Health Information and Quality Authority

An tÚdarás Um Fhaisnéis agus Cáilíocht Sláinte

Evidence summary of reinfection and the duration of antibody responses 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 and Youth Affairs, 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

COVID-19	Coronavirus disease 2019
CDC	Centers for Disease Control and Prevention
CI	confidence interval
COVID-19	Coronavirus disease 2019
C_t	cycle threshold
EAG	expert advisory group
ECDC	European Centre for Disease Prevention and Control
HIQA	Health Information and Quality Authority
HPSC	Health Protection Surveillance Centre
HSE	Health Service Executive
HTA	health technology assessment
IgA	immunoglobulin A
IgM	immunoglobulin M
IgG	immunoglobulin G
NPHET	National Public Health Emergency Team
N protein	nucleocapsid protein
RBD	receptor-binding domain
RNA	ribonucleic acid
RQ	research question

RT-PCR	reverse transcription polymerase chain reaction
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
S protein	spike protein
SNP	single nucleotide polymorphism
WHO	World Health Organization

Glossary of key terms

Clade	Related organisms descended from a common ancestor. For example, isolate M of HIV-1 (the human immunodeficiency virus) consists of at least ten clades
Convalescent period	The convalescent period is the time during which an individual has recovered from an infectious disease (i.e. COVID-19) and during which blood serum may contain antibodies against the infectious agent of the disease
Genome	The genetic material of an organism
IgG (Immunoglobulin G)	A class of immunoglobulins including the most common antibodies circulating in the blood that facilitate the phagocytic destruction of microorganisms foreign to the body
Immunocompetent	The capacity for a normal immune response
Immunocompromised	Having the immune system impaired or weakened (such as by drugs or illness)
Lineage	Descent in a line from a common progenitor. Viruses can be grouped into lineages, based on the evolutionary trajectories of the virions and their production mechanisms
Multiplex qPCR	In multiplex q-polymerase chain reaction (q-PCR), two or more target genes are amplified in the same reaction, using the same reagent mix
Nanopore sequencing	Nanopore sequencing is a unique, scalable technology that enables direct, real-time analysis of long DNA or RNA fragments. It works by monitoring changes to an electrical current as nucleic acids are passed through a protein nanopore
Neutralising antibodies (NAb)	A neutralising antibody (NAb) is an antibody that is responsible for defending cells from pathogens, which are organisms that cause disease. They are produced naturally by the body as part of its immune response, and their production is triggered by both infections and vaccinations against infections

Phylogenetic tree	A diagram that represents evolutionary relationships among organisms
Receptor-binding domain (RBD)	A receptor-binding domain (RBD) is a key part of a virus located on its 'spike' domain that allows it to dock to body receptors to gain entry into cells and lead to infection
Seroconversion	The production of antibodies in response to an antigen. Seroconversion timing refers to the first time an individual tests positive for antibodies (based on serial serological samples)
Seropositivity	Having or being a positive serum reaction especially in a test for the presence of an antibody
Single nucleotide polymorphisms (SNPs)	Single nucleotide polymorphisms (SNPs) are the most common type of genetic variation among people. Each SNP represents a difference in a single DNA building block, called a nucleotide
Single nucleotide polymer (SNP) phylogenetic tree	A type of analysis where the single nucleotide polymorphism (SNP) information is used to construct the phylogenetic tree, otherwise known as FST analysis
Titre(s)	The strength of a solution or the concentration of a substance in solution as determined by titration

Evidence summary of reinfection and the duration of antibody responses following SARS-CoV-2 infection

Key points

- This evidence summary reviewed the possibility of reinfection and the duration of antibody responses (beyond 60 days) following SARS-CoV-2 infection.
- Ten studies, representing seventeen patients, were identified that relate to reinfection following recovery from a laboratory-confirmed SARS-CoV-2 infection. Comparative whole genome sequencing demonstrated that fourteen patients had confirmed reinfections (whereby the first and second infections were from different viral strains), and three patients had strong evidence of reinfection.
- The average age of reinfected cases was 40 years and 71% were male. The time interval between initial infection and reinfection ranged between 13 and 142 days. Severity ranged from asymptomatic to severe in both first and second infections. There was one fatality in an elderly woman who was immunocompromised.
- In five reinfection cases, the first and second infections were caused by SARS-CoV-2 with different lineages or clades, strongly indicating that infections were caused by different viral strains.
- The number of single nucleotide polymorphisms was reported in eight studies. Between nine and 24 variants were discovered comparing the first and second genomes across all patients. With an average estimated SARS-CoV-2 mutation rate of 33 nucleotides per year (or 2-3 nucleotides per month), in all cases it is likely that the second infection was caused by a second strain of SARS-CoV-2, rather than prolonged shedding of viral RNA from the first infection.
- One patient had serial anti-SARS-CoV-2 IgG antibody testing on reinfection, with IgG seroconversion taking place, further strengthening the case for 'true' reinfection.
- Twenty-two studies were identified that examined the duration of antibody responses (Immunoglobulin G [IgG] and or neutralising antibodies) following SARS-CoV-2 infection for longer than 60 days. Maximum follow-up was 182 days in one study and mean maximum follow-up was 97 days across all studies. Mean sample size was 79 (range: 3-349).

- All studies that reported IgG seropositivity at 60-79 days post-infection reported 100% seropositivity (n=9 studies), which declined to 78%-100% at 80-99 days (n=6 studies). Of seven studies that reported data ≥100 days post-infection, four reported 100% seropositivity and one reported >70% up to 182 days.
- Twelve studies reported seropositivity rates for neutralising antibodies, with some reporting at more than one time point. Three studies reported 100% seropositivity rates at 60-79 days, which declined to 53%-100% at 80-99 days (n=4 studies). Three of seven studies reported 100% seropositivity at ≥100 days post-infection. One study reported a neutralising capacity of 70% up to 182 days and three studies reported a significant decline in seropositivity over time.
- In terms of the longitudinal analysis of anti-SARS-CoV-2 IgG antibody titres, just over half of studies (n=7/12) found that titres were maintained, or increased, until the end of follow-up, while five studies reported a reduction in IgG titres over time.
- All but one study that reported neutralising antibody titres reported a decline over time, in particular at the later stages of follow-up.
- A limitation of this review is the absence of data on cell-mediated immunity, as protection from reinfection or clinically significant disease involves both cell-mediated and antibody-mediated immune responses. Therefore, the detection of antibodies alone does not guarantee protective immunity (and their absence does not preclude it). Additionally, the strength of the response (in terms of antibody titres) needed to confer protective immunity is unknown.
- In summary, true reinfection can occur following SARS-CoV-2 infection. While evidence suggests antibody-mediated responses can be detected in most patients beyond two months and even up to six months post-symptom onset, the neutralising capacity declines substantially in the late convalescent period, raising concerns over the duration of protective immunity.

Evidence summary of the duration of immunity and reinfection following SARS-CoV-2 infection

Introduction

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 account of expert interpretation of the evidence by HIQA's COVID-19 Expert Advisory Group.This evidence summary was developed to address the following research question (RQ):

> What is the rate of reinfection/duration of immunity in individuals who recover from a laboratory-confirmed coronavirus infection?

The first report on this RQ was published on 13 May 2020 with subsequent updates on 9 June 2020 and 6 August 2020.

For the present update, the scope of the review was refined to focus on the following specific research questions:

For individuals who recover from a laboratory-confirmed coronavirus infection:

1. **Is reinfection with SARS-CoV-2 possible?**
2. **What is the long-term duration of the antibody response?**

The processes as outlined in HIQA's protocol (available on www.hiqa.ie) were followed. Relevant databases of published literature and pre-print servers were searched. For this update, studies on suspected cases of reinfection were only included if whole genome sequencing comparing the first and second infections was performed. For studies on the long-term duration of antibody responses, only studies that measured Immunoglobulin G (IgG) and or neutralising antibodies beyond 60 days post-infection were included.

Results

The database search (Pubmed, Embase and EuropePMC) retrieved 3,272 citations. This was supplemented by a limited grey literature search, including desktop searching (Google and online newspaper articles). Following screening of citations, 135 studies were included for full text review. These full texts were reviewed in duplicate and 28 studies met our inclusion criteria, including ten studies on reinfection[1-10] and 22 studies on the duration of antibody responses (IgG and or neutralising antibodies).[11-31] All included studies were case reports or case series.

Reinfection

Ten studies, representing seventeen patients, were identified that relate to reinfection following recovery from SARS-CoV-2 infection. Two case series reported on six patients in India,[1, 9] one case series reported on four patients in Qatar,[7] three case studies reported on patients in the US,[4, 8, 10] and four case studies reported on patients in Belgium,[6] Ecuador,[3] Hong Kong[5] and the Netherlands.[2]

The mean age of patients was 39.8 (range: 25 to 89) and 71% (N=12) were men. The time interval between RT-PCR confirmation of initial infection and reinfection ranged from 13 to 142 days. Six of the ten studies are as yet only published as pre-prints, so have not been formally peer-reviewed. Table 1 provides summary data on all seventeen cases and Appendix 1 provides details of the testing procedures and laboratory methods employed by each study.

Across all cases, severity ranged from asymptomatic to severe on initial infection and reinfection, without any clear pattern in terms of disease progression. There was one fatality[2] which occurred in a patient who was severely immunocompromised. This patient (89 years of age) was suffering from Waldenström's macroglobulinemia and treated with B-cell depleting therapy. All other patients were seemingly immunocompetent.

Initial infection and reinfection events were all confirmed by RT-PCR. Of the four studies that reported cycle threshold (C_t) values at both episodes of infection, three patients had higher viral loads on reinfection[1, 2] and two patients had lower viral loads.[4, 8]

Table 1 Summary of studies on possible reinfection cases

Author Study type	Patient demographics	Location	Severity of illness	Interval	Whole genome sequencing differentiation	
					Single nucleotide polymorphisms	**Lineage/ clade comparison**
Abu-Raddad 2020[7] **Case series (N=4 reinfection cases out of a cohort of N=133,266 infections)**	Patient 1: 20-29 year old male Patient 2: 40-49 year old male Patient 3: 40-49 year old female Patient 4: 20-29 year old male	Qatar	Most patients asymptomatic*, however clinical course of the reinfection cases not reported	Patient 1: 45 days Patient 2: 70 days Patient 3: 87 days Patient 4: 54 days	N=2 patients: authors consider confirmed reinfection cases due to "multiple changes in allele frequency" and presence of the D614G mutation (23403bp A>G) N=2 patients: authors consider 'supportive' evidence for reinfection including presence of D614G mutation	N/R
Goldman 2020[8] **Case study**	60-69 year old male	United States	Severe initial infection with hospitalisation, mild on reinfection	118 days	10 nucleotide differences (confirmed reinfection)	1st infection: clade 19B, 2nd infection: clade 20A
Gupta 2020[1] **Case series (N=2)**	Patient 1: 25 year old male	India	Patient 1: Asymptomatic on initial infection and reinfection	Patient 1: 108 days	Patient 1: 9 nucleotide differences (confirmed reinfection)	N/R
	Patient 2: 28 year old female	India	Patient 2: Asymptomatic on initial infection and reinfection	Patient 2: 111 days	Patient 2: 10 nucleotide differences (confirmed reinfection)	
Larson 2020[10] **Case study**	42 year old male	United States	Mild initial infection, severe reinfection	51 days	'Several variations' noter, however only partial genome recovered from 1st infection (supportive evidence)	2nd infection: Lineage B.1.26 (not available for 1st infection)

Mulder 2020[2] **Case study**	89 year old female	The Netherlands	Severe initial infection, more severe reinfection and subsequent death	59 days	10 nucleotide differences (confirmed reinfection)	Sequences did not cluster in phylogenetic tree
Prado-Vivar 2020[3] **Case study**	46 year old male	Ecuador	Mild initial infection, more severe reinfection	63 days	18 nucleotide differences (confirmed reinfection)	1st infection: lineage B.1.p9 lineage, clade 20A 2nd infection: lineage A.1.1, clade 19B
Shastri 2020[9]shas **Case series (n=4)**	Patient 1: 27 year old male	India	Patient 1: Mild initial infection, mild/moderate reinfection	Patient 1: 60 days	8 nucleotide differences (confirmed reinfection)	3 patients infected on both occasions with lineage B.1.1, clade A2a One patient had a shift in lineage from B.1 to B In terms of subclades, one patient clustered in different subclades on reinfection
	Patient 2: 31 year old male		Patient 2: Asymptomatic initial infection, mild reinfection	Patient 2: 59 days	9 nucleotide differences (confirmed reinfection)	
	Patient 3: 27 year old male		Patient 3: Asymptomatic initial infection, mild reinfection	Patient 3: 13 days	9 nucleotide differences (confirmed reinfection)	
	Patient 4: 24 year old female		Patient 4: Mild intial infection, mild/moderate reinfection	Patient 4: 48 days	12 nucleotide differences (confirmed reinfection)	
Tillett 2020[4] **Case study**	25 year old male	US	Mild initial infection, severe reinfection with hospitalisation	48 days	11 nucleotide differences (confirmed reinfection)	1st and 2nd infections from same clade (20C)
To 2020[5] **Case study**	33 year old male	China	Mild initial infection, asymptomatic reinfection	142 days	24 nucleotide differences (confirmed reinfection)	1st infection: GISAID clade V, Nextstrain clade 19A, Pangolin lineage B.2 2nd infection: GISAID clade G, Nextstrain clade 20A, Pangolin lineage B.1.79

Van Elslande 2020[6] **Case study**	51 year old female	Belgium	Moderate initial infection, mild reinfection	93 days	11 nucleotide differences (confirmed reinfection)	1st infection: lineage B.1.1 2nd infection: lineage A

*most of those infected were identified through random testing campaigns, surveys or contact tracing

N/R – not reported

In terms of antibody testing, while a number of studies sampled patients at either initial infection or reinfection, only two studies performed IgG testing at both infection events. In the first study, involving a 46 year old male, IgG was negative at the initial infection, four days post-symptom onset, and positive on reinfection, 30 days post-symptom onset.[3] The timing of testing may have impacted the findings, however, as the first sample may have been taken prior to seroconversion taking place. In the second, involving a 33 year old male, IgG was negative at the initial infection, 10 days post-symptom onset.[5] Seroconversion occurred following the reinfection event – IgG was negative at serial testing days 1-3 post-hospitalisation, and positive on day five post-hospitalisation.

Fourteen reinfection cases were confirmed by whole genome sequencing, confirming that the first and second infections were caused by different viral strains, although the degree of separation between both strains varied (Table 1). Three reinfection cases could not be confirmed by sequencing due to insufficient genetic material extracted from the initial infection. However, strong genetic evidence for reinfection was still present.

With the exception of the study by Abu-Raddad et al.,[7] all confirmed cases included a quantification of the number of single nucleotide polymorphisms comparing first and second infections (range: eight[9] to 24[5] nucleotide differences, Table 1 and Appendix 2). In addition, five studies presented stronger evidence of reinfection through phylogenetic analysis; in each case the first and second infections belonged to different lineages or clades.[3, 5, 6, 8, 9]

The presence of rare mutations in one viral strain strengthens the case for reinfection. For example, in the case presented by To et al.,[5] the second genome also contained the mutation nsp6 L142F, which is rarely found (only 0.009% of genomes deposited into GISAID, an open-access database of genomic data on influenza and coronaviruses, contained this mutation as of 20 August 2020[32]).

Mutations that confer the D614G amino acid change in spike protein was found on reinfection in a number of studies.[5, 7, 8, 10] This mutation defines the SARS-CoV-2 strain with greater replicative fitness[33] and is now present in most circulating SARS-CoV-2 strains.[5] Another genetic variation, 22882T>G (S:N440K) within the receptor-binding domain of the spike protein which possibly confers resistance to neutralising antibodies, was detected in one study.[1]

Only one study assessed reinfection rate.[7] In this study, potential reinfection cases were identified among a larger cohort of 133,266 laboratory-confirmed SARS-CoV-2 cases, which encompassed all RT-PCR samples in Qatar between 28 February and 12 August 2020. Reported cases were mainly among young and healthy men; no

deaths occurred and of nine hospitalisations at any time, only one occurred during reinfection with most for isolation purposes. Of the initial cohort of 133,266 cases, 15,808 had multiple RT-PCR swabs and of these, 243 people had at least one positive swab that was ≥45 days from the first positive swab. Fifty four of the 243 cases had 'strong' or 'good' evidence of reinfection in accordance with the prespecified criteria. These criteria included the pattern and magnitude of the change in PCR cycle threshold (C_t) value across repeated samples, time interval between samples, purpose of PCR testing (i.e., symptoms, contact tracing, or survey/testing campaign), and case severity (by WHO classification). Ultimately, only 12 cases were suitable for whole genome sequencing, two of which were considered 'confirmed' reinfection based on genomic analysis, with a further two cases considered 'supportive' of reinfection (partial genomes of inferior quality were retrieved, limiting analysis) (Table 1). Overall, comparing these four cases with all the patients with adequate serial sampling data, the authors estimated the risk of reinfection to be 0.01% (95% CI: 0.01-0.02%) and the incident rate of reinfection to be 0.36 (95% CI: 0.28-0.47) per 10,000 person weeks.

Duration of immune response following SARS-CoV-2 infection

Twenty-two studies were identified that examined the duration of antibody responses (IgG and or neutralising antibodies) following SARS-CoV-2 infection longitudinally for 60 days or longer (Table 2).[11-31, 34] Maximum follow-up was 182 days in one study[29] and mean maximum follow-up was 97 days across all studies (standard deviation=30.2). Mean sample size was 81 individuals (range: 3 to 349). Seven studies were conducted in China, three in Germany, three in the UK, two in the US, and one each in Australia, Canada, France, India, Italy, Portugal, and Sri Lanka. All studies were case series and 86% were published as pre-prints (19/22). A wide variety of testing platforms were used (Appendix 1).

Table 2 provides IgG and neutralising antibody detection rates at 60-79, 80-99 and ≥100 days follow-up. Data are truncated by maximum study follow-up, and in most instances only a minority of participants in individual studies were followed for this length of time.

Twenty studies presented data on IgG seropositivity (mean sample size 75; range 3-349). Nine studies reported a 100% seropositivity rate at 60-79 days post-infection.[11, 12, 16, 20-22, 24, 30, 31] This subsequently declined to 77.8%[14] or was maintained at 100%[12, 24-26] at 80-99 days (n=6 studies). Of the seven studies with the longest follow-up (≥100 days), four studies reported 100% seropositivity rate.[15, 17, 18, 22] Another study did not report seropositivity rate, however noted a significant reduction in IgG titres beyond 100 days.[28] One study reported greater than 70%

seropositivity up to 182 days (6 months)[29] and another reported that a 'large proportion' of 271 participants were seropositive up to 150 days post-infection.[13]

Twelve studies reported seropositivity rates for neutralising antibodies (mean sample size 100; range 3-349). Three studies reported 100%[21, 24, 27] seropositivity rates at 60-79 days post-infection, which declined to 53.3%[19] or was maintained at 100%[24, 25] at 80-99 days (n=4 studies). At ≥100 days, three studies reported 100% seropositivity;[15, 17, 27] one study reported a neutralising capacity of 70%[29] up to 182 days while three studies reported a significant decline in seropositivity over time.[13, 18, 28]

In terms of the longitudinal analysis of anti-SARS-CoV-2 IgG antibody titres, just over half of studies (n=7/12) found that titres were maintained, or even increased, until the end of follow-up.[14, 15, 17, 21, 22, 25, 26] This finding includes the study with the longest follow-up (Wu et al.[29]). In this study, titres of anti-SARS-CoV-2 IgG-N and IgG-S reached their peaks at weeks 4 and 5, respectively. After a contraction phase, in which titres constantly decreased during weeks 6 to 14, IgG-N and IgG-S titres stabilised and were maintained at high levels until the end of the observation period of 26 weeks post-symptom onset.

Other studies reported a reduction in IgG titres over time.[11, 12, 18, 23, 28] This observation was most pronounced at the later stages of follow-up; in the study by Isho et al. the IgG response against receptor-binding domain (RBD) of the spike protein showed approximate decreases of 25% and 46% by day 105 and day 115, respectively.[18]

On the other hand, neutralising antibody titres declined over time in nine studies,[15, 17, 18, 21, 23, 26-29] with only one study reporting sustained levels.[25] Again, the decline in titres was most notable at the later time points; in the study by Isho et al. titres dropped most drastically in samples 106-115 days post-symptom onset.[18] One study reported a gradual reduction until the average value fell below the cut-off value (limit of detection for the assay to determine a positive result) at week 12.[29] Another study found a reduction in titres only in patients with asymptomatic illness (levels were maintained in mild cases).[21] The study by Gontu et al. reported a notable acceleration in decline in virus neutralisation titres above 1:160 starting 60 days post-symptom onset (titres above 1:160 are commonly necessary for donors of convalescent plasma).[17]

Table 2 Summary of studies of SARS-CoV-2-specific IgG and neutralising antibodies ≥60 days

Immunoglobulin G (IgG)	
60-79 days post-onset	
Adams 2020	50-60+ days post-onset: N=9/9 seropositive; including N=2/2 positive at ≥60 days.*
Dittadi 2020	47-72 days post-onset: 100% sensitivity by Abbott and Maglumi (N=between 15 and 18 patients)
Gallais 2020	47-69 days post-onset: N=9/9 seropositive by Abbott and Euroimmun ELISA; N=7/9 seropositive by Biosynex lateral flow assay
Kreer 2020	69 days post-onset: N=1/1 seropositive
Lei 2020	≥65 days post-onset: N=3/3 seropositive
Liu 2020b	Day 61-65 post-onset: Mild: 2/2 seropositive for total antibodies (IgA/IgG/IgM) Severe: 14/14 seropositive for total antibodies (IgA/IgG/IgM) (at 61-65 days, it is presumed IgG is the prevailing antibody detected)
Nayak 2020	≥60 days post-onset: N=4/4 seropositive
Yang 2020b	Of N=55 patients: N=1 at 76 days post-discharge seropositive N=8 at 60-75 days post-discharge seropositive N=10 at 50-60 days post-discharge seropositive N=55 ≥28 days post-discharge seropositive
Xiao 2020	64-85 days post-onset: N=17/17 seropositive
80-99 days post-onset	
Dittadi 2020	81-109 days post-onset: Sensitivity was 100% by Abbott and 87.5% by Maglumi (N=between 15 and 18 patients)
Fill Malfertheiner 2020	60-90 days post-onset: N=21/27 (77.8%) seropositive
Muecksch 2020	≥81 days post-onset: sensitivity ranged from 97.6% (Roche and Siemens) to 70.7% (Abbott); N=41
Nayak 2020	84 days post-onset: N=2/2 seropositive
Pepper 2020	Median 86 days post-onset: 100% of N=15 seropositive
Seow 2020	100% (63/63) seropositive after 8 days post-symptom onset, including 1 patient at 94 days post-symptom onset
≥100 days post-onset	
Figueiredo-Campos 2020	Up to 150 days post-onset, "large proportion" of N=271 seropositive
Flehmig 2020	106-109 days post-onset, 100% of N=3/3 seropositive
Gontu 2020	100-138 days post-onset, 100% sampled seropositive; only N=4 of 175 had undetectable levels at initial sampling
Isho 2020	63-112 days post-onset: N=11/11 seropositive**

Liu 2020a	Median 122 days post-onset (range: 60 to 136 days): N=30/30 seropositive
Wheatley 2020	≥100 days post-onset, significant reduction in IgG titres (total N=64 patients)
Wu 2020	Up to 26 weeks (182 days/6 months): >70% seropositive at end of follow-up. Total sample: N=349
Neutralising antibodies (NAbs)	
60-79 days post-onset	
Lei 2020	≥65 days post-onset: N=3/3 detectable
Nayak 2020	≥60 days post-onset: N=4/4 had neutralising antibodies
Wang 2020	60-90 days post-onset: N=30/30 developed a neutralising antibodies response; titres declined gradually over the 3-month study period
80-99 days post-onset	
Jeewandara 2020	≥90 days post-onset, 53.3% of N=15 had neutralising antibodies. Decline over time, from 100% day 29-36. All below the cut-off value were those who had mild or asymptomatic illness
Muecksch 2020	≥81 days post-onset: a broad range of SARS-CoV-2 neutralising titres were evident in individual sera, that decreased over time in the majority of participants
Nayak 2020	84 days post-onset: N=2/2 had neutralising antibodies
Pepper 2020	Median 86 days post-onset: 100% of N=15 developed SARS-CoV-2-specific neutralising plasma
≥100 days post-onset	
Figueiredo-Campos 2020	Up to 150 days post-onset, level of SARS-CoV-2 neutralisation activity was found to be proportional to IgG titre
Flehmig 2020	106-109 days post-onset, 100% of N=3/3 detectable
Gontu 2020	100-138 days post-onset: robust viral neutralisation responses persisted for at least 100 days, however a notable decline in virus neutralisation titres after 60 days
Isho 2020	106-115 days post-onset: significant drop in neutralising capacity in N=9 sampled
Wang 2020	≥105 days post-onset: N=2/2 had neutralising antibodies
Wheatley 2020	≥100 days post-onset, significant reduction in neutralising capacity (total N=64 patients)
Wu 2020	Up to 26 weeks (182 days/6 months): >70% had significant neutralising capacity. Total sample: N=349

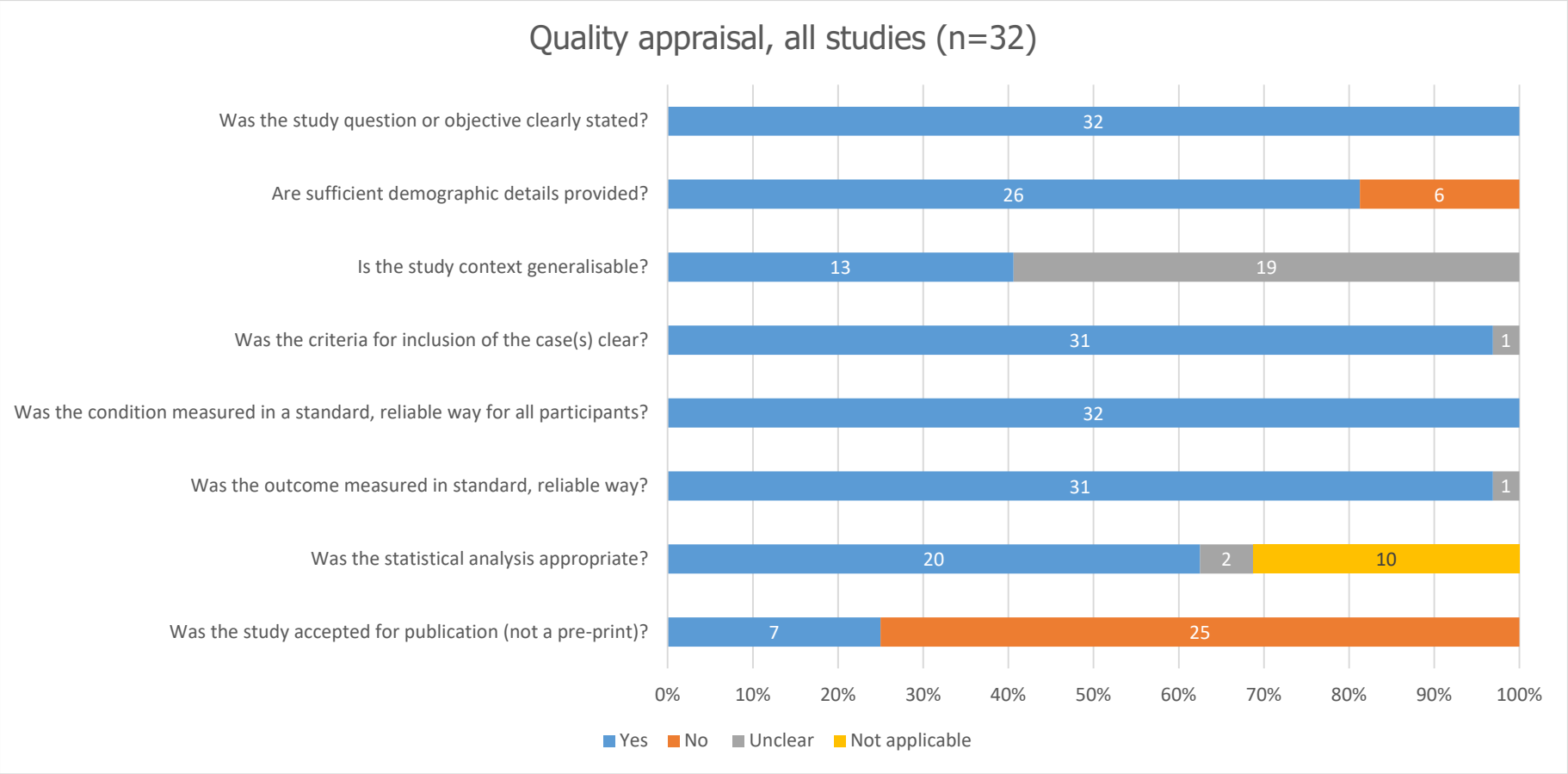
Note – duration denotes longest follow-up in included studies. Duration of immune response inconsistently reported as either duration from symptom onset, post-PCR diagnosis, post-admission or post-discharge.
*Data derived from graph (Figure 1 in Adams 2020)
**Data derived from graph (Figure 3 in Isho 2020)
N/R – not reported

Methodological quality

Figure 1 provides details of the quality appraisal of all 32 included studies, across eight critical domains. The overall quality of evidence was low due to the inherent biases in included study designs.

In general, study questions were clearly stated (n=32/32) and the reporting of the condition (n=32/32) and outcomes (n=31/32) were conducted in a standard, reliable way. Sufficient demographic details were provided in 26 of the 32 studies. Regarding studies on the duration of antibody responses, concerns exist regarding the variability in the diagnostic test accuracy and limits of detection of testing platforms used. Studies employing tests not available in Ireland limit the generalisability of the findings. Overall, 78% of studies (n=25) included in this review were published as pre-prints that have not yet been formally peer-reviewed raising additional concerns about overall quality and the potential for results to change prior to formal publication. Pre-prints were checked to see if published prior to final publication.

Figure 1 Quality assessment domains



Notes:
Data presented for all included studies (n=32); numbers on bars indicate number of studies that were deemed yes/no/unclear/not applicable for each question.
The same risk of bias tool was used across all study designs due to the lack of clarity in some studies regarding the distinction between cohorts and case series. For the purposes of this assessment, all were considered as case reports or case series.
In terms of context generalisability/applicability, some studies on the duration of antibody responses were deemed 'unclear' due to the use of testing platforms that may not be available in Ireland.

Discussion

Reinfection

In previous reviews conducted by HIQA on immunity following SARS-CoV-2 infection, no true cases of reinfection were identified, and all were classified as 'redetection' based on intermittently positive RT-PCR testing. In this review, based on comparative genomic sequencing, fourteen cases of confirmed reinfection and three cases of probable reinfection were identified.

Accurate epidemiological and virological information, including genomic analysis, is needed to differentiate cases of prolonged viral RNA shedding (resulting in cases of apparent 'redetection') from true reinfection. Laboratory confirmation of two infections by two different strains, supported by phylogenetic data, provides the best evidence of two distinct infections caused by different SARS-CoV-2 viral strains. This can be accomplished with comparative genomic analyses, whereby polymorphisms in nucleotide sequences can provide evidence of differentiation between the viral strains. In addition to the number of polymorphisms, the elucidation of minority variants and rare mutations increase the probability that the viral strains are distinct.

Strong evidence of reinfection also exists if sequences recovered from the two infectious events belong to different genetic clades or lineages.[35] However, even if viral strains are from the same clade or lineage, differences in the number of single nucleotide polymorphisms may indicate different viral strains. While there is currently no clear definition of the phylogenetic differences that are required to consider viruses from two separate episodes as 'different', the greater the number of single nucleotide polymorphisms between the two infections over a given timeframe, the greater the likelihood that the episodes are caused by different viral strains. As the virus is expected to mutate by approximately two to three single nucleotide polymorphisms per month, samples with differences greater than this increase the likelihood that the infections are from different viral strains.[36, 37] In this review, for 14 of the included cases greater number of variants were recorded than would be expected to occur naturally through viral evolution; based on the quality of comparative genomic analysis they could therefore be considered as 'confirmed' reinfections. The remaining three cases were supportive of reinfection; confirmation was not possible due to insufficient genetic material (partial genomes retrieved) during the first infection.

While genomic analysis provides the best evidence for reinfection, other clinical and epidemiological factors strengthen the case for reinfection. In this review, all

patients had distinct clinical courses whereby there was a symptom-free interval (range: 13 to 142 days).

In addition, laboratory testing can support reinfection. Antibody testing was performed in many identified cases, however serial testing to identify seroconversion timing was only performed in one patient.[5] This patient seroconverted for IgG following the second episode, indicating a lack of protective IgG thus increasing the vulnerability to reinfection. Seroconversion for anti-SARS-CoV-2 IgG also supported the hypothesis that the second infection was SARS-CoV-2-related and not as a result of another infectious virus.

As well as the published and pre-print studies discussed in this review, a website that is updated with potential SARS-CoV-2 reinfection cases was identified.[38] An additional seven cases of reinfection were listed (Appendix 4). Sources included news articles and personal communication between reporters and physicians. The cases orginated from Sweden, Spain, Belgium and The Netherlands and concerned two females aged 25 and 53 years, four males aged 30, 62, 60s and 80s and one person whose gender was not reported aged over 60 years. All reinfections were confirmed by sequencing. The interval between positive tests was between 23 and 147 days and severity varied on initial and reinfection events. While these reports cannot be verified as they are not yet formally published, they point to additional reinfection cases outside what is currently known in the scientific literature.

The primary limitation of our findings is the inability to calculate a population-level reinfection rate due to the extremely low number of confirmed reinfected cases identified, given that there have been over 50 million SARS-CoV-2 infections recorded globally at the time of writing.[39] This is an underestimation of all reinfection cases, as confirmation necessitates RT-PCR testing along with whole genome sequencing of both events. In the early stages of the pandemic, asymptomatic or mild disease courses did not meet most national criteria for RT-PCR testing, while at present whole genome sequencing is largely confined to the epidemiological surveillance of circulating strains and for other research purposes. Only one study included in this review sought to estimate the risk of reinfection, and reported a rate of 0.01% (95% CI: 0.01-0.02%) over approximately 5.5 months.[7] These data suggest that reinfection can occur, but it is a rare phenomenon, suggestive of a strong protective immunity following the initial infection.

The phenomenon of reinfection has significant policy implications and confirms that immunity following SARS-CoV-2 is not universal, that is, individuals that have been infected once cannot be definitively considered to be immune. While rarely reported, more evidence and longer follow-up is required to better understand the implications of reinfection. Although there are no documented cases of onward transmission from

a reinfected case, or even from re-detected cases,[40] knowledge is evolving and infection prevention and control, isolation and contact tracing considerations are not likely to differ for the reinfection compared with the first infection.

There are also policy implications for testing in reinfected cases (RT-PCR, antibody or antigen testing). Some jurisdictions only test potential cases of reinfection if a minimum time period has elapsed (for example, in the serial testing of healthcare workers), and others only recommend testing potential cases of reinfection when there is severe illness and or hospitalisation. Existing policies may need to be considered in context of emerging evidence of a real, if possibly rare, potential for reinfection. However, as RT-PCR testing may simply detect viral remnants from the initial infection, it is possible that antigen testing or potentially cell culture testing could potentially be used to identify live virus in the case of suspected reinfection. The optimal testing would include phylogenetic testing, such as whole genome sequencing. Population surveillance through whole genome sequencing of common strains (for example, using the clade or lineage system) is of importance for the epidemiological analysis of suspected reinfections, as well as the analysis of cluster investigations and the assessment of potential nosocomial outbreaks.

Duration of antibody responses

This review found 100% anti-SARS-CoV-2 IgG seropositivity at 60-79 days post-infection, which remained detectable in 78%-100% of patients at 80-99 days follow-up. Greater variability in seropositivity rate was evident in studies that reported data with follow-up duration of 100 days or more, although some studies still reported 100% seropositivity. As for neutralising antibodies, 100% seropositivity rates were reported at 60-79 days post-infection, declining to 53% or maintained at 100% at 80-99 days post-infection. Beyond 100 days follow-up, a number of studies reported a significant decline in seropositivity rates.

In terms of the longitudinal analysis of anti-SARS-CoV-2 IgG antibody titres, the majority of studies (n=7/12) found that titres were maintained, or even increased, until the end of follow-up, while five studies reported a reduction in IgG titres over time. All but one study that reported neutralising antibody titres reported a decline over time, in particular at the later stages of follow-up.

Functional neutralising antibodies specific to SARS-CoV-2 that are produced following infection or vaccination are considered important for viral neutralisation and viral clearance. Even though the functional capacity and titres of neutralising antibodies necessary for viral clearance is not fully understood, many current vaccine design efforts focus on eliciting a robust neutralising antibody response to provide protection from infection.[41] The reduction in neutralising capacity in the later stages

of the convalescent period suggests immunity may not be long-term. If vaccination results in a similar immune trajectory, consideration may be given to the need for repeat vaccine administrations ('booster' doses), or focussing on vaccines that co-target other immune processes.

Our findings of declining neutralising antibodies also has implications for immunotherapy. A notable acceleration in decline in virus neutralisation titres ≥1:160 was observed starting 60 days after first symptom onset in one study,[17] a value that is suitable for potential donors for convalescent plasma therapy.[42] The optimal window for donating convalescent plasma should therefore take into consideration neutralising antibody kinetics. Research in convalescent plasma donation is still evolving and additional studies are needed to validate the findings of this review.

Due to the recent emergence of SARS-CoV-2, the longest follow-up data on the immune response reported in studies included in this review was 182 days (six months). While studies consistently demonstrated anti-SARS-CoV-2 IgG and neutralising antibody detection in most patients beyond 60 days, limitations of this review included potential variability in the accuracy of tests used across studies, poor reporting of the limits of detection employed and small numbers of participants from the original sample who were followed until the study end (without clear criteria as to why follow-up was longer for these individuals). Additionally, while 100% seropositivity was reported in most studies in this review, enrolment in longitudinal cohorts often required seropositivity at baseline. Therefore, these data fail to capture those who never seroconverted (prior evidence summaries by HIQA found that a small number of individuals never develop an antibody response[43]). The limits of detection for SARS-CoV-2-specific antibodies were not uniform across studies, and frequently not reported. Differences in test accuracy, levels of detection, and the use of non-validated tests may partly explain differences observed. While this review was limited by small sample sizes in a number of studies, it is notable that more recent studies typically included a larger number of participants with longer follow-up periods.

An important limitation of this review is that it is known that protective immune responses involve both cell-mediated (through T-cell responses) and antibody-mediated immunity. Therefore, the detection of antibodies alone does not guarantee protective immunity, nor does their absence preclude it. That is, it is currently unknown whether antibody responses or T-cell responses in infected people confer immunity, and it is unknown how strong a response is needed (in terms of antibody titres) for this to occur.

Cell-mediated immunity is still poorly understood in the context of SARS-CoV-2, although research in this area is rapidly evolving. One study that was identified after the date of our database search, and has yet to be peer-reviewed, investigated T-cell, B-cell and immunoglobulin antibody responses in a cohort of COVID-19 patients from the Lombardy region in Italy (with mild to critical disease) and Swedish volunteers (with mild symptoms).[44] Similar to our findings, anti-SARS-CoV-2 IgG titres remained stable up to six months post-symptom onset. This response was still present in 80% (12/15) of patients who were followed 6-8 months post-symptom onset, the longest duration of follow-up we have identified. While IgG titres were maintained up to six months, a significant decline was observed at 6-8 months post-symptom onset. In terms of cell-mediated immunity, T-cell responses against at least one of the SARS-CoV-2 peptide pools was detectable in all patients (n=6) tested at 1-2 months post-symptom onset, and this response was maintained in 96% (22/23) of patients 3-8 months post-symptom onset. Notably, the only patient who had no T-cell response at four months had a detectable memory B-cell response. IgG-producing B-cells were detected in 33% (2/6) and 96% (25/26) of patient samples collected 1-2 months and 3-8 months post-symptom onset, respectively. This study provides additional evidence for a decline in antibody titres beyond six months, with preservation of B-cell and T-cell responses in most patients up to eight months post-symptom onset.

Two other studies were identified that also looked at T-cell responses.[45, 46] The first study found that nearly all patients mounted a T-cell response in the early convalescent period.[45] The second, that has not yet been peer-reviewed, investigated the magnitude of the cellular immune response in 100 donors at six months following primary infection.[46] All patients had a cellular response, and median T-cell responses were 50% higher in donors who had experienced an initial symptomatic infection. These responses were also strongly correlated with peak antibody levels, highlighting the interplay between cell-mediated and antibody-mediated responses.

T-cell studies provide additional evidence regarding pre-existing immunity to SARS-CoV-2 due to cross-protection from endemic coronaviruses, through T-cell recognition. In one study, SARS-CoV-2-reactive CD4+ T cells were investigated in SARS-CoV-2-infected as well as unexposed individuals.[45] Reactive CD4+ T cells were detected in 40%-60% of unexposed donors (serum samples provided between 2015 and 2018), suggesting cross-reactive T cell recognition between seasonal human coronaviruses and SARS-CoV-2. Pre-existing immunity from prior exposure to SARS-CoV-2, or cross-protection from exposure to other related coronaviruses, may partly explain the inter-individual variations in clinical and immunological responses to SARS-CoV-2 infection.

Finally, an important limitation of this review was the large number of pre-print studies retrieved (25 of 32 included studies). The methodological quality of studies that have not undergone a formal peer review process may be of concern. Additionally, as all studies were case reports or case series, concerns may also exist regarding the inherent biases associated with observational study design.

Conclusions

Evidence from seventeen individual patients (across ten studies) indicates that true reinfection can occur following recovery from SARS-CoV-2 infection. Limited evidence from 22 studies suggest anti-SARS-CoV-2 IgG is maintained in most patients beyond two months and even up to six months post-symptom onset, while neutralising antibody titres decline substantially in the late convalescent period.

Protection from reinfection or clinically significant disease involves both cell-mediated and antibody-mediated immunity. Therefore, the detection of antibodies alone does not guarantee protective immunity (and their absence does not preclude it). Additionally, the strength of the response (in terms of antibody titres) needed to confer protective immunity is unknown. For these reasons, the documentation of confirmed reinfection cases, through genetic sequencing, is crucial for our understanding of protective SARS-CoV-2 immunity.

Appendix 1. Reinfection studies

Author DOI Country Study design	Population Patient demographics	Test parameters	Clinical description	Whole genome sequencing	Antibody testing	Peer review status
Abu Raddad 10.1101/2020.08.24.20179457 Qatar Cross sectional analysis	Initial cohort: N=243 SARS-CoV-2 lab-confirmed infected persons with at least one subsequent positive swab ≥45 days after the first positive swab. Of this cohort, 54 had some evidence of reinfection based on timing of tests and symptoms. N=12/54 had paired specimens of first positive and reinfection retrieved for genomic sequencing. Of these 12, four were confirmed or	For N=12 pairs available for genomic sequencing; PCR tested using AccumPower SARS-CoV-2 RT-PCR kit (Bioneer, Korea) or Roche cobas® SARS-CoV-2 test Serological testing: Roche Elecsys® Next-generation sequencing (NGS) library construction was performed using the CleanPlex SARS-CoV-2 Panel (Paragon Genomics, USA; SKU: 918012). NGS libraries quantified using KAPA Library Quantification Kit (Roche, USA; KK4824), and normalized,	Specific to the 12 cases available for genomic sequencing – N/R Overall, of 54 showing evidence of reinfection, nine were hospitalised at any time; all but one occurred following 1st infection and mostly for isolation purposes. No deaths recorded. Of note, the vast majority of infections in Qatar occurred in young and healthy men and had limited severity.	Of the 12 cases with comparative genomic analysis, four cases had conclusive or supporting evidence of reinfection. N=2 cases conclusive evidence for reinfection due to multiple changes of allele frequency and presence of the D614G mutation (23403bp A>G) N=2 cases with supporting evidence for reinfection: D614G mutation present; 'sufficient evidence for differences', however one genome of inferior quality	Of the four cases with conclusive or 'sufficient' evidence of reinfection, antibody test result available for only one, which was negative	Not peer-reviewed (pre-print)

	supporting evidence of reinfection. They were: • Male aged 20-29 • Male aged 40-49 • Female aged 40-49 • Male aged 20-29	pooled, and sequenced on an Illumina MiSeq instrument				
Goldman 2020 **10.1101/2020.09.22.2019 2443** **US** **Case report**	60-69 years. Sex N/R Nursing home resident with history of severe emphysema with home O_2	PCR testing using Xpert® Xpress SARS-CoV-2 test on the GeneXpert Infinity (Cepheid, Sunnyvale, CA) Serological testing for anti-spike, anti-RBD, and anti-N IgG, IgM and IgA using ELISA Whole genome sequencing: Rapid metagenomic next-generation sequencing and in modified multiplexed PCR amplicon method using the ARTIC V3 primers. Clade designations: using NextStrain	1st infection: Severe, requiring hospitalisation. Episode of unstable atrial fibrillation treated with cardioversion and anticoagulation 2nd infection: Less severe, but still requiring hospitalisation. Treated with remdesivir and dexamethasone	Both viral strains were from different clades. 1st infection: clade 19B and 2nd infection: clade 20A Nucleotide difference: 10 high confidence; 5 of these type the March sequence to clade 19B, and 5 type the July sequence to 20A The A23403G mutation was found on reinfection, which confers the D614G amino acid change in spike protein Of note, C_t values higher on reinfection.	1st infection: N/R 2nd infection: Positive IgG decreasing from day 14 to 42	Not peer-reviewed (pre-print)

Gupta 2020 10.1093/cid/ciaa1451 India Case series	**Patient 1:** 25 year old male 2nd infection 108 days after first **Patient 2:** 28 year old female 2nd infection 111 days after first Both healthcare workers	**Patient 1 and Patient 2:** RT-PCR confirmation of SARS-CoV-2 infection. Whole genome sequencing: Capture based* (TWIST biosciences) and amplicon-based** (COVIDSeq, Illumina) Antibody testing: N/R	Both patients asymptomatic on initial infection and 2nd infection. **Patient 1:** C_t value 1st infection: 36; 2nd infection: 16.6 (higher viral load) **Patient 2:** C_t value 1st infection: 28.16; 2nd infection: 16.92 (higher viral load)	**Patient 1:** 9 unique variant differences between initial and 2nd infections **Patient 2:** 10 unique variant differences between initial and 2nd infections. Genetic variation 22882T>G (S:N440K) within the receptor binding domain found in 2nd infection Mutation during 2nd infection conferring resistance to neutralising antibodies was discovered 22882T>G (S: N440K)	Initial infection: N/R 2nd infection: N/R	Published in *Clinical Infectious Diseases*
Larson 2020 **DOI 10.1093/cid/ciaa1436** **USA** **Case report**	42 year old male healthcare worker	RT-PCR test: N/R Serologic test: N/R Whole genome sequencing: ARTIC nCoV-2019 Sequencing protocol, YouSeq SARS-CoV-2 Coronavirus NGS Library prep kit, and SuperScript IV (ThermoFisher Scientific). Global lineage was determined using a subset of SARS-CoV-2 genomes available from the Global Initiative on Sharing All Influenza	1st infection: Cough, subjective fever and myalgia on initial infection. 2nd infection: Fever, cough, shortnesss of breath, gastrointestinal symptoms	Only partial genome recovered from 1st infection, while 2nd infection yielded a nearly complete coding genome. Nucleotide difference: Number of SNP's N/R, although numerous variants noted, one of which was a high confidence variation. Lineage information: 2nd infection: lineage B.1.26 with spike variant D614G	Initial infection: N/R 2nd infection: SARS-CoV-2 spike IgG present after 2 weeks	Published in *Clinical Infectious Diseases*

		Data repository (GISAID accessed, Jun 24, 2020)				
Mulder 2020 10.1093/cid/ciaa1538 The Netherlands Case report	89-year old woman, suffering from Waldenström's macroglobulinemia, treated with B-cell-depleting therapy (immunocompromised) 2nd infection 59 days after first	RT-PCR confirmation of SARS-CoV-2 infection: E-gen. Whole genome sequencing: SARS-CoV-2-specific multiplex qPCR and Nanopore sequencing Antibody testing: WANTAI SARS-CoV-2 Ab and IgM ELISA	1st infection: Patient presented with fever, cough, dyspnoea. Patient discharged after 5 days and symptoms subsided completely. 2nd infection: Two days after a new chemotherapy treatment, patient presented with fever, cough and dyspnoea with SpO_2 90% and RR 40/min. Patient deteriorated and died two weeks later. E-gen C_t value 1st infection: 26.2; 2nd infection: 25.2	The 2 strains differed at 10 nucleotide positions in the ORF1a (4), ORF (2), Spike (2), ORF3a (1) and M (1) genes. Sequences did not cluster in the phylogenetic tree. **Authors' conclusions:** With an average estimated SARS-CoV-2 mutation rate of 33 nucleotides per year (or 5-6 nucleotides per 2 months) it is likely that the 2nd episode was a reinfection rather than prolonged shedding. **Limitations:** PCR negative samples were not retrieved between episodes	At days 4 and 6 IgM negative	Letter to the editor, Published in *Clinical Infectious Diseases*
Prado-Vivar 2020 10.2139/ssrn.3686174 Ecuador Case report	46 year old male 2nd infection 63 days after first	RT-PCR confirmation of SARS-CoV-2 infection: Veri-Q PCR 316 kit (Mico Biomed, South Korea), that target ORF3a and N genes RNA extraction: Quick-RNA™ Viral Kit (Zymo, USA)	Initial infection: Mild (headache, drowsiness). RT-PCR: gene ORF3a, C_t: 36.85 2nd infection: Mild (fever, dyspnoea); symptoms were more severe than initial infection and included odynophagia, nasal congestion, fever of 38.5°C, strong back pain,	Initial infection: 20A clade according to NextClade, and to the B1.p9 lineage in GISAID 2nd infection: 19B clade according to NextClade, and the A.1.1 lineage in GISAID	Initial infection: IgG negative 4 days post-symptom onset; IgM positive 2nd infection: IgM and IgG positive 30 days post-	Not peer reviewed (pre-print)

		Whole genome sequencing: Oxford Nanopore MinION using a tilling PCR protocol developed by ARTIC-Network, and the reads were analysed using the artic-medaka consensus generation tool*** To generate consensus genomes, reads were mapped against reference strain Wuhan-Hu-1 Antibody testing: 1st infection: Qualitative antibody IgG/IgM Rapid Test (SAFECARE BIO-TECH, China). 2nd infection: NovaLisa® SARS-CoV-2 IgG and NovaLisa® SARS-CoV-2 IgM (NovaTec Immundiagnostica GmbH, Germany)	productive cough and dyspnoea. RT-PCR C_t N/R	Therefore viral genomes of initial and 2nd infections belonged to different clades When compared to the Wuhan-Hu-1 reference genome; 8 single nucleotide polymorphism (SNP) in 1st infection and 10 in 2nd. No shared mutations further suggesting that both variants resulted from distinct evolutionary trajectories.	symptom onset Anti SARS-CoV2 IgG: 34.1 Anti SARS-CoV2 IgM: 54.2	
Shastri 2020 10.2139/ssrn.3688220 India Case series	Patient 1: 27 year old male Patient 2: 31 year old male Patient 3: 27 year old male	RT-PCR confirmation of SARS-CoV-2 infection: Patients 1 to 3- TaqPath™ COVID19 CEIVD RT PCR (Applied Biosystems). Patient 4- using the	1st infection: Patients 1 and 4 had mild symptoms; Patients 2 and 3 were asymptomatic 2nd infection: All developed mild symptoms	Across 4 patients, total of 39 mutations identified within the eight genomes, including 22 non-synonymous, 16 synonymous, and 1 stop-coding substitutions.	Patients 1, 2 were tested for IgG after 2nd infection – both negative Patient 3 – not tested	Not peer-reviewed (pre-print)

	Patient 4: 24 year old female	above and Xpert® Xpress SARS-CoV-2 Serology: chemiluminescence (CLIA) with Abbott Architect SARS-CoV-2 anti-NC IgG for patient 1 and 2 and with Roche SARS-CoV-2 anti-NC total antibody (IgM+IgG) for patient 4 Whole genome sequencing: Oxford Nanopore MinION using a tilling PCR protocol developed by ARTIC-Network SARS-CoV-2 genomes were assigned lineages using the package PANGOLIN		Nucleotide differences comparing 1st & 2nd infections: Patient 1: 8 SNPs Patient 2: 9 SNPs Patient 3: 9 SNPs Patient 4: 12 SNPs Lineage/clade information: Patient 1: 1st infection lineage B.1, 2nd lineage B; clade A2a Patient 2: 1st and 2nd infections lineage B.1.1; clade A2a Patient 3: 1st and 2nd infections lineage B.1.1; clade A2a Patient 4: 1st and 2nd infections lineage B.1.1; clade A2a	Patient 4 tested after 2nd infection for total antibody IgM/IgG – negative	
Tillett 2020 10.2139/ssrn.3680955 USA Case report	25 year old male 2nd infection 48 days after first	RT-PCR confirmation of SARS-CoV-2 infection: 1st infection: Taqpath COVID-19 (EUA) Multiplex assay or CDC Real-Time PCR Whole genome sequencing: RNA extraction using QIAGEN DNase I; PCR amplification using KAPA	Initial infection: Mild (sore throat, cough, headache, nausea, diarrhoea) RT-PCR C_t 35.24 2nd infection: Severe (initial fever and respiratory symptoms followed by hypoxia and hospitalisation. Supplemental O_2 delivered	First and second viral genomes belonged to the same clade (20C; with all 5 mutations that are clade-defining) Nucleotide difference: 1st infection had 4 single nucleotide variants (SNVs) absent from the 2nd; 2nd infection had 7 SNVs absent from the 1st	Initial infection: No testing performed 2nd infection: IgM and IgG positive 7 days post-symptom onset	Not peer reviewed (pre-print)

		HiFi HotStart. Following enrichment, libraries were pooled and sequenced with an Illumina NextSeq 500 Phylogenetic analysis of WGS isolates were made in comparison with 171 contemporaneous sequences from Nevada (reference strain MN908947.3) Antibody testing: N/R	and myalgia, cough and shortness of breath reported) RT-PCR C_t 35.31	**Author's conclusion:** Viruses possessed a level of genetic discordance that cannot be explained by short term *in vivo* evolution		
To 2020 10.1093/cid/ciaa1275 China Case report	33 year old male from Hong Kong 2nd infection 142 days after first	RT-PCR confirmation of SARS-CoV-2 infection: LightMix® E-gene kit IgG against SARS-CoV-2 nucleoprotein was performed using Abbott SARS-CoV-2 IgG assay Whole genome sequencing: RNA extraction using Qiagen Viral RNA Mini Kit. Bioinformatics analysis of nanopore sequencing data was performed using the workflow from ARTIC network. Maximum-likelihood whole genome phylogenetic tree was constructed using IQ-	Initial infection: Fever, cough, sore throat, sputum, headache 2nd infection: Asymptomatic (tested positive by SARS-CoV-2 RT-PCR on the posterior oropharyngeal saliva taken for entry screening at the Hong Kong airport). CRP elevation consistent with acute infection: 8.6 mg/L	Initial infection: GISAID clade V, Nextstrain clade 19A, and Pangolin lineage B.2 with a probability of 0.99; phylogenetically closely related to strains collected in March/April 2020 (US or England) 2nd infection: GISAID clade G, Nextstrain clade 20A, and Pangolin lineage B.1.79 with a probability of 0.70; phylogenetically closely related to strains collected in July/August 2020 (Switzerland and England) Nucleotide difference: 24; the virus genome from the	Initial infection: IgG negative 10 days post-symptom onset 2nd infection: Seroconversion occurred. IgG negative at 10 days post-symptom onset (1 day post-hospitalisation) and serial samples negative (1-3 days post-hospitalisation)	Published in *Clinical Infectious Diseases*

		TREE2, with substitution model TIM2+F as the best predicted model by BIC. Clade information described using GISAID, Nextstrain, and Pangolin nomenclatures. Nucleotide position was numbered according to the reference genome Wuhan-Hu-1 (GenBank accession number NC_045512.2) Antibody testing (IgG): Abbott SARS-CoV-2 IgG assay or microsphere-based antibody		1st episode contained a stop codon at position 64 of ORF8, leading to a truncation of 58 amino acids. Another 23 nucleotide and 13 amino acid differences located in 9 different proteins, including positions of B and T cell epitopes, were found between viruses from the 1st and 2nd episodes. The 2nd genome contains the mutation nsp6 L142F, which is rarely found **Author's conclusions:** Viral genomes from 1st and 2nd episodes belong to different clades/lineages	However IgG became positive on day 5 post-hospitalisation	
Van Elslande 2020 10.1093/cid/ciaa1330 Belgium Case report	51 year old female 2nd infection 93 days after first	RT-PCR confirmation of SARS-CoV-2 infection. Whole genome sequencing: Full-length genome sequencing with ONT MinION	Initial infection: Moderate infection with typical COVID-19 symptoms including headache, fever, myalgia, coughing, chest pain, dyspnoea, anosmia and a change in taste. Persistent symptoms of tiredness, muscle pain and dyspnoea at home for 5 weeks 2nd infection:	Initial infection: lineage B.1.1 SARS-CoV-2 virus 2nd infection: lineage A Nucleotide difference: 11 mutations were identified across the genome of the two strains (11/29903 differences, 99.7% identity)	Initial infection: Not performed 2nd infection: IgG positive	Letter to the editor, Published in *Clinical Infectious Diseases*

			Mild (headache, cough, fatigue and rhinitis)			

***Capture-based technologies** or target enrichment allows for simultaneous sequencing of sets of genomic regions of interest in several individuals in the same sequencing run, therefore reducing sequencing costs. This technique uses custom RNA probes which are complementary to the target genomic regions

** **Amplicon-based technologies** are used in Next Generation Sequencing (NGS) as methods for target enrichment and amplification with speed, accuracy, and sensitivity. One example of amplicon-based technology uses multiplex polymerase chain reaction (PCR) and amplifies multiple genomic targets through a simple PCR experiment.

***Artic-medaka consensus generation tool** is a tool to create consensus sequences and variant calls from nanopore sequencing data. This task is performed using neural networks applied a pileup of individual sequencing reads against a draft assembly. It outperforms graph-based methods operating on base called data, and can be competitive with state-of-the-art signal-based methods whilst being much faster

PANGOLIN - Phylogenetic Assignment of Named Global Outbreak LINeages

Appendix 2. Additional comparative genomic sequencing data

Study	Whole genome sequencing
Goldman et al.[8]	Comparative genomic analysis demonstrated that viral strains from each infection were from different clades (clades 19B and 20A). Additionally, there were ten high confidence nucleotide differences (five of which type the March sequence to clade 19B, and five type the July sequence to 20A). Additionally, the A23403G mutation was found on reinfection, which confers the D614G amino acid change in spike protein.
Gupta et al.[1]	Comparative genomic analysis demonstrated that there were nine and ten variant differences in the first and second patients, respectively. A genetic variation 22882T>G (S:N440K) within the receptor-binding domain of the spike protein was detected in the sample from the second infection in the second patient, which possibly confers resistance to neutralising antibodies.
Larson et al.[10]	only a partial genome was recovered from the initial infection, limiting comparative analysis. Numerous nucleotide differences were recorded however, one of which was a high confidence variation. The lineage of the second infection was determined as B.1.26 with spike variant D614G.
Mulder et al.[2]	Comparative genomic analysis demonstrated that the two strains differed at ten nucleotide positions in the ORF1a (4), ORF (2), Spike (2), ORF3a (1) and M (1) genes. Sequences did not cluster in the phylogenetic tree
Prado-Vivar et al.[3]	Comparative genomic analysis demonstrated that the initial infection belonged to the 20A clade according to NextClade, and to the B1.p9 lineage in GISAID. The second infection belonged to 19B clade according to NextClade, and the A.1.1 lineage in GISAID. Therefore, viral genomes of both infections belonged to different clades. When compared with the Wuhan-Hu-1 reference genome; eight single nucleotide polymorphisms were found in the first infection and 10 in the second. No shared mutations were found, further suggesting that both variants resulted from distinct evolutionary trajectories.
Shastri et al.[9]	Across all four patients, a total of 39 mutations were identified within the eight genomes, including 22 non-synonymous, 16 synonymous, and 1 stop-coding substitutions. Of the non-synonymous mutations, the nucleotide difference comparing first and second infections was eight, nine, nine and twelve for each of the four patients. While three patients were infected on both occasions by SARS-CoV-2 with lineage B.1.1, clade A2a, one patient had a shift in lineage from B.1 to B. In terms of subclades, one patient clustered in different subclades on reinfection.
Tillett et al.[4]	Comparative genomic analysis demonstrated that the first and second viral genomes belonged to the same clade (20C). In terms of nucleotide

	differences, the initial infection had four single nucleotide polymorphisms absent from the second and the second had seven single nucleotide polymorphisms absent from the first.
To et al.[5]	Comparative genomic analysis demonstrated that the initial infection belonged to GISAID clade V, Nextstrain clade 19A, Pangolin lineage B.2 and phylogenetically closely related to strains collected in March or April 2020 in either the US or England. The second infection belonged to GISAID clade G, Nextstrain clade 20A, Pangolin lineage B.1.79 and phylogenetically closely related to strains collected in July or August 2020 in Switzerland and England. Nucleotide difference was 24. The virus genome from the first episode contained a stop codon at position 64 of ORF8, leading to a truncation of 58 amino acids. Another 23 nucleotide and 13 amino acid differences located in nine different proteins, including positions of B and T cell epitopes, were found between viruses from the first and second episodes. The second genome also contained the mutation nsp6 L142F, which is rarely found (only 0.009% of genomes deposited into GISAID, an open-access database of genomic data on influenza and coronaviruses, contained this mutation as of 20 August 2020[32]).
Van Elslande et al.[6]	Comparative genomic analysis demonstrated that the first virus was from lineage B.1.1 and the second was from lineage A. There were 11 single nucleotide polymorphisms.

Appendix 3. Studies on duration of antibody responses

Author DOI Country Study design	Population Patient demographics Clinical characteristics	Test parameters	Primary outcome results	Peer review status
Adams 2020 10.1101/2020.04.15.20066407 UK Case series	SARS-CoV-2 ELISA and RT-PCR (used as reference test) Compared to 9 commercially available lateral flow immunoassay (LFIA) devices Plasma samples. RT-PCR from upper respiratory tract (nose/throat) swab Acute samples were collected from patients a median 10 (range 4-27) days from symptom onset (n=16), and from recovering healthcare workers median 13 [range 8-19] days after 1st symptoms; (n=6).	N=40 adult positive for SARS-CoV-2 by RT-PCR. N=142 controls For SARS-CoV-2 patient: Age mean 60 (range 22-95) Severity: Mild 26(65%), Severe 4(10%), critical 9(22.5%), 1 asymptomatic (2.5%) N=18 convalescent cases (>28 days from symptom onset). N=16 case (≤ 28 days from symptom onset). N=6 convalescent health care worker (≤ 28 days from symptom onset)	**Duration of detection of serum immunoglobulin levels:** 40 SARS-CoV-2 samples and 50 controls tested by ELISA. 34/40 positive for IgG, other 6 where taken within 9 days of symptom onset. All samples taken >= 10 days after symptom onset positive for IgG. IgM positive in 28/40 samples (70%). No patient was IgM positive and IgG negative. N=9 patients had samples from between 50 and 60 days after onset of symptoms. In these 9 patients IgM (5 out of 9) and IgG (9 out of 9) still present. N=2 patients had samples ≥60 days, both were still positive. **Serum titres of IgG over time (typically expressed as Geometric Mean Titres [GMTs]):** Considering the relationship between IgM and IgG titres and time since symptom onset, univariate regression models showed IgG antibody titres rising over the first 3 weeks from symptom onset. The lower bound of the pointwise 95%CI for the mean expected titre crosses OD threshold between days 6-7. However, given sampling variation, test performance is likely to be optimal from several days later. IgG titres fell during the second month after symptom onset but remained above the OD threshold (at 60 days from symptom onset). No temporal association was observed between IgM titres and time since symptom onset.	Not peer reviewed; medRxiv

	Convalescent samples were collected from adults a median 48 [range 31-62] days after symptom onset and/or date of positive throat swab (n=18)		**Other outcome:** There was no evidence that SARS-2-CoV severity, need for hospital admission or patient age were associated with IgG or IgM titres in multivariable models	
Dittadi 2020 10.20944/preprints202008.0114.v1 Italy Case series	N=55 patients; N=98 samples Time from the onset of symptoms: 3 to 109 days N=46 males, N=9 females Median age: 63 years, minimum 28, maximum 89	Two test kits: **Abbott SARS-COV-2 IgG** ▪ 2 two-step chemiluminescence microparticle immunoassay ▪ Nucleoprotein based antigen ▪ Analyser: Architect I2000sr ▪ Abbott SARS-COV-2 IgG assay is calibrated against an internal standard and the results are expressed as Index (ratio between the sample result and the calibrator result). The samples are considered reactive with an index >1.4. **MAGLUMI 2019-nCoV IgG** ▪ 2 two-step chemiluminescence microparticle immunoassay ▪ S1, S2 and N proteins	All specimens IgG positive after 17 days post-symptom onset **<u>Sensitivity >60 days post-symptom onset:</u>** **46-72 days:** 100% by both tests (N=between 15 and 18 patients) **81-109 days:** 100% by Abbott; 87.5% by Maglumi (N=between 15 and 18 patients) **Maglumi:** Median concentrations of IgG, after a rapid increase up to about 20 days, quickly decreased to 15% of the maximum **Abbott:** Constant IgG trend up to 80 days, then a moderate decline **Authors' conclusions:** The titre of IgG against SARS-CoV-2 in patients exposed to COVID-19 may significantly and rapidly decrease, with a different time-course depending on the method used for determination	Not peer reviewed (pre-print)

		▪ The MAGLUMI 2019-nCoV IgG was calibrated against an internal standard and the results were expressed as Arbitrary Units/mL (AU/mL). The samples are considered reactive with a concentration >1.0 AU/mL Analyser: Maglumi 800		
Figueiredo-Campos 2020 DOI: 10.1101/2020.08.30.20184309 Portugal Case series	N=187 plasma donors N=271 samples in longitudinal analysis of anti-SARS-CoV-2 antibodies N=307 samples from COVID-19 hospital patients and healthcare workers, N=2500 University staff (seroprevalence study) and 187 post-COVID19 volunteers Demographics of COVID-19 patients: Mean age=63.23 (20-93) N=88 male N=97 female	ELISA, following a protocol developed by Stadlbauer 2020 (https://pubmed.ncbi.nlm.nih.gov/32302069/) Immunologic antigens: Spike and N-protein Assay validation: 100 pre-COVID-19 sera from healthy volunteers collected between Oct 2012 and Nov 2017 as negative controls. Furthermore, 19 sera from PCR positive hospital healthcare workers with mainly mild symptoms, just over 30 days since first symptoms and the positive SARS-CoV-2 PCR result. Receiver operating characteristic curve determined sensitivity and specificity and the assays cut-off, at 0.4171 and 0.4816 for RBD and S protein respectively, corresponding to 100% specificity and 99% sensitivity for RBD and 94.74% specificity	Anti-SARS-CoV-2 antibody responses followed a classic pattern with a rapid increase within the first three weeks after symptoms. **Duration and titres of IgG** From the second month after disease onset IgG and IgA antibody levels remained readily detectable in most people up to 5 months (150 days) after first symptoms. Geometric means of IgM, IgG and IgA titres at day 91-120 were 96, 533 and 141, respectively. **Neutralising activity** Neutralisation activity in all tested sera in which anti-SARS-CoV-2 IgG was determined, across 2-5 months after initial SARS-CoV-2 PCR-positive testing. The level of SARS-CoV-2 neutralisation activity was found to be proportional to the anti-SARS-CoV-2 RBD IgG titre determined.	Not peer reviewed (pre-print)

		and 98% sensitivity for Spike in this initial analysis.		
Fill Malfertheiner 2020 10.1016/j.jcv.2020.104575 Germany Case series	N=27 patients PCR-positive (at least 1 positive RT-PCR test); N=25 followed longitudinally N=22 female, N=3 male 18–35 years N=10; 36–50 years N=8; 51–65 years N=7. Serologic testing was performed 2–4 weeks after initial outbreak and again 8–12 weeks after the outbreak in 166 individuals, including 27 COVID-19 patients who had also participated in the 1st testing. Median time span between the 1st and 2nd serologic tests was 38 days (range 29–47)	Two kits: Anti-SARS-CoV-2 IgG and IgA ELISA (EUROIMMUN AG, Lübeck, Germany; https://www.euroimmun.com) Elecsys Anti-SARS-CoV-2 (Roche Diagnostics, Rotkreuz, Switzerland; https://diagnostics.roche.com) According to manufacturer's recommendations, for the IgA and IgG assay an OD ratio of >1.0 was considered positive. The qualitative Elecsys Anti-SARS-CoV-2 Antibody immunoassay analyzer was used. The assay does not discriminate between the antibody type(s) present and can detect IgA, IgM, and IgG. The test is based on a recombinant nucleocapsid (N) antigen and has a threshold value of 1.0. Accordingly, all samples with a value<1.0 were considered negative.	N=21 (77.8 %) of COVID-19 study subjects developed a specific IgG-response over the course of 12 weeks The values of detectable IgG-responses significantly increased over time as confirmed with both tests When analysed on an individual level, a majority of COVID-19 cases (n=18) showed an increase of IgG values over time (72 %) while only three individuals with initially positive IgG values (>1.0) showed decreased levels at follow up and two did neither	Peer reviewed (Journal of Clinical Virology)
Flehmig 2020	N=3	**Immunoglobulin test:** ELISA test system E 111-IVD	**IgG**	Not peer reviewed (pre-print)

DOI: 10.1101/2020.08.20.20174912 Germany Case series	Maximum follow up: Patient 1: 109 days; Patient 2: 107 days; Patient 3: 106 days. N=2 adult females and N=1 adult male.	developed by Mediagnost GmbH, Reutlingen (Germany) **Virus neutralisation assay:** Investigators quantified *in vitro* the ability of human sera from patients to inhibit the infection of human cells Caco-2 (human colorectal adenocarcinoma) with a strain of SARS-CoV-2 virus. The SARS-CoV-2 strain icSARS-CoV-2-mNG6 was obtained from the World Reference Center for Emerging Viruses and Arboviruses (WRCEVA) of the UTMB (University of Texas Medical Branch). To generate icSARS-CoV-2-mNG stocks, Caco2 cells were infected, the supernatant was harvested 48 hours post infection, centrifuged and stored at -80°C. For MOI (multiplicity of infection) determination, a titration using serial dilutions of the virus stock was conducted.	Anti-SARS-CoV-2 S1-RBD IgG: Detectable in all patients (n=3) at maximum follow-up (106-109 days). Increase in titres followed by a plateau (approx. 6 weeks later) **Neutralising antibody** Detectable in all patients at maximum follow-up. Increase followed by a decrease in titres over time. Peak at approx. 4 weeks.	
Gallais 2020 France Case series 10.1101/2020.06.21.20132449	SARS-CoV-2 At least 1 index case in each household had positive RT-PCR and /or serological evidence (contacts did not have RT-PCR testing)	N=7 households, comprising N=9 index patients and N=8 close contacts N=10 healthy controls The median age of index patients was 45 years (range, 34-65 years) and 4 were male	**IgG** N=9/9 positive for IgG 47-69 days after symptom onset by Abbott and Euroimmun ELISA N=7/9 positive by Biosynex lateral flow assay Authors' Conclusions: Anti-SARS-CoV-2 antibodies and a significant T cell response detectable up to 69 days after symptom onset Contacts:	Not peer reviewed

	3 serological tests: 1. The Abbott Architect SARS-CoV-2 IgG chemiluminescent microparticle immunoassay for detection of IgG against the SARS-CoV-2 nucleoprotein 2. The Euroimmun Anti-SARS-CoV-2 Assay, an ELISA for the detection of IgG against the SARS-CoV-2 S1 domain of the spike protein including the immunologically relevant receptor binding domain (RBD) 3. Biosynex, a lateral flow assay for detection of IgM and IgG against the SARS-CoV-2	Blood samples were collected from 47 to 69 days post symptom onset	N=6/8 contacts reported COVID-19 symptoms within 1 to 7 days after the index patients but all were SARS-CoV-2 seronegative. N=6/8 had SARS-CoV-2-specific T cell response, however	

	RBD of the Spike protein S (Abbott Architect assay: sensitivity 100% and specificity 100%; Euroimmun assay: sensitivity 100% and specificity 97.7%; Biosynx assay: sensitivity 95.6% and specificity 99.4%)			
Gontu 2020 10.1101/2020.08.21.261909 USA Case series	N=540 convalescent plasma samples obtained from N=175 COVID-19 plasma donors N=88 females (50.3%) and 87 males (49.7%) Age: median 46, IQR: 36-54 Follow-up: 17-142 days post-symptom onset (median 68 days, IQR: 48-93)	Plasma from donors was collected with the transfusion apheresis system (Trima Accel® Terumo BCT) and standard blood banking protocols were followed. An aliquot of collected plasma was tested for antibodies by ELISA and/or virus neutralisation assays. **Antibody assay:** SARS-CoV-2 antibodies in plasma samples were detected and quantified against purified recombinant SARS-CoV-2 spike ectodomain (S/ECD) or receptor-binding domain (S/RBD) proteins using in-house indirect Fab antibody-based or isotype-specific (IgM and IgG) ELISA assays.	Only N=4 of 175 [2.3%; 95% confidence interval (CI): 0.9-5.7%] individuals had undetectable levels of IgG, IgM, or total antibody to S/RBD or S/ECD at initial sampling, whereas a significantly higher fraction (29 of 114; 25.4%; 95% CI: 18.3-34.1%) had undetectable virus neutralisation titres (z-score=6; P<0.01). Thus, ~75% of RT-PCR-confirmed symptomatic individuals were serologically positive for anti-spike protein antibody AND their convalescent plasma had demonstrable ability to neutralise SARS-CoV-2 in VN assays. Maximum duration IgG response: N=55/55 tested >100 days had detectable levels; max follow up: 138 days Robust IgG and viral neutralisation responses to SARS-CoV-2 persisted, in the aggregate, for at least 100 days post-symptom onset. IgG: Titres peaked at approximately 30 days post-symptom onset and persisted through 140 days post-symptom onset. However, a notable acceleration in decline in virus neutralisation titres ≥160, a value suitable for convalescent	Not peer reviewed (pre-print)

		Virus neutralisation assay: The neutralisation titres of the plasma samples were quantified on a cell-based assay using SARS-CoV-2 strain USA-WA1/2020 (NR-52281-BEI Resources, USA) using Vero E6 cells (CRL-1586, ATCC, USA)	plasma therapy, was observed starting 60 days after first symptom onset. Author's conclusion: Together, these findings better define the optimal window for donating convalescent plasma useful for immunotherapy of COVID-19 patients.	
Isho 2020 10.1101/2020.08.01.20166553tients Canada Case series	N=496 serum samples and N=90 saliva samples obtained from acute (<21 days PSO) and convalescent (>21days PSO) patients identified in surveillance by the Toronto Invasive Bacterial Diseases Network. Consecutive, consenting patients admitted to 4 hospitals enrolled	ELISAs used for the detection in serum of anti-spike trimer and anti-spike RBD antibodies. For manual and automated IgG assays, sensitivities of 95.6% and 95.5% for spike and 93.8% and 91.3% for RBD respectively, at a false positive rate of ≤ 1%, were obtained in these cohorts. The AUCs were ≥0.97 in all cases. Automated assays for the detection of IgA and IgM were also developed. To evaluate the neutralisation potential, protein-based surrogate neutralisation ELISA (snELISA) approach used.	▪ In serum, IgG response to spike trimer was sustained over 115 days (7.3% change in the median as compared to the max). However, IgG response against RBD showed a ~25.3% decrease by day 105 and a ~46.0% decrease by day 115. ▪ N=11/11 positive days 63-112 (interpreted from Figure 3). ▪ Antigen specific IgM and IgA were rapidly induced and reached a max day 16-30. By day 115, anti-spike and anti-RBD IgA levels were ~74.1% and ~84.2% of their respective max levels, while IgM were ~66.2% and ~75.1% respectively. ▪ Multivariable analyses adjusting for severity of disease, sex and patient age did not change conclusions about the relationships between time PSO and anti-RBD IgM, anti-spike IgM, anti-RBD IgA, anti-spike IgA and anti-RBD IgG, however the modest decline in anti-spike IgG after day 35 was statistically significant. ▪ The neutralisation reaches its max in the 31-45 day PSO bin, and decreases to an intermediate median plateau in the 46-105 day PSO bins before more drastically dropping in the 106-115 day PSO samples (fewer samples are in this time bin (n=9) compared to other bins (n=20)). ▪ Analysis of paired serum samples from hospitalised patients were analysed to dissect the results above. These results depict the relative stability of the IgG anti-	Pre-print on MedRvix

			spike trimer levels, a partial decrease in the anti-RBD IgG and anti-spike IgA levels, and a near loss of the anti-RBD IgM and IgA levels over time. ▪ Levels of anti-NP antibodies closely resembled those for anti-spike and anti-RBD IgG and IgM/IgA response, namely a relative stability in the IgG and a more rapid decline in IgA/IgM levels.	
Jeewandara 2020 DOI: 10.21203/rs.3.rs-47016/v1 Sri Lanka Case series	N=261 Disease severity: severe pneumonia (n=10), moderate illness (n=19), mild illness (n=150) and prolonged shedding (n=82) Age, sex N/R	**Assay to measure Neutralising antibody** Recently developed surrogate virus neutralisation test which measures the percentage of inhibition of binding of the RBD of the S protein to recombinant ACE210 (Genscript Biotech, USA). Inhibition percentage ≥ 25% in a sample was considered as positive for neutralising antibodies. Neutralising antibodies were measured on day 14 to 21 (n=98), day 22 to 28 (n=100), day 29 to 36 (n=132), day 37 to 42 (n=32), day 43 to 49 (n=16), day 50 to 70 (n=29) and >90 days (n=15).	**Positivity rate (days since illness onset):** Day 14 to 21 = 79.8%, Day 22 to 28 =88.9%, Day 29 to 36 =100%. (begins to decline thereafter, particularly in asymptomatic/mild cases) Day 37 to 42 = 90.6% Days 50 to 70 = 65.5% >90 days = 53.3% **Association between positivity rate and disease severity:** Mild/asymptomatic illness: All those who had neutralising antibodies below the cut-off value were those who had mild/asymptomatic illness. Moderate/severe illness: Those with moderate/severe illness and individuals with prolonged shedding were positive for neutralising antibodies after day 90 of illness. Patients with severe or moderate COVID-19 had earlier appearance of Nabs at higher levels compared to those with mild or asymptomatic illness.	Not peer reviewed (pre-print)
Kreer 2020 Germany Case series 10.1101/2020.06.12.146290.	SARS-CoV-2 ELISA for IgG Multiple antibody and cell responses tested using a variety of platforms	N=12 patients Mean age: 48.8 years (range: 28-59 years) 50% male	**IgG** For longitudinal analysis, n=5 patients sampled at 3 time points between 8–69 days post-diagnosis SARS-CoV-2 neutralization values of plasma IgG ranged from 78.8 to 1500 μg/ml, respectively At 69 days one person still positive for IgG	Not peer-reviewed

		N=5 patients for longitudinal analysis Mean age: 46.4 years (range: 28-58 years) 60% male		
Lei 2020 DOI 10.1101/2020.07.09.20149633 China Case series	N=177 total participants: n = 63 asymptomatic cases (among 11,766 individuals returning to work screened; n = 12 by RT-qPCR and n = 51 by ELISA), n= 63 healthy contacts (negative NAAT and antibody test) n=51 mild COVID-19 cases (among 1056 hospitalised patients; no pre-existing conditions; hospitalised). Exposure history: Clear exposure history or days after symptoms onset were only obtained from 48/63 healthy contacts, 36/63 asymptomatic	**RNA detection** SARS-CoV-2 infection was confirmed using TaqMan One-Step RT-qPCR Kits (DAAN Gene, Guangzhou, China) **Antibody detection** Validated assay for IgG antibodies against recombinant N and S protein of SARS-CoV-2 in serum specimens were detected by commercial kits according to the manufacturer's instructions (YHLO Biotech, Shenzhen, China). Positivity threshold: The antibody levels ≥ 10 AU/mL are reactive (positive), and the results < 10 AU/mL are negative. Antibody responses to 23 proteins of SARS-CoV-2 were further detected in parallel using a proteome microarray. **Neutralisation detection using pseudotyped virus neutralizaion assay**	**IgG** Asymptomatic patients (n=36): Day 17 to 25 = S1 and N specific IgM and IgG responses peaked (n=6). Decline thereafter. 2 months after exposure = N-specific IgG antibodies were detectable (n = 5). Mild patients (n=51): Elicited higher levels of N-specific IgM and IgG responses, which maintained for at least 65 days (n=3). **Neutralising Antibodies** Asymptomatic individuals (n=63): 36.5% (23/63) asymptomatic individuals (mainly NAAT positive (8/12)) did not produce neutralizing antibody. 63.5% (40/63) asymptomatic infections only induced low titers of neutralizing antibody (mean IC50 1:24). nAb response in asymptomatic individuals was produced on 7d after exposure and peaked on days 10 to 25, then declined. Healthy contacts (n=63): 19% (12/63) healthy contacts only induced low titers of neutralizing antibody (mean IC50 1:13). Mild patients (n=51): Stimulated the highest levels of neutralising antibody with the mean IC50 1:269. Only 11.8% (6/51) mild patients (mainly NAAT alone positive (4/6)) did not elicit neutralising antibodies.	Not peer reviewed (pre-print)

	infections and 51 mild patients. Only mild patients had confirmed RT-qPCR at initial infection; data not available for asymptomatic and healthy controls (initial infection not confirmed). Mean age (SD) of entire sample: 44.6(19.25) Male: 48%, Female: 52%	nAb titres in sera were determined using a Fluorescence Activated Cell Sorting (FACS)-based neutralisation assay. Neutralisation rate: The half-maximal inhibitory concentration (IC50) of each serum sample was determined as the highest dilution ratio of serum with 50% neutralization rate. Note: sera samples were not collected for all patients at each time point. Healthy controls: Day 3: n =2; Day 7-9: n = 8; Day 10-16: n =11; Day 17-25: n =11; Day 26-34: n =3; Day 35-39: n =4; Day 65+: n =9. Asymptomatic: Day 7-9: n =4; Day 10-16: n = 14; Day 17-25:n = 6; Day 35-39: n = 4; Day 40-45: n =3; Day 65+: n = 5. Mild (IgG and IgM/nAb): Day 1-7: n = 7/4 ; Day 8-14: n = 17/6; Day 15-21: n =9/6; Day 22-28: n = 13/6; Day 29-35: n = 13/8; Day 36-42: n = 17/11; Day 43-49: n = 8/7; Day 65+: n = 3.	Mild patients produced the neutralising antibody early to 1d after symptom onset, and the titre rose persistently until 22 days and maintain for at least 65 days (n=3).	

Liu 2020a DOI: 10.1101/2020.08.21.20179358 China Case series	N=49 COVID-19 convalescent individuals (CI) in comparison to N=27 matched SARS-CoV-2 unexposed individuals (IU) Two cohorts, Chinese and German Chinese cohort: 21 UIs and 30 CIs German cohort: 6 UIs and 19 CIs	▪ EUROIMMUN ELISA (for German cohort), not reported for Chinese cohort. ▪ Chinese cohort: Median time between the first diagnosis of COVID-19 and blood sampling was 122 days (range: 60 to 136 days).	▪ In the Chinese cohort, at the time of blood sampling: ○ 0% IgM single positive ○ 80% IgG single positive (N=24/30) ○ 20% IgM and IgG positive (N=6/30) ○ 90% neutralising antibody (N=27/30).	Not peer reviewed (pre-print)
Liu 2020b China Case series 10.1093/clinchem/hvaa137	SARS-CoV-2 spike protein receptor binding domain (RBD)-specific IgM or total antibodies (IgA/IgG/IgM) using 2 commercial microparticle chemiluminescence immunoassays	N=192 PCR confirmed patients N=1,019 serum samples Of 192 patients, 83 (43%) classified as severe cases Demographic details not given	Total antibodies IgA/IgG/IgM seropositivity over time (from symptom onset) in mild and severe cases: **Day 31-36** Mild: 12/18 Severe: 103/103 **Day 37-42** Mild: 19/24 Severe: 79/80 **Day 43-48** Mild: 36/42 Severe: 86/86 **Day 49-54** Mild: 20/23 Severe: 54/54 **Day 55-60** Mild: 7/7 Severe: 39/39 **Day 61-65** Mild: 2/2 Severe: 14/14 After 25-30 days, all sampled severe patients (115/115) seropositive. At end of follow-up (61-65 days), both mild (2/2) and severe (14/14) all positive.	Letter to editor
Muecksch 2020 DOI: 10.1101/2020.08.05.20169128	N= 97 participants, who were not hospitalised during the course of their illness (mild cases)	**Antibody detection** 4 commercially available qualitative serology test kits were used. Assays were performed on the Abbott Architect and Diasorin	**Sensitivity of assays over time** The Abbott, Roche and Siemens assays all had sensitivities of 95 to 100% at 21-40 days post PCR-positive test, while the Diasorin assay had a lower sensitivity of 85%. The relative sensitivities of the assays changed with time. Specifically, the sensitivity of the Abbott assay	Not peer reviewed (pre-print)

UK Case series	Serum samples were taken at a baseline visit and three further visits (number of days between baseline visit and follow-up). Visit 1 (baseline;~3.5 to ~8.5 weeks post-diagnosis),40.8 days (24 – 61 days); Visit 2 (2 weeks post-baseline), 55.1 days (40 – 79 days); Visit 3 (4 weeks post-baseline), 69.8 days (55 – 95 days); Visit 4 (8 weeks post-baseline), 98.4 days (85 – 110 days). Mean age 44.2 years (21 – 65 y); n=27 male, n=70 female (72%)	Liason platforms (NHS Lothian), and the Roche Elecsys (NHS Lanarkshire) and Siemens Atellica (NHS Tayside) platforms. The **Abbott** SARS-CoV-2 IgG assay detects anti-N IgG using a 2-step chemiluminescent microparticle immunoassay (CMIA) method with an acridinium-labelled anti-human IgG. The **DiaSorin** SARS-CoV-2 IgG assay is also a 2-step CMIA method targeting undisclosed epitopes in the SARS-CoV-2 S protein and employs an isoluminol conjugated anti-human IgG. The **Roche** Anti-SARS-CoV total antibody assay is a 2-step bridging electrochemiluminesent immunoassay (ECLIA) using ruthenium-labelled and biotin conjugated N protein. The **Siemens** SARS-CoV-2 total antibody assay is a 1-step bridging CLIA method that detects antibodies against the RBD of the S protein, using acridinium and biotinylated S1 RBD. **Pseudovirus neutralisation:**	declined to 85% in the 61-80 day window, and 71% at >81 days post-diagnosis. Conversely, the sensitivities of the other assays were maintained or increased over time. **Sensitivity at longest follow up (>81 days; N=41)** Abbott 70.73% (54.5-83.9) Roche 97.56% (87.1-100.0) Siemens 97.56% (87.1-100.0) Diasporin 92.68% (80.1-98.5) **Titres** Mean antibody titres decreased in the Abbott assay at visits 2 and 3 compared to visit 1 but increased in the Diasorin and particularly the Roche assays and remained approximately constant in the Siemens assay. Notably, 79 out of 97 (81%) of participants showed a decrease in antibody titre on the Abbott platform, while 82/97 (85%) showed an increase on the Roche assay, despite the fact that both assays detect N-specific antibodies **Neutralising capacity (n=80/97)** A broad range of SARS-CoV-2 neutralising titres were evident in individual sera, that decreased over time in the majority of participants. The majority of participants exhibited a similar relative decrease in neutralising activity over time (regardless of the initial NT50 values or the number of days post PCR at visit 1) suggesting exponential decay. Visit 1: In samples collected at visit 1, the neutralising activity ranged from <30 to 4300, with a geometric mean of 234 (arithmetic mean was 411). 34/80 (42%) had NT50 of < 250 11/80 participants (14%) had NT50 values >1000. Visits 2 and 3: Overall, the decrease in median NT50 was ~25% per two-week sampling interval, the median neutralisation titre in the	

		Serum neutralising antibody titres were measured using a validated pseudotyped virus SARS-CoV-2 neutralisation assay with a luciferase reporter for luminescence detection. Neutralisation activity: Half-maximal neutralising titre (NT50).	cohort decreased by 45% over 4 weeks between visit 1 and 3. **Comparison in rate of decline between IgG antibodies and nAb** In most participants, the decline in serum neutralising activity was greater than the decline in antibody titre measured using any serological assay.	
Nayak 2020 DOI: 10.1101/2020.08.31.276675 India Cross-sectional	N=42 patients; N=4 tested > 60 days post-diagnosis Mean age 39.4 years, range 15 – 70 years Samples taken 34-84 days post PCR diagnosis. (n = 3/42 individuals with data >77 days post follow-up)	**IgG measurement** SARS-CoV-2 RBD-specific antibody titres were measured with direct ELISA validated for clinical testing. LOD = 100 SARS-CoV-2 whole virus-specific IgG was detected using a commercially available assay (COVID-Kavach ELISA tests kit, Zydus diagnostics). Assay cut-off = 1.5. **Virus neutralisation** Focus-reduction neutralisation mNeonGreen (FRNT-mNG) assay was used for determination of neutralisation titres. Neutralisation: nAb iters calculated based on the plasma dilution that neutralized 50% of the virus. The neutralization potency of the plasma sample was measured by the reduction in	N=2 patients tested at 84 days post-diagnosis: • Both RBD-specific IgG antibody seropositive (titres: 2220 and 354; ELISA limit of detection=100) • Both whole-virus-specific IgG antibody seropositive (titres: 26 and 3; ELISA commercial kit limit of detection = 1.5) • Neutralisation titre (FRNT-mNG$_{50}$): 39 and 26 [limit of detection for FRNT-mNG$_{50}$: 20] N=4 patients tested >60 days: all four had detectable IgG and neutralising titres above limit of detection. **RBD-specific IgG and IgA titres** Four individuals had undetectable RBD-specific IgG and IgA titres (these patients had samples taken at days 34, 45, 45 and 47) Therefore N=38/42, 90.47% had detectable levels (34-84 days post diagnosis). **Whole virus-specific IgG titres** 9 individuals had undetectable whole virus-specific IgG at days 34 (n=2), 41 (n=2),45 (n=4) and 47. **Virus neutralisation:** Only half of the COVID-19 recovered individuals showed 50% or more neutralisation even at a 1:20 dilution of plasma (lowest dilution).	Not peer reviewed (pre-print

		virally-infected foci - counted using Viridot. Neutralisation titres were performed using a 3 fold dilution of plasma – starting at 1:20 up to 1:43740.	RBD-specific IgG titres correlated with neutralising antibody titres as well as with RBD-specific memory B cell frequencies.	
Pepper 2020 DOI: 10.21203/rs.3.rs-57112/v1 USA Case series	N=15. Samples drawn by N=14 at 2 visits and N=1 at 1st visit only. Mean age: 47 years (28 – 71) 27% Male, 73% Female Samples taken at 2 visits. Mean time from symptom onset to Visit 1 (days) 35.5 (19 – 44); mean time from symptom onset to Visit 2 (days): 86 (73 – 110)	Methodological steps included the following: ▪ SARS-CoV-2 Protein Production and Purification ▪ Plasmid construction ▪ Constructs produced in Expi293F cells ▪ Purification of His-tagged proteins ▪ Tetramer generation ▪ ELISA ▪ Receptor-binding inhibition assay ▪ Plaque reduction neutralisation test (PRNT) ▪ Immunophenotyping and sorting RBD-specific B cells ▪ Monoclonal antibody generation.	Overall, individuals developed SARS-CoV-2-specific IgG antibody and neutralising plasma, as well as virus-specific memory B and T cells that not only persisted, but in some cases increased numerically over three months following symptom onset. Furthermore, the SARS-CoV-2-specific memory lymphocytes exhibited characteristics associated with potent antiviral immunity: memory T cells secreted IFN-γ and expanded upon antigen re-encounter, while memory B cells expressed receptors capable of neutralising virus when expressed as antibodies.	Not peer reviewed (pre-print)
Seow 2020 10.1101/2020.07.09.20148429 UK Case series	N=65 N=59 admitted patients and 6 staff Average age 55.2 years (range 23-95 years) 77.2% male	RT-qPCR confirmed ELISA for IgG, IgM and IgA response against spike (S), the receptor binding domain (RBD) and nucleocapsid (N) Neutralising antibodies: SARS-CoV-2 neutralisation potency using HIV-1	**IgM, IgA and IgG: seroconversion** ▪ N=2/65 individuals (3.1%) did not generate a detectable antibody response against any of the antigens; however samples only available up until 2- and 8-days post-symptom onset for these 2 individuals and the mean time to seroconversion against at least 1 antigen was 12.6 days post-symptom onset. ▪ IgG responses against S, RBD and N antigens were observed in 92.3%, 89.2% and 93.8% of individuals respectively.	Not peer reviewed; medRxiv

	A severity score was assigned to patients (ranged from asymptomatic to ECMO), score ranged from 0 to 5	based virus particles, pseudotyped with SARS-CoV-2 S in a HeLa cell line stably expressing the ACE2 receptor	▪ The frequency of individuals generating an IgM response ▪ was similar to IgG, with 92.3%, 92.3% and 95.4% seropositive against S, RBD and N respectively. ▪ The frequency of individuals with an IgA response to RBD and N was lower, with only 72.3% and 84.6% seropositive respectively. **IgM, IgA and IgG: longitudinal analysis** ▪ Longitudinal analysis across sequential samples (number followed N/R) highlighted the rapid decline in the IgM and IgA response to all 3 antigens following the peak OD between 20- and 30-days post-symptom onset. ▪ In individuals sampled at time points >60 days post-symptom onset, the IgM and IgA responses were approaching baseline. ▪ The IgG OD (as measured at 1:50 dilution) remained high in the majority of individuals, even up to 94 days. **Neutralising antibodies: titres and seroconversion** ▪ The average time to detectable neutralization was 14.3 days post-symptom onset (range 3-59 days). ▪ Increased neutralization potency was observed with increasing days post-symptom onset with each individual reaching a peak neutralization titre (ranging from 98 to 32,000) after an average of 23.1 days (range 1-66 days). ▪ Only 2 individuals (3.1%) did not develop a response (ID50 <50) which was consistent with their lack of binding antibodies at the time points tested (<8 days post-symptom onset). ▪ At peak neutralization, 7.7% had low (50-200), 10.8% medium (201-500), 18.5% high (501-2000) and 60.0% potent (2001+) neutralizing titres. ▪ For serum samples collected after 65 days, the percentage of donors with potent neutralising antibodies (ID50>2000) had reduced to 16.7%.	

			Neutralising antibodies: longevity of response ▪ Following peak neutralisation, a waning in ID50 was detected in individuals sampled at >40 days. ▪ Comparison of the ID50 at peak neutralization and ID50 at the final time point collected showed a decrease in almost all cases. ▪ For some individuals with severity score 0, where the peak in neutralisation was in the ID50 range 100-300, neutralisation titres became undetectable (ID50 <50). **Severity & neutralising kinetics** ▪ ID50 values between individuals with 0-3 disease severity was compared with those in the 4/5 group. ▪ Magnitude of the neutralising antibody response at peak neutralization was significantly higher in the severity 4/5 group. ▪ Time taken to measure detectable titres and the time of peak neutralization did not differ between the 2 groups. ▪ This suggests disease severity enhances the magnitude of the antibody response but does not alter the kinetics.	
Wang 2020 DOI: 2020.07.14.20151159 China Case series	N=30 patients (recovered and discharged from hospital); N=173 samples 10% categorised as severe based on national treatment guidelines. N=12 males, N=18 females Age N/R	SARS-CoV-2-specific Neutralising antibodies measured using the lentiviral pseudotype assay. Pseudovirus (3·8 × 104 copies) was incubated withserial dilutions of serum samples from patients. Titers of NAbs were calculated as the 50% inhibitory dose (ID50).	All patients (30/30) developed a Neutralising antibody response, including N=2 patients sampled at >105 days post-symptom onset. **Neutralising antibodies** The median peak time for Neutralising antibodies was 33 days (IQR 24-59 days) after symptom onset. Neutralising antibody titres in 93.3% (28/30) of the patients declined gradually over the 3-month study period, with a median decrease of 34.8% (IQR 19.6-42.4%). **Correlation between nAb titres and IgG titres** NAb titers increased over time in parallel with the rise in IgG antibody levels, correlating well at week 3 ($r = 0.41$, $p < 0.05$)	Not peer reviewed (pre-print)

	Sequential serum samples were collected from patients in the acute phase (5–6 samples) and the convalescent phase (2 follow-up points: 60 days (54–63) and 96 days (90–99) after symptom onset			
Wheatley 2020 10.1101/2020.09.09.20191205 Australia Case series	N=64 patients N=158 samples. Samples collected between day 26 and 149 post-symptom onset with samples nominally denoted as early (≤50days), 73 intermediate (50-100 days) and late (≥100 days) convalescence Median age 55 (62, 49); Female 43.8% (28); Disease severity: Mild 68.8% (44); Moderate 23.4% (15 7.8% (5)	For all participants, whole blood was collected with sodium heparin anticoagulant. Plasma was collected and stored at -80oC, and PBMCs were isolated via Ficoll-Paque separation, cryopreserved in 10% DMSO/FCS and stored in liquid nitrogen. **Further steps included:** ▪ **Microneutralisation assays**: SARS-CoV-2 isolate CoV/Australia/VIC01/2020 was passaged in Vero cells and stored at -80C, all samples assessed in 2 independent micro-neutralisation assays ▪ **Expression of SARS-CoV-2 proteins:** A set	▪ In early convalescence (<= 50 days), neutralisation activity was widespread with a median serological titre of 52, which declined to 34 in late convalescence (>=100 days). ▪ Rapid decay evident over the first half of time-series (half-life ($t_{1/2}$) prior to day 70 = 55 days), compared with slower decay in the second half ($t_{1/2}$ from day 70 = 519 days). ▪ Similar response in immune plasma to inhibit interaction of the SARS CoV-2 receptor binding domain (RBD) with soluble hACE2 receptor19 waned with a similar two-phase decay, dropping more rapidly before day 70 ($t_{1/2}$ = 238 days) and slowing after day 70 ▪ Decay of S-specific IgG was best fit by a model of constant decay over the period of observation ($t_{1/2}$ = 229 days), with rates of decay divergent for antibodies binding S1 ($t_{1/2}$ =115 days), S2 ($t_{1/2}$ = 344 days) and RBD antigens ($t_{1/2}$ = 126) ▪ N-specific IgG decays significantly more rapidly than S-specific IgG ($t_{1/2}$ = 71 and 229 days respectively) ▪ IgG, S-specific IgM and IgA1 fit a two-phase decay, with a	Not peer reviewed (pre-print)

		of proteins was generated for serological and flow cytometric assays ▪ **SARS-CoV-2 bead-based multiplex assay:** The isotypes and subclasses of SARS-CoV-2 specific antibodies including trimeric S, S1 (Sino Biological), S2 (ACROBiosystems), NP (ACROBiosystems,) and RBD46 were coupled to magnetic COOH- bioplex beads (Biorad) using a 2-step carbodiimide coupling reaction ▪ **RBD-ACE2 binding inhibition multiplex bead-based assays:** were repeated independently twice. ▪ **Flow cytometric detection of S- and RBD-specific memory B cells:** Probes for delineating SARS-CoV-2 S-specific B cells within cryopreserved human PBMC were generated by sequential addition of streptavidin-PE (Thermofisher) to trimeric S protein	▪ more rapid early decay ($t_{1/2}$ = 55 and 42 days respectively) followed by a slower decay in late convalescence ($t_{1/2}$ = 118 and >1000 days respectively) ▪ SARS-CoV-2-specific B cell responses were measured longitudinally in *31 subjects where sufficient cells were available. Following infection, frequencies of IgG+ S-specific memory B cells increased over time irrespective of disease severity ▪ Overall, the authors found that both neutralising and binding antibody responses decay as expected after recovery from COVID-19. Binding and neutralising antibody responses, together with individual serum clonotypes, decay over the first 4 months post-infection, as expected, with a similar decline in S-specific CD4+ and circulating T follicular helper (cTFH) frequencies. In contrast, S-specific IgG+ memory B cells (MBC) consistently accumulate over time, eventually comprising a significant fraction of circulating MBC. Modelling of the concomitant immune kinetics predicts maintenance of serological neutralising activity above a titre of 1:40 in 50% of convalescent subjects to 74 days, with probable additive protection from B and T cells.	

		biotinylated using recombinant Bir-A (Avidity). ▪ Mass spectrometry (MS)-based quantitative proteomics of serum anti-S1 antibodies		
Wu 2020 DOI: 10.1101/2020.07.21.20159178 China Case series	N=349 symptomatic patients N=585 samples collected up to 26 weeks after disease onset Characteristics of hospitalised (N=149 non severe cases and N=60 severe cases) patients with complete medical records are presented (*not for the entire cohort) Age in years non-severe: 60 (43.5-68), severe: 60 (47.3-67.8) Female sex: non-severe: 76 (51%) severe: 23 (38.3%)	▪ Samples were analyzed for IgM and IgG recognizing the RBD of the spike protein (denoted IgM-S and IgG-S, ▪ respectively) as well as IgM and IgG binding the nucleocapsid protein (IgM-N and IgG-N, ▪ respectively). ▪ Blood samples were collected and separated by centrifugation at 3000g for 15 min within ▪ 4-6 h of collection, followed by 30 min inactivation at 56°C and storage at -20°C for further analyses. ▪ ▪ Capture chemiluminescence immunoassays (CLIA) by MAGLUMI™ 2000 Plus (Snibe, Shenzhen, China) were used as the test system.	Week 1 after symptom onset, the 4 antibodies tested positive with different frequencies: IgM-S (66%) > IgG-N (33%) > IgM-N (22%) > IgG-S (11%) **<u>Antibody response weeks 8-26 post symptom onset</u>** **IgM-S:** 69% at week 8 and then rapidly decreased to 0% at week 13 fluctuating below 35% thereafter and 20% at week 26. **IgM-N:** detected in 23%% of the patients at week 8. Afterwards, this number rapidly declined and became undetectable at week 10 and 12, followed by negligible fluctuations at very low positive rates (0% at week 26). **IgG-S**: positive in 92% of the patients at week 8 and remained at a relative high percentage until the end of the observation period at week 26 (100%). **IgG-N**: positive in 9"% of the patients at week 82 and stayed at very high levels thereafter (100% at week 26). **Neutralizing antibody titers response weeks 8-26 post symptom onset** The titer of IgM-S reached its peak at week 4, and then slowly decreased until the average value fell below the cut off value at week 12. After reaching the peak at week 3, the titers of IgM-N dropped rapidly below the cutoff value after around 9 weeks. The titers of IgG-N and IgG-S reached their peaks at week 4 and 5, respectively. After a contraction phase, in which titers constantly decreased during week 6 to 14, IgG-N and IgG-S titers stabilized and were maintained at high levels until the	Not peer reviewed (pre-print)

			end of the observation period of 26 weeks post symptom onset. Thus, SARSCoV-2-specific IgG responses were very similar to antibody responses against many other viruses with a peak activity a few weeks after infection, which was followed by a contraction phase over several weeks, but finally resulting in a stabilized antibody response that could be detected for at least 6 months. **Authors conclusions**: Taken together, the data indicate sustained humoral immunity in recovered patients who suffer from symptomatic COVID-19, suggesting prolonged immunity	
Yang 2020 China Cross-sectional 10.1101/2020.07.01.20144030	Assay for IgM/IgG not described	N=72 clinically recovered patients, of which N=55 patients included with serology samples ≥28 days post-discharge Mean age: 48.8 years (range: 27-70 years) ▪ 62% female	IgG seropositive in 55 patients; (13 patients seronegative for IgG and IgM, 3 patients re-detected positive and 1 patient with a serious chronic condition all excluded from study). Of the 55 patients: N=1 at 76 days post-discharge (61-year old female) N=8 at 60-75 days post discharge N=10 at 50-59 days post discharge N=55≥28 days post discharge	Not peer-reviewed
Xiao 2020 DOI: 10.1111/irv.12798 China Case series	N=31 lab-**confirmed COVID-19** cases N=70 sera samples collected from COVID sample between days 0 and 85 post-symptom onset Age in years: 26 to 82 (median = 58) N=80 **non-COVID-19** healthy	The archived SARS sera were tested for SARS-CoV spike (Sc) and nucleocapsid (Nc)-specific IgG antibodies using an ELISA kit that was provided by Autobio Diagnostics Co. Ltd (Zhengzhou, China). Recombinant SARS-CoV-2 S and N proteins (Sino Biological Inc, China) were used to coat 96-well plates at 0.5μg/ml overnight at 4°C. After washing and blocking, serially diluted sera (at a starting dilution of	**<u>COVID 19 sample (N=31)</u>** 'SARS-CoV spike 'S' **Sample:** 50 to 63 days **No of sera:** 3 **No seropositive:** 3 (100%) GMT (SD): 3.90 (1.12) **Sample:** 64-85 days **No of sera:** 17 **No seropositive:** 17 (100%) GMT (SD): 3.65 (1.23) 'SARS-CoV spike 'N' **Sample:** 50 to 63 days **No of sera:** 3	Peer reviewed (Published in Influenza and Other Respiratory Viruses Journal)

	Elderly (between 60 to 89 years old), N=28 adults and N=30 children with lab-confirmed influenza and N=35 adults and N=30 children that submitted sera for non-respiratory illness testing at an independent clinical diagnostic laboratory. N=30 archived sera from SARS-CoV during the 2003 outbreak in Guangdong were screened for activity, and 27 were included in the study. **Total:** 261	1:100) were added to the plate and incubated for 2 hours at 37°C. Plates were washed and added with an anti-human IgG horseradish peroxidase-conjugated secondary antibody (Sigma). Colorimetric reaction was developed using 3,3′,5,5′-Tetramethylbenzidine (TMB) substrate (Gcbio Technologies, China), stopped using 0.5 mol/L sulphuric acid and the absorbance read at 450 nm. Endpoint titers were determined to be the last reciprocal dilution with a positive/negative optical density (O.D) ratio ≥2.	**No seropositive:** 3 (100%) GMT (SD): 3.86 (1.21) **Sample:** 64-85 days **No of sera:** 17 **No seropositive:** 17 (100%) GMT (SD): 3.89 (1.23) **Non COVID 19 sera sample: SARS adults 2003 (N=27)** 'SARS-CoV spike 'S' **No of sera:** 27 **No seropositive:** 8 (30%) GMT (SD): 3 (1.45) 'SARS-CoV spike 'N' **No of sera:** 27 **No seropositive:** 10 (27%) GMT (SD): 2.77 (1.52) **Non COVID 19 sera sample: Healthy Elderly 2015 (N=80)** 'SARS-CoV spike 'S' **No of sera:** 80 **No seropositive:** 5 (6.3%) GMT (SD): 1.73 (1.09) 'SARS-CoV spike 'N' **No of sera:** 80 **No seropositive:** 5 (6.3%) GMT (SD): 1.73 (1.09) **Non COVID 19 sera sample: Non-respiratory testing adults March 2020 (N=35)** 'SARS-CoV spike 'S' **No of sera:** 35 **No seropositive:** 0 (0%) GMT (SD): 1.69 (1.00) 'SARS-CoV spike 'N' **No of sera:** 35	

			No seropositive: 1 (2.9%) GMT (SD): 1.71 (1.05) **<u>Non COVID 19 sera sample: Influenza confirmed adults Jun-Jul 2017 (N=28)</u>** 'SARS-CoV spike 'S' **No of sera:** 28 **No seropositive:** 0 (0%) GMT (SD): 1.69 (1.00) 'SARS-CoV spike 'N' **No of sera:** 28 **No seropositive:** 0 (0%) GMT (SD): 1.69 (1.00) **<u>Non COVID 19 sera sample: Non respiratory testing children Dec 2019 (N=30)</u>** 'SARS-CoV spike 'S' **No of sera:** 30 **No seropositive:** 0 (0%) GMT (SD): 1.69 (1.00) 'SARS-CoV spike 'N' **No of sera:** 30 **No seropositive:** 0 (0%) GMT (SD): 1.69 (1.00) **<u>Non COVID 19 sera sample: Influenza confirmed children Dec 2019 (N=30)</u>** 'SARS-CoV spike 'S' **No of sera:** 30 **No seropositive:** 0 (0%) GMT (SD): 1.69 (1.00) 'SARS-CoV spike 'N' **No of sera:** 30 **No seropositive:** 0 (0%) GMT (SD): 1.69 (1.00)	

			Author conclusions: Although the average SARS-CoV-2 S and N-IgG titers were comparable, N-responses were more variable among individuals. S- and N-assay specificity tested with non-COVID-19 sera were comparable at 97.5% and 97.0%, respectively. Therefore, S will make a better target due to its lower cross-reactive potential and its' more consistent frequency of detection compared to N.	

Appendix 4. Additional sources

Summary of reinfection cases from news articles or personal communication

Item	Source of publication	Patient demographics	Location	Severity of 1st infection	Severity of 2nd infection	Interval
1.	News article in Business Insider (https://www.businessinsider.com/four-coronavirus-reinfection-cases-reported-in-the-netherlands-2020-8?international=true&r=US&IR=T)	Over 60 years of age, sex N/R	Netherlands	N/R	N/R	60 days
2.	News article in De Standaard (https://www.standaard.be/cnt/dmf20200929_97735497)	30 year old male	Belgium	Mild infection	Mild infection	143 days
3.	News article in De Standaard (https://www.standaard.be/cnt/dmf20200929_97735497)	25 year old female	Belgium	Mild infection	Mild infection	115 days
4.	News article in El País (https://elpais.com/ciencia/2020-10-13/seis-personas-entre-40-millones-de-casos-el-misterio-de-los-reinfectados-por-el-coronavirus.html)	62 year old male	Spain	Mild infection	Severe infection requiring hospitalisation	147 days
5.	Unpublished (https://bnonews.com/wp-content/uploads/2020/10/10182020SwedenReinfection.png)	53 year old female	Sweden	Mild infection	Mild infection (less severe)	120 days
6.	Unpublished	Male aged 60-69	Netherlands	Mild infection	Severe infection	12 days

	(https://bnonews.com/wp-content/uploads/2020/09/9232020TilburgReinfectionCases1.png)					
7.	Unpublished (https://bnonews.com/wp-content/uploads/2020/09/9232020TilburgReinfectionCases1.png)	Male aged 80-89	Netherlands	Mild infection	Mild infection	23 days

CXR – chest x-ray
N/R – not reported
HCW – healthcare worker
SSRN - Social Science Research Network

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For further information please contact:

Health Information and Quality Authority
George's Court
George's Lane
Smithfield
Dublin 7
D07 E98Y

Phone: +353 (0) 1 814 7400
info@hiqa.ie
www.hiqa.ie