknowns	10.1038/s41577-020-0389-z	Exclusion reason: Wrong study design
Sette 2021	Adaptive immunity to SARS-CoV-2 and COVID-19	10.1016/j.cell.2021.01.007	Exclusion reason: Wrong study design
Sewell 2020	Cellular immune responses to covid-19	10.1136/bmj.m3018	Exclusion reason: Review
Stephens 2020	COVID-19 and the Path to Immunity	10.1001/jama.2020.16656	Exclusion reason: Wrong study design
Stephenson 2021	Single-cell multi-omics analysis of the immune response in COVID-19	10.1038/s41591-021-01329-2	Exclusion reason: Wrong follow-up period
Tavukcuoglu 2021	Functional responsiveness of memory T cells from COVID-19 patients	10.1016/j.cellimm.2021.104363	Exclusion reason: Wrong follow-up period
Thieme 2021	Detection of SARS-CoV-2-specific memory B cells to delineate long-term COVID-19 immunity	10.22541/au.161074580.02596064/v1	Exclusion reason: Wrong follow-up period
Tong 2021	Memory B cell repertoire for recognition of evolving SARS-CoV-2 spike	10.1101/2021.03.10.434840	Exclusion reason: Wrong follow-up period
Wang 2020	Serological Responses to Human Virome Define Clinical Outcomes of Italian Patients Infected with SARS-CoV-2	10.1101/2020.09.04.20187088	Exclusion reason: Wrong follow-up period
Wang 2021	Exposure to SARS-CoV-2 generates T-cell memory in the absence of a detectable viral infection	10.1038/s41467-021-22036-z	Exclusion reason: Wrong follow-up period;
Wheatley 2021	Evolution of immune responses to SARS-CoV-2 in mild-moderate COVID-19	10.1038/s41467-021-21444-5	Exclusion reason: Wrong follow-up period
Wilson 2020	Distinct B cell subsets give rise to antigen-specific antibody responses against SARS-CoV-2	10.21203/rs.3.rs-80476/v1	Exclusion reason: Wrong follow-up period
Wilson 2020	Distinct B cell subsets give rise to antigen-specific antibody responses against SARS-CoV-2	10.21203/rs.3.rs-80476/v1	Exclusion reason: Wrong follow-up period
Wu 2020	Persistence of humoral and cellular immune response after SARS-CoV-2 infection: opportunities and challenges	10.1007/s11684-020-0823-4	Exclusion reason: Review
Wu 2020	Persistence of humoral and cellular immune response after SARS-CoV-2 infection: opportunities and challenges	10.1007/s11684-020-0823-4	Exclusion reason: Review
Yang 2006	Long-lived effector/central memory T-cell responses to severe acute respiratory syndrome coronavirus (SARS-CoV) S antigen in recovered SARS patients	10.1016/j.clim.2006.05.002	Exclusion reason: Wrong exposure (SARS-CoV)
Yang 2006	Long-lived effector/central memory T-cell responses to severe acute respiratory syndrome coronavirus (SARS-CoV) S antigen in recovered SARS patients	10.1016/j.clim.2006.05.002	Exclusion reason: Wrong exposure (SARS-CoV)

Yang 2007	Persistent memory CD4+ and CD8+ T-cell responses in recovered severe acute respiratory syndrome (SARS) patients to SARS coronavirus M antigen	10.1099/vir.0.82839-0	Exclusion reason: Wrong exposure (SARS-CoV)
Yang 2007	Persistent memory CD4+ and CD8+ T-cell responses in recovered severe acute respiratory syndrome (SARS) patients to SARS coronavirus M antigen	10.1099/vir.0.82839-0	Exclusion reason: Wrong exposure (SARS-CoV)
Yang 2020	Longitudinal Characteristics of T Cell Responses in Asymptomatic SARS-CoV-2 Infection	10.1007/s12250-020-00277-4	Exclusion reason: Wrong study design
Yang 2020	Longitudinal Characteristics of T Cell Responses in Asymptomatic SARS-CoV-2 Infection	10.1007/s12250-020-00277-4	Exclusion reason: Wrong study design
Zhang 2020	Adaptive immune responses to SARS-CoV-2 infection in severe versus mild individuals	10.1038/s41392-020-00263-y	Exclusion reason: Wrong follow-up period
Zheng 2020	A human circulating immune cell landscape in aging and COVID-19	10.1007/s13238-020-00762-2	Exclusion reason: Wrong follow-up period

Appendix 2: Data extraction

Appendix 2.1: Part 1 – reinfection

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	**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	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-

	reinfection swab (to rule out persisting PCR positivity due 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)	applying the confirmation rate obtained from viral genome sequencing analysis. **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	2.22%) and incidence rate of infection was 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.
Breathnach 2021 UK	N=10,727 PCR or antibody positive at baseline Median f/u: N/R	**Primary endpoint:** PCR-confirmed SARS-CoV-2 reinfection. **Time interval:** Cases where the second	**Risk of reinfection:** 0.07% (with ≥90 days between infection events) **Relative risk of reinfection:** 0.058 (95% CI: 0.029 to 0.116)

DOI: 10.1016/j.jinf.2021.01.005 Published	Maximum f/u: Approx. 11 months (February to December 2020) **Analysis period:** Minimum interval between tests: 90 days. Study period: February to December 2020. Those who had evidence of COVID-19 in the first wave of infections in the UK (February to July 2020, with a peak in early April), as shown either by a positive SARS-CoV-2 PCR or a positive antibody test were identified. Their risk of having a positive SARS-CoV-2 PCR assay in the first five months of the second wave (August to December 2020) was compared with patients who had a previous negative PCR or antibody test. **Demographics:** Mean age 50; 60% Female	positive result was < / = 90 days after the first were excluded. **Test parameters:** Antibody samples were tested on either the Roche Elecsys or the Abbot Architect according to manufacturers guidelines. PCR assays were performed on the Roche 6800 or the Altona Diagnostics Real-Star.	Of note, there were no reinfections in the first seven months after the peak of the first wave; all eight patients with likely reinfections were diagnosed in December, the last month of the study period; reinfections accounted for 1.69% of all infections in that month
Hanrath 2020 10.1016/j.jinf.2020.12.023 Prior SARS-CoV-2 infection is associated with protection against symptomatic reinfection	**Analysis period and time interval:** ▪ Two periods for analysis: 1st wave: 10 March - 6 July 2020; 2nd wave: 7 July - 20 November. ▪ Follow-up: median 5.8 months (173 days, IQR: 162–229 days, between first	**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 date of first positive PCR/antibody result to the end of the analysis period. **Test parameters:**	**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. ▪ 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%

UK Retrospective cohort study Published (Journal of Infection)	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)	▪ 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	[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 positivity to SARS-CoV-2 is associated with a	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 (the latter excluded from follow-up)	**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). **Test:** Antibody test and/or diagnostic nucleic acid amplification test (NAAT). NAAT is considered a proxy representing a new infection or may represent	**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: ▪ 12.4% (n=1,227) tested negative when retested within 0-30 days

decreased risk of future infection USA Retrospective cohort study Published	**Demographics:** (negative index test group/positive index test group) Mean age =47.66/44.34 years; Female 56.7%/54.1%	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.	▪ 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 2021 UK 10.1016/S0140-6736(21)00675-9	N=8,278 **Median f/u:** 275 days (9.1 months) (IQR 218–291 days) for the positive cohort and 195 days (6.5 months) (IQR 131–214 days) for the negative cohort. **Maximum f/u:** >11 months	▪ Questionnaires on symptoms and exposures were sent electronically at baseline and every 2 weeks. ▪ SARS-CoV-2 antibody testing and Nucleic Acid Amplification Testing (NAAT) with real-time PCR (rtPCR) was done at enrolment and at regular intervals (PCR every 2 weeks, antibody testing every 4 weeks).	**Incidence density:** 7.6 reinfections per 100,000 person-days in the positive cohort compared with 57.3 primary infections per 100,000 person-days in the negative cohort **Adjusted incidence rate ratio of reinfection comparing antibody or PCR-positive group with negative group**

SARS-CoV-2 infection rates of antibody-positive compared with antibody-negative health-care workers in England: a large, multicentre, prospective cohort study (SIREN) Prospective cohort Published Health care workers UK	**Study period (reinfection f/u):** 18 June 2020 to 31 Dec 2020 Participants were assigned to the positive cohort if they met one of the following criteria: antibody positive on enrolment or antibody positive from previous clinical laboratory samples, with or without a previous positive PCR test; antibody negative on enrolment with a positive PCR result before enrolment. Participants were assigned to the negative cohort if they had a negative antibody test and no documented previous positive PCR or antibody test.	▪ Most sites used rtPCR; however, a small number of sites used Loop-mediated isothermal amplification testing or Rapid Testing with rtPCR to confirm positive results. ▪ The B.1.1.7 variant emerged and spread during the study period, and the effect of this variant was included in the analysis by creating a binary variable of when the S-Gene Target Failure (SGTF) PCR, used to identify the B.1.1.7 variant in the laboratory network, accounted for 50% or more of the positive results for each region. The SGTF PCR testing was introduced to specific laboratories in England only, termed Pillar 2 laboratories, which are large hospital laboratories established specifically for the COVID-19 response for the purpose of community testing.	▪ All events (possible and probable reinfections): 0.159 (95% CI: 0.13–0.19) ▪ Symptomatic reinfections only (with COVID-19 symptoms): 0.074 (95% CI: 0.06–0.10) ▪ Asymptomatic reinfections only: 0.484 (95% CI: 0.37–0.63) ▪ Probable reinfections only: 0.002 (95% CI: 0.00–0.01) Author conclusions: A previous history of SARS-CoV-2 infection was associated with an 84% lower risk of infection, with median protective effect observed 7 months following primary infection.
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	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	**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:	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)

	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. **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+.	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 the 90 days changed an individual's status from uninfected to previously infected. Additional cohort analysis was then expanded 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 outbreaks in care homes, September and October 2020 UK Retrospective cohort Published Eurosurveillance	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 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.	**RT-PCR testing** Nasal swabs were subjected to SARS-CoV-2 RT-PCR at the Public Health England (PHE) national reference Laboratory. **Antibody testing** 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.	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) 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.

	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 (assumed to have seroconverted).		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 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	**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	**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)*

	weekly in staff and monthly in residents. ▪ Patients were followed between Oct 2020 and 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).	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 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	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:** 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.
Leidi 2021 10.1101/2021.03.19.21253889 Risk of reinfection after seroconversion to SARS-CoV-2: A population-based propensity-score matched cohort study Switzerland	N=498 **Mean f/u:** 249 days (8.3 months) **Maximum f/u:** Approx. 10 months **Duration:** Serological status assessment in April-June 2020 to the end of the second pandemic wave (January 2021). **Demographics**	**Primary endpoint:** newly acquired SARS-CoV-2 infections in seropositive individuals from a population-based sample as compared to seronegative controls. **Antibody testing:** Seropositivity was defined by the detection of anti-S1 domain of spike protein IgG antibodies using a two-step sequential strategy. Antibodies were first detected by a commercially available ELISA (Euroimmun, Lübeck, Germany #EI 2606-9601 G). All potentially indeterminate (IgG ratio for detection ≥0.5) and positive results were confirmed by a recombinant immunofluorescence assay (rIFA), as this technique was considered the	**Seropositive group:** 5/498 reinfections; incidence: 0.3 per 1,000 person-weeks ('likely' reinfections) **Seronegative group:** 154/996 infections; incidence: 4.8 per 1,000 person-weeks **Hazard ratio for reinfection**: 0.06, 95% CI 0.02 to 0.14, p<0.001 (PM matching)

Retrospective matched cohort study Preprint	Among 8,344 serosurvey participants, 498 seropositive individuals were selected and matched with 996 seronegative controls. Age range: 20 to 74 years old	reference method in the laboratory of virology of Geneva University Hospitals (WHO Swiss reference lab) at the time the seroprevalence survey took place. **Reinfection definition:** Two independent adjudicators with experience in clinical management of SARS-CoV-2 infected patients evaluated suspected cases via hospital electronic health records or phone interview with participants. Adjudication was based on clinical judgement and criteria included, when available, reason for testing, subject's illness history (including date of symptom onset) and the value and temporal evolution in RT-PCR cycle threshold (Ct). The purpose of this investigation was to differentiate clinical reinfections from protracted RNA detection. Cases of suspected reinfections were classified as likely or unlikely. Conflicts were solved by a third person.	
Lumley 2021 UK 10.1101/2021.03.09.21253218 An observational cohort study on the incidence of SARS-CoV-2 infection and B.1.1.7 variant infection in healthcare workers by antibody and vaccination status Prospective cohort study Preprint Health care workers	N=1,273 **F/u:** 216 days (7.2 months) (13,109 individuals contributed 2,835,260 person-days follow-up) Of the 13,109 HCWs participated; 8,285 received the Pfizer-BioNTech vaccine (1407 two doses) and 2,738 the Oxford-AstraZeneca vaccine (49 two doses). 11 HCWs received another vaccine or could not recall the manufacturer. Staff members were classified into five groups: 1. unvaccinated and consistently seronegative during follow-up	▪ Antibody status was determined using an anti-trimeric spike IgG ELISA ▪ SARS-CoV-2 infection diagnosed with RT-PCR ▪ B.1.1.7 variant: PCR-positive results from symptomatic community testing were recorded; from November 2020, Oxford University Hospitals used the Thermo Fisher TaqPath PCR assay as their first-line diagnostic assay, which includes orf1ab, S and N gene targets. As such SGTF indicative of theB.1.1.7 variant could be identified, i.e. orf1ab-positive/N-positive only. Oxford Nanopore sequencing was undertaken of all stored PCR-positive primary samples from 1 December 2020 onwards to identify the infecting lineage.	▪ Compared to unvaccinated seronegative HCWs, natural immunity and two vaccination doses provided similar protection against symptomatic infection: no HCW with two vaccines doses had symptomatic infection, and incidence was 98% lower in seropositive HCWs (adjusted incidence rate ratio 0.02 [95%CI <0.01-0.18]). ▪ Two vaccine doses or seropositivity reduced the incidence of any PCR-positive result with or without symptoms by 90% (0.10 [0.02-0.38]) and 85% (0.15 [0.08-0.26]) respectively. ▪ Single-dose vaccination reduced the incidence of symptomatic infection by 67% (0.33 [0.21-0.52]) and any PCR-positive result by 64% (0.36 [0.26-0.50]).

	2. unvaccinated and ever seropositive 3. vaccinated one dose, always seronegative prior to vaccination 4. vaccinated two doses, always seronegative prior to first vaccination 5. vaccinated (one or two doses and ever seropositive prior to first vaccination. The latter group were combined as relatively few staff were previously seropositive and received two vaccine doses. Vaccinated groups were considered at-risk of infection >14 days after each vaccine dose.		There was no evidence of differences in immunity induced by natural infection and vaccination for infections with S-gene target failure and B.1.1.7.
Manica 2021 10.1101/2021.04.14.21255502 The risk of symptomatic reinfection during the second COVID-19 wave in individuals previously exposed to SARS-CoV-2 Cohort study Preprint Italy	N=1,402 **Maximum f/u:** 8 months Overall seroscreening population: 7,979. This represented five Italian municipalities within the Autonomous Province of Trento, Italy, where an IgG serological screening aimed at covering the entire adult resident population was conducted between 5 May and 15 May 2020.	**Serological tests:** performed using Abbott SARS-CoV-2 IgG chemiluminescent assays and analyzed on the Abbott Architect i2000SR automated analyzer **Reinfection cases:** Positive cases were ascertained by using either RealTime SARS-CoV-2 assay on naso-oropharyngeal swabs (detectability per ml of UTM buffer 250 copies) or rapid antigenic test (sensitivity >90%, specificity >97%). Out of 221 confirmed cases, 124 were symptomatic. **Symptomatic infections:** Defined as positive participants having fever and either cough or at least two of the following symptoms: widespread myalgia, headache, dyspnoea, pharyngodynia, diarrhea, nausea/vomiting, anosmia/ageusia, asthenia.	Cumulative incidence of symptomatic infections in seropositive group: 0.14% (95%CI: 0.04% to 0.58%) Cumulative incidence of symptomatic infections in seronegative group: 2.67% (95% CI: 2.12% to 3.37%) **Adjusted odds ratio** of developing symptomatic infection: 0.05 (95% CI: 0.01 to 0.17) Four cases were identified among participants who tested positive to IgG in May 2020; two of them were symptomatic. Both these cases were males ascertained in December 2020, who requested to be tested after symptoms onset. The older patient (88 years) was admitted to a hospital but did not require mechanical ventilation or admission to an intensive care unit. The younger patient (52 years)

			was a mild case who was isolated and treated at home.
Masia 2021 10.1016/j.jinf.2021.03.020 Incidence of delayed asymptomatic COVID-19 recurrences in a 6-month longitudinal study Published Spain	N=146 **Maximum f/u:** 6 months Median age was 64 years, 88 (60.3%) were male, and 72.6% had coexisting comorbid diseases.	**Primary endpoint:** Reinfection rate **Serology:** IgG antibody plasma levels against the SARS-CoV-2 internal nucleocapsid protein (N-IgG) and the spike protein (S-IgG) (Anti-SARS-CoV-2 IgG ELISA, Euroimmun, Lubeck, Germany) **Reinfection:** SARS-CoV-2 RNA was detected by RT-PCR (AllplexTM 2019-nCoV Assay, Seegene, Seoul, Korea) which targeted the E, RdRP, and N genes. **WGS:** Genome sequencing of SARS-CoV-2 was performed on nasopharyngeal samples following ARTIC amplicon sequencing protocol for MinIon version V3- Phylogenetic analysis was done using webserver Nextstrain (https://nextstrain.org/), with the SARS-CoV-2 database Nextclade (https://clades.nextstrain.org/).	**Reinfection rate based on whole genome sequencing:** 1 confirmed reinfection out of 146 primary infections (0.68%) Overall, 5 patients with positive RT-PCR occurring more than 90 days since first COVID-19 diagnosis were identified. Median (range) time from diagnosis to new detection of SARS-CoV-2 RNA was 183 (167–204) days. Cases included 3 men, with ages ranging from 44 to 73 years, and 3 of them had subjacent comorbidity. Two patients were readmitted to hospital at re-positivity, and 3 patients remained asymptomatic. Only one patient had a Ct<33, and in the other four patients the Cts ranged from 33 to 38. Genomic sequencing was performed in 4 individuals with available paired samples. In the three patients with Ct≥33, all of them asymptomatic, the same clade 20B was detected. In two of them, the clade showed the same hallmark single nucleotide variants. In the third patient, the follow-up sample showed two new mutations, a K374R substitution in the N gene and an A222V substitution in the S gene, probably reflecting adaptive viral changes associated to persistent infection. Genomic sequencing of the symptomatic patient with a Ct of 18 showed phylogenetically distinct genomic sequences; the first sample was member of the clade 20A, and the most recent sample was member of the clade 20B. The 3 patients with asymptomatic recurrence and

			the symptomatic patient with no sequencing data showed detectable antibody levels at the time of SARS-CoV-2 RNA re-positivity, ranging from 3.01 to 6.01 S/CO for S-IgG and 2.6 to 2.46 S/CO for N-IgG. The patient with symptomatic reinfection had no detectable antibody levels at the time of re-positivity.
Mohamadreza 2021 10.21203/rs.3.rs-262191/v1 COVID-19 Re-infection or Relapse? A Retrospective Multi Center Cohort Study From Iran Preprint Retrospective cohort study Iran	N=1,899 **Maximum f/u:** 6 months **Demographic/clinical criteria:** The majority of patients were male and nurses. The mean age was 37.54 ±15.16 years old. Weakness, myalgia, and fever were the most clinical presentation symptoms in both episodes. Chest Computed Tomography scan showed pneumonia in 56.8% of cases and 43.2% of cases in the first and second episodes respectively Mean duration between discharge and second presentation was 117±61.42 days.	Details of testing methodology not reported.	Symptomatic reinfection rate: 1.9% (37/1,899) Phylogenic sequencing of SARS-CoV-2 and viral culture was not possible.
Papasavas 2021 10.1016/j.jhin.2021.04.021 Seroprevalence of SARS-CoV-2 antibodies, associated epidemiological factors	N=433 Median f/u: 5.5 months Maximum f/u: 196 days (6.5 months) The average age of participants was 43.2 ± 12.9 years (median 43, range 18-81). Of the 6,811	Participants completed a questionnaire on REDCap Three blood draws were completed (initial visit; 2-4 weeks after initial visit; 3-6 months after initial visit)	0/35 seropositive participants who had a subsequent PCR test at least 30 days following the positive antibody test had a positive test 1.3% (29/2,173) seronegative participants had a subsequent positive PCR test

and antibody kinetics among healthcare workers in Connecticut Healthcare workers Published USA	participants who reported gender, there were 5,387 females (79.1%). Based on initial testing, 433 (6.3%; 95% CI: 5.7%-6.9%) participants were seropositive (out of a total of 8,663 HCWs provided electronic consent and 6,863 (23% of the entire employee population) provided an initial sample)	Anti-SARS-CoV-2 IgG Antibody Detection: Abbott Architect i2000 platform. Seropositivity was defined as IgG Index (Signal/Cutoff (S/C)) ≥1.4. SARS-CoV-2 diagnosis: RT-PCR testing	
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	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	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).	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.

SARS-CoV-2 re-infection risk in Austria Austria Retrospective observational study Published	all the remaining general population from Austrian Epidemiological Reporting System. Of those with tentative reinfections, 62.5% were women; median age (IQR) = 39.8 (25.9 to 54.5). **Median f/u:** 210 days (7 months) **Maximum f/u:** 300 days (10 months)	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. 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	OR was estimated at 0.09 (95% CI: 0.07 to 0.13)
Qureshi 2021 Re-infection with SARS-CoV-2 in Patients Undergoing Serial Laboratory Testing 10.1093/cid/ciab345 Retrospective Preprint USA	N=9,119 **Mean** interval between positive tests: 116 days (3.9 months) **Maximum f/u:** N/R; time period applied to dataset: 1 December 2019 to 13 November 2020.	Data were obtained from the Cerner de-identified Coronavirus Disease 2019 (COVID-19) dataset. The methodological aspects of the dataset are available in other publications. Patients with a positive laboratory test for SARS-CoV-2 were identified based on Logical Observation Identifiers Names and Codes; these codes denote detection of SAR-CoV-2 ribonucleic acid in respiratory (nasopharyngeal swabs, bronchoalveolar lavage, sputum) and other specimens or detection of SARS-CoV-2 N gene or RdRp gene in respiratory secretions, all by nucleic acid amplification with probe detection.	Reinfection rate: 63/9,119; 0.7% (95% CI: 0.5%-0.9%) The mean period (±standard deviation [SD]) between two positive tests was 116 ± 21 days. A logistic regression analysis identified that asthma (odds ratio [OR] 1.9, 95% CI 1.1-3.2) and nicotine dependence/tobacco use (OR 2.7, 95% CI 1.6-4.5) were associated with re-infection. There was a significantly lower rate of pneumonia, heart failure, and acute kidney injury observed with re-infection compared with primary infection among the 63 patients with reinfection. There were two deaths (3.2%) associated with reinfection.
Sheehan 2021	N=8,845 with history of prior infection at baseline	Main outcome was risk of reinfection, defined as a positive PCR test ≥90 days after initial testing.	**Risk of reinfection** N=974 (11%) of the positive patients were retested after 90 days and 56 had possible

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	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)	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	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.
Shields 2021 10.1101/2021.02.24.21252368 Longitudinal protection following natural SARS-CoV-2 infection and early vaccine responses: insights from a cohort of	N=246 (dental practitioners) Maximum f/u: 6 months Baseline seroprevalence was 16.3% in overall cohort of 1,507 individuals	**Serological analysis:** A 'commercially available, CE marked' IgGAM ELISA was used that measures the total antibody response (IgG, IgA and IgM simultaneously) against the spike glycoprotein (Product code: MK654, The Binding Site (TBS), Birmingham) **Reinfection:** RT-PCR was used	**Adjusted risk ratio** for reinfection: 0.26 (95% CI 0.11 to 0.63) The risk of infection was 9.6% in participants who were seronegative at baseline, compared to 2.8% in individuals who were seropositive (p=0.001) Reinfections only occurred in the absence of specific, detectable anti-spike IgG response

community based dental health care professionals Preprint Healthcare workers UK		NIBSC and WHO standards: NIBSC 20/136, the first World Health Organization International Standard for anti-SARS-CoV-2 immunoglobulin and NIBSC 20/162 were employed.	Serological analysis: there were no PCR-proven infections in 64 individuals with a baseline anti-SARS-CoV-2 IgG level greater than 147.6 IU/ml (with respect to the WHO international standard NIBSC 20/136). Notes on vaccination: ▪ It is notable that 51.5% (n=450/873) had received a single dose of a SARS-CoV-2 vaccine (Oxford/AstraZeneca, n=17; Pfizer-BioNTech, n= 429; Unknown, 222 n=4) during follow up. Estimates on reinfection risk, however, relate to baseline antibody status prior to vaccination. ▪ Of those vaccinated with a single dose of the Pfizer-BioNTech SARS-CoV-2 were analysed based on prior exposure to the virus - defined by either positive baseline serology, or PCR-proven infection during the follow up period, vaccination on the background of prior exposure to the virus was associated with a more rapid and quantitatively greater total antibody response against the SARS-CoV-2 spike glycoprotein, consistent with the boosting of immunological memory.

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 2.2: Part 2 – immune memory

Study characteristics	Patient demographics Clinical characteristics Test parameters	Primary outcome results
Author: **Abayasingham** *Country:* Australia *DOI:* 10.1016/j.xcrm.2021.100228 *Study design:* Cohort study *Setting:* Collection of Coronavirus COVID-19 Outbreak Samples in New South Wales (COSIN) *Publication status:* Published	*Patient demographics:* Participants in COSIN *Participants*: n=15 were screened for memory B-cells with n=6 healthy negative controls; n=5 were screened for RBD memory B-cells with neutralising activity (NA) *Median age:* Range 23 to 84 years (memory B-cell subgroup); NR for NA group *Female:* 7/15 (memory B-cell group); NR for NA group *Type of test:* ▪ SARS-CoV-2 infection confirmed by NAT ▪ flow cytometry for detection of memory B-cells; ELISA for investigation of neutralising capacity. *Follow-up duration/range/intervals:* Memory B-cell analysis at two time points, t1 and t2, calculated from the number of days post-onset of symptom (DPS). ▪ t1 ranged from 30 to 87 DPS (median 68 days) ▪ t2 ranged from 110 to 181 DPS (median 132 days) NA analysis at 3 time points with low EPT and 3 time points with above-average EPT	*Immune memory component reported:* Memory B-cells 1. RBD-specific memory B-cells 2. RBD-specific memory B-cells with neutralising capacity *Results:* 1. RBD-specific memory B-cells ▪ The healthy participants had a high CD27+RBD+IgD+ frequency but low CD27+RBD+IgD-IgG+ and CD27+RBD+IgD-IgG- frequencies ▪ The COSIN participants generally had higher mean frequencies of all 3 virus-specific subsets than the healthy participants ▪ 12 of the 15 participants had a CD27+RBD+IgD-IgG+ frequency above the healthy control derived cut-off at t2, and this was a significant increase from the same values observed at t1 ▪ Two participants who did not have a CD27+RBD+IgD-IgG+ B-cell response greater than the healthy cut-off were from the low end point titer (EPT) group and had mild or moderate clinical illnesses; the third was from the high EPT group and this individual had severe disease 2. RBD-specific memory B-cells with neutralising capacity ▪ Of the 50 monoclonal antibodies (mABs) that bound RBD, 14 mABs from 3 of the 5 participants had neutralising activity ▪ No neutralising were isolated from the two patients who did not seroconvert ▪ 6 potent mABs were sequenced analysed (2 highest from 3 patients) and these were a mix of IGHV genes and had undergone affinity maturation, as indicated by the somatic hypermutation level of 2% to 7%. *Author conclusions:* Despite declining anti-RBD antibody titers and neutralising activity in the serum at 6 months, the memory B-cells still contain anti-RBD-specific reactivity that have the capacity to generate antibodies that can neutralise SARS-CoV-2 in vitro
Author: **Anand**	*Patient demographics:* SARS-CoV-2 infected and pre-pandemic uninfected plasma donors	*Immune memory component reported:* RBD-specific memory B-cells

Study characteristics	Patient demographics Clinical characteristics Test parameters	Primary outcome results
Country: Canada *DOI:* https://doi.org/10.1016/j.xcrm.2021.100290 *Study design:* Cohort study *Setting:* Unclear; blood donors *Publication status:* Published	*Participants*: 101 blood samples from 32 COVID-19 convalescent patients; 10 pre-pandemic uninfected samples *Mean age*: 47 years (range 20 to 65 years) Male n= 17; female n=15 Type of test: ▪ COVID-19 infection confirmed by RT-PCR ▪ ELISA and flow-cytometry assay for antibodies (RBD and Spike) ▪ antibody-dependent cytotoxicity (ADCC) assay ▪ flow-cytometry to distinguish naïve and memory B-cells. RBD-specific naïve and memory B cells were characterized based on surface expression of CD21 and CD27. *Follow-up duration/range/intervals:* Sampled at four longitudinal time points between 16 and 233 days post-symptom onset (PSO) • 6 weeks (16-95 days; median: 43 days) n=32 • 11 weeks (48-127 days; median: 77 days) n=28 • 21 weeks (116-171 days; median: 145 days) n=28 • 88 and 31 weeks (201-233 days; median: 218 days) n=13	*Results:* ▪ Total RBD-specific memory B-cells were detected in 100% of the donors and the mean frequency remained stable between 6 and 31 weeks PSO (0.20% to 0.26%) ▪ IgG+ RBD-specific memory B-cells were detected in 100% of the donors and the frequency of this population modestly increased up to 31 weeks PSO ▪ IgA+ RBD-specific memory B-cells were low but stable over the 8 month period *Author conclusions:* ' … stabilization of antigen-specific B cells observed … (is) suggestive of decreased antibody production by B cells after resolution of infection or the gradual replacement of Ig-secreting short-lived plasma cell by memory B cells.' 'Furthermore, we show that COVID-19 patients generate RBD-specific memory B-cells and IgG+ memory cells that persist for over 8 months … Thus, the decline of antibody levels does not negate the protective potential because of the importance of cellular responses against SARS-CoV-2 infection …'
Author: **Breton** *Country:* USA *DOI:* 10.1084/jem.20202515	*Patient demographics:* Participants: n=41 SARS-CoV-2 infected participants or close contact who had seroconverted and n=20 SARS-CoV-2 unexposed (pre-pandemic)	*Immune memory component reported:* ▪ Circulating CD4+ and CD8+ T-cell compartments (CD4+ central memory T-cells (Tcm), CD8+ Tcm, CD8+ T effector memory cells (Tem). *Results:*

Study characteristics	Patient demographics Clinical characteristics Test parameters	Primary outcome results
Study design: Cohort *Setting:* Rockefeller University Hospital, New York *Publication status:* Published	Median age: 45 years in SARS-CoV-2 infected; 52.5 years unexposed Female: 36.6% in SARS-CoV-2 infected; 45%unexposed Type of test: ▪ COVID-19 infection confirmed by RT-PCR ▪ flow cytometry. Follow-up duration/range/intervals: Average of 1.3 and 6.1 months post-infection	▪ The relative distribution of all the clusters (CD4+ Tcm, CD8+ Tcm, CD8+ Tem) remained significantly different from HCs at the 6.1 month time point, indicating significant shifts in circulating CD4+ and CD8+ T-cell compartments that persist for 6.1 months after SARS-CoV-2 infection ▪ Central memory CD4+ and CD8+ decreased and this defect persisted throughout the observation period ▪ Antigen-specific CD4+ T-cells expressing memory markers as well as IL-2, IFN-ɣ, TNF-α and CD154 were markedly increased in COVID-19 recovered individuals compared to healthy donors but the relative frequency of these cells decreased at the 6.1 month time-point ▪ Polyfunctional antigen-specific CD4+ and CD8+ memory T-cells were elevated at the two time points *Author conclusions:* The data indicate that recovered individuals show persistent polyfunctional SARS-CoV-2 antigen specific memory that could contribute to rapid recall responses. In addition, recovered individuals show enduring immune alterations in relative numbers of CD4+ and CD8+ T-cells, expression of activation/exhaustion markers and cell division.
Author: **Cohen** *Country:* USA *DOI:* 10.1101/2021.04.19.21255739 *Study design:* Cohort study *Setting:* Two prospective cohorts from Seattle and Atlanta *Publication status:* Pre-print	*Patient demographics:* COVID-19 patients: 71% mild; 24% moderate; 5% severe Participants: n=111 patients; 29 healthy controls Median age: 48.5 years (range 18 to 82 years) Male: 45% Type of test: ▪ COVID-19 infection confirmed by RT-PCR ▪ flow cytometry. Follow-up duration/range/intervals: From early infection and for eight months thereafter. Blood samples collected at 2-3 time-points from 165 participants and 4-7 time-points from another 80 participants	*Immune memory component reported:* ▪ SARS-CoV-2 spike and RBD-specific memory B-cells ▪ Virus-specific memory CD4+ and CD8+ T-cells ▪ Age and disease severity association with immune response. *Results:* ▪ SARS-CoV-2 spike and RBD-specific memory B-cells increase for several months after infection and then plateau over 8 months ▪ Among COVID-19 patients, 89% (102/113) mounted CD4+ T-cells response; these were rarely detected in the uninfected group ▪ Among COVID-19 patients, 69% generated CD8+ T-cells in contrast to infrequent to rare responses in the uninfected group ▪ SARS-CoV-2-specific CD4+ T-cells were primarily central memory phenotype (CD45RA- CCR7+) and to a lesser extent effector memory (CCRA-CCR7-). This profile of the memory T-cell subsets was very consistent between subjects and stable over time ▪ The vast majority of SARS-CoV-2-specific CD8+ T-cells showed an effector memory phenotype during the early phase of the response, which contracted over time and simultaneously there was an increase in the

Study characteristics	Patient demographics Clinical characteristics Test parameters	Primary outcome results
	Note: This cohort will be followed for 2 to 3 more years.	proportion of the TEMRA (CD45RA+CCR7-) subset. A small but stable fraction of SARS-CoV-2-specific CD8+ T-cells expressed a central memory phenotype ▪ Increased age positively correlated with increased frequencies of spike and RBD-specific IgG+memory B-cells, with 1.19 to 1.24-fold higher responses per decade of age (controlling for severity). *Author conclusions:* This in-depth longitudinal study demonstrates that durable immune memory persists in most COVID-19 patients including those with mild disease.
***Author:* Dan** *Country:* USA *DOI:* 10.1126/science.abf4063 *Study design:* Cohort *Setting:* Blood samples collected at the Icahn School of Medicine at Mt. Sinai *Publication status:* Published	*Patient demographics:* COVID-19 patients Participants: N=188 overall cohort Median age: 40 years overall cohort Male: 43% overall cohort Type of test: ▪ COVID-19 infection confirmed by RT-PCR* ▪ flow cytometry. Follow-up duration/range/intervals: n=43 samples analysed at ≥6 months post-infection; time-points (TPs) were: ▪ TP1 36 to 136 days ▪ TP2 111-240 days *77% of participants were PCR positive; 1% were PCR negative; 22% were either of unknown PCR status or were untested (All had diagnosed or suspected COVID-19)	*Immune memory component reported:* ▪ Spike-specific memory B-cells ▪ CD4+ memory T-cell ▪ CD8+ memory T-cell ▪ Immune memory relationships. *Results:* 1. SARS-CoV-2 memory B-cells a. Frequencies of SARS-CoV-2 spike specific memory B-cells increased over the first 120 days and then plateaued b. Spike-specific memory B-cell frequencies from the first TP to the second from paired samples in 24 of 36 longitudinally tracked donors c. Spike-specific memory B-cells in SARS-CoV-2 unexposed subjects were rare d. RBD-specific memory B-cells appeared as early as 16 days post-symptom onset (PSO) and the frequency steadily increased in the following 4 to 5 months e. 29 of 36 longitudinally-followed individuals had higher frequencies of RBD-specific memory B-cells at TP2 f. ~10-30% of spike-specific memory B-cells from SARS-CoV-2 convalescent donors were specific for the RBD domain g. SARS-CoV-2 N-specific memory B-cell frequency steadily increased during the first ~4 to 5 months PSO h. During the earliest phase of memory (20-60 days PSO) IgM+ and IgG+ isotypes were similarly represented, but IgM+ memory B-cells then declined and IgG+ spike-specific memory B-cells dominated by 6 months. IgA+ spike-specific memory B-cells

Study characteristics	Patient demographics Clinical characteristics Test parameters	Primary outcome results
		were detected as a small fraction of total spike-specific memory B-cells (~5%)and remained low and stable over an 8 month period i. Similar patterns of increasing IgG+ memory, short-lived IgM+ memory, and stable IgA+ memory were observed for RBD- and N-specific memory B-cells over the 8 month period 2. SARS-CoV-2 memory CD8+ T-cells a. 70% of subjects had detectable circulating SARS-CoV-2 memory CD8+ T-cells at 1 month PSO and 50% at ≥6 months b. SARS-CoV-2 memory CD8+ T-cells declined with an apparent half-life ($t_{1/2}$) of 125 in the full cohort and in 190 days in paired samples c. Spike-specific memory CD8+ T-cells exhibited similar kinetics ($t_{1/2}$ 225 days for full cohort; 185 days among paired samples) d. The majority of SARS-CoV-2 memory CD8+ T-cells were terminally differentiated effector memory cells (T_{EMRA}), with small populations of central memory (T_{CM}) and effector memory (T_{EM}) 3. SARS-CoV-2 memory CD4+ T-cells a. SARS-CoV-2 memory CD4+ T-cells were identified in 169 subjects b. SARS-CoV-2 memory CD4+ T-cells declined with an apparent $t_{1/2}$ of 94 days in the full cohort and 64 days among 36 paired samples c. 93% of subjects had detectable circulating SARS-CoV-2 memory CD4+ T-cells at 1 month PSO and 92% at ≥6 months d. Spike-specific and M-specific memory CD4+ T-cells exhibited similar kinetics ($t_{1/2}$ of 139 days and 153 days respectively in the full cohort) e. A plurality of SARS-CoV-2 memory CD4+ T-cells present at ≥6 months had a T_{CM} phenotype f. Memory cT_{FH} specific for SARS-CoV-2 spike and MP_R were detected in the majority of subjects at early time points g. cT_{FH} memory appears to be stable, with almost all subjects positive for spike and MP_R memory cT_{FH} cells at 6 months PSO h. The percentage of PD-1^{hi} SARS-CoV-2 memory cT_{FH} dropped over time

Study characteristics	Patient demographics Clinical characteristics Test parameters	Primary outcome results
		i. A significant fraction of both spike-specific and MP_R memory cT_{FH} cells were CCR6+ and there were increases in these over time j. Overall , substantial cT_{FH} memory was observed after SARS-CoV-2 infection with durability ≥6 months PSO 4. Immune memory relationships (with sex and severity) a. No differences were observed in IgA or PSV neutralisation titers, SARS-CoV-2 memory B-cells, memory CD8+ T-cell or CD4+ T-cell frequencies between males and females b. Spike and RBD IgG titers and spike and RBD-specific memory B-cells were higher in hospitalised then non-hospitalised cases c. Memory CD8+ T-cell frequencies were not higher in hospitalised compared with non-hospitalised cases d. Memory CD4+ T-cells frequencies tended to be lower in hospitalised compared with non-hospitalised cases *Author conclusions:* (O)ur data show immune memory in at least three immunological compartments was measurable in ~95% of subjects 5 to 8 months PSO, indicating that durable immunity against secondary COVID-19 diseases is a possibility in most individuals.
Author: **Gaebler** *Country:* USA *DOI:* 10.1038/s41586-021-03207-w *Study design:* Cohort study *Setting:* Rockefeller University Hospital *Publication status:* Published	*Patient demographics:* Adults who were diagnosed with SARS-CoV-2 infection or close contacts who seroconverted Participants: N=87; n=21 randomly selected individuals were sampled for % RBD-binding memory B-cells at 1.3 and 6 months; n=6 to examine changes to antibodies produced by RBD-binding memory B-cells after 6.2 months Mean age: 45 years (range 18 to 78 years) Male: 60% Type of test: ▪ COVID-19 infection confirmed by RT-PCR	*Immune memory component reported:* 1. % of RBD-specific memory B-cells at 1.3 and 6 months 2. Changes in the antibodies produced by memory B-cells after 6.2 months *Results:* ▪ Memory B-cell response evolves between 1.3 and 6 months post-infection ▪ The % of RBD-binding memory B cells increased marginally between 1.3 and 6.2 months in 21 randomly selected individuals ▪ The number of RBD-specific memory B-cells remains unchanged at 6.2 months after infection ▪ There was a small but significant increase in the % of IgG-expressing anti-RBD memory cells from 49% to 58% from 1.3 to 6 months ▪ The average number of nucleotide mutations in IGH and IGL was only 4.2 and 2.8, respectively, at 1.3 months, these values increased to 11.7 and 6.5 at 6 months ▪ Expanded clones of memory B-cells were found at 6.2 months. Expanded clones accounted for 12.4% of all antibody sequences at 6.2 months, compared to 32% after 1.3 months.

Study characteristics	Patient demographics Clinical characteristics Test parameters	Primary outcome results
	▪ flow cytometry to isolate individual B lymphocytes with receptors that bound to RBD ▪ binding assays using control and mutant RBDs to examine breadth of antibodies expressed by memory B-cells. Follow-up duration/range/intervals: 1.3 months and 6 months following infection	▪ 43 expanded clones that were present at the earlier point were not detectable after 6.2 months and 22 new, expanded clones appeared. The relative distribution of clones that appeared at both times varied. *Author conclusions:* Memory B-cell response to SARS-CoV-2 evolves between 1.3 and 6 months after infection in a manner that is consistent with antigen persistence …. memory B-cells that evolved during the observation period express antibodies with increase neutralizing potency and breadth. … although the magnitude of the RBD-specific memory B-cell compartment is conserved between 1.3 and 6.2 months … there is extensive clonal turnover and antibody sequence evolution that is consistent with prolonged germinal centre reactions.
Author: **Hartley** *Country:* Australia *DOI:* 10.1126/sciimmunol.abf8891 *Study design:* Case control cohort study *Setting:* Alfred Health Human Research/Monash University *Publication status:* Published	*Patient demographics:* Patients with confirmed COVID-19 (6 severe; 3 moderate; 16 mild); healthy controls Participants: N=25 patients (n=36 blood samples, including 11 paired sample); N=36 healthy controls Mean age: 40 years (range 25 to 67 years) Female: 32% Type of test: ▪ COVID-19 infection confirmed by PCR ▪ flow cytometry. Follow-up duration/range/intervals: 4 to 242 days PSO. 11 patients were sampled twice, first between 21 to 106 days PSO and again at 116 to 242 days	*Immune memory component reported:* *Results:* ▪ RBD and NCP-specific MBCs predominantly expressed IgM+ or IgG1+ and continued to rise to 150 days ▪ RBD-specific IgG+ MBCs were predominantly CD27+ and numbers significantly correlated with follicular helper T-cell numbers ▪ RBD-specific memory B-cell numbers were highest between 100-150 days PSO ▪ Total and IgM+ MBCs in paired samples taken >200days were lower than in the corresponding first samples, whereas IgG+ MBCs remained stable ▪ NCP-specific memory B-cell numbers increased over the first 150 days as well, and in contrast to RBD-specific memory B-cells, they did not decline between 150-240 days *Author conclusions:* Thus, the SARS-CoV-2 antibody response contracts in convalescence with persistence of RBD and NCP-specific B memory cells
Author: **Kang** *Country:* South Korea *DOI:* 10.1093/infdis/jiab159/6184114	*Patient demographics:* PCR-confirmed positive COVID-19 patients; SARS-CoV-2 negative healthy controls (HCs); serum samples from those who had been infected with Middle East respiratory syndrome coronavirus (MERS-CoV) 5 years ago as second control group	*Immune memory component reported:* Memory T-cell *Results:* *Distribution of SARS-CoV-2-specific memory CD4+ or CD8+ T-cells* ▪ SARS-CoV-2-specific OX40+CD137+CD4+ T-cells and CD69+CD137+ CD8+ T-cells persisted at 8 months ▪ Antigen-specific cytokine-producing or polyfunctional CD4+ T-cells were maintained for up to 8 months

Study characteristics	Patient demographics Clinical characteristics Test parameters	Primary outcome results
Study design: Cohort study *Setting:* Seoul National University Hospital or community treatment centre in Daegu *Publication status:* Accepted manuscript;	Participants: n=7 asymptomatic patients; n=9 mildly symptomatic patients; n=8 severely symptomatic patients; n=6 HCs; n=7 MERS controls Median age: 25 years asymptomatic; 48 years mild; 63 years severe; 35 years HCs; 60 years MERS controls Male: 71.4% asymptomatic; 44.4% mild; 75% severe; 83.3% HCs; 85.7% MERS controls Type of test: ▪ COVID-19 infection confirmed by RT-PCR ▪ flow cytometry. Follow-up duration/range/intervals: Serum samples of patients collected at 2, 5 and 8 months after diagnosis or post symptom onset (PSO)	▪ The frequency of SARS-CoV-2-specific CD4+ memory T-cells was significantly higher in patients with severe disease than in the asymptomatic patients at 2 and 5 months POS. A similar, albeit non-significant trend was detected in comparison with the patients with mild disease. ▪ The frequency of SARS-CoV-2-specific memory CD4+ T-cells tended to decline over time in all severity groups and the significance of the differences between the groups decreased. ▪ SARS-CoV-2-specific CD69+CD137+ memory CD8+T-cells was also distinct when compared with the HCs at 8 months PSO. However, the level of SARS-CoV-2-specific memory CD8+T-cells was not significantly different among the severity of COVID-19 patients. ▪ Collectively, a broad memory T-cell response was induced after recovery from COVID-19 and persisted up to 8 months PSO. *Functionality of memory T-cells responding to SARS-CoV-2 antigens* ▪ The levels of at IFN-ɣ,TNF-α and IL-2 in memory CD4+ T-cells 2 and 5 months PSO in patients with COVID-19 tended to be higher than in HCs ▪ The proportion of IL-2 producing memory CD4+ T-cells responding to spike protein from patients with mild and severe disease was higher than from HCs even at 8 months PSO. ▪ IFN-ɣ,TNF-α, and IL-2 Ag-specific memory CD4+ T-cells in patients with severe disease was significantly increased compared to that of asymptomatic patients at 2 months PSO. ▪ The functionality of Ag-specific memory CD4+ T-cells declines over time and the significance of the differences according to disease severity. However, the proportions of cytokine-producing Ag-specific CD8+ T-cells were not significantly different according to disease severity. ▪ Therefore, the functionality of memory CD4+ T-cells responding to SARS-CoV-2 antigens was greatest in symptomatic patients. *Longitudinal analysis of polyfunctional memory T-cell response* ▪ The proportion of polyfunctional CD4+ T-cells tended to be higher in patients with severe disease than in those with mild disease or asymptomatic patients. *Author conclusions:* Memory T-cell response to SARS-CoV-2, based on the frequency and functionality, persists for 8 months post-symptom onset. Further investigations involving its longevity and protective effect from reinfection are warranted.

Study characteristics	Patient demographics Clinical characteristics Test parameters	Primary outcome results
Author: **Long** *Country:* China *DOI:* 10.1038/s41421-021-00250-9 *Study design:* Cohort study *Setting:* Unclear *Publication status:* Published	*Patient demographics:* Individuals recovered from symptomatic (RS) and asymptomatic SARS-CoV-2 infection (RA) and healthy controls (HCs) Participants; n= 20 RS; n=13 RA; n=10 HCs Median age: 49.5 years RS; 47 years RA; 38.5 years HC Male: 45.5% RS; 30.8% RA; 60% HC Type of test: ▪ COVID-19 infection confirmed by PCR ▪ flow cytometry and T-cell ELISpot. Follow-up duration/range/intervals: Mean follow-up for RS and RA were 169 days (IQR 164 to 174 days) - Approximately 6 months	*Immune memory component reported:* Memory B-cell and T-cell responses *Results:* ▪ Comparatively low frequencies of memory B-cells specific for the receptor-binding domain (RBD) of spike glycoprotein (S) persisted in the blood of individuals who recovered from infection (2/13 RAs and 10/20 RS). ▪ The distribution of naïve (CD54RA+CCR7+), central memory (CD54RA-CCR7+) and effector memory (CD45RA-CCR7-) in CD4+ and CD8+ T-cells was similar in convalescent and HCs. ▪ The S1 memory T-cell peptide pool had higher reactivity in patients than HCs. ▪ The magnitude of B-cell spot number was not correlated with the levels of virus-specific IgG in peripheral blood. ▪ In virus-specific memory B-cell functional test, the rate of individuals with positive B-cell ELISPOT results were higher in the RS group compared with the RA group (50% vs. 15.4%). Memory CD4+ or CD8+ T-cell frequencies showed no difference. Therefore, the long-term humoral immunity to SARS-CoV-2 was higher in individuals who experienced a severe COVID-19 disease course, while T-cell memory did not show a similar pattern.
Author: **Sandberg** *Country:* Sweden *DOI:* 10.1101/2021.03.17.435581 *Study design:* Cohort study *Setting:* Karolinska KI/K COVID-19 Immune Atlas project *Publication status:* Pre-print	*Patient demographics:* Convalescent patients followed-up at 5 and 9 months. Moderate patients had been treated in the infectious disease unit and severe patients had been treated in the ICU Participants: ▪ For 5 month follow-up n=8 moderate; n=9 severe convalescent patients ▪ For 9 month follow-up n=8 moderate; n=5 severe convalescent patients Mean age: ▪ At 5 month follow-up 58 years (moderate group); 56 years (severe group) ▪ At 9 month follow-up 58 years (moderate group); 61 years (severe group)	*Immune memory component reported:* ▪ S1 and N-specific B cell memory ▪ memory B-cell-derived antibody-secreting cells (mASCS) ▪ Polyfunctional SARS-CoV-2-specific T-cell memory *Results:* ▪ S1-specific IgG mASCs were detected in all patients at both 5 and 9 months ▪ N-specific IgG mASCs were detected in all but one patient who was below positive threshold at 9 months ▪ Very few patients had detectable S1 or N-specific IgA mASCs after stimulation ▪ There was a high variation in frequencies of S1- or N-specific cells between patients, ranging from 0.2% to 21% ▪ The numbers of S1-specific mASCs positively correlated with the numbers of N-specific mASCs at 5 months but not 9 months

Study characteristics	Patient demographics Clinical characteristics Test parameters	Primary outcome results
	Male: ▪ At 5 month follow-up 7/8 (moderate group); 6/9 (severe group) ▪ At 9 month follow-up 7/8 (moderate group); 4/5 (severe group) Type of test: ▪ COVID-19 infection confirmed by PCR ▪ Flow cytometry and Fluorospot Follow-up duration/range/intervals: 5 and 9 months	▪ Robust S1- and N-specific MBCs persist up to 9 months ▪ Polyfunctional SARS-CoV-2-specific T cell memory persists up to 9 months ▪ Magnitude of B-cell and T-cell memory did not differ between moderate and severe patients at 5 or 9 months *Author conclusions:* S1 and N-specific IgG memory B-cells are readily detectable in circulation at both 5 and even 9 months post-symptom onset in all patients within this cohort, although the magnitude of these responses is highly variable. A strong polyfunctional T-cell response was observed.
Author: **Sherina** *Country:* Italy and Sweden *DOI:* 10.1016/j.medj.2021.02.001 *Study design:* Cohort study *Setting:* Unclear; plasma samples *Publication status:* Published	*Patient demographics:* Convalescent blood donors who experienced mild to critical disease Participants: 32 samples from 24 patients (mild=11; moderate=4; severe=8; critical=1). 17 patients were sampled at a single time-point (TP), 6 at 2 TPs and 1 at 3 TPs; n=4 HCs Median age overall cohort: ▪ Italian cohort 63 years ▪ Swedish cohort 53.5 years Male overall cohort: ▪ Italian cohort 58% ▪ Swedish cohort 50% Type of test: ▪ COVID-19 infection confirmed by RT-PCR ▪ flow cytometry. Follow-up duration/range/intervals: ▪ TP1 2-4 weeks post-symptom onset (PSO) ▪ TP2 3-6 months PSO ▪ TP3 6-8 months PSO	*Immune memory component reported:* SARS-CoV-2-specific memory B-cells *Results:* ▪ SARS-CoV-2-specific memory B- and T-cells developed and remained present in 95% of the patients followed-up until the latest date of the study, regardless of disease severity. ▪ One patients who had no detectable T-cell response at 4 months had a detectable memory B-cell response. ▪ In patients with samples from >1TP, a clear shift from the production of specific antibodies at the early TP, to the generation of memory B and T-cells at later TP(s) was observed. ▪ Compared to the early TP samples, the number of S1-specific IL-2, IFNɣ, and IL-2/IFNɣ-producing T-cells was significantly higher in the later TP samples, especially those collected at 6 to 8 months. *Author conclusions:* SARS-CoV-2-specific memory B and T-cell responses developed with time and were persistent in all of the patients followed up for 6 to 8 months

Study characteristics	Patient demographics Clinical characteristics Test parameters	Primary outcome results
Author: Sokal Country: France DOI: 10.1016/j.cell.2021.01.050 Study design: Cohort Setting: Patients were recruited from Henri Mondor University Hospital and HD's samples were frozen at EFS Henri Mondor before 2019 Publication status: Published	*Patient demographics:* Patients with SARS-CoV-2 requiring oxygen (S-CoV) and mild ambulatory forms (M-CoV) and healthy controls (HDs) Participants; n=21 S-CoV; n=18 M-CoV; n=6 HDs Median age: 57 Years (S-CoV); 35.5 years (M-CoV) Male: 17/21 S-CoV; 4/18 M-CoV; 3/6 HD Type of test: ▪ COVID-19 infection confirmed by RT-PCR ▪ flow cytometry. Follow-up duration/range/intervals: First samples collected median 18.8 days (±SD; 8.8 days) and 35.5 days (±SD: 12.8 days) after disease onset. Two addition samples were collected at 3 months (M3) and 6 months (M6)	*Immune memory component reported:* Memory B-cells (MBCs) *Results:* ▪ At 6 months, following the resolution with time of the primary extra follicular response, the remaining B-cells were separated in three populations: a mixture of naïve/transitional B-cells; a resting MBC population and a CD95+ activated cluster. This activated cluster could be further subdivided into three distinct populations ○ $CD21^{low}CD27+CD38+CD71+$ activated B-cells ○ $CD21^{low}CD27^{low}CD38-CD71^{low}CD11c+FcRL5+$ cells, likely to correspond to atypical memory and or double negative (DN2) population ○ $CD21+CD27^{int/+}CD38-CD71^{low}CD95+$ cells corresponding to a cluster with intermediate characteristics between ABCs and MBCs ▪ Further analysis found that at 6 months only a few B cell clusters were related to the original antibody secreting cells (ASCs) (from the primary response), whereas they were increased in the MBC resting compartment by that time ▪ There was limited clonal overlap of S-specific MBCs with the initial ASC response, suggesting that two distinct, albeit synchronous, responses take place in COVID-19 patients ▪ In contrast with the rapid disappearance of S-specific ASCs, both the percentage and absolute number of S-specific CD27+IgD-B-cells appeared stable up to 6 months and even continuously increased up to that time point in a subset of convalescent S-CoV patients ▪ Most M-CoV patients still harboured a sizeable population of S-specific MBCs at M6 and only one out of 16 showed a frequency of S-specific switched CD27+ MBCs below that of pre-pandemic HCs ▪ Two M-CoV patients whose serum levels of S-Specific IgG dropped below detectable levels by M6 still harboured a clear population of S-specific MBCs at M6 demonstrating an induction of a robust and stable S-specific MBC population in both M- and S-CoV patients ▪ There was a positive, albeit modest, correlation between early CD27+CD71+ ABCs at baseline and S-specific MBCs at 6 months indicating that early B-cell activation does not prevent the development of B-cell memory against SARS-CoV-2

Study characteristics	Patient demographics Clinical characteristics Test parameters	Primary outcome results
		Author conclusions: These findings demonstrate that an antigen-driven activation persisted and matured up to 6 months after SARS-CoV-2 infection and may provide long-term protection.
Author: Tan Country: China DOI: 10.1007/s11684-020-0822-5 Study design: Cohort Setting: Unclear; blood donors Publication status: Published	*Patient demographics:* Blood donors infected with SARS-CoV-2 Participants: n=18; n=10 cases were assessed for memory T-cells at 6-7 months Mean age: 64.7% <60 years in overall sample at 6-8 months (NR for n=10 assessed for memory T-cells) Male: 47.1% in overall sample at 6-8 months (NR for n=10 assessed for memory T-cells) Type of test: ▪ COVID-19 infection confirmed by PCR* ▪ flow cytometry. Follow-up duration/range/intervals: 10 blood samples available at 6-7 months post-infection were used for memory T-cell tests *PCR results NR for five patients	*Immune memory component reported:* CD4+ and CD8+ Memory T-cells *Results:* ▪ Interferon γ-producing CD4+ and CD8+ cells were increased upon SARS-CoV-2 antigen stimulation as compared to non-stimulated samples. *Author conclusions:* These observations indicate that memory T-cells for SARS-CoV-2 can persist for up to 6–7 months post-infection, in agreement with the status of humoral immunity

Appendix 3: Quality Appraisal

Tool for reinfection studies: 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

Tool for immune memory studies: Joanna Briggs Institute. Critical Appraisal Checklist for Cohort Studies 2017. Available at: https://jbi.global/sites/default/files/2019-05/JBI_Critical_Appraisal-Checklist_for_Cohort_Studies2017_0.pdf

Appendix 3.1: Quality Appraisal table 1 of 2: reinfection studies (NIH tool)

	Abu-Raddad 2021 [assessment: 'fair']	Breathnach 2021 [assessment: 'fair']	Hall 2021 [assessment: 'good']	Hanrath 2021 [assessment: 'fair']	Hansen 2021 [assessment: 'good']	Harvey 2020 [assessment: 'poor']	Jefferey-Smith 2021 [assessment: 'fair']	Krutikov 2021 [assessment: 'good']	Leidi 2021 [assessment: 'fair']
1. Was the research question or objective in this paper clearly stated?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2. Was the study population clearly specified and defined?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3. Was the participation rate of eligible persons at least 50%?	Yes	Yes	Yes	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	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	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	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	Unclear	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., categories of exposure, or exposure measured as continuous variable)?	N/A	N/A	N/A	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	Yes	No – All had an antibody test in the database, but type of test and validity unknown	Yes	Yes	Yes
10. Was the exposure(s) assessed more than once over time?	Yes	Yes	Yes	Yes	Yes	No	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	No – All had NAAT, but type of NAAT cannot be determined	Yes	Yes	Yes
12. Were the outcome assessors blinded to the exposure status of participants?	No; Retrospective study	No; Retrospective study	Yes; Prospective study	No; Retrospective study	No; Retrospective study	No; Retrospective study	No; Retrospective study	Unclear; Prospective study	Unclear
13. Was loss to follow-up after baseline 20% or less?	Yes	Yes	Yes	Yes	Yes	Not Reported	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)?	Database analysis; unclear if all confounders measured	Unclear	Yes	No	Yes	Statistical analysis and adjustment for confounders not reported	No	Yes	Unclear

Quality Appraisal table 2 of 2: reinfection studies (NIH tool)

	Lumley 2020 [assessment: 'good']	Manica 2021 [assessment: 'fair']	Masia 2021 [assessment: 'fair']	Mohamadreza 2021 [assessment: 'poor']	Papasavas 2021 [assessment: 'fair']	Perez 2021 [assessment: 'fair']	Pilz 2021 [assessment: 'fair']	Qureshi 2021 [assessment: 'fair']	Sheehan 2021 [assessment: 'fair']	Shields 2021 [assessment: 'fair']
1. Was the research question or objective in this paper clearly stated?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2. Was the study population clearly specified and defined?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3. Was the participation rate of eligible persons at least 50%?	Yes	Yes	Yes	Yes	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	Unclear, Enrollment was not random	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	Yes	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	Unclear	Yes	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	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., categories of exposure, or exposure measured as continuous variable)?	N/A	N/A	N/A	Unclear	Yes	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	Unclear	Yes	Yes	Yes	Yes	Yes	Yes
10. Was the exposure(s) assessed more than once over time?	Yes	Yes	Yes	Yes	Yes	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	Testing methodology insufficiently reported	Yes	Yes	Yes	Yes	Yes	Yes
12. Were the outcome assessors blinded to the exposure status of participants?	Unclear; Prospective study	Unclear	Unclear	No; retrospective	Unclear	No; Retrospective study	No; Retrospective study	No; Retrospective study	No; Retrospective study	Unclear
13. Was loss to follow-up after baseline 20% or less?	Yes	Yes	Yes	Yes	Yes	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	No - only age standardisation in adjusted analyses	Unclear	Unclear	Unclear	No	No	Authors acknowledge confounding by the selection criteria of the analysis	No	Unclear

Appendix 3.2: Quality Appraisal table: immune memory studies (Joanna-Briggs Institute tool)

Study	Were the two groups similar and recruited from the same population?	Were the exposures measured similarly to assign people to both exposed and unexposed groups?	Was the exposure measured in a valid and reliable way?	Were confounding factors identified?	Were strategies to deal with confounding factors stated?	Were the groups/participants free of the outcome at the start of the study (or at the moment of exposure)?	Were the outcomes measured in a valid and reliable way?	Was the follow up time reported and sufficient to be long enough for outcomes to occur?	Was follow up complete, and if not, were the reasons to loss to follow up described and explored?	Were strategies to address incomplete follow up utilized?	Was appropriate statistical analysis used?	*Overall appraisal (include / exclude / seek further info)*
1. Abayasingham	Unclear	N/A	Yes	No	No	N/A	Yes	Yes	N/A	N/A	N/A	*Include*
2. Anand	Unclear	N/A	Yes	No	No	N/A	Yes	Yes	N/A	N/A	N/A	*Include*
3. Breton	Unclear	N/A	Yes	No	No	N/A	Yes	Yes	N/A	N/A	N/A	*Include*
4. Cohen	Unclear	N/A	Yes	No	No	N/A	Yes	Yes	N/A	N/A	N/A	*Include*
5. Dan	Unclear	N/A	Yes	No	No	N/A	Yes	Yes	N/A	N/A	N/A	*Include*
6. Gaebler	Unclear	N/A	Yes	No	No	N/A	Yes	Yes	N/A	N/A	N/A	*Include*
7. Hartley	Unclear	N/A	Yes	No	No	N/A	Yes	Yes	N/A	N/A	N/A	*Include*
8. Kang	Unclear	N/A	Yes	No	No	N/A	Yes	Yes	N/A	N/A	N/A	*Include*
9. Long	Unclear	N/A	Yes	No	No	N/A	Yes	Yes	N/A	N/A	N/A	*Include*
10. Sandberg	Unclear	N/A	Yes	No	No	N/A	Yes	Yes	N/A	N/A	N/A	*Include*
11. Sherina	Unclear	N/A	Yes	No	No	N/A	Yes	Yes	N/A	N/A	N/A	*Include*
12. Sokal	Unclear	N/A	Yes	No	No	N/A	Yes	Yes	N/A	N/A	N/A	*Include*
13. Tan	Unclear	N/A	Yes	No	No	N/A	Yes	Yes	N/A	N/A	N/A	*Include*

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