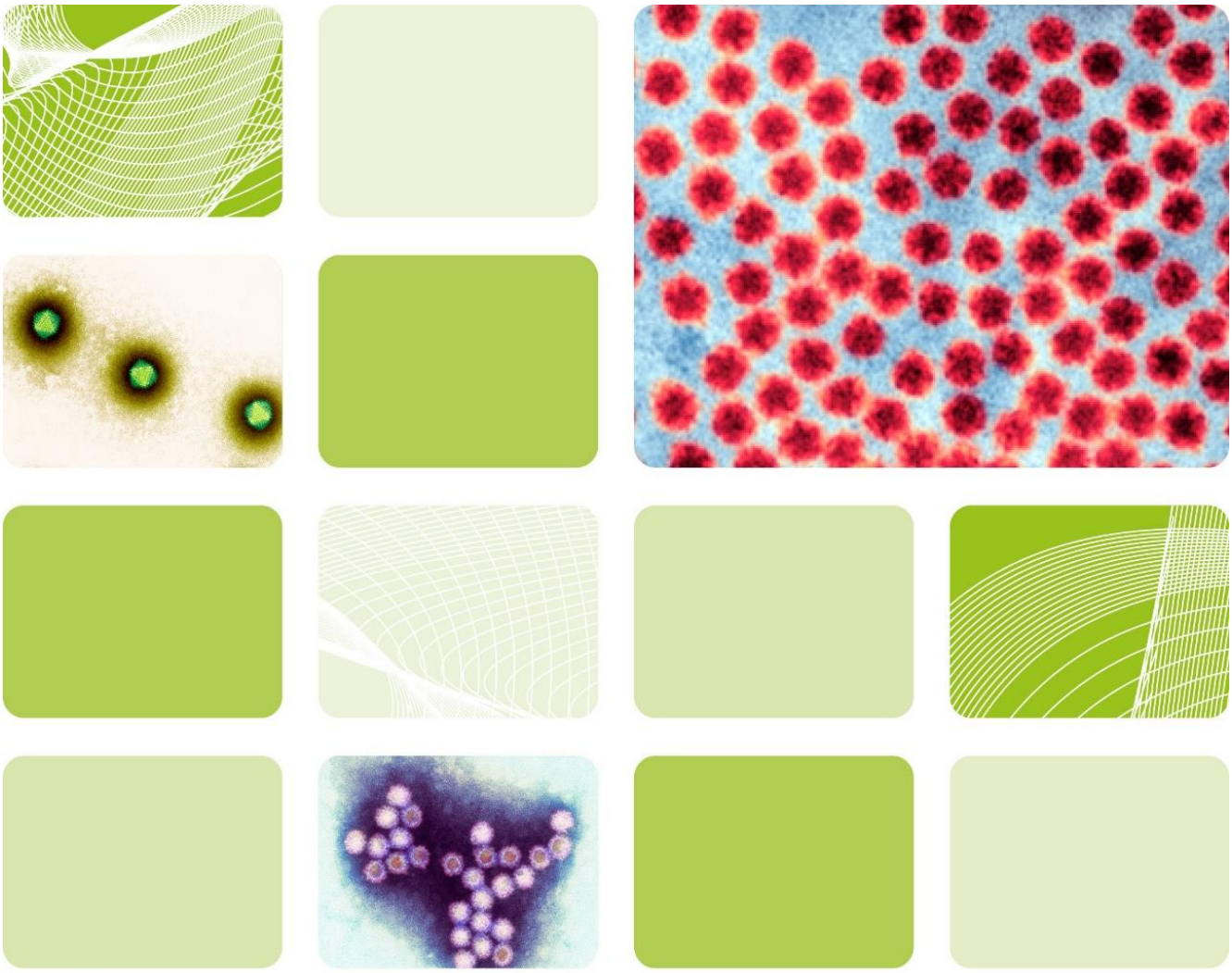
UK Health Security Agency

UK Standards for Microbiology Investigations

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) serology



Acknowledgments

UK Standards for Microbiology Investigations (UK SMIs) are developed under the auspices of UKHSA working in partnership with the partner organisations whose logos are displayed below and listed on the UK SMI website. UK SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee.

The contributions of many individuals in clinical, specialist and reference laboratories who have provided information and comments during the development of this document are acknowledged. We are grateful to the medical editors for editing the medical content.

UK SMIs are produced in association with:

Displayed logos correct as of December 2024

Contents

Amendment table

Each UK SMI document has an individual record of amendments. The amendments are listed on this page. The amendment history is available from standards@ukhsa.gov.uk.

Any alterations to this document should be controlled in accordance with the local document control process.

Amendment number/date	2/23.05.25
Issue number discarded	2
Insert issue number	2.1
Section(s) involved	**Amendment**
Whole document.	**This is an administrative point change.** **The content of this UK SMI document has not changed.** **The last scientific and clinical review was conducted on 09/06/2022.** Hyperlinks throughout document updated to Royal College of Pathologists website. Public Health England replaced with UK Health Security Agency throughout the document, including the updated Royal Coat of Arms Partner organisation logos updated. Broken links to devolved administrations replaced. References to NICE accreditation removed. Scope and Purpose replaced with General and Scientific information to align with current UK SMI template.
Section 10: Public health responsibilities of diagnostic laboratories	This section has been added to UK SMI templates to highlight the public health responsibilities that diagnostic laboratories have as part of their duties.

Amendment number/date	1/09 June 2022
Issue number discarded	1
Insert issue number	2

Anticipated next review date*	09 June 2025
Section(s) involved	Amendment
Whole document	Document presented in the new UK SMIs virology template. Document amended with up-to-date information and references on SARS-CoV-2 Information on therapeutic neutralising monoclonal antibodies added to the document.
Section 7 and 7.1	Flowchart and footnotes were updated
Section 8	Interpreting and reporting laboratory results table updated

*Reviews can be extended up to 5 years where appropriate.

1 General information

View general information related to UK SMIs.

2 Scientific information

View scientific information related to UK SMIs.

3 Scope of document

Coronavirus disease (COVID-19) is an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), discovered in late 2019 (1). Most people infected with SARS-CoV-2 will experience mild to moderate respiratory illness and recover without requiring treatment (1). Black, Asian and Minority Ethnic (BAME) patients, older people, and those with underlying medical problems such as cardiovascular disease, diabetes, chronic respiratory disease and cancer are more likely to develop serious illness. See COVID-19: the green book, chapter 14a for a full list of people considered clinically extremely vulnerable from COVID-19.

COVID-19 vaccines have been widely available since December 2020 with all vaccines currently licensed in the UK using a form of the S protein as the main target for generation of neutralising antibodies.

SARS-CoV-2 seroprevalence testing programmes have been rolled out across all four nations of the UK with different approaches for testing certain key workers or patients. These antibody testing programmes have aimed to provide information on:

- the prevalence of SARS-CoV-2 infection in different regions of the country (2);
- how the disease spreads amongst symptomatic and asymptomatic individuals (3);
- the protective immunity against reinfection (4);
- the persistence of antibodies (5), and;
- trends in natural infection transmission and vaccine induced immunity (6).

SARS-CoV-2 serology may also be used in the clinical management of inflammatory diseases, including paediatric multisystem inflammatory syndrome (PIMS), when temporally associated with COVID-19 (7). Serology is utilised in conjunction with PCR testing to determine whether there is evidence of current infection, previous infection, and/or previous vaccination.

Therapeutic neutralising monoclonal antibodies (nMABs) bind to specific sites on the spike protein of the SARS-CoV-2 virus particle, blocking cell entry and inhibiting the progression of infection. Serological testing for the presence of detectable SARS-CoV-2 anti-spike antibodies prior to treatment with neutralising monoclonal antibodies is advised where possible but is not necessarily a requirement for treatment with nMABs. Please refer to the latest guidance for nMABs treatment (8,9).

This UK SMI describes a diagnostic testing algorithm which supports and gives indications to the laboratories on how to interpret results from commercially available serological kits.

Refer to UK SMI Q1 – Evaluations, validations and verifications of diagnostic tests and UK SMI Q 7 – Good practice when undertaking serology assays for infectious diseases for information regarding good laboratory practice in serological testing.

In this rapidly evolving field, please consult national sources of up-to-date guidance and information about SARS-CoV-2 and COVID-19.

This UK SMI should be used in conjunction with other UK SMIs.

4 Background

Serological assays for SARS-CoV-2 may detect the humoral immune responses induced by the SARS-CoV-2 virus and/or SARS-CoV-2 vaccination. Unlike methods which detect the virus itself, antibody tests can demonstrate that an individual has been exposed to the virus or vaccine at some time prior to sampling, regardless of the current presentation. The detection of specific antibodies against different viral antigens, such as nucleocapsid or spike antigen, can differentiate vaccine response from natural exposure (assuming the vaccine antigen remains the spike protein alone).

A longitudinal study has reported that patients who recovered from mild SARS-CoV-2 infection developed SARS-CoV-2-specific IgG antibodies, a neutralising plasma response to the spike protein receptor binding domain RBD, and specific memory B and T cells, that were all detectable for at least 3 months (10). While there is an increase in evidence to demonstrate a multi-faceted cellular and humoral immune response following SARS-CoV-2 infection, correlates of immunity are not yet well defined (11). At present, serological assays cannot be used to infer the presence or absence of protective immunity against SARS-CoV-2, or as a sole method for the diagnosis of COVID-19 disease.

Serology is also useful in guiding epidemiological and public health control measures by providing information of the level and length of the immune response following SARS-CoV-2 viral infection. This information will help to determine the likelihood of reinfection and how rapidly new outbreaks may spread across the country, especially

in health and social care workers and those at higher risk of clinical complications. Healthcare workers from all regions of the UK participated in a study called SIREN (Sarscov2 Immunity and Reinfection Evaluation) to determine the impact of detectable SARS-CoV-2 antibody on the incidence of COVID-19. The study showed that previous SARS-CoV-2 infection protects most individuals against reinfection for a few months. Primary infection also reduces the risk of asymptomatic infection (12,13).

In symptomatic, immunocompetent individuals, SARS-CoV-2 infection will normally elicit the development of IgM and IgG antibodies. In the first 7 days of infection, the adaptive immune response begins to develop, and antibodies may not yet be detectable in peripheral blood. IgM and IgG antibodies are increasingly likely to be detected from 7 days after the onset of symptoms, at which time the majority of individuals will have a detectable antibody response (14). The detectable IgM response then begin to decline, reaching lower levels by week 5 and almost disappearing by week 7, while detectable circulating IgG normally persists beyond 7 weeks (15) (see Figure 1).

Asymptomatic and immunocompromised individuals may show a delayed or absent antibody response to SARS-CoV-2 infection (16). As more data becomes available, understanding of the antibody response will increase.

4.1 Antibody testing in the UK

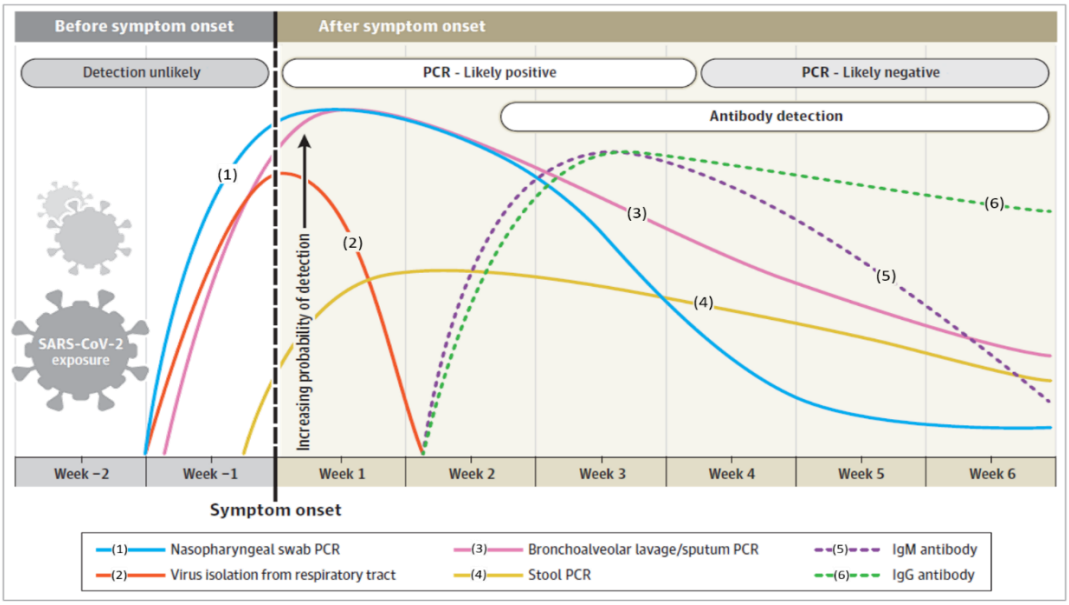
Coronaviruses have four structural proteins: the spike glycoprotein (S), nucleocapsid protein (N), envelope protein (E) and membrane protein (M). At the present time, some commercially-available antibody tests target the N protein, which is a relatively stable protein structurally associated with the viral genome, whereas others target the S protein, which contains some highly mutable regions and (along with the M and E proteins) is found on the surface of the virus. The nucleocapsid protein is highly immunogenic and induces an earlier antibody response than the spike protein during infection, making it an attractive protein for diagnostic assay design. The spike protein is also relatively immunodominant, consisting of 2 subunits: the S1 protein containing the receptor binding domain (RBD); and the S2 protein which mediates fusion of the virus particle to the cell membrane (17). To date, SARS-CoV-2 vaccines in the UK are based on the spike (S) protein thus detection of spike (S) antibody indicates past infection or past vaccination or both. SARS-CoV-2 spike protein is structurally similar to the SARS-CoV-1 spike protein, however cross-recognition of SARS-CoV-2 by SARS-CoV-1 monoclonal antibodies was not observed (18). See COVID-19: laboratory evaluations of serological assays for an evaluation of commercial kits in which cross-reactions to other *Betacoronavirus* also were not observed (19).

Commercially available serological assays can detect IgG alone, or both IgG and IgM (total antibody) (20). An evaluation, using serum samples from PCR-positive individuals, showed no substantive differences in sensitivity of assays whether they test for IgG alone, or both IgG and IgM (19).

Antibodies detected in an assay which includes spike proteins as an antigen may have a closer correlation with the presence of neutralising antibodies against SARS-CoV-2 (21).

The impact of infection with variant SARS-CoV-2 strains on the performance of serology tests is uncertain at present, but may not affect those commercial test kits and assays which detect a broad antibody repertoire.

Figure 1: Estimated variation over time in diagnostic tests for detection of SARS-CoV-2 infection relative to symptom onset (15).



Note: the dominant (or specific) viral variant; immunological competence of the host (including any response to specific SARS-CoV-2 vaccination); and use of SARS-CoV-2 antiviral therapies may all alter the time course shown above, both at the individual level and within populations.

Therapeutic interventions could also impact further evolution of variants. Significant protective levels could vary depending on evolving data

All laboratories performing serology testing should participate in a national external quality assurance (EQA) scheme and be aware of any sensitivity variance between assays. Refer to local validation data and use validated kits. Also refer to UK SMI Q 7: good practice when ordering and undertaking diagnostic tests for infectious disease serology for information regarding good laboratory practice in serological testing.

5 Safety considerations

The section covers specific safety considerations related to this UK SMI, and should be read in conjunction with the general safety considerations (22-41).

Refer to guidance on COVID-19: safe handling and processing for samples in laboratories (42) and Annex 2 of The approved list of biological agents (23).

This guidance should be supplemented with local COSHH and risk assessments. Refer to current guidance on the safe handling of all organisms documented in this UK SMI.

6 Specimen processing and procedure

6.1 Specimen type

Blood, serum or plasma (follow manufacturers' specifications).

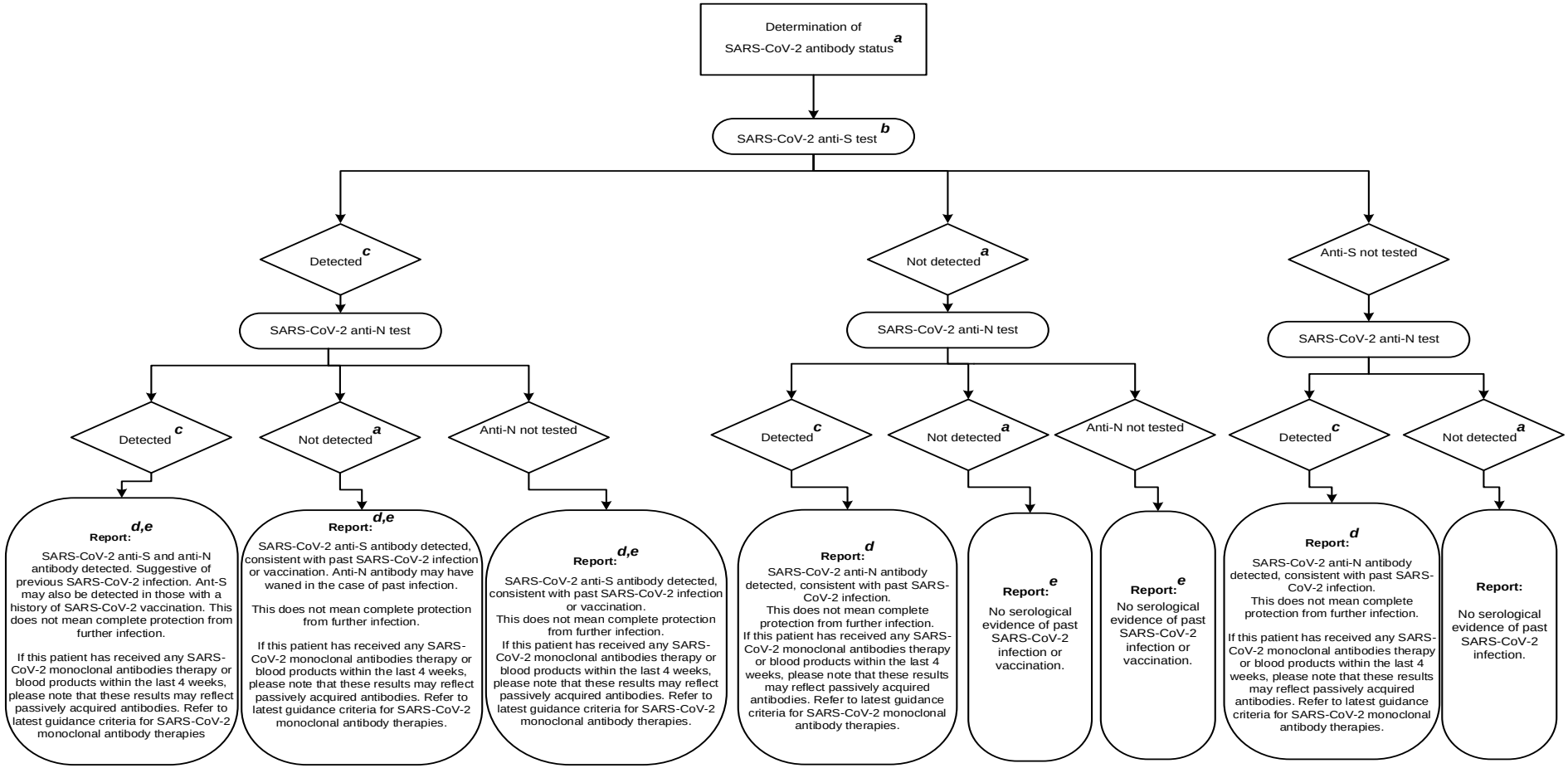
6.2 Specimen transport and storage conditions

Specimens should be collected in appropriate CE or UKCA marked leak proof containers and transported in sealed plastic bags according to UK regulations.

Specimens should be transported and processed according to manufacturers' instructions or local validation data (43).

Samples should be retained in accordance with The Royal College of Pathologists guidelines 'The retention and storage of pathological records and specimens'(38).

7 Investigation of SARS-CoV-2 antibodies



Note: It is important to follow current government guidelines for the latest advice on protection from SARS-CoV-2.

7.1 Footnotes relating to investigation of SARS-CoV-2 antibodies algorithm

a) Immunocompromised individuals may not mount a detectable antibody response or may show a delayed humoral immune response to infection and/or vaccination. The timing of sampling, in relation to any potential or known infection or vaccination, should be considered for all individuals, as circulating antibodies may not yet have developed, or may have waned to undetectable levels. Serology tests are unsuitable for detecting current or recently-acquired infection. An appropriate respiratory tract sample should be tested for SARS-CoV-2 RNA if current infection is suspected.

b) Anti-spike testing prior to treatment with nMABs is advised where possible but it is not a requirement for treatment with nMABs at the time of writing. Patients may be tested for anti-spike antibodies using any validated quantitative or qualitative anti-S assay that detects IgG alone, or both IgG and IgM (44).

c) Consideration should be given to the possibility of a false positive result. The likelihood of false reactivity depends on local seroprevalence (45).

d) Data is not currently available to understand how measurable circulating antibodies correlate to functional immunity, or the possibility of re-infection. Therefore, a reactive result cannot be interpreted to mean that the patient is immune, or that they are not currently infected or that they cannot transmit the virus to others.

e) Inference of vaccination history by detection or absence of anti-S antibodies is only applicable to individuals who have received those vaccines which target the spike protein.

Note: interpretation of equivocal or indeterminate results will depend on the assay manufacturer's instructions and on local validation data.

8 Interpreting and reporting laboratory results

Interpretation and reporting table for SARS-CoV-2 anti-S and anti-N testing:

	Anti-S	Anti-N	Interpretative Comment
1	Detected	Detected	SARS-CoV-2 anti-S and anti-N antibody detected. Suggestive of previous SARS-CoV-2 infection. Anti-S may also be detected in those with a history of SARS-CoV-2 vaccination. This does not mean complete protection from further infection. It is important to follow current government guidelines for the latest advice on protection from SARS-CoV-2. If this patient has received any SARS-CoV-2 monoclonal antibodies therapy or blood products within the last 4 weeks, please note that these results may reflect passively acquired antibodies. Refer to latest guidance criteria for SARS-CoV-2 monoclonal antibody therapies
2	Detected	Not detected	SARS-CoV-2 anti-S antibody detected, consistent with past SARS-CoV-2 infection or vaccination. Anti-N antibody may have waned in the case of past infection. This does not mean complete protection from further infection. It is important to follow current government guidelines for the latest advice on protection from SARS-CoV-2. If this patient has received any SARS-CoV-2 monoclonal antibodies therapy or blood products within the last 4 weeks, please note that these results may reflect passively acquired antibodies. Refer to latest guidance criteria for SARS-CoV-2 monoclonal antibody therapies.

3	Detected	Not tested	SARS-CoV-2 anti-S antibody detected, consistent with past SARS-CoV-2 infection or vaccination. This does not mean complete protection from further infection. It is important to follow current government guidelines for the latest advice on protection from SARS-CoV-2. If this patient has received any SARS-CoV-2 monoclonal antibodies therapy or blood products within the last 4 weeks, please note that these results may reflect passively acquired antibodies. Refer to latest guidance criteria for SARS-CoV-2 monoclonal antibody therapies.
4	Not detected	Detected	SARS-CoV-2 anti-N antibody detected, consistent with past SARS-CoV-2 infection. This does not mean complete protection from further infection. It is important to follow current government guidelines for the latest advice on protection from SARS-CoV-2. If this patient has received any SARS-CoV-2 monoclonal antibodies therapy or blood products within the last 4 weeks, please note that these results may reflect passively acquired antibodies. Refer to latest guidance criteria for SARS-CoV-2 monoclonal antibody therapies.
5	Not detected	Not detected	No serological evidence of past SARS-CoV-2 infection or vaccination. It is important to follow current government guidelines for the latest advice on protection from SARS-CoV-2.
6	Not detected	Not tested	No serological evidence of past SARS-CoV-2 infection or vaccination. It is important to follow current government guidelines for the latest advice on protection from SARS-CoV-2.

7	Not tested	Detected	SARS-CoV-2 anti-N antibody detected, consistent with past SARS-CoV-2 infection. This does not mean complete protection from further infection. It is important to follow current government guidelines for the latest advice on protection from SARS-CoV-2. If this patient has received any SARS-CoV-2 monoclonal antibodies therapy or blood products within the last 4 weeks, please note that these results may reflect passively acquired antibodies. Refer to latest guidance criteria for SARS-CoV-2 monoclonal antibody therapies.
8	Not tested	Not detected	No serological evidence of past SARS-CoV-2 infection. It is important to follow current government guidelines for the latest advice on protection from SARS-CoV-2.

9 Public health responsibilities of diagnostic laboratories

Diagnostic laboratories have public health responsibility as part of their duties. Amongst these are additional local testing, or referral to further characterise the organism as required, primarily for public health purposes e.g. routine cryptosporidium detection; serotyping or microbial subtyping; and a duty to refer appropriate specimens and isolates of public health importance to a reference laboratory.

Diagnostic laboratory outputs inform public health intervention, and surveillance data is required to develop policy and guidance forming an essential component of healthcare. It is recognised that additional testing and referral of samples may entail some costs that has to be borne by the laboratory but in certain jurisdictions these costs are covered centrally.

Diagnostic laboratories should be mindful of the impact of laboratory investigations on public health and consider requests from the reference laboratories for specimen referral or enhanced information.

References

An explanation of the reference assessment used is available in the scientific information.

1. World Health Organisation: Coronavirus https://www.who.int/health-topics/coronavirus 2021. **++**

2. Amirthalingam G, Whitaker H, Brooks T, Brown K, Hoschler K, Linley E et al. Seroprevalence of SARS-CoV-2 among Blood Donors and Changes after Introduction of Public Health and Social Measures, London, UK. Emerg Infect Dis 2021;27:1795-801.**2++** 10.3201/eid2707.203167

3. Bi Q, Lessler J, Eckerle I, Lauer SA, Kaiser L, Vuilleumier N et al. Insights into household transmission of SARS-CoV-2 from a population-based serological survey. Nat Commun 2021;12:3643.**2+** 10.1038/s41467-021-23733-5

4. Abo-Leyah H, Gallant S, Cassidy D, Giam YH, Killick J, Marshall B et al. The protective effect of SARS-CoV-2 antibodies in Scottish healthcare workers. ERJ Open Res 2021;7.**3+** 10.1183/23120541.00080-2021

5. Shrotri M, Harris RJ, Rodger A, Planche T, Sanderson F, Mahungu T et al. Persistence of SARS-CoV-2 N-Antibody Response in Healthcare Workers, London, UK. Emerg Infect Dis 2021;27:1155-8.**2+** 10.3201/eid2704.204554

6. Whitaker HJ, Elgohari S, Rowe C, Otter AD, Brooks T, Linley E et al. Impact of COVID-19 vaccination program on seroprevalence in blood donors in England, 2021. J Infect 2021;83:237-79.**2+** 10.1016/j.jinf.2021.04.037

7. Royal College of Paediatrics and Child Health. Guidance Paediatric multisystem inflammatory syndrome temporally associated with COVID-19 2020.**++**

8. NHS. Interim Clinical Commissioning Policy: Antivirals or neutralising monoclonal antibodies for non-hospitalised patients with COVID-19 2022.**++**

9. NHS. UK Interim Clinical Commissioning Policy: Therapies for symptomatic non-hospitalised patients with COVID-19 2022.**++**

10. Rodda LB, Netland J, Shehata L, Pruner KB, Morawski PA, Thouvenel CD et al. Functional SARS-CoV-2-Specific Immune Memory Persists after Mild COVID-19. Cell 2021;184:169-83 e17.**1+** 10.1016/j.cell.2020.11.029

11. Chen Z, John Wherry E. T cell responses in patients with COVID-19. Nat Rev Immunol 2020;20:529-36.**3+** 10.1038/s41577-020-0402-6

12. Hall VJ, Foulkes S, Saei A, Andrews N, Oguti B, Charlett A et al. COVID-19 vaccine coverage in health-care workers in England and effectiveness of

BNT162b2 mRNA vaccine against infection (SIREN): a prospective, multicentre, cohort study. Lancet 2021;397:1725-35.**1++** 10.1016/S0140-6736(21)00790-X

13. Hall VJ, Foulkes S, Charlett A, Atti A, Monk EJM, Simmons R et al. SARS-CoV-2 infection rates of antibody-positive compared with antibody-negative health-care workers in England: a large, multicentre, prospective cohort study (SIREN). Lancet 2021;397:1459-69.**1++** 10.1016/S0140-6736(21)00675-9

14. Zhao J, Yuan Q, Wang H, Liu W, Liao X, Su Y et al. Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019. Clin Infect Dis 2020.**2+** 10.1093/cid/ciaa344

15. Sethuraman N, Jeremiah SS, Ryo A. Interpreting Diagnostic Tests for SARS-CoV-2. JAMA 2020.**1+** 10.1001/jama.2020.8259

16. Burbelo PD, Riedo FX, Morishima C, Rawlings S, Smith D, Das S et al. Detection of Nucleocapsid Antibody to SARS-CoV-2 is More Sensitive than Antibody to Spike Protein in COVID-19 Patients. J Infect Dis 2020.**2+** 10.1093/infdis/jiaa273

17. Huang AT, Garcia-Carreras B, Hitchings MDT, Yang B, Katzelnick LC, Rattigan SM et al. A systematic review of antibody mediated immunity to coronaviruses: antibody kinetics, correlates of protection, and association of antibody responses with severity of disease. medRxiv 2020.**1+** 10.1101/2020.04.14.20065771

18. Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh C-L, Abiona O et al. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. Science 2020;367:1260-3.**2++** doi:10.1126/science.abb2507

19. Public Health England. COVID-19 laboratory evaluations of serological assays. 2020. **++**

20. MHRA - Target product profile: Enzyme Immunoassay (EIA) Antibody tests to help determine if people have antibodies to SARS-CoV-2; 2021. **++**

21. Kohmer N, Westhaus S, Ruhl C, Ciesek S, Rabenau HF. Brief clinical evaluation of six high-throughput SARS-CoV-2 IgG antibody assays. J Clin Virol 2020;129:104480.**1+** 10.1016/j.jcv.2020.104480

22. Home Office. Anti-terrorism, Crime and Security Act. 2001. **++**

23. Advisory Committee on Dangerous Pathogens. The Approved List of Biological Agents. Health and Safety Executive 2021. 1-39. **++**

24. Health and Safety Executive. Blood-borne viruses in the workplace. Guidance for employers and employees. HSE. 2001. **++**

25. British Standards Institution (BSI). BS 5726:2005 - Microbiological safety cabinets. Information to be supplied by the purchaser and to the vendor and to the installer, and siting and use of cabinets. Recommendations and guidance. 2005. 1-14. **++**

26. British Standards Institution (BSI). BS EN12469 - Biotechnology - performance criteria for microbiological safety cabinets 2000. **++**

27. Health and Safety Executive. Control of Substances Hazardous to Health Regulations. The Control of Substances Hazardous to Health Regulations 2002 (as amended). Approved Code of Practice and guidance L5 (sixth edition). HSE Books. 2013. **++**

28. Official Journal of the European Communities. Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on *in vitro* diagnostic medical devices 1998. 1-37. **++**

29. World Health Organization. Guidance on regulations for the transport of infectious substances 2019-2020. WHO. 2019. **++**

30. Centers for Disease Control and Prevention. Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories. MMWR Surveill Summ 2012;61:1-102.**+**

31. Department of Health. Health Protection Legislation (England) Guidance. 1-112. 2010. **++**

32. The Welsh Assembly Government. Health Protection Legislation (Wales) Guidance. 2010. **++**

33. Public Health England. Laboratory reporting to Public Health England: a guide for diagnostic laboratories. PHE. 2020. 1-31. **++**

34. Health and Safety Executive, Advisory Committee on Dangerous Pathogens. Management and operation of microbiological containment laboratories. HSE. 2019. **++**

35. Health and Safety Executive. Managing risks and risk assessment at work (accessed 28/07/2021). https://www.hse.gov.uk/simple-health-safety/risk/index.htm. **++**

36. Scottish Government. Public Health (Scotland) Act. 2008. **++**

37. Home Office. Public Health Act (Northern Ireland) 1967 Chapter 36. 1967. **++**

38. The Royal College of Pathologists. The retention and storage of pathological records and specimens (5th edition). 1-59. 2015. **++**

39. Health and Safety Executive. Risk assessment: A brief guide to controlling risks in the workplace. HSE. 2014. **++**

40. Health Services Advisory Committee. Safe working and the prevention of infection in clinical laboratories and similar facilities. Books. H 2003. **++**

41. Department for Transport, Maritime and Coastguard Agency, HSENI, Civil Aviation Authority. Transport of infectious substances UN2814, UN2900 and UN3373 Guidance note number 17/2012 (revision 7). 2013. **++**

42. Public Health England. COVID-19 safe handling and processing for samples in laboratories. 2021. **++**

43. Baron EJ, Miller JM, Weinstein MP, Richter SS, Gilligan PH, Thomson RB, Jr. et al. A Guide to Utilization of the Microbiology Laboratory for Diagnosis of Infectious Diseases: 2013 Recommendations by the Infectious Diseases Society of America (IDSA) and the American Society for Microbiology (ASM). Clin Infect Dis 2013;57:e22-e121.**+** cit278 [pii];10.1093/cid/cit278 [doi]

44. NHS. Interim Clinical Commissioning Policy: Antivirals or neutralising monoclonal antibodies in the treatment of COVID-19 in hospitalised patients (Version 5) 2022.**++**

45. Public Health England. Assurance of SARS-CoV-2 RNA positive results during periods of low prevalence 16 October 2020.**+**