Health Information and Quality Authority

An tÚdarás Um Fhaisnéis agus Cáilíocht Sláinte

Evidence summary of the immune response following infection with SARS-CoV-2 or other human coronaviruses

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Key points

- Sixty-seven studies were identified that investigated the immune response following coronavirus infections, including SARS-CoV-2 (n=39) (that causes COVID-19), SARS-CoV-1 (n=24) and MERS-CoV (n=4).
- Many studies have not yet been peer reviewed (n=25/67) and the overall quality of evidence was low.
- Five separate research questions were identified that focused on the rate and timing of antibody detection after infection, the duration of the immune response, the reinfection rate and the association between these responses and the severity of initial disease.
- The detection rate or timing of antibodies following acute SARS-CoV-2 infection was assessed in 23 studies. Immunoglobulin M (IgM) titres were typically the first to rise in acute infection, followed by immunoglobulin G (IgG), with IgG tending to persist for much longer in the body. The median time to antibody detection following symptom onset ranged from five to 13 days for IgM and 12 to 14 days for IgG. While the rate and timing of IgM and IgG detection were inconsistent across studies, SARS-CoV-2-specific IgG antibodies were detected in all individuals after approximately two weeks; however, the adequacy or duration of this response is not yet known. Three studies reported data in relation to neutralising antibodies, which were detected in all included patients.
- Eight studies were identified that reported the duration of the immune response following SARS-CoV-2 infection. Maximum follow up was seven-to-eight weeks, with IgG and neutralising antibodies detected up to two months after symptom onset. The full duration of the immune response is unknown.
- Due to the lack of long-term follow-up data relating to SARS-CoV-2, evidence on other coronaviruses was also retrieved, although the applicability of these to SARS-CoV-2 is unknown. Twenty-four studies reported on the duration of SARS-CoV-1-specific immunity. In general, SARS-CoV-1-specific IgG antibody levels were sustained for one to two years post infection, declining thereafter. Four studies on MERS-CoV suggest the immune response is less consistent than for SARS-CoV-1, although one study reported a sustained immune response up to 34 months in the majority of participants.

- Ten studies were retrieved that report re-detection of SARS-CoV-2 following recovery. An agreed definition for reinfection (as opposed to re-detection) was not identified. Technical issues in testing may underlie these possible reinfection cases, including intermittent false negatives from the inconsistent viral shedding in the later course of the disease, or the detection of dead viral remnants by RT-PCR when no viable virus is present. No patients who were re-detected positive showed obvious clinical symptoms or disease progression. Thus, it is not yet possible to conclude whether reinfection following recovery from SARS-CoV-2 occurs.
- Ten studies that investigated the association between severity of initial disease and immune responses found inconsistent findings. Four studies reported that patients with severe COVID-19 disease develop higher IgM/IgG antibody levels than those with moderate or mild disease, whereas three found no such association.

Evidence summary of the immune response following infection with SARS-CoV-2 or other human coronaviruses

1. Introduction

The Health Information and Quality Authority (HIQA) has developed a series of 'Evidence Summaries' to assist the Clinical Expert Advisory Group (EAG) in supporting the National Public Health Emergency Team (NPHET) in their response to COVID-19. These summaries are based on specific research questions. This evidence summary was developed to address the following research question:

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

The objective of this review is to summarise the evidence on the immune response following acute coronavirus infections, including SARS-CoV-2.

To do this, the following sub-questions were addressed:

1. What proportion of confirmed cases develop specific antibodies to SARS-CoV-2 (seroconversion rate)?
2. How quickly does one develop specific antibodies to SARS-CoV-2 (seroconversion timing)?
3. What is the duration of detection of serum antibodies and antibody titres over time associated with infection with SARS-CoV-2 or other coronaviruses?
4. What is the reinfection rate following recovery from acute SARS-CoV-2 infection?
5. Does the seroconversion rate and or timing, and duration of immunity, depend on the severity of the initial infection?

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. Below is the summary of all relevant evidence from 1 January 2000 until 1 May 2020. Data published by national agencies were not included. As the focus of the review is SARS-CoV-2, evidence was only considered for other coronaviruses where there was limited SARS-CoV-2 evidence available.

2. Results

In total, 67 studies were identified, including 54 case series,[1-54] seven case reports,[55-61] five cohort studies[62-66] and one cross-sectional study.[67] Fifty studies were conducted in China,[3-6, 8, 9, 12-19, 21, 23-26, 29-32, 35, 37-42, 44-53, 59-63, 66, 68, 69] three in South Korea,[10, 27, 57] three in Taiwan,[7, 22, 56] two in Germany,[11, 43] two in Saudi Arabia,[2, 54] and one each in Finland,[55] France,[34] Italy,[58] Jordan,[36] the Philippines,[28] Singapore[33] and the UK.[1] SARS-CoV-2 was investigated in 39 studies,[1, 3, 9, 11-14, 19, 23-27, 29, 30, 34, 35, 40-43, 45, 46, 48, 50-53, 55, 56, 58-63, 66, 67] SARS-CoV-1 in 24[4-8, 15-18, 20-22, 28, 31-33, 37-39, 44, 49, 64, 65, 70] and MERS-CoV in three.[2, 10, 36]

2.1 Research questions 1 and 2: Seroconversion rate and timing

2.1.1 Characteristics of included studies

Seroconversion is the transition from a seronegative (no detectable coronavirus-specific antibodies in the serum sample) to a seropositive condition (detectable coronavirus-specific antibodies in the serum sample). In total, 23 studies were identified that assessed the rate and or timing of immunoglobulin M (IgM) and or immunoglobulin G (IgG) antibody detection in patients with acute SARS-CoV-2 infection, including 16 case series,[14, 19, 23, 24, 26, 29, 30, 34, 35, 40, 41, 43, 46, 51, 52, 68] four case reports,[55, 56, 58, 61] two cohort studies[62, 66] and one cross-sectional study.[69] Due to the abundance of data relating to SARS-CoV-2, evidence relating to other coronaviruses was not considered.

The number of participants in included cohort studies or case series ranged from three to 380 individuals, and the number of samples taken ranged from 10 to 535. The median age of individuals ranged from 40 to 68, and a similar number of males and females were followed across studies. A diverse range of serological tests were used, including chemiluminescent immunoassay (CLIA),[19, 51, 61] enzyme-linked immunosorbent assay (ELISA),[19, 26, 34, 46, 51, 62, 68] enzyme immunoassay (EIA),[66] gold immunochromatographic assay (GICA),[19] immunofluorescence assays (IFA),[23, 43, 55, 58] immunochromatography (ICG) strip assay,[35] lateral flow immunoassay (LFIA),[62] magnetic chemiluminescence enzyme immunoassay (MCLIA),[67] modified cytopathogenic assay (MCA),[42] proteomic microarrays[24] and SARS-CoV-2 antibody detection kits.[30, 40, 52] One study used a rapid test (ALLTEST 2019-nCoV IgG/IgM Rapid Test Cassette).[56] Table 2 (Section 6) summarises the characteristics and primary outcome findings of the included studies.

2.1.2 Seroconversion rate

Seroconversion rate (proportion of individuals who seroconvert) for coronavirus-specific antibodies varied across studies and stage of disease. As few studies measured serial antibody samples to identify the point at which a patient

seroconverts,[55, 56] the proportion of patients that tested positive at a specific time point was reported as a proxy for the seroconversion rate.

Three studies investigated the detection rate for immunoglobulin M (IgM) and immunoglobulin G (IgG) at three different stages of the disease.[19, 35, 62] The detection rate for IgM ranged between 11.1% and 60% at the early stage (1-7 days) after symptom onset, between 53.8% and 86.7% at the intermediate stage (8-14 days), and between 74.2% and 96.7% after 14 days. The detection rate for IgG ranged between 3.6% and 50% at the early stage, between 57.1% and 76.9% at the intermediate stage, and between 93.3% and 100% after 14 days. Figures 1 and 2, below, illustrate these findings.

Figure 1 Immunoglobulin M detection rate over time

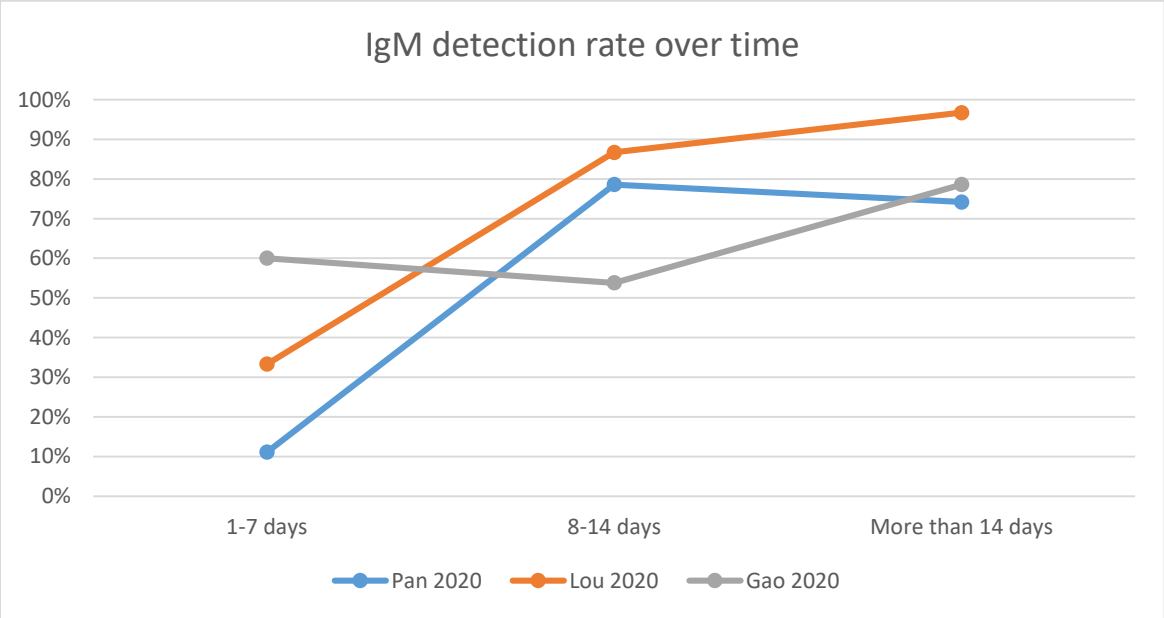
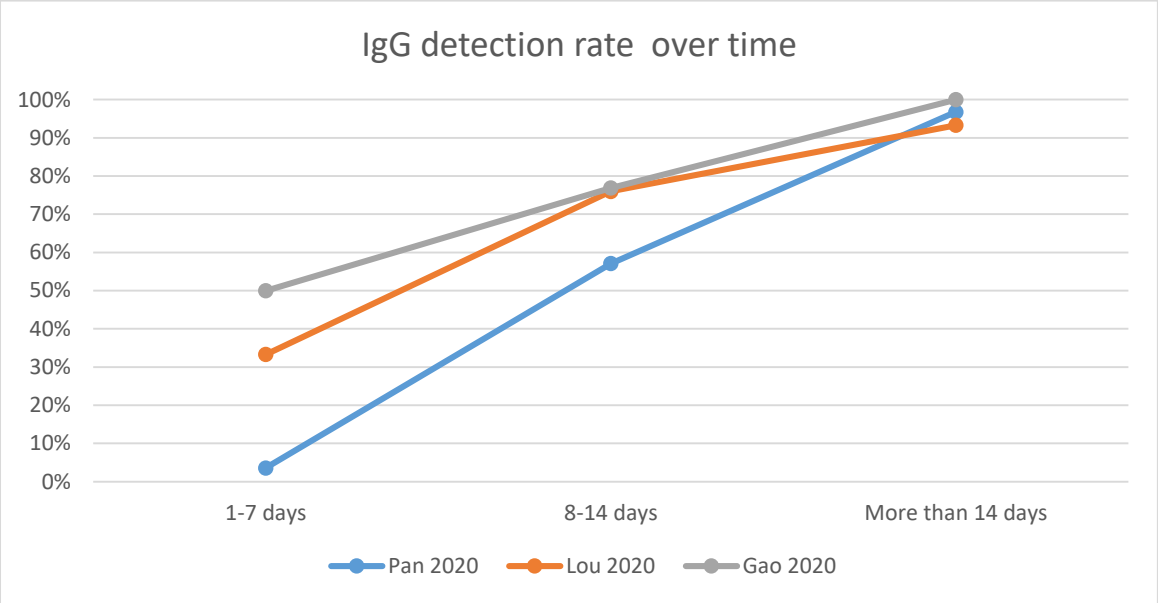


Figure 2 Immunoglobulin G detection rate over time



One study (n=34) evaluated antibody detection at two points in time;[46] at week three all patients tested positive for IgG and IgM, whereas at week five, all tested positive for IgG and 83% for IgM.

Six studies reported the antibody detection rate at one point in time.[14, 23, 24, 34, 66, 69] This ranged from 78% to 100% for IgM and from 64.7% to 100% for IgG; however, the timing of samples varied widely (from one day to 6-7 weeks post symptom onset). The IgM detection rate was lowest at the later time-points, whereas the vast majority were reported to have seroconverted for IgG when samples were taken after 14 days.

2.1.3 Seroconversion timing

Across studies, IgM titres were typically the first to rise in acute infection, followed by IgG, with IgG tending to persist for much longer in the body. However, the timing for IgM and IgG detection varied significantly across studies with virus-specific antibodies detected at an early stage after symptom onset in some cases, but not until the intermediate or late stage in others.

The median time to antibody detection following symptom onset ranged from 12 days[62] to 14 days[68] for IgG and from five days[68] to 13 days[69] for IgM. In three studies, the antibody detection timing was reported to be shorter for IgM than for IgG,[19, 35, 62] whereas one study found IgG seroconversion before IgM.[69] While steady decreases in IgM titres after one week were reported in most studies, IgG titres did not wane and remained positive for up to seven weeks in the two studies with the longest follow up.[14, 40]

One study also reported immunoglobulin A (IgA) antibody detection; approximately 90% seroconverted by two weeks post symptom onset, with a median of five days (IQR: three to six).[68]

Three studies reported neutralising antibody data. The first found that all patients tested positive for neutralising antibodies by day 14,[43] the titres of which did not suggest close correlation with clinical courses. Additionally, one patient who had the lowest virus neutralisation titre at end of week two seemed to shed virus from stool over a prolonged time. A second study found a neutralising antibody detection rate of 100% within 20 days of symptoms onset, and which remained at 100% for the duration of follow up (day 41-53).[42] In a third study, IgG and IgA responses detected by different assays correlated strongly with neutralising antibody response, with all patients eventually developing neutralising antibodies.[71]

Finally, a case series involving nine COVID-19 cases measured antibody titres (by immunofluorescence), viral load (by RT-PCR) and infectivity (live virus isolation).[43] In this study, live virus isolation was attempted on multiple occasions from clinical samples. While the virus was readily isolated during the first week of symptoms from a considerable proportion of samples (16.7% in swabs, 83.3% in sputum samples), no isolates were obtained from samples taken after day eight despite persistent high viral loads. Seroconversion was detected by IgG and IgM immunofluorescence using cells expressing the spike protein of SARS-CoV-2 and a virus neutralisation assay using SARS-CoV-2. Antibody detection (IgM and or IgG) in 50% of patients occurred by day seven, and in all by day 14. All patients showed detectable neutralising antibodies, the titres of which did not suggest close correlation with clinical courses. This study supported the hypothesis that an appropriate antibody response results in the clearance of infectious virus.

2.2 Research question 3: Duration of immune response

As SARS-CoV-2 was first identified in December 2019, there is a lack of evidence on the long-term duration of antibody responses following infection. However, other similar coronaviruses, particularly SARS-CoV-1 and MERS-CoV, may be of interest as the immune response may follow a similar trajectory. Details of study characteristics can be found in Tables 3 to 5, Section 6.

2.2.1 SARS-CoV-2

Eight studies were identified that examined the duration of the immune response in SARS-CoV-2 infection.[1, 13, 14, 25, 34, 42, 43, 72] Follow-up time ranged between two and eight weeks. Five studies were conducted in China[3, 13, 14, 25, 42, 72] and one each was conducted in Germany,[43] France[34] and the UK.[1] A number of different methods were used to determine immune response, including ELISA,[1, 13, 34] neutralising assay,[13, 42, 43] plaque reduction neutralisation test (PRNT),[34] ELISpot,[13] chemiluminescence immunoassay kits (CLIA),[25] as well as rapid tests such as lateral

flow immunoassay devices (LFIA).[1] All studies were either case series or case reports. Four of these studies were published as pre-prints and have not yet undergone peer review.[1, 13, 42, 72]

Four studies reported on the duration of immunoglobulin antibody responses following infection (maximum follow-up: 50-60 days post-infection). In the first study, nine patients had serology data from 50 and 60 days post symptom onset.[1] IgM and IgG were detected in five (56%) and nine (100%) patients, respectively. The second case series comprised 12 patients discharged from hospital (length of stay 11-37 days) following acute infection with SARS-CoV-2.[13] Serology testing was undertaken either at discharge or two weeks after discharge.[13]An IgG and IgM response to nucleocapsid protein (NP) and spike protein receptor binding domain (S-RBD) was detected in 100% of patients and the IgG response was maintained for at least two weeks post discharge (the end of the study). The third study reported serology results for a case series of 60 patients who were tested at six-to-seven weeks from symptom onset.[14] IgM and IgG were detected in 47 (78%) and 60 (100%) patients, respectively. Serology was repeated in 10 patients one week later (week seven-to-eight) with a decline in titres noted for both antibodies, which was greater for IgG than IgM. In the fourth study, 98 serology measurements from 43 patients indicted that the positivity rate for IgG reached 100% by 11-15 days after onset of symptoms and remained at this level 31-55 days after symptom onset.[25]

Four case series (range: 3-70 patients) reported neutralising antibody serology data, with the longest follow-up 41-53 days post-symptom onset. One study found that half of all patients produced neutralising antibodies by day seven, and all (n=9) by day 14. [43] The second case series comprised 12 patients discharged from hospital following acute infection with SARS-CoV-2.[13] Serology testing was undertaken either at discharge (n=6, length of stay 17-37 days) or within two weeks of discharge (n=6, length of stay 11 to 19 days). Four (out of six) of the recently discharged patients had high neutralising antibody titres; the titres in five out of six of the patients who were two weeks post discharge were positive, but in four of these the titres were lower than in the recently discharged patients. In the third case series that included 117 samples from 70 patients, a 100% seropositivity rate was reported at 41-53 days after symptom onset (based on 29 samples). The highest antibody titres were reported to be between days 31-40; titres then decreased slightly between days 41-53.[42] In a small case series (n=3) comprising two mild and one severe case, the authors reported detection of neutralising antibodies in all three cases 20-30 days after symptom onset.[34]

Only one study reported on T-cell responses.[13] The authors found that compared with healthy donors, the number of IFN-gamma secreting NP specific T-cells in four (out of 6) recently discharged patients suggested that they had developed a SARS-CoV-2 specific T-cell response.[13] Only one (out of 6) of the patients who had serology testing two weeks after discharge had a high number of IFN-gamma

secreting T-cells suggesting anti-viral T-cells may not be maintained at high numbers in recovered patients. Table 1, below, summarises the duration of immune responses following SARS-CoV-2 infection.

Table 1 Summary of studies on maximum duration of SARS-CoV-2 immune response

IgG positivity	**Adams 2020(1)**	50-60 days post symptom onset 9/9 patients positive for IgG
	Dong 2020(13)	25–33 days post admission to hospital. 6/6 patients positive for IgG
	Du 2020(14)	49-56 days post symptom onset IgG positive in 10/10 but titres declining
	Jin 2020(25)	31-55 days post symptom onset 100% IgG positive (based on 8 serology measurements at 31-55 days)
Neutralising assays	**Dong 2020(13)**	25-33 days post admission to hospital. 5/6 positive for neutralising antibodies
	Okba 2020(34)	20-30 days post symptom onset 3/3 patients positive for neutralising antibody
	Wang 2020(42)	41-53 days post symptom onset. 29/29 samples were positive for neutralising antibodies
	Wolfel 2020(43)	14 days post symptom onset. 9/9 patients neutralising antibodies
T-cells	**Dong 2020(13)**	4/6 recently discharged positive for T-cells. 1/6 tested 14 days post discharge positive for T-cells

Note – duration denotes longest follow-up in included studies. Duration of immune response inconsistently reported as either duration from symptom onset, post-admission or post-discharge.

2.2.2 SARS-CoV-1

Twenty four studies provided data on the duration of the immune response to SARS-CoV-1; maximum follow up was up to 12 years in two studies,[20, 33] between one and six years in 12 studies,[4, 5, 8, 17, 28, 31, 32, 37, 39, 44, 49, 65] and up to one year in 10 studies.[6, 7, 15, 16, 21, 22, 38, 47, 64, 73] A further seven studies were identified as potentially relevant; however, these studies were only available in Chinese and it was not possible to locate full text copies of these studies.[18, 70, 74-78]

All studies were conducted in China apart from two in Taiwan,[7, 22] one in the Philippines[28] and one in Singapore.[33] All studies were case series or prospective cohort studies, with sample sizes ranging from two[28] to 311[18] participants. Table 3 provides additional details of included studies.

For studies with less than one year follow up, IgM antibodies were reported to begin to decline two-to-three weeks after the onset of symptoms[7, 15, 21, 22, 75] and had disappeared by three to 12 months after infection.[7, 16, 22] In all studies, IgG antibodies were detectable at the end of follow-up, which ranged from 12 weeks to one year.[6, 7, 15, 16, 18, 21, 22, 75] Two studies reported on the magnitude and duration of T-cell immunity one year after the onset of symptoms.[15, 47] T-cell populations were said to be decreased in convalescent patients compared with healthy controls in the early post-infection period in both studies.[15, 47] In the second study with longer follow up, T-cell populations later rapidly recovered, but at one year T-cell counts were still reduced compared with healthy controls. The number of CD8+ T-cells recovered significantly faster than CD4+ T-cells.[47]

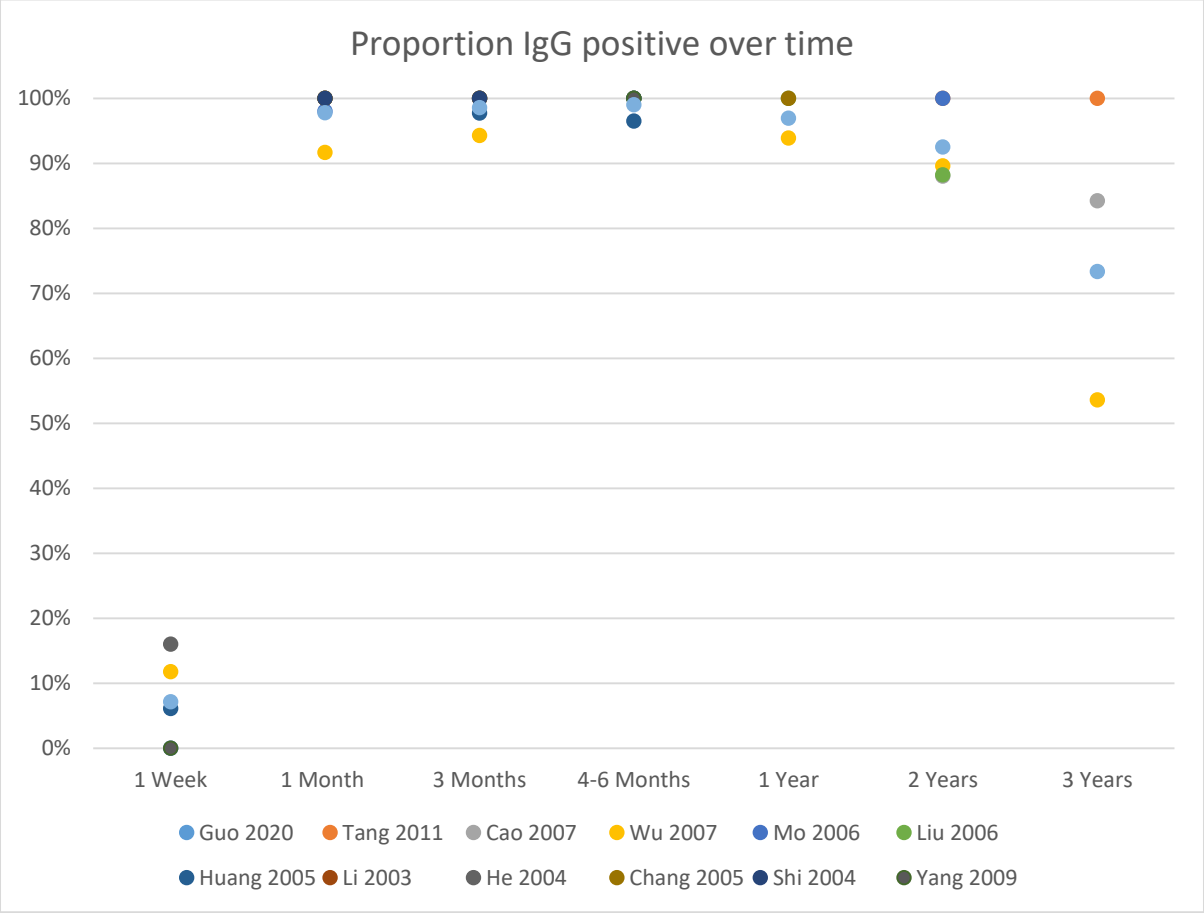
For studies with one-to-two years follow up, IgG antibodies were still detectable at the study end point.[49, 65] Additionally, SARS-CoV-1 infection was reported to induce a strong memory T-cell response approximately one year after infection in both studies.[8, 49] Furthermore, cross-reactive memory T-cells to SARS-CoV-1 may exist in the T-cell repertoire of a small subset of healthy individuals in one study.[8]

Five studies reported follow-up data at approximately two years after SARS infection.[17, 31, 32, 37] In the first study, SARS-specific IgG and neutralising antibodies were detectable at the end of the study in 30 patients.[17] High and sustainable levels of immune responses were found to be strongly correlated with disease outcome.[17] In a second study, IgG antibody and neutralising antibody titres were found to be highly correlated.[31] Neutralising antibodies were detectable in all patients at 24 months; however, 11.8% of serum samples were negative for SARS-CoV-1-specific IgG antibodies at the final visit. A third study reported that IgG and neutralising antibodies were still detectable at 720 days; however, titres were close to the cut-off point for positivity.[32]

In addition to evidence of persistent humoral immunity at two years post-infection, three of these studies investigated T-cell-mediated immunity in recovered SARS patients up to 30 months after infection. In the first study, despite the potent immune responses and clinical recovery observed in patients, peripheral lymphocyte counts were not restored to normal levels compared with matched controls at 24 months,[17] in line with findings previously reported at one year follow up. A second study reported that SARS-CoV-1 N-protein-specific memory CD4+ and CD8+ T-cells were maintained for two years after SARS-CoV-1 infection,[37] while in the final study, T-cell cytotoxic activity could be detected after *in vitro* stimulation at 12 months, but not at 24 and 30 months.[28]

Figure 3 illustrates the proportion of patients detected to be IgG positive over the first three years post-symptom onset.

Figure 3 Proportion IgG positive over time following SARS-CoV-1 infection



In the four studies that followed patients for three to six years, in general, antibody levels were reported to decrease over time. One study reported a decline in SARS-specific IgG antibody titres and neutralising antibodies with IgG GMTs dropping from 244 at month four to 28 at month 36 (that is, study end point) and neutralising antibodies dropping from 1,232 at month four to 32 at month 36.[4] Another study reported that SARS-CoV–specific IgG antibodies were detectable in >90% of patients at two years follow up, but approximately 50% of the convalescent population had no detectable SARS-CoV-1–specific IgG at three years. IgM became undetectable at approximately 90 days.[44] In another study, only two of 23 patients maintained a low level of SARS-CoV-1-specific IgG antibodies at six years post-infection.[39] However, memory T-cell responses to a pool of SARS-CoV-1 S peptides were identified in the majority (60.9%) of recovered patients. There was evidence to suggest that the memory T-cell response was correlated with clinical severity.[39] No SARS-CoV-1 antigen-specific memory B cell responses were detected. Of note, a fourth study reported that SARS-CoV-1-specific antibodies could be detected at high titres at three years follow up using ELISA with RBD-based ELISA, while the positivity rate was only 42% using a commercially available viral lysate-based ELISA

kit.[5] This suggests that differences in positivity rates reported across studies may be attributable to differences in the sensitivity of the tests used.[5]

Of the two studies with the longest (at least 12 years) follow up, the first reported that anti-SARS-CoV-1 IgG antibodies against the whole virus were present in 81% (26/32) of recovered SARS-CoV-1 patients during the first year after infection.[79] In general, IgG levels peaked at 100% (32/32) in 2004 (one-to-two years after the outbreak), declined quickly from 2004 to 2006, and subsequently continued to decline at a slower rate, decreasing to 69% (18/26) in 2015 (approximately 12 years after infection).[79] The second study reported on the response of memory T-cells, and found that SARS-CoV-1-specific memory T-cells targeted against SARS-CoV-1 structural proteins persisted up to 11 years post-infection in all (N=3) recovered patients.[33] SARS-specific T-cells were not activated by MERS-CoV peptides suggesting that T-cell immunity against SARS-CoV-1 is highly specific and SARS-specific T-cells are unlikely to provide cross-protection against infection with other distantly related coronaviruses.

2.2.3 MERS-CoV

Four case series examining the duration of the immune response following MERS-CoV infection were identified, with the longest follow-up 34 months post-symptom onset.[2, 10, 36, 54]

One study, with nine patients, reported a rigorous antibody response in all survivors who had severe disease, but not in survivors of mild disease.[2] In this study, patients with severe MERS-associated pneumonia had a persistent antibody response detected for more than 18 months after infection, whereas patients with disease confined to the upper respiratory tract or who were asymptomatic had no detectable MERS-CoV antibody response. Similar findings were reported in another study of 11 patients (five with severe disease and six with mild disease) who were followed up for one year.[10] While all had an initial antibody response, the majority of those with mild disease (four out of six) had negative results for antibodies using four different assays at one year follow up, and all five patients with severe disease had positive antibody tests. MERS antibody titres waned during the first six months after disease onset, especially in patients who had had high antibody titres at 21-50 days after onset. The waning of antibody titres between six months and one year after disease onset was less pronounced.

The third study included 21 patients (14 had samples taken at six months and seven at 24 months), antibody responses were present, but at a lower titre at 24 months compared with those who had samples taken at six months, although the difference was not statistically significant.[54] Virus-specific CD8+ and CD4+ T-cell responses were present at six months and 24 months even in those with mild or subclinical illness. A final study on MERS-CoV followed seven patients with probable MERS (not confirmed by RT-PCR) up to 34 months, using three different techniques to measure

the immune response.[36] At 34 months, of the seven participants for whom immunofluorescence assay results were positive for anti-MERS-CoV antibodies at 13 months, four (57%) had positive results at 34 months. During this time (13 to 34 months), the anti-MERS-CoV nucleocapsid ELISA titres decreased for all but one person, for whom the titre remained the same. One patient never developed neutralising antibodies. Of the six that did, antibodies were still detectable at 34 months, albeit with a decrease in titres over time in two of these six.

2.3 Research question 4: Reinfection rate

No agreed definition for what constitutes 'reinfection' was identified in the literature; however, 10 studies were retrieved that relate to re-detection of viral RNA following a negative RT-PCR sample, comprising seven cases series,[3, 12, 27, 41, 45, 48, 50] two case reports[57, 59] and one cohort study.[66] All were conducted in China apart from two in South Korea.[27, 57] Characteristics of included studies are provided in Table 6, Section 6.

All studies report cases of re-detected SARS-CoV-2 following recovery; however, the testing methodology, location of specimen, timing of testing (both recovery and re-detection times) and criteria for discharge from hospital varied across studies. The maximum sample size was 262 patients[3] and the median age of patient cohorts ranged from 41.5[41] to 62 years.[66] For studies conducted in China, patients were discharged in accordance with the Chinese Clinical Guidance for COVID-19 Pneumonia Diagnosis and Treatment: (1) normal temperature for three days or more, (2) significant improvement in respiratory symptoms, (3) chest radiology findings show substantial improvement of acute exudative lesions, (4) two consecutive negative nucleic acid tests using respiratory tract samples (taken at least 24 hours apart).[80]

In terms of estimating the rate of re-detected positive specimens, individual case studies do not provide meaningful data. Of the studies that followed a cohort of recovered patients (defined as at least two upper respiratory tract samples negative for SARS-CoV-2 collected at ≥ 24-hour intervals), five studies provided a rate of re-detection via RT-PCR of respiratory samples.[3, 12, 41, 45, 48] In these studies, the re-detection rate ranged from 3% (2/62 cases)[48] to 21% (15/70 cases).[45] In all studies, those re-detected were asymptomatic at the time of the positive test. Additionally, three studies reported re-detected positive anal or faecal samples in asymptomatic patients.[12, 41, 50]

An agreed definition for reinfection (as opposed to re-detection) with SARS-CoV-2 was not identified, possibly due to the limited number of such events described in the literature. The following two definitions for 'possible reinfection' were developed internally by HIQA's review team; one was stringent and the other was less stringent.

For the stringent criterion, 'possible reinfection' was defined as:

> 'A positive viral respiratory RT-PCR sample for SARS-CoV-2 following recovery, defined as at least two negative upper respiratory tract samples for SARS-CoV-2, collected at ≥ 24-hour intervals at a minimum of 14 days after the initial positive test AND a minimum of 14 days between recovery (for example, symptom resolution, afebrile) and onset of new symptoms.'

For the less stringent criterion, 'possible reinfection' was defined as:

> 'A positive viral respiratory RT-PCR sample for SARS-CoV-2 following recovery, defined as at least two negative upper respiratory tract samples for SARS-CoV-2, collected at ≥ 24-hour intervals. For symptomatic patients, samples should be collected at least seven days after symptom onset or after three days without fever. For asymptomatic SARS-CoV-2-infected persons, the tests to document virus clearance should be taken at a minimum of 14 days after the initial positive test.'

Using the stringent criterion, none of the cases described in the included studies can be defined as 'possible reinfection'. However, using the less stringent criteria, the majority of patients with re-detected viral RNA would be defined as 'possible reinfection', although not all studies provided sufficient information (for example, the duration of time between 'recovery' and re-detected positive for each case).

2.4 Research question 5: Immune response and severity of initial disease

Ten studies were retrieved that described the impact of the severity of initial infection with SARS-CoV-2 and the immune response.[1, 9, 11, 24, 34, 35, 42, 53, 63, 67] Unsurprisingly, as the virus has only recently been identified, none described how initial severity impacted the duration of immunity. Table 7, Section 6, summarises study characteristics and primary outcome data of included studies.

Four studies reported that antibody titres were higher in severe compared with mild cases. The first reported that among 285 patients, whose serum samples were taken in three-day intervals during their hospital stay, IgG and IgM titres in the severe group were higher than in the non-severe group, although statistical difference was only observed in IgG levels at two weeks.[67] The second reporting on one 'mild' case and two 'severe' cases found that antibody levels were higher following severe infection compared to the mild.[34] Specifically, antibody responses to spike (S), spike S1 subunit (B), spike N=terminal (S1A) domain, receptor bindings domain (E) nucleocapsid were higher in the severe cases than the mild. The third reported on 70 COVID-19 patients, 12 of whom were inpatients and 58 'convalescent' patients.[42] After adjusting for other factors associated with antibody levels, patients

with more severe symptoms tended to have higher antibody titres than those who were classified as moderate.

The fourth study reported detailed findings for 67 hospitalised SARS-CoV-2 infected patients with 'severe' and 'non-severe' disease.[63] Patients were classified as 'strong responders' if their peak titre was greater than 2-fold of the cut-off point, 'weak responders' if their peak titres were 1-2 fold of the cut-off point and 'non-responders' if their peak titre was below the cut-off point. The proportion of strong responders was significantly higher and proportion of weak responders significantly lower in patients with severe disease than patients with non-severe disease. IgM and IgG appeared earlier and were continuously significantly higher in patient with severe disease compared with those with non-severe disease. A higher proportion of non-severe patients had cleared the virus at day seven than severe patients (by RT-PCR). IgM was detectable in severe patients at 11.6 days (+/- 3 days) after illness onset compared with 14 days (+/- 5.3 days) in non-severe patients, and IgG was detectable in severe patients 13.4 days (+/- 4 days) after illness onset compared with 15.3 days (+/- 5.7 days) in non-severe patients.

Three studies reported antibody findings that were inconsistent with this general trend. One case series compared a 'more severe' case with a 'mild' case as well as three controls (a 'mild', a 'mild/moderate' and a 'negative' control).[11] Patients with mild symptoms displayed a much stronger IgA response soon after onset of symptoms that decreased during the course of disease seven to 14 days later, with the more severe case showing a delayed, but eventually very strong SARS-CoV-2 specific IgA response. A similar, but less pronounced trend was observed for IgG antibodies. The memory B-cell population increased after approximately 15 days post onset in both cases, but persisted in the severe case to day 32. The second study investigated seroconversion rates (or detection rates at specific time points) among mild, severe and critical disease states, and found no correlation between disease severity and antibody detection rates.[63] The third study found that there was no association between antibody titres (IgM/IgG) and disease severity or need for hospital admission-based on multivariable modelling on 40 convalescent patients.[1]

The association between lymphocyte counts (CD4+ and CD8+ subsets) and the severity of infection was investigated in one study.[53] Study authors reported that CD4+ T-cell and CD8+ T-cell counts were inversely associated with disease severity; the more serious the disease was, the lower were the T-cell, CD4+ T-cell and CD8+ T-cell counts on admission.

The association between the detection rate of viral RNA in blood and anal swab specimens and disease severity (patients classified as either mild or severe) was investigated in one study.[9] In the blood detection cohort, six cases had detectable virus in the blood, all of which were classified as severe; 51 had no virus detectable in the blood of which only 12 (23.5%) were classified as having severe disease. In

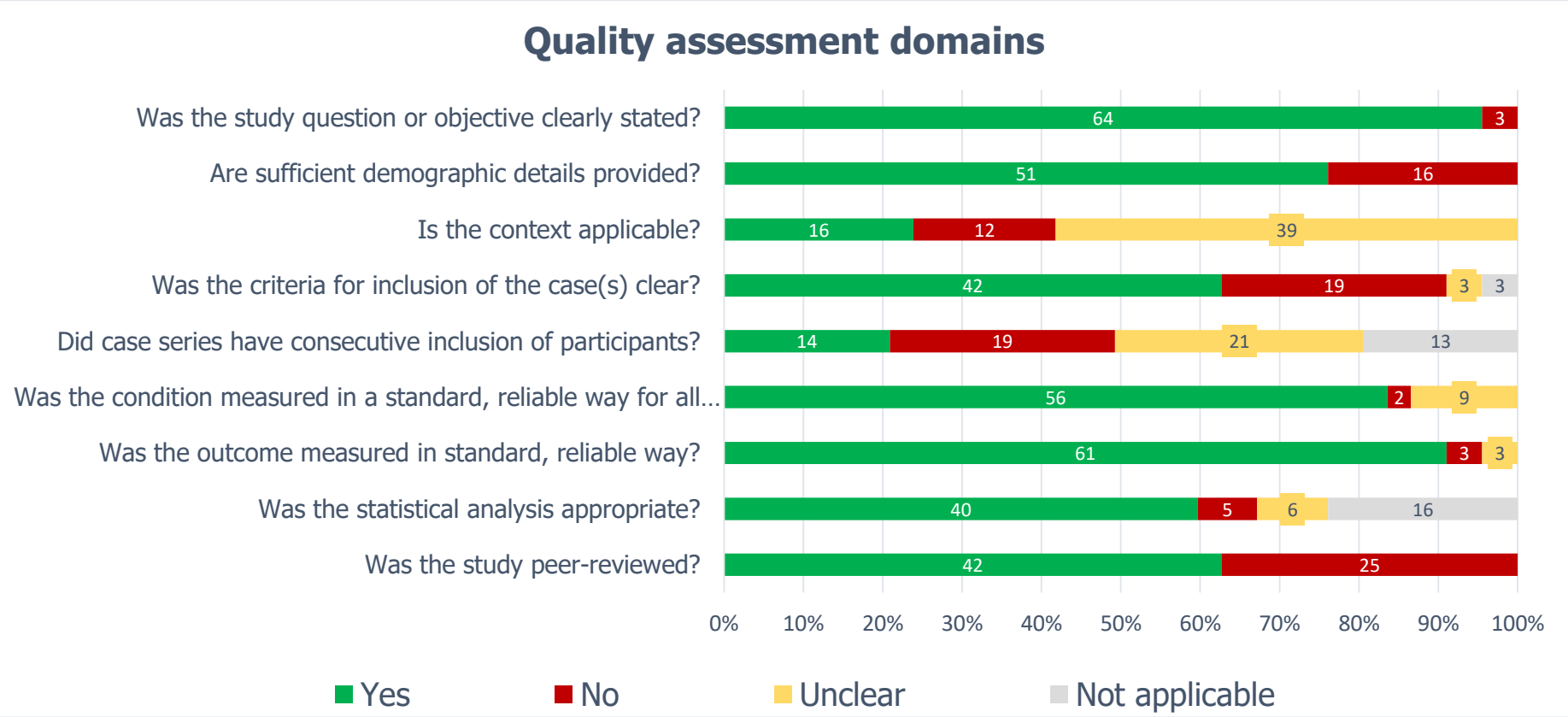
the anal swab cohort, 11 of 28 were anal swab positive, eight of which (72.7%) were classified as having severe disease. This was significantly higher than those who were anal swab negative (n=17), only four (23.5%) of which were classified as severe disease. The authors noted that detectable SARS-CoV-2 viral RNA in blood is a strong indicator for the further clinical severity.

Finally, the association between re-detection positive (or possible reinfection) and severity of initial disease was investigated in one study.[3] Authors found that 36.7% (11/38) of re-detected positive patients were characterised by mild initial symptoms. The percentage was significantly higher than what was seen among non-re-detectable positive patients (12.7%, 19/204, $p<0.01$). Additionally, there were no re-detected positive cases in patients with severe initial infection.

3 Methodological quality

Figure 4, below, provides details of the quality appraisal of all included studies, across nine critical domains. In general, study questions were clearly stated (n=64/67) and the reporting of the condition (n=56/67) and outcomes (n=61/67) were conducted in a standard, reliable way. Sufficient demographic details were provided in 51/67 studies. Of concern was how applicable some studies were to the Irish context (n=11/67 were not applicable, and it was 'unclear' in n=40/67 studies). Nineteen case series chose non-consecutive cases (out of 54), while it was unclear in n=21/54. Approximately two-thirds of studies (n=42/67) were peer-reviewed.

Figure 4 Quality assessment domains



Notes:

Data presented for all included studies (n=66); 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 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 / case series.

4 Discussion

In this review, the evidence on the immune response following coronavirus infections was summarised, including the rate and timing of antibody detection, the duration of immune responses following seroconversion, the reinfection rate among those recovered, and the association between these immune responses and the severity of initial infection. As the focus of the review is SARS-CoV-2, evidence was only considered for other coronaviruses where there was limited SARS-CoV-2 evidence available. Due to the recent emergence of this virus, no studies are yet available on the long-term immune response. Therefore, evidence was also retrieved and summarised on SARS-CoV-1 infection and MERS-CoV; however, the applicability of this to SARS-CoV-2 is unknown.

The overall quality of evidence was low based on pre-defined quality appraisal criteria. In general, study objectives and methods for outcome measurement were well reported across studies. However, the applicability of the majority of studies to the Irish context was uncertain. Concerns also exist regarding the small sample size in many studies and the methodological quality of preprint studies that have not undergone a formal peer review process. The evidence available to answer these research questions is evolving. Large-scale studies of population-based antibody responses with appropriate sample sizes and extended follow-up periods, and correlation with immunity and protection against reinfection, are not yet possible.

4.1 Seroconversion rate and or timing following coronavirus infection

Twenty-three studies were identified that described the initial immune response to SARS-CoV-2. Most studies used the first detection of IgM and or IgG as a proxy for seroconversion. The rate and timing of first detection of IgG or IgM antibodies differed across studies due to differences in the timing and sampling methods used. However, in general, a majority of patients tested positive for IgM within two weeks, and all patients tested positive for IgG in studies that followed patients for longer than two weeks. The median time to first detection of IgM and IgG ranged from five to 13 days and 12 to 14 days, respectively. In studies that measured serial titres in patients from the time of diagnosis, IgM was typically the first antibody to rise, followed by IgG; IgM titres then waned over time while IgG titres remained positive for up to seven weeks in two studies that had the longest follow up. Three studies reported on the response of neutralising antibodies; in all studies all participants developed antibodies within two-to-three weeks.

A major limitation of studies that investigate antibody detection rate or timing is that, as of yet, there is no reference antibody standard for SARS-CoV-2.[52] Validation of tests is therefore particularly difficult. This may partly explain differences observed across studies. Additionally, a wide variety of testing platforms were used, and test accuracy differs significantly depending on the type of test used. Sample size was also an issue in many of the studies. The seroconversion rate and

timing may become more consistent when studies that use validated tests on larger sample sizes are conducted.

4.2 Duration of immune response

As SARS-CoV-2 is a new virus, there are limited data on the duration of the immune response associated with infection and similar coronaviruses, such as SARS-CoV-1 and MERS CoV, may provide some insight.

For SARS-CoV-2, the maximum follow up was seven-to-eight weeks in identified studies. While IgG and neutralising antibody titres appear to be maintained in most patients over this time period, further studies will be needed to determine if these levels are maintained for longer periods of time. As with the studies on seroconversion, small sample sizes and the unknown accuracy of the tests involved were an issue.

SARS-CoV-1-specific IgG antibodies were detectable for three years post-infection in five studies. However, there is considerable uncertainty regarding the duration of the immune response beyond this time point. The two studies that investigated the persistence of SARS-CoV-1 IgG antibody levels beyond three years post-infection presented discordant findings, with the positivity rate reported to be 8.7% at six years in one study, and 69.2% at 12 years in the second study. Another study reported a significant reduction of SARS-CoV-1-specific IgG antibodies two-to-three years after infection, concluding that SARS-CoV-1 recovered patients might be susceptible to reinfection more than three years after initial exposure.

Differences in the positivity rate between studies may be attributable to IgG antibody levels falling below the limit of detection of the tests at follow up, or cross-reactivity with other common human respiratory pathogens. Two studies reported considerable differences in the positivity rate at follow up using different diagnostic tests. Moreover, in the absence of data on reinfection, the levels of peptide-specific CD4+ memory T-cells or anti-SARS-CoV-1 IgG associated with effective SARS-CoV-1 immunity are unknown.

Four studies were identified on the immune response to MERS-CoV. Studies suggest that there is a greater and more sustained response in patients with severe disease compared with mild disease, however the duration of the response is unclear. Although one study suggested antibody titres were still detectable in the majority of patients at almost three years after disease onset (34 months) and that T-cells responses may be present at 24 months even in those with mild or subclinical disease.

Based on data from SARS-CoV-1 and MERS CoV, it is possible that a specific immune response can be maintained for more than two years after infection. However, even if an immune response is maintained for this level of time, it is not known if the

antibody response is sufficient to ensure full protection against reinfection by the same virus. It is possible that the antibody response would result in a less severe, or possibly asymptomatic infection, with the associated risk of transmission to others.

4.3 Reinfection

It is not yet possible to conclude that reinfection following recovery from SARS-CoV-2 occurs, although 10 studies report on re-detection of SARS-CoV-2 following recovery. Only a short time elapsed between confirmatory negative tests and subsequent redetection positive, with no patients that were re-detected positive showing obvious clinical symptoms or disease progression. Using the stringent criterion described previously, none of the cases in these studies can be defined as 'possible reinfection'. However, using the less stringent criteria, the majority of patients with re-detected viral RNA would be defined as 'possible reinfection'. Possible technical and or testing errors include intermittent false negatives or false positives, and may underlie apparent 'reinfection' cases and a better understanding of the pathogenesis of how patients might become reinfected is required in order to develop a more robust definition for reinfection. Greater understanding of the potential for false positive and negative PCR tests is also needed to inform this question.

In all identified cases of re-detectable PCR following previous negative results, all patients were asymptomatic. These cases are unlikely to be clinically or epidemiologically important, unless evidence emerges that these re-detected cases are themselves infectious to others. Due to the relatively short period between the two consecutive negative test results and the subsequent positive test result (<14 days in most studies), and the lack of symptoms or disease progression, it appears more likely that patients in these studies experienced re-detection of the virus rather than reinfection. None of the included studies sequenced and compared the genomes of the first and second infections, or attempted culture of viable virus in addition to RT-PCR testing. Therefore re-detection could reflect detection of non-viable viral material (which is being inconsistently shed) rather than viable virus. Additionally, no study provided serial viral load data. For other viral infections, it has been demonstrated that risk of transmission is highly correlated with viral load (for example, in the transmission of HIV[81]). In the absence of serial viral load data, estimating the risk of transmission in those who are re-detected positive is challenging.

It is also noteworthy that previous evidence summaries conducted by HIQA's research team found substantial discordance between different sample sites used for SARS-CoV-2 testing,[82] along with differences in viral kinetics.[83] In particular, viral RNA from faecal samples has been found to be detected for a prolonged period after symptom resolution,[84] and hence may not be the most appropriate sample for determining reinfection. It is not entirely clear what specimens were used to

determine discharge criteria in some of these studies, so the potential for false negative test results upon discharge cannot be ruled out. The World Health Organization (WHO) recommends that:

> 'if a negative result is obtained from a patient with a high index of suspicion for COVID-19 virus infection, particularly when only upper respiratory tract specimens were collected, additional specimens, including from the lower respiratory tract if possible, should be collected and tested.'[85]

Hence it may be appropriate, if there is suspicion of ongoing infection, for clinicians to consider additionally testing lower respiratory tract specimens prior to discharge in order to reduce the potential for a false negative.

4.4 The association between severity of initial disease and immune response

Data relating disease severity to immune responses were inconsistent across studies. While four studies found that those with severe illness had higher antibody levels than those with moderate or mild illness, four found no association. One study found that CD4+ and CD8+ T-cell counts were inversely related to disease severity. A final study reported on cases that re-detected RT-PCR, and found that a higher proportion of re-detected cases were characterised by mild symptoms. Small sample sizes and short follow-up periods limit the conclusions that can be drawn, and further research is needed to assess the associations between disease severity and immunologic responses in affected patients.

5 Conclusion

Seroconversion studies on SARS-CoV-2 found that while the rate and timing of IgM and IgG detection varied across studies, most individuals displayed an IgG SARS-CoV-2-specific antibody response within two weeks. However, the adequacy or duration of this response is not yet known.

While long-term immunological data relating to SARS-CoV-2 are not yet available, evidence from studies of SARS-CoV-1 suggested that SARS-CoV-1-specific IgG antibody levels are sustained for one-to-two years post-infection and decline thereafter. The applicability from SARS-CoV-1 to SARS-CoV-2 is unknown. It is unclear if reinfection can occur following recovery from SARS-CoV-2. Noting that, as yet, there does not appear to be an agreed definition for reinfection (as opposed to re-detection), the limited data to date are more suggestive of re-detection. Due to the relatively short testing period, and the lack of symptoms or disease progression in these cases, re-detection could reflect detection of non-viable viral material (which is being inconsistently shed) rather than viable virus.

6 Tables of study characteristics and primary outcomes

Table 2 Rate and or timing of IgG/IgM detection following acute SARS-CoV-2 infection

Author DOI Country Study design	Virus type Test performed	Population Patient demographics	Primary outcome results	Comments
Rate/timing of seroconversion				
Du 2020(14) DOI: 10.1002/jmv.25820 China Case series/follow up study	SARS-CoV-2 Testing details not reported	N=60 patients N=10 had repeat samples No further patient demographics reported	**IgM** Approx. 6-7 weeks after symptom onset: 47/60 were positive (78%) **IgG** Approx. 6-7 weeks after symptom onset: 60/60 were positive (100%) IgG titres higher than IgM titres Serial samples (approximately 6-7 and 7-8 weeks after symptom onset): 10 patients were tested twice (1 week apart); both titres showed a decrease, with the IgG titre being greater than the IgM titre.	Letter to the editor
Gao 2020(19) DOI: 10.1097/CM9.0000000000000820	**SARS-CoV-2** Chemiluminescent immunoassay (CLIA), Gold immunochromatographic assay	N=22 Median age: 40 years (4-72) Female n=8; Male n=14	**Number of serum samples and time of sampling** N=37 (note: some missing) days 1-7 after onset: n=10 days 8-14 after onset: n=13 days 14-24 after onset: n=14 **IgM (at least 1 positive by CLIA/GICA/ELISA)**	Accepted to Chinese Medical Journal (publish before print)

China Case series	(GICA), and Enzyme-linked immunosorbent assay (ELISA)		Seroconversion rate and timing: Early (1-7 days): 60% (6/10) Middle (8-14 days): 54% (7/13) Late (14-24 days):79% (11/14) **IgG (at least 1 positive by CLIA/GICA/ELISA)** Seroconversion rate and timing: Early (1-7 days): 50% (5/10) Middle (8-14 days): 77% (10/13) Late (14-24 days): 100% (14/14)	
Guo 2020(79) DOI: 10.1093/cid/ciaa310 China Case series/follow up	**SARS-CoV-2** Deep sequencing or a qPCR assay for diagnosis of cases Antibody testing by ELISA-based assay on the recombinant viral nucleocapsid protein ELISA cut-off values: Authors determined the mean values and SDs of plasma from healthy individuals. The optimal coating	N=101 Two cohorts: confirmed positives (N=48) [deep sequencing or a qPCR assay] and probable positive (N=59) [suspected to be infected with SARS-CoV-2 based on clinical manifestation, chest radiography imaging, and epidemiology but no virus were detected by deep sequencing or a qPCR assay] 208 plasma samples collected	**Timing of samples (confirmed or probably positive):** Total samples=208 Day 1-7: N=41 Day 8-14: N=84 After day 14: N=83 The appearance of IgM, IgA, and IgG antibodies against SARS-CoV-2 was positive as early as day 1 after the symptom onset The times of detection of IgM, IgA, and IgG against SARS-CoV-2 ranged from day 1 to 39 post symptom onset <u>Seroconversion rate & timing</u>: **IgM** and **IgA**: 188/208 (90.4 %) Of acute phase samples, IgM and IgA antibodies were both detectable at a median of 5 days (interquartile range [IQR], 3–6 days) **IgG**: 162/208 (77.9 %) Median seroconversion timing post symptom onset: Day 14 (IQR, 10–18 days)	*Clinical Infectious Diseases* Corrected proof

	concentration of antigen and optimal plasma dilutions were 0.1 µg/mL and 1:200, respectively. The cutoff values were determined by calculating the mean absorbance at 450 nm (A450) of the negative sera plus 3-fold the SD values, which were 0.13, 0.1, and 0.30 for IgM, IgA, and IgG, respectively			
Han 2020(52) doi: 10.1016/j.clim.2020.108413 Case series	The SARS-COV2 nucleic acid test was conducted via real-time RT-PCR according to the protocol of the nucleic acid kit (Kangwei Century Biotechnology Company, China). The SARS-CoV2 antibody kit was used to test for	3 cases who were all from the same family	**Case 1** ▪ 47-year-old female ▪ PMHx: Systemic lupus erythematosus and had been taking oral prednisone (7.5 mg/d) since her diagnosis ▪ Admitted for testing due to close contact testing positive for SARS-CoV-2 ▪ SARS-CoV2 nuclei acid test from nasopharyngeal swabs was negative, but her IgM and IgG antibodies were positive ▪ She was given antiviral treatment, including 0.2 g BID of Abidol orally and 5 million IU of interferon nebulisation.	Clin Immunol Peer reviewed

	specific IgM and IgG antibodies (Guangzhou Wonfo Biological Technology Co, Ltd., China) via colloidal gold immunochromatography		▪ Ground-glass opacity changes were found in the right upper lung. She was given extra piperacillin sodium tazobactam sodium (4.5 TID), and then glycyrrhizin (150 mg QD). CT showed improvements and she was discharged **Case 2** ▪ 81-year-old male ▪ Symptomatic ▪ SARS-CoV-2 nucleic acid test was positive by both nasopharyngeal swabs and sputum on 27 February ▪ IgM and IgG specific antibodies were positive 10 days post symptom onset **Case 3** ▪ 44-year-old female ▪ Symptomatic ▪ SARS-CoV-2 nucleic acids and specific IgG and IgM antibodies positive 10 days post symptom onset	
Haveri 2020(55) DOI: 10.2807/1560-7917.ES.2020.25.11.2000266 PMCID: PMC7096774 Case study	**SARS-CoV-2/Finland/1/2020 virus strain** Immunofluorescence assays (IFA)	Female Chinese tourist in her 30s	While the antibodies were undetectable on Day 4 after onset of symptoms, IgG titres rose to 80 and 1,280 and IgM titres to 80 and 320 on Days 9 and 20, respectively.	Published in Eurosurveillance

Finland				
Jia 2020(23) DOI: 10.1101/2020.02.28.20029025.t China Case series/follow up study	Primary screening of pharyngeal swab nucleic acid amplification was performed by 2 kits of 6 companies (DAAN, Sansure Biotech, BGI, ShangHai ZJ Biotech, Geneodx, Biogerm) IgM/IgG antibodies kit were detected on Time-Resolved Immunofluorescence Analyzer by Fluorescence immunochromatographic assay method (Beijing Diagreat Biotechnologies Co., Ltd, Lot: 20200214) Cutoff of IgM and IgG were 0.88 and 1.02 fluorescence	N=24 patients tested positive for SARS-CoV-2 Other demographic details not provided	From the time of the first exposure to COVID-19 infection to the nucleic acid test, the time ranged from 1 day to 34 days **IgM** Positivity rate = 79% (19/24) (once-off, time range: 1 to 34 days) **IgG** Positivity rate = 67% (16/24) (once-off, time range: 1 to 34 days)	Pre-print Not peer reviewed

	intensity (Flu) units			
Jiang 2020(24) https://doi.org/10.1101/2020.03.20.20039495. China Case series	**SARS-CoV-2** Proteome microarrays	N=29 (and 21 controls) Mean age: 42.3 (SD: 13.8) Female: 16; Male: 13. Severity: 3 mild cases; 26 'common cases'	**Samples:** N=29 (patient group); Collected mean 22 days after onset. **Results:** 100% seroconversion for IgG and IgM. The level of S1 IgG positively correlates to age and level of lactate dehydrogenase, especially for women. The level of S1 IgG negatively correlates to lymphocyte percentage.	Not peer-reviewed
Ju B 2020(26) DOI: 10.1101/2020.03.21.990770 Prospective Case series China	**SARS-CoV-2** ELISA	N=8 patients infected with SARS-CoV-2 in January 2020 Age range: 10 to 66 years	▪ The isolation and characterisation of 206 viral Spike protein receptor-binding domain (RBD)-specific monoclonal antibodies (mAbs) derived from single B cells of eight SARS-CoV-2 infected individuals was performed ▪ Both clone types demonstrated impressive binding and neutralising activity against pseudovirus and live SARS-CoV-2 ▪ No cross-reactivity with SARS-Cov-1 or MERS was found.	Not peer-reviewed. Preprint.
Lee 2020(56) DOI: 10.1016/j.jmii.2020.03.003 Case study Taiwan	**SARS-CoV-2** ALLTEST 2019-nCoV IgG/IgM Rapid Test Cassette, Hangzhou ALLTEST Biotech Co., Ltd. Hangzhou, China	One 46-year old woman after returning from Macau to Taiwan	IgG antibody was measured in seven serum samples (obtained on the hospital day 2, 3, 7, 9, 13, 20, and 23) from the patient. The SARS-CoV-2 IgG antibody was detected in five serum samples since the hospital day 7 (illness day 11) IgM not reported/not tested	Journal of Microbiology, Immunology and Infection Short communication

Liu 2020(29) DOI: 10.1101/2020.03.06.20031856 China Case series/follow up study	**SARS-CoV-2** SARS-CoV-2 RNA was detected by real time RT-PCR on pharyngeal swab specimens ELISA assay for IgM and IgG antibodies against N protein of SARS-CoV-2 using ELISA kit (Lizhu, Zhuhai, China)	N= 238 admitted hospital patients with confirmed or suspected SARS-CoV-2 infection Among the 238 recruited patients, 153 patients were laboratory-confirmed cases. The median age was 55 years (IQR, 38.3-65), and 138 (58.0%) of the patients were men	IgM and or IgG seropositivity rate in confirmed patients = 83.0% (127/153) Seroconversion timing: After 10 days, seroconversion rate rose to >80% (IgM and or IgG)	
Liu 2020(30) doi: https://doi.org/10.1101/2020.03.28.20045765 Case series China	**SARS-CoV-2** SARS-CoV2 antibody detection kit	N=133 Median age: 68 Female: 63; Male: 70 44 moderate cases (22 males and 22 females, median age was 67.5 [IQR 64-71.75]), 52 severe cases (28 males and 24 females, median age was 68 [IQR 61.25-74]), and 37 critical cases (20 males and 17 females, median age was 70 [IQR 60-76.5])	**IgM** Seroconversion rate by severity of disease: Moderate: 79.55% Severe: 82.69% Critical:72.97% **IgG** Seroconversion rate by severity of disease: Moderate: 93.18% Severe:100% Critical: 97.30%	Not peer-reviewed

Long 2020(69) DOI: 10.1101/2020.03.18.20038018 China Multi-centre cross-sectional study and a single-centre follow-up study	RT-PCR assay for nasal and pharyngeal swab specimens IgG and IgM antibody against SARS-CoV-2 in plasma samples were tested using Magnetic Chemiluminescence Enzyme Immunoassay (MCLIA) kit supplied by Bioscience (Chongqing) Co., Ltd, China	N=285 patients in multi-centre cross sectional study including N=63 patients in single-centre follow-up study Median age: 47 years (IQR, 34-56 years) 55% were males 262/285 patients had clear records of time of symptom onset 39/285 cases were classified as severe or critical illness condition	**Seroconversion rate & timing** Of 262 cases with clear records on symptom onset: ▪ IgG seroconversion rate reached 100% at around 17-19 days after symptoms onset ▪ IgM seroconversion rate reached its peak of 94.1% approx. 20-22 days after symptoms onset **Titres:** ▪ During the first 3 weeks of symptoms onset, there was an increase in the titre of IgG and IgM antibodies. However, the antibody level IgM showed a slight decrease after 3 weeks ▪ Severe cases (N=20) had higher antibody titres than non-severe <u>Follow-up study</u> (N=63 patients) The median day of seroconversion for both lgG and IgM was 13 days (after symptoms onset)	Not peer-reviewed
Lou 2020(62) doi: https://doi.org/10.1101/2020.03.23.20041707 Cohort study China	**SARS-CoV-2** ELISA, LFIA, and CMIA assays	N=80 cases and N=300 controls Median age: 55 (range: 45-64) Female proportion: 38.7%	**IgM** Seroconversion rate & timing: 0-7 days: 33.3% 8-14 days: 86.7% 15-24 days: 96.7% Median seroconversion time: 18 days post exposure; 10 days post onset **IgG** Seroconversion rate & timing: 0-7 days: 33.3%	Not peer-reviewed

			8-14 days: 76.0% 15-24 days: 93.3% Median seroconversion time: 20 days post exposure; 12 days post onset	
Nicastri 2020(58) doi.org/10.2807/1560-7917.ES.2020.25.11.2000230 Italy Case report	Two real-time RT-PCR on a nasopharyngeal swab confirmed SARS-Cov-2 In house-prepared immunofluorescence (IF) slides and neutralisation test as confirmatory test for antibodies	Italian man in his late 20s Patient isolated for clinical assessment after travel to Wuhan, China. He was in Wuhan from 20 January to 3 February and isolated in Italy on 6 February. Patient was asymptomatic (or paucisymptomatic, only had transient mild conjunctivitis and a body temperature of 37.3).	**Seroconversion** Patient was asymptomatic. Exposure could be as early as 20 January. Retrospective analysis of admission sample (17 days after first travel to Wuhan): IF results showed positivity for both IgG and IgM (≥ 1:640 and 1:80, respectively) at the same time point of the first viral RNA positive result. **Re-detectable positive** Nasopharyngeal swab was positive every day until day 11, negative day 12 and 13, positive day 14 to 16 and negative day 17 and 18.	Eurosurveillance
Okba 2020(34) DOI: 10.3201/eid2607.200841 Multisite (Samples from France & Germany) Case series	Anti-SARS-CoV-2 S1 IgG and IgA: ELISAs by using β-versions of 2 commercial kits (EUROIMMUN Medizinische Labordiagnostika AG, https://www.euroimmun.comExternal Link) Optical density (OD) detected at 450 nm	Serum samples (n=10) collected from 3 PCR-confirmed patients: 2 with mild COVID-19 and 1 with severe COVID-19 in France. For validation testing, samples from Wolfel 2020(43) included (n=31)	▪ SARS-CoV-2–specific antibody responses in severe and mild cases was detected by using serum samples collected at different times post-onset of disease from 3 PCR-confirmed COVID-19 patients from France ▪ After infection, all 3 patients seroconverted between days 13 and 21 after onset of disease (IgG/IgA) ▪ When tested in a PRNT, serum samples from all 3 patients neutralised SARS-CoV-2 infection. Antibody responses detected by different assays correlated strongly with neutralising antibody response	In press Emerging Infectious Diseases

	Virus-neutralising antibodies were tested by using a PRNT50			
Pan 2020(35) doi: https://doi.org/10.1101/2020.03.13.20035428 Case series China	**SARS-CoV-2** ICG strip assay	N=105 patients 48 male, 57 female) Median age: 58 years (range 20-96 years) 134 samples from 105 patients taken	Samples taken at early stage (1-7 days from onset), intermediate stage (8-14 days) and late stage (more than 14 days) **IgM** Seroconversion rate & timing: 1-7 days: 11.1% 8-14 days: 78.6% ≥15 days: 74.2% In total: 55.8% **IgG** Seroconversion rate & timing: 1-7 days: 3.6% 8-14 days: 57.1% >15 days: 96.8% In total: 54.7%	Not peer-reviewed
To 2020(66) DOI: 10.1016/S1473-3099(20)30196-1. Cohort study Hong Kong, China	**SARS-CoV-2** Antibody levels detected by Enzyme Immunofluorescence Assay (EIA)	N=23 Median age: 62 years (range 37–75)	For 16 patients with serum samples available 14 days or longer after symptom onset, rates of seropositivity were: ▪ 94% for anti-NP IgG (n=15) ▪ 88% for anti-NP IgM (n=14) ▪ 100% for anti-RBD IgG (n=16) ▪ 94% for anti-RBD IgM (n=15)	Lancet J Infectious Disease Peer-reviewed

Wang 2020(40) DOI: 10.1101/2020.04.13.20040980 China Case series/follow-up study	**SARS-CoV-2** SARS-CoV-2–specific antibodies were detected using "New Coronavirus 164 (2019-nCoV) Antibody Detection Kit" (INNOVITA, China)	N=26 15 Female, 11 Male Median age not reported; range was 5 to 72 years All cases mild/moderate	**IgG seroconversion timing:** Mean seroconversion timing: 15.7 days Earliest seroconversion was in 7 days Two patients remained IgG positive at 50 days One COVID-19 patient who did not produce any SARS-CoV-2-bound IgG successfully cleared SARS-CoV-2 after 46 days of illness, revealing that without antibody-mediated adaptive immunity, innate immunity may still be powerful enough to eliminate SARS-CoV-2.	Pre-print Not peer reviewed
Wang 2020(42) doi.org/10.1101/2020.04.15.20065623 China Follow-up study/case series	The presence of neutralising antibody was determined with a modified cytopathogenic assay based on live SARS-CoV-2	N=70 patients N=117 serum samples Mean age: 45.1 years (range 16.0-84.0) Female proportion: 58.6% Of the 70 patients enrolled into this study, 58 were recovered and discharged from hospital One (1.4%) patient was asymptomatic infected, 22 (31.4%) had mild clinical manifestations, 43 (61.5%) were moderate, and the remaining 4 (5.7%) were in severe condition	**Neutralising Antibodies:** ▪ Seropositivity rate reached 100% within 20 days post onset, and remained 100% until day 41-53 ▪ Antibody level was highest during days 31-40 post onset, and then decreased slightly ▪ No difference in titres between males and females ▪ Multivariate analysis: ▪ Patients aged 31-84 had a higher antibody level than those at age of 16-30 ▪ Patients with a worse clinical classification had a higher antibody titre	Pre-print Not peer reviewed
Wölfel 2020(43)	**SARS-CoV-2** Seroconversion was detected by	N=9 hospitalised patients Sex of participants not reported	**Seroconversion rate & timing: IgM and or IgG** Day 7: 50% of patients by day 7 Day 14: 100% of patients by day 14	Nature Peer-reviewed

DOI: 10.1038/s41586-020-2196-x. Munich, Germany Case series	IgG and IgM immunofluorescence using cells expressing the spike protein of SARS-CoV-2 and a virus neutralisation assay using SARS-CoV-2 Testing for virus by RT-PCR	All cases had comparatively mild courses	▪ Seroconversion was not followed by a rapid decline in viral load ▪ No viruses were isolated after day 7 ▪ All patients showed detectable neutralising antibodies, the titres of which did not suggest close correlation with clinical courses ▪ Of note, case #4, with the lowest virus neutralisation titre at end of week 2, seemed to shed virus from stool over prolonged time ▪ Results on differential recombinant immunofluorescence assay indicated cross-reactivity or cross-stimulation against the four endemic human coronaviruses in several patients	
Xiao 2020(46) DOI: 10.1016/j.jinf.2020.03.012 Case series China	**SARS-CoV-2** Chemiluminescent Immunoassay (CIA), Shenzhen Yahuilong Biotechnology Co., Ltd	N=34 Mean age: 55 (range: 25-87) Female: 12; Male: 22	**IgM** In week 3 after symptoms onset, all patients tested positive for IgM In week 5, 2 patients (16.7%) were negative **IgG** In week 3 and week 5 all patients were positive for IgG	Pre-proof Accepted to Journal of infection
Zhao 2020(51) DOI: 10.1093/cid/ciaa344 Case series	**SARS-CoV-2** Enzyme Linked Immunosorbent Assay (ELISA) kits supplied by Beijing Wantai	N=173 Median age: 48 (IQR: 35-61) Female proportion: 51.4%	n=535 samples **IgM** In week 3 after symptoms onset, all patients tested positive for IgM In week 5, 2 patients (16.7%) were negative **IgG**	Published by Oxford university press for the Infectious Disease Society of America

China	Biological Pharmacy Enterprise Co.,Ltd		In week 3 and week 5 all patients were positive for IgG Note: The reason for the negative antibody findings in 12 patients might due to the lack of blood samples at the later stage of illness.	
Zhao 2020(61) DOI: 10.1093/cid/ciaa408 China Case study	**SARS-CoV-2** Total antibody and IgM specific for SARS-CoV-2 was measured with chemiluminescence kits supplied by Beijing Wantai Biological Pharmacy Enterprise Co., Ltd., China	38-year-old man Co-infected with HIV and HCV ▪ Patient had 3 serial negative tests for SARS-CoV-2 RNA from nasopharyngeal swabs ▪ Patient had pneumonia on CT ▪ 42 days from the onset of his illness, his immune response was evaluated	<u>At 42 days post-symptom onset:</u> **IgM**: 49.5 cut-off index (COI) **Total antibody**: 13.2 COI ▪ These were significantly lower and higher, respectively, than those in patients with COVID-19 who had recovered from the illness who are not HIV/HCV positive. ▪ At this time, SARS-CoV-2 RNA was still negative from nasopharyngeal and anal swabs. <u>At 49 days post-symptom onset</u>**:** IgM remained at similar levels with 54 COI Total antibody rose to 523.8 COI Note: ▪ Patient was taking lamivudine, tenofovir and efavirenz daily since 2016 ▪ In 2017, he took antiviral agents (DAA) against HCV for 3 months by himself, and HCV became persistently negative ▪ On admission his CD4 and CD8 T-cell counts in peripheral blood were 216 and 584	*Clinical Infectious Diseases* Accepted manuscript

Table 3 Duration of immune response: SARS-CoV-2

Author DOI Country Study design	Virus type Test parameters	Population Patient demographics Clinical characteristics	Primary outcome results	Comments
Adams 2020(1) 10.1101/2020.04.15.20066407 UK Case series	**SARS-CoV-2** ELISA and RT-PCR (used as reference test) Compared to nine 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)	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	**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. **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, univariable 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	medRxiv – not peer reviewed

	days from symptom onset (n=16), and from recovering healthcare workers median 13 [range 8-19] days after first symptoms; (n=6). 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)	days from symptom onset)	association was observed between IgM titres and time since symptom onset. **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	
Dong 2020(13) 10.1101/2020.03.17.20036640 China Case series	**SARS-CoV-2** RT-PCR and CT to confirm infected. ELISA for IgG/IgM (not commercial) Neutralising antibody assay	N=12 COVID-19 patients recently virus free and discharged from hospital. 6 were recently discharged and 6 had been discharged for 2 weeks(follow-up patients) n=4 controls 2 patients showed lymphopenia. Seven	**Duration of detection of serum immunoglobulin levels:** COVID-19 patients mounted IgG and IgM responses to SARS-CoV-2 proteins, especially NP and S-RBD, and also suggest that infected patients could maintain their IgG levels, at least for two weeks. **Duration of detection of neutralising antibodies:** Four of the recently discharged patients had high neutralising antibody titres. All bar one of the follow-up patients had lower lowers of neutralising antibody titres than the recently discharged patients, although all except one was positive.	medRxiv not peer reviewed

	Interferon gamma ELISpot FACS staining	patients were female. Age mean 41 years (range 26 to 68)	**B-cell/T-cell responses:** Compared to discharged patients, there was a trend towards an increased frequency of NK cells in the follow-up patients. However, there was no significant difference in terms of the percentages of T cells among those two groups (discharged and follow-up) and the healthy donors. Compared to healthy donors, the number of IFN-gamma secreting NP specific t-cells in four of the recently discharged patients suggests that they had developed a SARS-CoV-2 specific T cell response. Only one of the follow-up patients (with lymphopenia) had a high number of IFN-gamma secreting T cells in response to NP, main protease and S-RBD, suggesting anti-viral T cells may not be maintained at high numbers in the PBMCs in the recovered patients. This suggests they may enter a quiescent state.	
Du 2020(14) 10.1002/jmv.25820 China Case series	**SARS-CoV-2** Unclear which test performed, but IgG and IgM measured using a kit of some sort Doesn't specifically state if RT PCR used to confirm cases	N=60 convalescent patients (onset time of 6-7 weeks). N=10 patients tested at two time points (6-7 weeks after onset of symptoms and 7-8 weeks after the onset of symptoms)	**Duration of detection of serum immunoglobulin levels:** All patients tested positive for the IgG against the virus, 13 patients tested negative for immunoglobulin M (IgM), with the immunoglobulin G (IgG) titre being greater than the IgM titre. The IgM and IgG titres in 10 convalescent patients were tested twice (1 week apart); both titres showed a decrease, with the IgG titre being greater than the IgM titre. (drop also greater) **Other outcomes:** Antibody detection could act as an indicator of the stage of COVID-19 progression and that the antibodies in	Published in journal of medical virology as a letter to the editor. Unsure if peer reviewed

			convalescent patients are not always maintained at a high level.	
Jin 2020(25) 10.1016/j.ijid.2020.03.065 China Case series (retrospective)	**SARS-CoV-2** IgM and IgG chemiluminescence immunoassay (CLIA) kits (commercially available) SARS-CoV-2 confirmed by RT-PCR Serum taken before and after conversion to virus negative. Duration from first symptoms to hospital admission, to laboratory confirmation, and to first serological test in the COVID-19 group patients was 3 days (IQR 2–7 days), 3 days (IQR 2–7 days) and 18 days (IQR 11–23	N=43 COVID-19 patients. N=33 controls (control group suspected of having COVID 19, but did not) Median age of the COVID-19 patients was 47.0 years (IQR 34.0–59.0 years), ranging from 7 years to 74 years, and 39.5% were male. All cases were non-severe cases. Chronic disease: hypertension (10, 23.3%), diabetes (3, 7.0%), and liver disease (2, 4.7%). Fever was present in 62.8% of COVID-19 patients before or on admission. The second most common symptom was cough (60.5%) Similarly, fever and cough were also the most common	**Duration of detection of serum immunoglobulin levels:** COVID-19 group: 27 patients tested for viral antibody before becoming virus-negative. Median duration from first symptoms to serological testing in these 27 patients was 16 days (IQR 9–20 days). 13 were IgM-positive (48%) and 24 were IgG-positive (89%). Three IgG-negative patients were also IgM-negative (these patients were test 0, 5 and 8 days from symptom onset). Days from laboratory confirmation to serological test: IgM-positive rate increased slightly at first (day 1-20) and then decreased as the number of days from laboratory confirmation to serological detection increased (up to 32 days); in contrast, the IgG-positive rate increased to 100% (by day 16-20) and was higher than IgM at all times. It remained at 100% by day 26-32. Meanwhile, the virus-positive rate tended to decrease over time. As the duration from symptom onset to serological testing increased. It was found that both IgM and IgG levels were not high during the first 5 days following symptom onset. IgG positive rate reached 100% by day 11-15, and remained there by 31-55 days. IgM positive rate increased until days 16-20 and started to decrease around 26-30 days after symptom onset By 31-55 days after symptom onset less than half of the patients were IgM positive. In summary: The IgM-positive rate showed a trend to increase at first and then decline; however, the IgG-	Published in international Journal of infectious diseases

	days), respectively	symptoms in the control group	positive rate increased and then became stable over time. Furthermore, the IgG-positive rate was consistently higher than the IgM-positive rate. **Other outcomes:** According to molecular detection as the gold standard, the sensitivities of serum IgM and IgG antibodies to diagnose COVID-19 were 48% (13/27) and 89% (24/27), respectively, and the specificities were 100% (33/33) and 91% (30/33).	
Okba 2020(34) 10.3201/eid2607.200841 Samples collected from France, the Netherlands, Germany Case series	**SARS-CoV-2** Samples confirmed with RT-PCR as SARS-CoV-2 A plaque reduction neutralisation test (PRNT) was used as a reference for this study ELISA (developed in house and two commercially available ones) Serum samples taken between	N=10 samples from 3 COVID-19 cases from France (2 mild cases and 1 severe). N=31 serum samples from COVID-19 cases from Berlin). N=31 controls from Berlin (controls were infected with other coronaviruses) Control samples from individuals infected with other coronaviruses (HCoV-229E, NL63 or OC43, SARS-CoV-1, MERS-CoV or other respiratory viruses)	**Duration of detection of neutralising antibodies:** With PRNT and all 3 ELISA kits the more severe case had higher response than the two mild cases. Based on PRNT results, the severe sample was positive 5-10 days after symptom onset. The titre peaked around 10-15 days after onset and declined gradually up to 30 days after symptom onset when the experiment ended. In the mild cases, the titres increased more gradually and were positive at 10-15 days after symptom onset and still increasing at the end of the experiment (20-25 days after onset). **Other:** The aim of this study was to test in house ELISA kits. Antibody levels were higher following severe infection compared to the mild ones	Published in Emerging Infectious Diseases

	day 6 and 27 in mild and severe cases, days not specified but noted samples were taken 'at different time points' over this period			
Wang 2020a(60) 10.21203/rs.3.rs-23009/v1 China Case report	**SARS-CoV-2** RT-PCR to confirm SARS-CoV-2. Throat and nasopharyngeal swabs	N=1 COVID-19 patient. Age 37 years old. Patient had fever, dry cough, fatigue, dizziness, runny nose and diarrhoea. Chest CT scan showed multiple nodules and mixed ground-glass opacification with consolidation in both lungs Laboratory findings showed that his lymphocyte and CD4+ counts were below the normal range	**Duration of detection of serum immunoglobulin levels:** In total the patient was monitored for 50 days from illness onset. New coronavirus-specific IgG antibody levels significantly increased by more than 3 times above those at illness onset, accompanied by decreased IgM levels. IgM and IgG measured 5 days after symptom onset were low (around 5 S/CO), IgM decreased to 0 by 12 days after illness onset, while IgG was still increasing by 31 days after illness onset (over 30 S/CO). **Other outcomes:** Treatment: antiviral treatment, including arbidol, lopinavir, IFN-α, and traditional Chinese medicine CD4+ T cell increased from around 260 c/μl to more than 400 c/μl from 5 days post symptom onset to 31 days after symptom onset.	Pre-print, not peer reviewed
Wang 2020b(42)	**SARS-CoV-2** Neutralising antibody	N=70 COVID-19 inpatients (n=12) and convalescent patients (n=58). Patients for	**Duration of detection of neutralising antibodies:** Seropositivity reached 100% within 20 days since illness onset and remained 100% until day 41-53. Based on 117 samples taken from 70 patients	medRxiv not peer reviewed

10.1101/2020.04.15.20065623 China Case series	determined using cytopathogenic assay. Neutralising antibody test of 1st sample since onset in this study, the median time was 33.0 days (range 10.0-53.0). The time of convalescent patients (35.0 days) were longer than inpatients (13.5 days).	longitudinal changes in n= 8 convalescent patients (4 mild, 4 moderate in severity) The mean age of the patients was 45 years (range 16-84). A total of 59% were female. The number of patients having history of cardiovascular disease, diabetes, and hypertension was 2 (2.8%), 5 (7.1%) and 9 (12.9%), respectively. One (1.4%) patient was asymptomatic infected, 22 (31.4%) had mild clinical manifestations, 43 (61.5%) were moderate, and the remaining 4 (5.7%) were in severe condition	**Serum titres of neutralising antibodies over time:** The antibody level was highest during day 31-40 since onset, and then decreased slightly by day 41-53. The total GMT was 1:163.7 (95% CI, 128.5 to 208.6), of which 52.1% (61/117) had a titre between 1:64 and 1:512. The GMT of day 31-40 since onset (1: 271.2, 95% CI, 175.8 to 418.5) reached the highest, and decreased slightly after that time period (1:201.7, 96% CI, 144.1-282.2). Univariate GEE analysis showed that the antibody level during day 31-40 was significantly higher than other phases. **Other outcomes:** In multivariate GEE analysis, patients at age of 31-60 and 61-84 had a higher antibody level than those at age of 16-30 (β=1.0518, P=0.0152; β=1.3718, P=0.0020). Patients with a worse clinical classification had a higher antibody titre (β=0.4639, P=0.0227).	
Wölfel 2020(43) doi: 10.1038/s41586-020-2196-x.	**SARS-CoV-2** Seroconversion was detected by IgG and IgM immunofluoresce	N=9 hospitalised patients	**Duration of detection of neutralising antibodies:** ▪ Seroconversion in 50% of patients occurred by day 7, and in all by day 14, but was not followed by a rapid decline in viral load. ▪ No viruses were isolated after day 7	Nature Peer-reviewed

Munich, Germany Case series	nce using cells expressing the spike protein of SARS-CoV-2 and a virus neutralisation assay using SARS-CoV-2 Testing for virus by RT-PCR		▪ All patients showed detectable neutralising antibodies, the titres of which did not suggest close correlation with clinical courses **Other outcomes:** ▪ Of note, case #4, with the lowest virus neutralisation titre at end of week 2, seemed to shed virus from stool over prolonged time ▪ Results on differential recombinant immunofluorescence assay indicated cross-reactivity or cross-stimulation against the four endemic human coronaviruses in several patients	

Table 4 Duration of immune response: SARS-CoV-1

Author DOI Country Study design	Virus type Test performed Location of sample Timing of sample	Population Patient demographics	Primary outcome results
SARS-CoV-1			
Cao 2010(5) DOI: 10.1186/1743-422x-7-299 China Case series	**SARS-CoV-1** Clinical case definition: WHO criteria **Testing:** ELISA (BJI-GBI Biotechnology, Beijing, China) and micro-neutralization assays **Sample:** Serum **Timing:** 3 year follow-up; sampling at	N = 19 recovered SARS patients. Control: N = 25 healthy blood donors	**Duration of detection of serum immunoglobulin levels:** 3 years **Duration of detection of neutralising antibodies:** *RBD-based ELISA:* Year2/3 = one sample became undetectable. Positive rate of 94.74%. *Lysate-based ELISA kit:* Year 2/3 = OD values for all samples dropped dramatically. Positive percentage of the year 3 samples was 42.11% (8/19) **Other outcome:** Viral lysate-based ELISA kit had much low sensitivity than the RBD-based ELISA

	month 3, 12, 18, 24, and 36 after the onset of clinical symptom		
Cao 2007(4) DOI: 10.1056/NEJMc070348 China Case series Peer-reviewed; N Engl J Med 357;11	**SARS-CoV-1** **Testing:** ELISA, Neutralising antibodies: conventional neutralisation assay. Reference value for positive result: 1:10 **Sampling:** Serum **Follow-up:** 3 years after disease onset (samples taken at 1, 4, 7, 10, 16, 24, 30, 36 months)	N = 56 positive for serum IgG and neutralising antibodies at recovery.	**Duration of detection of serum immunoglobulin levels:** 36 months **Serum titres of IgG over time (typically expressed as Geometric Mean Titres [GMTs]):** GMTs: 244 at month 4; 34 at month 30; 28 at month 36. IgG antibodies were undetectable in 19.4% of serum samples at month 30, and in 25.8% at month 36. **Duration of detection of neutralising antibodies:** 36 months **Serum titres of neutralising antibodies over time:** GMTs: 1232 at month 4; 32 at month 30; 32 at month 36. Neutralising antibodies were undetectable in 11.1% of serum samples at month 30 and in 16.1% at month 36. **Other outcome:** The titres of IgG and neutralising antibodies were significantly correlated during the 3-year follow-up period (Spearman's correlation coefficient, 0.905; P = 0.002). Femoral neck necrosis: patients with femoral neck necrosis had significantly lower neutralising antibody levels (P<0.001, from mixed-linear random-effects models. No significant differences in the kinetics of specific antibodies according to disease severity, duration of hospitalization, type and number of coexisting conditions, or use or non-use of corticosteroids.

			Treatment: Not reported.
Chan 2005(6) China DOI: 10.1128/cdli.12.11.1317-1321.2005 Peer-reviewed: Clin Diagn Lab Immunol. 2005 Nov; 12(11)	**SARS-CoV-1** Serological and RT-PCR confirmation of SARS CoV infection with an epidemiological link and clinical features compatible with SARS. **Testing:** neutralization tests and subclass-specific IF tests. Neutralization titer was determined as the highest dilution of serum which completely suppresses the cytopathic effect in at least half of the infected wells. **Samples:** Sera	N = 20 SARS patients. Age: mean age of 39.8 years (range, 20 to 65). Sex: male-to-female ratio was 11:9 Follow-up sera at 7 months available for 11 patients. N = 2 chronic hepatitis B carriers. Patients infected with other human coronaviruses: Acute- and convalescent-phase sera from patients with recent OC43 infection (N = 11) and patients with recent 229E infection (N = 3)	**Duration of detection of serum immunoglobulin levels:** IgG: Detectable at 7 months. IgM: Detectable 8/11 patients at 7 months (GMT at 7 months = 19). IgA: GMT at 7 months = 35 Total immunoglobulin (IgGAM) titers at 7 months decreased in one patient, increased in two patients. and remained stable in eight patients. **Serum titres of IgG over time:** Time to seroconversion - 17.2 days (range of 13 to 28). Month 1: GMT = 206 Month 7: GMT = 34 IgG antibody titers remained stable at seven months in 7 patients. IgG continued to increase in three patients. One patient showed a fourfold or greater decrease in SARS-CoV-1 IgG at 7 months. **Duration of detection of neutralising antibodies:** 7 months **Serum titres of neutralising antibodies over time:** The mean time to developing neutralizing antibody was 15.4 days (range of 11 to 21). Month 7: Titres decreased in two patients, increased in two patients, and there was no significant change in seven patients. Month 1 and 7: neutralisation titres remained unchanged at 124. **Other outcome:** **Time to seroconversion:** No difference in time to seroconversion between the patients who survived (n = 14) and those who died (n = 6).

	Timing: collected during illness and convalescence up to 7 months postinfection		**Crossreactivity:** SARS-CoV-1 antibody response was sometimes associated with an increase in pre-existing IgG antibody titers for human coronaviruses OC43, 229E, and NL63. N = 12 (60%) of SARS patients had fourfold rising titers to OC43, 229E, or both. **Mortality:** N = 6 patients had a fatal outcome.
Chang 2005(7) doi: 10.1128/CDLI.12.12.1455-1457.2005 Taiwan Prospective follow-up	**SARS-CoV-1** SARS was diagnosed based on a positive RT-PCR result for SARS-CoV-1 on their initial throat swabs and/or the seroconversion of the IgG specific antibody to SARS-CoV IgM and IgG measured with indirect immunofluorescent assay (IFA) (Euroimmune, Lübeck, Germany)	Of 76 SARS patients hospitalised with pneumonia, 18 were followed for 1 year. For the 18 patients who were examined for 1 year, male-to-female ratio of this group was 7:11. Their ages ranged from 24 to 71 years, with a median age of 45.5 years.	**IgM** 15 patients had detectable IgM to SARS-CoV in their sera collected at 1 month after disease onset With the exclusion of one patient, whose serum samples were not collected at 3, 6, and 9 months after the disease onset, IgM antibodies were undetectable in 2 patients at 1 month after the disease onset, in 10 patients at 3 months, in 16 patients at 6 months, and in all 17 patients at 12 months **IgG** All of the patients except one, whose serum sample was not collected at 12 months after the disease onset, had detectable IgG antibodies in their sera 12 months after disease onset. **Disease severity:** Patients who developed respiratory failure during their SARS disease courses did not have significantly higher IgG titres than those who did not develop respiratory failure. There was no correlation between the IgG titre checked 1 month after disease onset and the patients' ages, initial CRP levels, peak CRP levels, or development of respiratory failure as determined by statistical analysis.
Chen 2005(8) **DOI:** 10.4049/jimmunol.175.1.591	**SARS-CoV-1** **Testing:** Flow cytometry, ELISPOT assays	N = 13 HLA-A*0201 subtype positive recovered SARS patients.	**Duration of detection of T-cells:** 12 – 14 months **Detection of CD8+ T-cells:**

Peer reviewed; J Immunol. 2005;175(1) China Case series	**Sample:** Blood **Timing:** 12-14 months after recovery	Sex: 8 females and 5 males. N = 12 HLA-A*0201 subtype negative recovered SARS patients. Sex: 5 females and 7 males. Controls: N = 36 healthy donors. Sex: g 21 females and 15 males. All donors aged 18 to 61 years.	Inactivated SARS-CoV-1 elicited an Ag-specific recall CTL response to spike protein-derived epitopes (SSp-1, S978, and S1202) in PBMCs of recovered SARS patients. **Other outcome:** **Cytokine production:** Cross-reactive memory T-cells to SARS-CoV-1 may exist in the T-cell repertoire of a subset of healthy individuals and can be reactivated by SARS-CoV-1 infection *in vitro*. SSp-1-specific CTLs derived from healthy donors demonstrated reduced cytotoxic activity and low levels of IFN-g production in comparison with those of CTLs from recovered SARS patients
Fan 2005(18) China Case series Peer reviewed; Zhonghua Liu Xing Bing Xue Za Zhi. 2005;26(3)	**SARS-CoV-1** **Testing:** ELISA. Cut-off value = 0.11 + negative control A **Sample:** Sera. Each patient was tested at least twice (Total 912 sera) **Timing:** 12 months. Sampling	N = 311 SARS patients from hospitals in Beijing (N = 258 cases in Xiaotangshan Hospital; N = 21 cases in Armed Police General Hospital, N = 9 cases in the Civil Aviation General Hospital; N = 23 cases in the PLA General Hospital) Sex: 132 males, 179 females. Age: Males 18 to 67 years, with an average of 37 years ± 13.	**Duration of detection of serum immunoglobulin levels:** 12 months **Serum titres of IgG over time (typically expressed as Geometric Mean Titres [GMTs]):** Peak titre 35 days after discharge. Then levels began to decline. IgG antibody level showed a 35.8% decrease within one year.

	every 2 - 4 weeks.	Females aged 18 to 74 years, with an average of 38 years ± 13	
Guo 2020(79) doi.org/10.1101/2020.02.12.20021386. China Long-term prospective follow-up study	**SARS-CoV-1** **Testing:** ELISA kit using whole virus (BGI-GBI Biotech Co. Ltd., Beijing, China) and an in-house recombinant SARS-CoV-1 N199 antigen assay. Any result Higher than the cut-off value considered positive. **Sampling:** Sera (Total 362 samples) **Timing:** Sampling in 2003 at hospital admission. Yearly sample collection until 2015.	34 SARS-CoV-infected healthcare workers during the 2002-2003 SARS outbreak were followed. The majority of the participants were aged between 20 and 30 in 2003, and 94.11% (32/34) of them were females. Serum samples were collected annually from 2003-2015.	Anti SARS-CoV IgG was found to persist for up to 12 years IgG titres typically peaked in 2004, declining rapidly from 2004-2006, and then continued to decline at a slower rate. Patients treated with corticosteroids at the time of infection were found to have lower IgG titres than those without. *ELISA commercial kit:* 2003: IgG titer against whole virus was 81.25% (26/32). 2007: Peaked at 100.00% (32/32). 2015: Decreased to 69.23% (18/26). *In-house recombinant SARS-CoV-1 N199 antigen assay:* 2003: IgG antibody against N199, the initial positive was 59.38% (19/32). 2005: Peaked at 87.50% (28/32). 2015: Decreased to 19.23% (5/26). **Conclusion:** IgG antibodies against SARS-CoV can persist for at least 12 years
He 2004(21) China	**SARS-CoV-1** Clinical case definition: fever	N=271 laboratory-confirmed (RT-PCR) SARS cases. Age: 36 ± 16 years	**Duration of detection of serum immunoglobulin levels:** SARS CoV IgG: 95 days.

DOI: 10.1128/CDLI.11.4.792-794.2004 Peer-reviewed; Clin Diagn Lab Immunol. 2004;11(4):792–794 Case series	of ≥ 38°C, cough or shortness of breath, new pulmonary infiltrates on chest radiography, and close contact with a person with a suspected or probable case **Testing:** IFA (Euroimmun AG, Lu¨beck, Germany), ELISA (Wantai Biological Pharmacy Enterprise Company, Ltd., Beijing, China) **Sample:** Serum (total number, 530; 1 to 5 samples per patient) **Timing:** 1-95 days after the onset of illness.		SARS CoV IgM: SARS-CoV-specific IgM levels dropped as early as 2 or 3 weeks after the onset of illness. Days 60-95 (study end-point) = 58/70 (83%). SARS CoV IgA: Days 60-95 = 54/70 (77%). **Serum titres of IgG over time (typically expressed as Geometric Mean Titres [GMTs]):** Days 1-14 = 140 (59.1%); Days 15-29 = 182/188 (96.9%); Days >25 = 165/165 (100%); .Days 60 to 95 = 70/70 (100%) with 58/70 (83%) showing titres >100. **Other outcome:** Diagnostic test accuracy SARS CoV IgG detection: IFA: Sensitivity 98%, specificity 98%. ELISA: Sensitivity 81%, specificity 99%. Diagnostic test accuracy SARS-CoV-IgM detection: IFA: Sensitivity 79%, specificity 100%. ELISA: Sensitivity 90%, specificity 99%.

Hsueh 2004(22) Taiwan DOI: 10.1111/j.1469-0691.2004.01009.x Peer-reviewed; Clin Microbiol Infect. 2004 Dec;10(12) Case series	**SARS-CoV-1** positive RT-PCR and real-time RT-PCR assays from respiratory or serum samples **Testing:** IFA (In-house assay and commercial kit). The Cut-off values for a positive result were 1:25 for the in-house IFA and 1:10 for the commercial IFA kit. Indirect ELISA. Cut-off value for a positive IgG result by ELISA was 0.26. Neutralisation assay. **Sample:** serum samples (6–12 samples from each patient)	N = 30 patients with SARS Age: 25–80 years (mean 43 years) Four patients had underlying disease, namely diabetes mellitus (n = 2), hypertension (n = 1) and chronic hepatitis B virus carriage (n = 1). Controls: N = 200 paired sera from patients with community-acquired pneumonia, N = 70 sera from hospitalised patients with acute respiratory distress syndrome, N = 10 sera from ten pregnant women obtained during routine pre-labour check-ups.	**Duration of detection of serum immunoglobulin levels:** IgG: > 3 months. IgM and IgA: Started to decline after 3–4 weeks, and remained at low levels (1:40–1:80) at 12 weeks. **Serum titres of IgG over time (typically expressed as Geometric Mean Titres [GMTs]):** Tests for IgG were negative until at least 3 days after the onset of illness. All patients were positive for IgG for > 28 days (1:400–1:1600). Peak titre = 1:6400. N = 1 had a high level of IgG (1:800) at 100 days after the onset of illness. **Duration of detection of neutralising antibodies:** 2-3 months **Serum titres of neutralising antibodies over time:** Days 10–12 = appeared (mean 1:32), increased thereafter. Days 18-24 = peaked (1:128– 1:256). N = 4 titre remained at 1:32 or 1:64 at 2 months after onset, and was 1:64 on day 100 of the illness. **Other outcome:** Seroconversion of IgG (mean 10 days). **Treatment:** In addition to treatment with ribavirin (29/30 patients), N = 28 patients received IV methylprednisolone (1–11 days, mean 6 days, and 2–4 days before any IgG response), N = 21 received IV immunoglobulin (2–12 days, mean 6 days), and N = 9 were given mechanical ventilation (4–12 days, mean 8 days) following respiratory failure.

	Timing: <7 days to 2–3 months after the onset of illness.		No significant differences in the kinetics of the IgG, IgM and IgA response between patients with or without underlying medical disease, steroid or IV immunoglobulin therapy, or mechanical ventilation.
Huang 2005(15) China DOI: 10.1016/j.micinf.2004.11.017 Peer-reviewed; *Microbes Infect*. 2005;7(3):427–436 Case series	**SARS-CoV-1** Case definition of SARS-CoV-1 based on the Chinese Ministry of Health on April 14, 2003. **Testing:** Lymphocyte analysis: Flow cytometry. Humoral response: ELISA. Reference OD = 0.030 **Sample:** Blood **Timing:** 5 months follow up. Sampled at 1, 2, 3 and 4 weeks, and 2, 3, 4 and 5 months	Exposed population: N = 95 healthcare workers with SARS; *Sex:* Male = 19 (20%), *f*emale = 76 (80%) Mean age: 28.7 ± 9.5 years Controls: N = 60 healthy adults. Sex: Male = 13 (21.6%), female = 47 (78.4%), Mean age: 29.5 years old	**Duration of detection of serum immunoglobulin levels:** Specific IgG positive rate remained stable at around 96.5% at days 121-140 (study end-point). Specific IgM positive rate dropped to 54.5% at days 121-140 (study end-point). **Serum titres of IgG over time (typically expressed as Geometric Mean Titres [GMTs]):** General IgG antibodies: Month 1 = significant increase (Peak at week 3); 2 months = Decreased gradually to normal levels. Specific IgG antibodies: Days 1-5 = OD 0.069; Days 41-60 = OD 1.477 (peak); Day >60 = decreasing titres; Day >101 = increase in titres. **Duration of detection of T-cells:** CD4+ and CD8+ T lymphocytes decreased significantly over the 5 months. CD3+CD8+ memory T lymphocytes were decreased by 36.78% (P = 0.040) and CD3+CD4+ memory T lymphocytes by 19.65% in convalescent patients. **Other outcome:** **Cytokine production:** IL-10 and TGF-b were continuously overproduced for the entire course of SARS infection. **Treatment:** antiviral regimens, gamma globulin and/or corticosteroids
Li 2006(17)	**SARS-CoV-1**	N = 30 recovered SARS patients;	**Duration of detection of serum immunoglobulin levels:** 24 months

China DOI: 10.1371/journal.pone.0000024 Peer-reviewed; *PLoS One*. 2006;1(1):e24. Case series	Case definition of SARS-CoV-1: WHO clinical criteria **Test:** Lymphocyte analysis: Flow cytometry Humoral responses: ELISA (No S20030004, HuaDa Comp, Beijing, China), ELISPOT-based technique (Diaclone, France), neutralisation assay **Sample type:** Blood **Timing:** 2 years follow-up; Samples collected at 1, 3, 6, 12 and 24 months after the onset of symptoms.	Sex: 13 male and 17 female. Age: 37 ± 11 years antibody and antigen negative for HIV-1, CMV, and EBV Controls: N = 70 normal healthy age matched individuals. Sex: 36 male and 34 female. Age: 39 ± 10 years. .	**Serum titres of IgG over time (typically expressed as Geometric Mean Titres [GMTs]):** Months 1-3 = significant increase in Total IgG; Months 3-12 = gradual decrease; Months 12-18 = significant decrease; Months 18-24 = no significant decrease. **Duration of detection of neutralising antibodies:** N protein-specific Nab detectable at 24 months S protein-specific Nab detectable at 24 months. **Serum titres of neutralising antibodies over time:** Trend towards decrease Nab titres over time. N protein-specific Nab: <6 month = antibody remained relatively high. Months 6 -12 = significant decrease in titres; Months 12-24 = no significant decrease. S protein-specific Nab: No significant decrease between sample measurements. **Detection of T-cells/B memory cells or other:** Total lymphocytes, CD3, CD4, and CD8 T lymphocytes, B lymphocytes and NK cells: Months 1-3 = increase in cell populations; Months >3 = decline in rate of lymphocyte population recovery; Month 24 = mean absolute numbers of lymphocytes remained statistically different from that in normal healthy age-matched controls. **Other outcome:** INF-g releasing cells detected at month 3, 12 and 18 after onset of symptom.

Li 2003(16) China DOI: 10.1056/NEJM20030731349052 0 *Peer reviewed; N Engl J Med*. 2003;349(5) Case series	**SARS-CoV-1** **Testing:** Test not reported. Cut-off for a positive result 1:10 **Sample:** Serum **Timing:** Weeks 1-12. Measured at weeks 1, 2, 3, 4, 8, and 12.	Exposed group: N = 20 patients with SARS Controls: N = 103 healthy volunteers	**Duration of detection of serum immunoglobulin levels:** IgG peak titre at 12 weeks. IgM titres disappeared by the end of week 12. Controls tested negative for IgM and IgG. **Serum titres of IgG over time (typically expressed as Geometric Mean Titres [GMTs]):** Week 2 = mean titre 1:40; Week 3 = 1:256 (12/12 (100%) seropositive); Week 4 = 1:368; Week 8 = 1:640 (peak titre); Week 12 = 1:640. **Other outcome:** 20/20 100% seroconversion rate
Libraty 2007(28) Philippines. DOI: 10.1016/j.virol.2007.07.015 Peer reviewed: Virology, 368(2)	**SARS-CoV-1** **Testing:** ELISA, IFN-γ ELISPOT assays **Sample:** Blood **Timing:** 6–30 months after infection	N = 2 recovered SARS healthcare workers. N = 16 healthy contacts.	**Duration of detection of serum immunoglobulin levels:** 12 months **Serum titres of IgG over time:** The waning of anti-SARS CoV IgG levels paralleled the waning of S protein-specific memory T-cells at 12 months (N = 1). Anti-SARS-CoV-1 IgG levels were 4-fold lower in patient #2 than patient #1 at 6 months. **Duration of detection of T-cells:** 12 months **Detection of CD4+ T-cells:** S protein-specific memory CD4+ T-cells greatest 6 months after SARS-CoV-1 infection (N=1), and decreased to near the limit of detection by 12 months onward. S protein-specific CTL activity could be detected after in vitro re-stimulation at 12 months, but not at 24 and 30 months (N=1).

			Other outcome: **Cytokine production:** IFN-γ+ production to peptide S729–745 was greatest 6 months after SARS-CoV-1 infection, and decreased to near the limit of detection by 12 months onward (N=1). **Individual variation in immune responses:** CD4+ T-cell responses to any SARS-CoV-1 structural protein epitopes were weaker or decreased more rapidly in SARS patient #2 compared to patient #1 suggesting that in some individuals humoral and CD4+ T-cell immunity to SARS-CoV-1 may wane rapidly.
Liu 2006 DOI: 10.1086/500469 China Prospective follow-up study	**SARS-CoV-1** Serum samples were collected from each patient at regular intervals (at 1, 4, 7, 10, 16, and 24 months after disease onset) Serum titres of IgG were measured using a commercially available ELISA kit Neutralising antibodies (NAbs) were measured by neutralisation assay	A total of 63 patients recruited; N=56 participants contributed at least 3 blood specimens during the follow-up. Mean age 29 years (range, 18–59 years); 27 patients were men. Nine patients had underlying disease and seven patients had a severe clinical condition (such as oxygen ventilation and transfer of the patient to an intensive care unit)	The number of study participants tested at each follow-up visit varied from 32 to 41 IgG serological findings remained positive throughout follow-up for all patients, except at the last visit (at month 24), when findings for 4 (11.8%) of 34 serum samples changed from positive to negative findings. Peak GMT occurred at month 4, before a significant decrease occurred over time until month 24 All samples tested positive for neutralising antibodies at all visits. GMTs peaked at month 4, decreased at month 7, and decreased again at month 24 Neutralising antibody and IgG antibody titres were strongly correlated

Mo 2006(32) China DOI: 10.1111/j.1440-1843.2006.00783.x Peer reviewed; *Respirology*. 2006;11(1) Case series	**SARS-CoV-1** Case definition of SARS-CoV-1: WHO clinical criteria **Testing:** ELISA (GBI Biotech, Beijing China) and IFA. Reference value for positive result: OD 0.13 + A negative control. Neutralisation assay. **Sample type:** Blood sample **Timing:** 7 to 720 days after the onset of symptoms. Serial blood samples were taken on days 7, 15, 30, 60, 90, 180, 270, 360, 450, 540 and 720.	Exposed group: N = 98 patients with SARS (N = 18 completed follow-up), Sex: 43 men and 55 women, Age: 20–75 years (mean 37.8 ± 12.2 years), Average duration of hospitalization was 23.1 ± 12.3 days. Control: N = 10 healthy volunteers, Sex: four men and six women, Age:17–58 years (mean 35.6 ± 12.2 year)	**Duration of detection of serum immunoglobulin levels:** **Ratios of positive IgG/IgM:** 0/0, 45.4/39.4, 88.6/71.4, 96/88, 100/48.6, 100/30.9, 100/17.1, 100/0 per cent, respectively, on 1–7, 8–14, 15–21, 22–28, 29–60, 61–90, 91–180 and 181–720 days. IgM was undetectable on day 180. IgG was still detectable at day 720. **Serum titres of IgG over time (typically expressed as Geometric Mean Titres [GMTs]):** IgG titres: Day 7 = not detected; Day 15 = increasing titres; Day 60 = 1:670 (peak); day 180 = 1:670 (plateaued); Day 540 = titres had rapidly declined; day 720 = average titre was close to the cut-off value for positivity (1:10). **Duration of detection of neutralising antibodies:** 17/18 detectable at 720 days **Serum titres of neutralising antibodies over time:** Day 15 = increasing titres; Day 30 = 1:590 (peak); Days 540 and 720 = 1/18 no detectable neutralising antibodies, 17/18 low titre (average of 1:10). Neutralising antibodies were not detectable in normal control sera. **Other outcome:** **Treatment:** Combination of antibiotics (cephalosporin and erythromycin) and antiviral agents (ribavirin or traditional Chinese medicine). Patients whose fever persisted for >3 days or who showed a progressive deterioration in their CXR (79.6%), received methylprednisonlone. **Seroconversion:**

			Earliest seroconversion occurred on day 10 after the onset of the disease.
Ng 2016(33) doi: 10.1016/j.vaccine.2016.02.063 Singapore Prospective follow-up study/case series	**SARS-CoV-1** (ELISpot) assays Intracellular cytokine staining (ICS) and degranulation assays and flow cytometry. Screening for the presence of SARS-specific T-cells was performed by a number of different testing methods	N=3 SARS-recovered individuals Follow up at 9 or 11 years post-infection	All memory T cell responses detected target the SARS-CoV structural proteins. Two CD8+ T cell responses targeting the SARS-CoV membrane (M) and nucleocapsid (N) proteins were characterized by determining their HLA restriction and minimal T cell epitope regions. These responses were found to persist up to 11 years post-infection. An absence of cross-reactivity of these CD8+ T cell responses against MERS-CoV was also demonstrated. **Interpretation:** Persistence of SARS-specific cellular immunity targeting the viral structural proteins in SARS-recovered individuals was demonstrated up to 11 years post-infection. The persistence of T cell responses suggests that SARS-recovered patients could be protected from reinfection.
Peng 2006(37) China DOI: 10.1016/j.virol.2006.03.036 Peer reviewed; Virology. 2006 Aug;351(2)	**SARS-CoV-1** Diagnostic criteria for SARS-CoV-1 infection: WHO clinical criteria **Testing:** Cytokine production: ELISA (R&D) and ELIspot assay (BD Biosciences)	Exposed group: N = 14 recovered SARS Individuals Sex: 7 men and 7 women, Age: 20 to 37 Control: N = 3 subjects without any contact history with SARS patients.	**Duration of detection of T-cells:** 2 years SARS-CoV N-protein-specific memory CD4+ and CD8+ T cells were maintained for 2 years after SARS-CoV infection. **Other outcome:** **Cytokine production** PBMCs produced IFN-γ and IL-2 following stimulation with a pool of overlapping peptides from the SARS-CoV N protein sequence.

Case-control study	**Sample type:** venous blood **Timing:** 2 years		
Shi 2004(38) China DOI: 10.1016/j.jcv.2004.05.006 Peer reviewed; Journal of Clinical Virology : the Official Publication of the Pan American Society for Clinical Virology. 2004 Sep;31(1) Case series	**SARS-CoV-1** probable SARS patients based on WHO criteria **Testing:** IFA, ELISA and viral neutralisation. ELISA cut-off value for a positive result = 0.15. Neutralisation titre = the highest dilution of the serum at which 50% of the wells were protected from viral cytopathic effect. **Sample:** Serum	N = 14 probable SARS patients. Age: 22 to 73 years old (median of 45 years).	**Duration of detection of serum immunoglobulin levels:** IgG antibody was detectable for 210 days. IgM was shown to be negative in 4, 8, 12 and all 14 patients by day 60,120,180 and 210 days post disease onset, respectively. **Serum titres of IgG over time (typically expressed as Geometric Mean Titres [GMTs]):** anti-viral IgG peak titre = 120 days; 120-210 days = decreasing titres; 210 days = high antibody titres. Duration of detection of neutralising antibodies: 210 days (peak at 180 days) **Serum titres of neutralising antibodies over time:** The geometric means of the neutralisation titres on day 20, 30, 60, 120 and 210 was 1:150, 1:475, 1:400, 1:200 and 1:200, respectively. **Other outcome:** IgG seroconverion 13/14 patients IgM seroconversion 13/14 patients

	Timing: Samples for ELISA were collected at 7 to 210 days after the onset of the symptoms. Samples for neutralisation assays collected at 20, 30, 60, 120, and 210 days post disease onset.		
Tang 2011(39) doi: 10.4049/jimmunol.0903490 China Prospective follow-up study	**SARS-CoV-1** The specific memory B cell and T cell responses to SARS-CoV-1 were measured by means of ELISPOT assay. IgG was measured with commercially available ELISA kits	N=23 patients Mean age 31.7 ± 8.3 years (range, 20–51 years) 17 (73.9%) were females. 9 patients had underlying disease and 7 patients had a severe illness	Six years postinfection, specific IgG to SARS-CoV-1 became undetectable in 21 of the 23 former patients. No SARS-CoV-1-specific memory B cell response was detected in either 23 former SARS patients or 22 close contacts of SARS patients and 20 health controls. Memory T cell responses to a pool of SARS-CoV S peptides were identified in 14 of 23 (60.9%) recovered SARS patients, whereas there was no such specific response in either close contacts or healthy controls. Patients with more severe clinical manifestations seemed to present a higher level of Antigen-specific memory T cell response. **Interpretation:** SARS-specific IgG may eventually vanish and peripheral memory B cell responses are undetectable in recovered SARS patients. In contrast, specific T cell anamnestic responses can be maintained for at least 6 years.
Tso 2004(64) China	**SARS-CoV-1** **Testing:** IFA	N= 62 survivors of SARS and N = 1 asymptomatic	**Duration of detection of serum immunoglobulin levels:** 1 year

DOI: 10.1086/424573 Peer-reviewed; *J Infect Dis*. 2004;190(9) Prospective cohort study	**Sample:** Serum **Timing:** 1 year. SARS survivors: Sampling on day of hospital admission, 15 days, 1 month, 3 months, 6 months, 9 months, and 12 months after the onset of SARS symptoms. HCW: samples collected 1, 3, 6, 9, and 12 months after the first day of deployment to the SARS ward	infected healthcare worker. Sex: male:female ratio 0.82. Age: mean age 37.07 years (SD, 12.96). Baseline SARS CoV immunoglobulin titre <25 at hospital admission.	**Serum titres of Ig over time (typically expressed as Geometric Mean Titres [GMTs]):** SARS survivors: SARS-CoV Ig mean titre at baseline = <25; Day 15 = 252.8; Months 1 = 613.3; Month 3 = 880.3; Months 3-12 = gradual decrease in the mean SARS CoV Ig titre; 12 months = 167.7 (i.e. 5.3-fold decrease in mean titre at 12 v 3 months). Asymptomatic HCW: 1 month mean SARS CoV Ig titre = 400; Month 3 and 6 = 50 (i.e., an 8-fold decrease). Month 9 and 12 = 25. **Other outcome:** 100% rate of seroconversion.
Wu 2007(4) doi: 10.3201/eid1310.070576 China Prospective follow-up	**SARS-CoV-1** Serum antibody titres measured by ELISA kit (BJI-GBI Biotechnology, Beijing, China)	A total of 176 cases that met the World Health Organization (WHO) SARS case definition Sex/age of cohort not reported	**IgG** 7 days after the onset of symptoms, the percentage who were IgG positive was ≈11.8%. This percentage continued to increase, reached 100% at 90 days, and remained largely unchanged up to 200 days. After 1 and 2 years 93.88% and 89.58% of patients, respectively, were IgG positive, which suggests that the immune responses were maintained in >90% of patients for 2 years. 3 years later, ≈50% of the convalescent population had no SARS-CoV–specific IgG.

			IgM The percentage of patients who were IgM positive within the first 7 days was 21.4% and peaked at 76.2% after 21–30 days. For most samples the IgM readings had reached background levels on day 90. **Interpretation**: SARS-specific antibodies were maintained for an average of 2 years, and significant reduction of IgG positive percentage and titres occurred in the third year. Thus, SARS patients might be susceptible to reinfection >3 years after initial exposure.
Yang 2009(65) China DOI: 10.1080/00365540902919384. Peer reviewed; Scandinavian Journal of Infectious Diseases. 2009 ;41(6-7) Retrospective seroepidemiological cohort study.	**SARS-CoV-1** All recovered cases were post-hoc confirmed by SARS-CoV. A probable SARS case was a patient with SARS contact history, high fever (>38°C), and radiographic evidence of infiltrates consistent with pneumonia or respiratory distress syndrome.	N = 67 confirmed SARS patients with >9 serum measurements during follow-up. 37.3% were men. Age: 16 to 57 years; mean age: 35.5 years (SD = 10.59). N = 688 non-SARS controls: Low risk/non-exposed controls (n = 200); high risk healthcare workers (n = 488).	**Duration of detection of serum immunoglobulin levels:** IgG: 82 weeks after onset of illness (study endpoint) **Serum titres of IgG over time (typically expressed as Geometric Mean Titres [GMTs]):** OD = 0.7 at week 82 (approx) **Other outcome:** Low risk controls: No positive antibody test High risk controls: 3 people (0.61%) with a positive IgG using ELISA; 1 (0.21%) confirmed using IFA Treatment: Corticosteroid treatment

	Testing: IgG: ELISA (Beijing GBI company, patch no. 200305). Positive samples confirmed with IFA (Huada Diagnostics Ltd, Beijing, China) Reference value for positive test: OD > 0.18 or OD > 0.13 above negative controls. **Sample type:** Serum **Timing** Intervention: Blood sampling every 3 weeks; 16 month follow up. Controls: 2 serum samples were collected during the		

	SARS outbreak and 6 months post-outbreak.		
Yang 2006(49) China DOI: 10.1016/j.clim.2006.05.002 Peer reviewed; *Clin Immunol*. 2006;120(2) Case-control study	**SARS-CoV-1** **Testing:** Cytokine production: ELISA (BD Pharmingen, San Diego, CA) and ELISpot (BD Pharmingen) assays. Lymphocyte analysis: Flow cytometry **Sample type:** peripheral blood **Timing:** >1 year after SARS-CoV infection	Exposed group: N = 8 recovered SARS patients Sex: 5 male and 3 female, Age: 25 to 34 years Control: N = 5 healthy donors, Sex: 3 male and 2 female, Age: 27 to 33 years,	**Duration of detection of T-cells:** >1 year after infection. SARS-CoV S-specific memory T cells were persistent in peripheral blood of recovered SARS individuals. **Other outcome:** **Cytokine production** Antigen-specific memory T cells of secreted high levels of IFN-g upon stimulation in vitro with a pool of SARS-CoV S peptides.
Xie 2006(47) China Peer reviewed; Acta Acad Med Sin, 2006, 28(2)	**SARS-CoV-1** **Testing:** Flow cytometry **Sample:** Blood	N = 62 seropositive SARS cases Sex: 21 males and 41 females, Age: average age 38 ± 1 years	Duration of detection of T-cells: *Total lymphocytes and T cells* Week 1: Total lymphocytes and T cells counts decreased significantly. Week 2: Numbers continued to decline. Months 1-3: Trend of rapid increase. Month 12: Significant differences between total lymphocyte and T cell count in SARS patients (Total lymphocyte 1,807 ± 473; T cell 1,285 ±

Case control study	**Timing:** 1 year follow-up. Sample collection at 1 week, 2 weeks, 1 month, 2-3 month and 1 year.	Controls: N = 56 healthy individuals Sex: 30 males, 26 females. Age: average age 36 ± 10 years	367) and normal controls (Total lymphocyte 2,254 ± 541; T cell 1,545 ± 394) at 1 year follow-up. *CD4 + T cells, CD8 + T cells, naive and memory CD4 + T cells* Week 1: Numbers decreased significantly. Week 2: Numbers continued to decrease. Month 2/3: Increased rapidly. 1 year of follow-up: Memory CD4 + T cells recovered to normal levels (SARS patients 438 ± 140 v controls 495 ± 203). Average CD4 + T cells and naive CD4 + T cells were reduced compared to normal patients (SARS patients v controls: CD4 + T cells, 672 ± 192 v 870 ± 299; Naive CD4 + T cells, 200 ± 108 v 320 ± 121). CD8 + T cells recover significantly faster than CD4+ T cells. At 2-3 months the number of CD8 + T had returned to normal levels (SARS patients 578 ± 395 v controls 580 ± 174).

Table 5 Duration of immune response: MERS-CoV

Author DOI Country Study design	Virus type Test parameters	Population Patient demographics Clinical characteristics	Primary outcome results	Comments
Alshukairi 2016(2) DOI: 10.3201/eid2206.160010 Jeddah, Saudi Arabia Prospective follow-up	**MERS-CoV** ELISA for MERS-CoV S gene antibody; IFA (immunofluorescence assay) for MERS-CoV IgG	▪ N=9 healthcare workers who survived MERS. ▪ Four of the 9 patients were women; 2 of them were 32 weeks and 20 weeks' pregnant. ▪ Average patient age was 38 years (range 27–54 years). ▪ Patients were classified into 4 categories according to their clinical presentation: asymptomatic, upper respiratory tract infection, pneumonia, or severe pneumonia. Patients with severe pneumonia were those	**Duration of detection of antibodies:** ▪ Of the 9 patients, 2 had severe pneumonia, 3 had milder pneumonia not requiring intensive care, 1 had upper respiratory tract disease, and 3 remained asymptomatic. All patients recovered without sequelae. ▪ The 2 patients with severe pneumonia had the highest antibody titres detected among all patients and remained MERS-CoV-antibody–positive at 18 months after illness onset and had prolonged viral shedding documented by persistent positive rRT-PCR results for 13 days (patient 1) and 12 days (patient 2) ▪ When tested at 18 months after illness onset both severe patients had positive antibodies. Asymptomatic/URT patients did not demonstrate positive ELISA for IgG at any point	Peer reviewed *Emerging Infectious Diseases*

		who required mechanical ventilation	Conclusion: Results indicate that the longevity of the MERS-Cov antibody response correlated with disease severity. Accordingly, 2 patients with severe MERS-associated pneumonia had a persistent antibody response detected for >18 months after infection, whereas patients with disease confined to the upper respiratory tract or who had no clinical signs had no detectable MERS-CoV antibody response.	
Payne 2016(36) DOI: 10.3201/eid2210.160706 Jordan Case series	**MERS-CoV** Anti-MERS-CoV nucleocaspid Indirect ELISA and MERS-CoV indirect IFA Neutralisation titres were determined by microneutralisation with live MERS-CoV	N=7 cases 13 and 34 month follow up Cases not confirmed by RT-PCR, were probably cases as had acute respiratory infection during outbreak and had exposure to at least one person with laboratory confirmed MERS-CoV. Cases aged from 31- 60 years of age (mean 42.4 years). Two cases had hypertension, one had atrial septal defect, one was pregnant and three reported no underlying conditions.	**Duration of detection of antibodies:** AT 34 months of the 7 participants for whom IFA results were positive at 13 months, 4 (57%), had positive results at 34 months. Six out of 7 (86%) had positive neutralising antibody titres. ELISA titres were from <400 to 1600. Overall, one of the 7 patients were considered overall as being serological negative at 34 months. **Serum titres** Between 13 months and 34 months the ELISA titres decreased (from 400 - >6,400 to <400 to 1600) for all but one person, where the titre remained the same (1600) between 13 and 34 months) Of the 7 participants, 6 (86%) had neutralising antibody titres ranging from 20 to 80 at 34 month follow up evaluation. Two (29%) had any decrease in neutralising antibody titres over time. One participant had no detectable neutralising antibodies.	Published in Emerging Infectious Diseases

			Overall summary: Antibodies against MERS-CoV, including neutralising antibodies, persisted in 6 (86%) of 7 persons 34 months after the 2012 MERS-CoV outbreak in Jordan. **Other outcome:** Any association between our MERS-CoV antibody results and clinical severity is therefore difficult to assess. Nonetheless, of the 5 persons for whom chest radiographs showed substantial changes within 3 days of symptom onset, each remained positive by microneutralisation (>20) 34 months after the outbreak.	
Choe 2015(10) DOI: 10.3201/eid2307.170310 Seoul, South Korea Case series	**MERS-CoV** MERS confirmed by RT-PCR MERS S1 ELISA (commercially available EUROIMMUN, Germany) Neutralising antibody assay Plaque-reduction neutralisation tests (PRNTs)	N=11 confirmed MERS-CoV patients Samples collected at 21-50 days after disease onset and at 1 year follow-up. N=5 had severe disease, n=6 had mild disease	**Duration of detection of antibodies:** All 5 patients with severe disease, but only 2 (33%) of 6 with mild disease, had PRNT90 antibody titres $\geq$40 at the 1-year follow-up. These patients also had positive microneutralisation assays, S1 ELISA assays and pseudoparticle neutralisation tests (ppNT), 1 year after illness onset. At 1 year after infection, the 4 patients who had mild disease (or who did not require supplemental oxygen or mechanical ventilation) all had negative results by micro-neutralisation assay and S1 ELISA, but 1 was positive by ppNT (titre of 10) and 2 by PRNT90 (titre 1:10). All bar one of these patients had chest infiltrates on x-ray.	Yes (emerging infectious diseases, CDC)

	Serum samples collected at approx. 6 and 12 months		**Serum titres** All 5 patients with severe disease, but only 2 (33%) of 6 with mild disease, had PRNT90 antibody titres ≥40 at the 1-year follow-up. Two of the severe patients who had acute-phase antibody titres of ≥320, declined ≥4-fold 1 year later. Four patients with acute phase peak antibody titres in the range of 80–160 only had ≤2-fold declines in titre. MERS antibody titres waned during the first 6 months after disease onset, especially in patients who had had high antibody titres. The waning of antibody titres between 6 months and 1 year after disease onset was less steep. **Other outcome:** **Antibody titres in 4 of 6 patients who had mild illness were undetectable even though most had evidence of pneumonia** The kinetics of antibody production seen with the PRNT90, ppNT, microneutralisation test, and S1 ELISA were comparable, suggesting that any of these tests could be used for detection of MERS-CoV antibodies in patients with past infection. The authors found strong positive correlations between duration of virus detection and antibody titres	

			Because of the poor antibody response that resulted from symptomatic disease, persons with asymptomatic or mild infection without severe lung parenchymal disease are not expected to develop detectable MERS-CoV antibodies	
Zhao 2017(54) DOI:10.1126/sciimmunol.aan5393 Saudi Arabia Case series	**MERS-CoV** MERS confirmed by RT-PCR Anti-MERS-CoV antibody titres measured by ELISA and IFA Microneutralisation assay MERS-CoV PRNT50 assay	N=21 MERS patients (n=7 of these patients had sample taken at 24 months, while 14 had sample taken at 6 months post infection) N=4 controls Detailed demographic and clinical information provided in a table. In brief, 9/21 female, age range 25 to 59, and seven had co-morbidities including diabetes mellitus, chronic heart disease, pregnancy, ESRD, organophosphate poisoning and pregnancy. Of 18 patients who provided PBMCs, 3 patients were asymptomatic, 6 patients had	**Duration of detection of antibodies:** Based on PRNT antibody responses tended to be present but lower (but not significantly different) in patients at 24 months compared to patients at six months after infection. **T-Cell response:** Both CD4+ and CD8+ T-cells responses were present but lower at 24 month post infection compared with 6 months post infection, however the difference was not statistically significant.	Published in Science Immunology

		pneumonia, and 9 patients had severe pneumonia		

Table 6 Study characteristics: reinfection rate

Author DOI Country Study design	Virus type Test parameters	Population Patient demographics Clinical characteristics	Primary outcome results	Comments
Reinfection rate				
An 2020(3) https://doi.org/10.1101/2020.03.26.20044222. China Retrospective Case series	**SARS-CoV-2** The discharge criteria of the recovered patients included: temperature returned to normal for >3 days, respiratory symptoms significantly improved, and significant absorption of pulmonary lesions of chest CT imaging, and at least 2 consecutive negative RNA test results at least 24 hours apart. RT-PCR was performed using a China Food and Drug Administration (CFDA) approved commercial kit specific for 2019-nCoV detection (GeneoDX Co., Ltd.,	N=262 confirmed COVID-19 patients discharged from Shenzhen Third People's Hospital. Among them, mild, moderate and severe patients accounted for 11.4% (n=30), 81.0% (n=212) and 7.6% (n=20), respectively.	**Redetectable Positive (RP)/Reinfection rate** Up to March 10, 14.5% of convalescent patients (n=38) were re-detected to be SARS-CoV-2 respiratory RNA positive during their followed-up period. ▪ The vast majority of RP patients (97.4%, n=37) were younger than 60 years of age. Among them, patients younger than 14 years old were more common compared with those between the ages of 14 and 60 years (35.0% vs 16.0%, p<0.01) ▪ In addition, 36.7% (11/38) of RP patients were characterised by mild symptoms. The percentage was significantly higher than what was seen among non-RP patients (12.7%, 19/204, p<0.01). ▪ There was no significant difference in the gender distribution ▪ There were no RP cases in severe patients	Not peer reviewed (pre-print)

	Shanghai, China) or Sherlock kit gifted from Feng Zhang lab. The redetectable positive (RP) patients were confirmed by digestive (anal swab) and respiratory positive RT-PCR tests. All patients followed for minimum of 14 days.		▪ RP patients showed no obvious clinical symptoms and disease progression upon re-admission	
Deng 2020(12) China Case series https://europepmc.org/article/PPR/PPR122436	**SARS-CoV-2** RT-PCR (device NR) using NP and anal swabs Discharge criteria: 2 negative RTPCR test results at least 1 day apart (sample site for discharge unclear) 3 days after discharge, patients were re-detected via NP swabs for 3 patients and via anal swabs for 1 patient Viral RNA was not consistently detected in subsequent tests in 3 of 4 patients.	4 discharged patients with re-detected SARS-Cov-2 RNA 3 days after discharge **Demographics:** Case 1: 29-year old male Case 2: 49-year old female (mother of case 1) Case 3: 12-year old female Case 4: 38-year old male **Clinical characteristics:** Initial Presentation: Case 1: Fever and cough	**Redetectable Positive (RP)/Reinfection rate** 17.6% (3/17) patients were found to be re-detectable positive by viral RNA RT-PCR of nasophayngeal swabs. 4 patients from a total of 17 cases (23.5%) were found to be re-detectable positive by any means (nasopharyngeal or anal swab) ▪ 3 patients showed nasopharyngeal swabs result positive after 3 days of discharge. The remaining one showed anal swab result positive after 3 days of discharge. ▪ No patient presented with symptoms upon re-detection ▪ 3 patients returned to the designated hospital for quarantine again. Two patients were discharged again from the hospital on March 2nd, 2020, and tested negative.	Not peer-reviewed (pre-print)

		Case 2: Cough Case 3: No symptoms Case 4: Fever, fatigue and cough Re-admission Case 1: No symptoms Case 2: No symptoms Case 3: No symptoms Case 4: No symptoms **COVID-19 Clinical syndromes (National Health Commission of the People's Republic of China definition):** Case 1: NR Case 2: NR Case 3: Mild Case 4: NR	▪ The other (case 4) was still under medical observation at the time of writing. ▪ The third case was quarantined in the hospital due to positive results of anal swab.	
To 2020(66) Hong Kong, China Cohort study	**SARS-CoV-2** qRT-PCR (QuantiNova Probe RT-PCR Kit (QIAGEN, Hilden, Germany)) using blood, urine, posterior	**Population setting:** 23 patients at 2 hospitals in Hong Kong **Demographics:**	▪ One patient (of 23) with complete resolution had undetectable viral load on days 21 and 22 after symptom onset, with rebound of viral load on days 23 and 24, followed by 5 days of undetectable viral load	Published The Lancet Infectious Diseases

http://www.sciencedirect.com/science/article/pii/S1473309920301961	oropharyngeal saliva, and rectal swab samples **Discharge criteria** A criterion for discontinuation of transmission-based precautions is a negative RT-qPCR result from two sets of nasopharyngeal and throat swab specimens. Other criteria not specified. Re-detected via rectal swab	13 male, 10 female Median age 62 years (range 37–75) **Clinical characteristics:** Fever, 22 (96%), cough, 5 (22%), chills, 4 (17%), dyspnoea, 4 (17%) **COVID-19 Clinical syndromes (author definitions):** Severe disease, 10 (43%), Mild disease, 13 (57%) Severe disease defined as the need for supplemental oxygen, admission to ICU, or death.		
Kim 2020(27) South Korea Case series https://www.ncbi.nlm.nih.gov/pmc/articles/PMC	**SARS-CoV-2** rRT-PCR (Thermo Fisher Scientific, MA, USA) using URT, LRT, serum, plasma, urine, stool samples. Discharge criteria not provided, as patients remained in-patients for the duration of the study	2 hospitalised patients **Demographics:** Patient 1: 35 year old woman Patient 2: 55 year old man	▪ Patient 2 had undetectable virus RNA across all tested samples for 7 consecutive days (from days 18-24 post symptom onset inclusive) having had several days of consecutively positive test results across multiple sample sites ▪ Patient 2 subsequently tested positive one more time via both URT (on day	Published J Korean Med Sc

7036338/pdf/jkms-35-e86.pdf	Redetected using URT and LRT samples	**Clinical characteristics:** **Presentation:** Patient 1: fever, chills, and myalgia Patient 2: sore throat and intermittent myalgia **COVID-19 Clinical syndromes**: Patient 1: Moderate Patient 2: Mild (not defined)	25) and LRT samples (on day 26), while an in-patient. ▪ Patient was discharged on day 27 post symptom onset. ▪ Patient 1 experienced relatively stable patterns of virus detection from admission through to discharge	
Lim 2020(57) South Korea Case report https://www.ncbi.nlm.nih.gov/pubmed/32056407	**SARS-CoV-2 RT-PCR** (Quantstudio 1 Applied Biosystems, Foster City, CA, USA) and PowerCheck™ SARS-CoV-2 Real-Time PCR kit, KogeneBiotech, Seoul, Korea) using sputum sample. Discharge criteria not provided, as patient remained in-patients for the duration of the study Redetected using sputum samples	**Population setting:** 1 patient admitted to hospital **Demographics:** 54 year old man **Clinical characteristics:** Presentation: Chills and muscle pains **COVID-19 Clinical syndromes (WHO definition):** Pneumonia	▪ Patient experienced 2 consecutive days of undetectable virus RNA from sputum samples on days 11 and 12 since symptom onset, having had 2 previous days of positive test results. ▪ Patient subsequently had 4 more consecutive days of positive test results	Published J Korean Med Sc
Qu 2020(59) China	**SARS-CoV-2** real-time RT-PCR (device NR) using throat swabs and sputum	**Population setting:** 1 patient admitted to hospital	▪ After the active treatment, the patient recovered from fever and other respiratory symptoms on February 4 (day 13 of hospitalisation). On	Published

Case report http://www.sciencedirect.com/science/article/pii/S1477893920300879	**Discharge criteria:** 2 successive negative results of Sars-Cov-2 nucleic acid detection, in addition to normal body temperature for 3 days as well as obvious improvement in respiratory symptoms and CT scan Redetected by throat and sputum samples	**Demographics:** 49 year old man **Clinical characteristics:** Presentation: Fever **COVID-19 Clinical syndromes:** NR	February 9 and February 10 (days 18 and 19 of hospitalisation), the SARS-CoV-2 nucleic acid detection was successively negative in throat swab samples. CT scan result showed that the inflammation was significantly decreased in both lungs. Both the results of SARS-CoV-2 nucleic acid detection and CT scans indicated a recovery trend, and the patient was ready for discharge ▪ On February 13 (Day 22 of hospitalization), the throat swab and sputum by nebulization were collected before the patient was discharged. Notably, SARS-CoV-2 nucleic acid was still detected in sputum from the patient although negative result of throat swab detection	Travel Medicine and Infectious Disease Journal
Wang 2020(41) China Case series https://europepmc.org/article/PPR/PPR150648	**SARS-CoV-2** RT-PCR (BioGerm) using NP and anal swabs **Discharge criteria:** 1. Temperature below 37 degrees lasting at least 3 consecutive days; 2. Resolved respiratory symptoms; 3. Substantially improved in chest lesions CT images, and	**Population setting:** 182 post-discharge patients recovering from COVID-19 under medical isolation **Demographics** (n=20 re-detected patients): Mix of children and adults Sex:	▪ 20 patients (11%) re-tested positive for SARS-CoV-2 within 14 days of meeting discharge criteria ▪ patients that were re-detected for SARS-CoV-2 had significantly shorter lengths of stay during their index admission than patients who were not re-detected ▪ Fourteen of the 20 (70%) re-detected patients tested positive from nasopharyngeal swabs and the other six patients (30%) tested positive from anal swabs. No patient tested positive from both samples	Not peer-reviewed (Pre-print)

	4. 2 consecutively negative RT-PCR test results with at least 1 day interval (sample site not reported) Fourteen of the 20 (70%) re-detected patients tested positive from nasopharyngeal swabs and the other six patients (30%) tested positive from anal swabs. No patient tested positive from both samples	Male, 7 (35%) Female, 13 (65%) **Age:** Median, 41.5 (Range 1-72) **Clinical characteristics:** *Initial presentation:* NR *Upon re-admission:* No symptoms, 20 (100%) **COVID-19 Clinical syndromes (n=20 re-detected patients) (Definition not reported):** Non-severe, 20 (100%)		
Xiao 2020(45) China Case series	Throat swab samples or deep nasal cavity swab samples were collected from patients on different dates after the onset of symptoms SARS-CoV-2 were detected by RT-PCR assay using a COVID-19 nucleic acid	N=70 patients Age (median): 57 (IQR 44-65) Male proportion: 44% All patients were mild to moderate	▪ 15 (21.4%) patients experienced a positive of nucleic acid detection by RT-PCR test for SARS-CoV-2 after 2 consecutive negative results ▪ Authors report this may be related to false negative RT-PCR tests	Letter to the editor Peer-reviewed In: Journal of Medical Virology.

	detection kit (Shanghai Huirui Biotechnology Co., Ltd)	Time from onset of symptoms to nucleic acid conversion (2 negative RT-PCR): median 36 days (IQR: 28-40)		
Xing 2020(48) doi: 10.2807/1560-7917.ES.2020.25.10.2000191 China COVID-19 case follow-up surveillance (case series)	**SARS-CoV-2** RT-PCR assay for SARS-CoV-2 SARS-CoV-2 nucleic acid in throat swab samples were taken according to the manufacturer's protocol (Shanghai BioGerm Medical Technology, Shanghai, China).	N=62 SARS-CoV-2 cases among medical personnel, of which 2 were repeat positive after discharge. All confirmed cases were hospitalised and isolated for treatment. The discharge criteria were: (i) afebrile for at least 3 days, (ii) obvious alleviation of respiratory symptoms, (iii) improvement in radiological abnormalities on chest computed tomography (CT) or X-ray and (iv) 2 consecutive negative detections of SARS-	▪ Case 1 was a male doctor in his 40s After discharge on 10 February, he was kept under surveillance and quarantined at home. He did not experience discomfort during the follow-up period. The results of consecutive throat swab tests were negative on 13 February, weakly positive on 14 February, positive on 15 February, negative on 16 February, weakly positive on 18 February, negative on 20 February and negative on 22 February. ▪ Case 2 was a female nurse in her 20s. After discharge on 13 February, Case 2 was kept under surveillance and quarantined at home. She did not experience discomfort during the follow-up. The results of consecutive throat swab tests were weakly positive on 14 and 15 February, negative on 16, 17 and 18 February, positive on 19 February and negative on 20, 21 and 22 February.	Eurosurveillance Peer-reviewed

		CoV-2 at least 24 h apart		
Zhang 2020(50) China Case series https://doi.org/10.1101/2020.03.28.20043059	**SARS-CoV-2** rRT-PCR (Mabsky Biotech Co., Ltd) using upper respiratory (nasal-throat mixed), faeces, urine, plasma samples Discharge criteria not provided	**Population setting:** 23 patients treated in hospital in Beijing **Demographics:** Adults Age: 48 years (IQR 40 to 62) Sex: Male, 12 (52%); Female, 11 (48%) **Clinical characteristics:** Presentation: Fever 20 (87%), cough 13 (57%), weakness 9 (39%), myalgia 5 (22%), pharyngalgia 5 (22%), headache 3 (13%) **COVID-19 Clinical syndromes (National Health Commission of the People's Republic of China definition):** Severe, 2 (9%)	▪ At 26 days after discharge, 1 case was detected positive again in faeces samples, but appeared healthy and negative for respiratory swabs.	Not peer-reviewed (Pre-print)

		Mild-to-moderate, 21 (91%)		

Table 7 Study characteristics: severity of initial disease

Author DOI Country Study design	Virus type Test performed Location of sample Timing of sample	Population Patient demographics	Primary outcome results	Comments
Adams 2020(1) 10.1101/2020.04.15.20066407 UK Case series	SARS-CoV-2 ELISA and RT-PCR (used as reference test) Compared to nine 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 first symptoms; (n=6). Convalescent samples were collected from adults a median 48 [range 31-62] days after	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. **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, univariable 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	medRxiv – not peer reviewed

	symptom onset and/or date of positive throat swab (n=18)		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. **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	
An 2020(3) https://doi.org/10.1101/2020.03.26.20044222. China Retrospective Case series	**SARS-CoV-2** The discharge criteria of the recovered patients included: temperature returned to normal for >3 days, respiratory symptoms significantly improved, and significant absorption of pulmonary lesions of chest CT imaging, and at least 2 consecutive negative RNA test results at least 24 hours apart. RT-PCR was performed using a China Food and Drug Administration	N=262 confirmed COVID-19 patients discharged from Shenzhen Third People's Hospital. Among them, mild, moderate and severe patients accounted for 11.4% (n=30), 81.0% (n=212) and 7.6% (n=20), respectively	Up to March 10, 14.5% of convalescent patients (n=38) were re-detected to be SARS-CoV-2 respiratory RNA positive during their followed-up period. **Rate of seroconversion** 36.7% (11/38) of RP patients were characterised by mild symptoms. The percentage was significantly higher than what was seen among non-RP patients (12.7%, 19/204, p<0.01). There were no re-detected positive cases in severe patients. **Timing of seroconversion** RNA negative conversion occurred mostly within 2-3 weeks since onset of illness among 63.6% of mild and within 1-2 weeks since onset among 22.2% moderate RP patients. By contrast, there were more NRP patients who displayed RNA	Not peer reviewed

	(CFDA) approved commercial kit specific for 2019-nCoV detection (GeneoDX Co., Ltd., Shanghai, China) or Sherlock kit gifted from Feng Zhang lab. The redetectable positive (RP) patients were confirmed by digestive (anal swab) and respiratory positive RT-PCR tests. All patients followed for minimum of 14 days.		negative conversion after 3 weeks since onset regardless of mild or moderate status. **Duration of immunity** Not reported **Other**	
Chen 2020(9) https://www.tandfonline.com/doi/pdf/10.1080/22221751.2020.1732837 China Cross-sectional	-SARS-CoV-2 Blood, pharyngeal and anal swabs Nucleic Acid Isolation Kit (Da'an Gene Corporation, Cat: DA 0630)	57 patients; 2 cohorts ▪ blood detection cohort (n=57) ▪ anal swab cohort (n=28) Patient diagnosed as severe if they had at least one of the following (1) respiratory distress; rate >= 30/min (2) oxygen saturation <= 93% in the rest state; (3) arterial oxygen tension over inspiratory oxygen fraction of less than 300mm Hg	**Rate of seroconversion:** ▪ In blood detection cohort, 6 cases had detectable virus in the blood (10.5%); 51 had no virus detectable in the blood (89.5%) ▪ In anal swab cohort, 11 of 28 were anal swab positive (39%) **Timing of seroconversion:** Not reported. **Duration of immunity:** Not reported **Other:** ▪ In blood detection cohort, 6 cases had detectable virus in the blood, all of which were classified as severe; 51 had no virus detectable in the blood and only 12 (23.5%)	

			were classified as severe. The ratio of severe symptoms between these two groups was statistically significant (p=0.0001) ▪ In anal swab cohort, 11 of 28 were anal swab positive, 8 of them (72.7%) classified as severe, which was significantly higher than that 4 (23.5%) of the remaining 17 cases were classified as severe	
Dahlke 2020(86) 10.1101/2020.04.14.20059733 Germany Immunological case series	SARS-CoV-2 Peripheral Blood mononuclear Cell immunotyping (PBMC) IgG, IgM and IgA serum antibody interactions differentially detected with fluorescently labelled secondary antibodies **Day of serum collection after symptom onset:** Patient 1: 6, 10 and 22 Patient 2: 3,15 and 24 Patient 3: day 12 Patient 4: days 4 and 11 Patient 5: N/A	4 patients and 1 healthy control Patient 1: 64-year old male defined as a 'more severe' case than the others Patient 2: 62-year old female (mild) Patient 3: Female; age not reported (mild), included as control Patient 4: Male; age not reported (mild/moderate) included as control Patient 5: age and gender not reported, included as negative control	**Rate of seroconversion:** 100% **Timing of seroconversion:** Memory B-cell population (CD19+CD24+cd38-/low) increased after approx. 15 days post disease onset in patients 1 (more severe) and 2 (mild) and persisted in the severe case to day 32 Expansion of plasmablasts(CD19+CD27+CD38+) detected in the mild case day3 and in the severe case as symptoms began to resolve but early time points were not analysed by flow cytometry from this patient Patient 1 (more severe) showed few IgA and IgG reactive peptides (above control sample threshold) at day 6, which considerably increased towards day 22 after virus clearance. Mild case had higher number of IgA reactive peptides already at day 3 post onset of symptoms and showed a decreasing number of reactive peptides from day 3 to 24. At this early time point, defined IgA epitopes were detected in the spike protein, while patient 1 developed	MedRvix

			these only at day 22. The trend of early IgA and IgG antibody response was also observed in control patient 4 (moderate case, day4 and day12) Patient 1 on day 6, IgA only target the ORF1ab polyprotein, at day 10 IgA response still low and at day 22 it turns into a broad response targeting the spike (S), membrane (M), ORF8, and nucleocapsid (N) proteins. While most IgA ORF1ab signals increase over time in patient 1, three signals decrease considerably. In contrast, some IgG responses were already present on day 6, targeting the S and M protein. In patient 2 a stronger and more focused IgA response was observed at day 3 against the S,E, N and ORF1ab proteins compared to patient 1, whereas in the IgG response only one stronger response was observed in towards the S protein. **Duration of immunity:** Not reported	
Liu 2020a(53) https://www.journalofinfection.com/article/S0163-4453(20)30182-1/pdf China	COVID-19 Test type and location of sample not stated Tests undertaken on admission to hospital	39 hospitalised patients; mean age 53 (IQ, 41 to 61); 20 women, 19 men; median time from onset to admission 5 days (IQR, 3-7); 38.5% had co-morbidities. 21 (53.8%) mild and moderate infection 18 (46.2%) severe and critical infection (according to	**Rate of seroconversion:** Not reported. **Timing of seroconversion:** Not reported **Duration of immunity:** Not reported **Other:** CD4+T cell and CD8+ T cell counts were closely related to disease severity and clinical outcome. The more serious the disease and the worse the	Letter to editor

Letter to editor describing retrospective cross-sectional		Guidelines for Diagnosis and Treatment of COVID-19 (Trial version 6))	prognosis, the lower were the T cell, CD4+ T cell and CD8+ T cell counts on admission.
			• T cells (x10^6/L) *p*=0.004
			  ○ mild/moderate; 914.0 (468.0-1214.0)
			  ○ severe/critical; 343.5 (237.0-730.3)
			• CD4+ T cells (x10^6/L) *p*=0.006
			  ○ mild/moderate; 591.0 (266.0-718.5)
			  ○ severe/critical; 217.5 (112.8-324.5)
			• CD8+ T cells (x10^6/L) *p*=0.011
			  ○ mild/moderate; 288.0 (165.0-414.5)
			  ○ severe/critical; 122.5 (76.0-256.8)
			• CD4+/CD8+ *p*=0.447
			  ○ mild/moderate; 1.780 (1.305-2.330)
			  ○ severe/critical; 1.345 (0.930-2.413)
			• B cells(x10^6/L) *p*=0.360
			  ○ mild/moderate; 174.0 (69.5-306.5)
			  ○ severe/critical; 105.0 (55.8-235.5)
			• NK cells (x10^6/L) *p*=0.352
			  ○ mild/moderate; 149.0 (58.8-240.5)
			  ○ severe/critical; 123.5 (44.5-177.8)	

Liu 2020b(30) doi: https://doi.org/10.1101/2020.03.28.20045765 Case series China	**SARS-CoV-2** SARS-CoV2 antibody detection kit	N=133 Median age: 68 Female: 63; Male: 70 44 moderate cases (22 males and 22 females, median age was 67.5 [IQR 64-71.75]), 52 severe cases (28 males and 24 females, median age was 68 [IQR 61.25-74]), and 37 critical cases (20 males and	**Rate of seroconversion** IgM Seroconversion rate by severity of disease: Moderate: 79.6% Severe: 82.7% Critical:73.0% IgG Seroconversion rate by severity of disease: Moderate: 93.2%	Not peer-reviewed

		17 females, median age was 70 [IQR 60-76.5])	Severe:100% Critical: 97.3% **Timing of seroconversion** Not reported **Duration of immunity** Not reported	
Long 2020(69) 10.1101/2020.03.18.20038018 China Multi-centre cross sectional study with single centre follow-up	SAR-CoV-19 Magnetic Chemiluminescence Enzyme Immunoassay (MCLIA) (Bioscience Chongqing Co. Ltd., China, CFDA approved) Serum samples taken at 3-day intervals from February 8th 2020 to hospital discharge.	285 patients in mulit-centre cross sectional study and 63 patients in single-centre follow-up Median age 47 years old (IQR, 34-56 years): 55.4% males 39 of 285 classified as severe or critical condition according to the guidelines	**Rate of seroconversion:** Overall 96.8% (61/63). Two patients, a mother and daughter, lost to follow-up maintained IgG and IgM negative status during hospitalisation Not reported stratified by severity of disease **Timing of seroconversion:** Not reported stratified by severity of disease **Duration of immunity:** Not reported **Other:** IgG and IgM titres in severe group was higher than those in the non-severe group, although significant statistical difference is only observed in IgG level of 2 weeks (p=0.001)	medRVIX
Okba 2020(34) Samples collected from France, the	SARS-CoV-2 PRNT was used as a reference for this study ELISA	10 samples from France were stratified as 'mild infection' (6 samples from 2 patients at different time points) or severe infection' (4 samples from 1 patient at different time points)	**Rate of seroconversion:** 100% of 2 cases that are stratified by severity **Timing of seroconversion:** Figure 1 shows antibody responses to spike (S), spike S1 subunit (B), spike N-terminal ($S1^A$) domain, receptor bindings domain (E)	MedRvix

Netherlands, Germany, 10.3201/eid2607.200841	Serum samples taken between day6 and 27 in mild and severe cases, days not specified but noted samples were taken 'at different time points' over this period		nucleocapsid of two mild with one severe case. This figure appears to show a higher response in the severe case to all proteins. **Duration of immunity:** Not reported **Other:** Antibody levels were higher following severe infection compared to the mild ones	
Tan 2020(35) China https://www.medrxiv.org/content/medrxiv/early/2020/03/26/2020.03.24.20042382.full.pdf Prospective cohort study	SARS-CoV-2 Serum ELISA kits (Livzon Diagnostics Inc. Zhuhai, China)	67 hospitalised SARS-CoV-2 infected patients with342 sequential serum samples. Median age 49 years (range 10-77 years); 35 (52.2%) male; 25 (37.3%) had underlying diseases; 29 were classified as severe pneumonia (9 critical), including all 3 children,	**Rate of seroconversion:** ▪ Of severe patients 53.6% were positive for IgM, 44.4% negative ▪ Of non-severe patients, 41.9% were positive for IgM, 58.1% negative ▪ Of severe patients 82.1% were positive for IgM, 17.9% negative ▪ Of non-severe patients, 84.6% were positive for IgG, 15.4% negative **Timing of seroconversion:** Minimum required observation period for IgM 18 days and for IgG 21 days. • Days of antibody first detectable in positive severe patients IgM 11.6 +/-3 days • Days of antibody first detectable in positive non-severe patients IgM 14 +/-5.3 days	MedRvix

			• Days of antibody first detectable in positive severe patients IgG 13.4+/- 4 days • Days of antibody first detectable in positive non-severe patients IgG 15.3 +/- 5.7 days **Duration of immunity:** Not reported **Other:** Patients were classified as strong responders (peak titre >2-fold of cut-off value), weak responders (peak titre 1-2 fold of cut-off value) and non-responders (peak titre below cut-off value). ▪ Proportion of strong responders is significantly higher and the proportion of weak responders is significantly lower in severe patients than in non-severe patients, IgM (p=0.017) and igg (p=0.032). ▪ Titres of IgM and IgG were continuously significantly higher in severe patients than in those in non-severe patients along with time (IgM, p=0.008; igg p=0.009). ▪ Proportion for viral clearance at day 7 after antibodies appearance was significantly higher in non-severe patients than in severe patients (for IgM, 81.8% vs. 7.7%, p=0.001; for igg, 60.0% vs. 26.3%, p=0.048).	

			Furthermore, the weak responders for IgG antibodies had a significantly higher viral clearance rate (56.5%) than that (9.1%) of strong responders (p=0.011)	
Wang 2020(42) 10.1101/2020.04.15.20065623 China Case series	SARS-CoV-2 Modified cytopathogenic assay. Indicators for immunogenicity assessment included seropositivity rate and determination of GMT. Neutralising antibody titre calculated by Reed-Meunch method on day 5. Blood samples collected from 2, 3 and 4 time points in 19, 8 and 4 patients, respectively. 39 patients had one blood sample only. Total 117 blood samples were analysed. Mean neutralising antibody test of 1st sample since onset of this study was 33 days (range 10 to 53 days) and 'the time of convalescent patients (35 days) was longer	70 Covid-19 Patients (12 inpatients and 58 convalescent patients). Mean age 45.1 years (range 16 to 84 years). 2 patients had history of CVD, 5 of diabetes, 9 of hypertension. • 1 patient asymptomatic • 22 mild • 43 moderate • 4 severe (1 inpatient and 3 convalescent) 117 blood samples	**Rate of seroconversion:** 100% **Timing of seroconversion:** Not reported stratified by severity **Duration of immunity:** Seropositivity reported up to day 53 of study, not stratified by severity **Other:** Compared to the patients with asymptomatic or mild manifestations (GMT 1:141.9, 95% CI, 79.5 to 235.2), the antibody levels were similar to patients with moderate or severe condition (GMT 1:199.5, 95% CI, 141.8 to 280.5). However, after adjusting other factors, patients with more severe symptoms tended to have a higher antibody titre (β=0.4639, (SE 0.2036; CI 95%, 0.0649 to 0.8630, P=0.0227)). The GMT of convalescent patients was 1:212.7 (95% CI, 157.5 to 287.3), and was higher than inpatients (1:76.1, 95% CI, 33.5 to 172.9; P=0.0055)	MedRvix

	than inpatients (13.5 days)'			

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