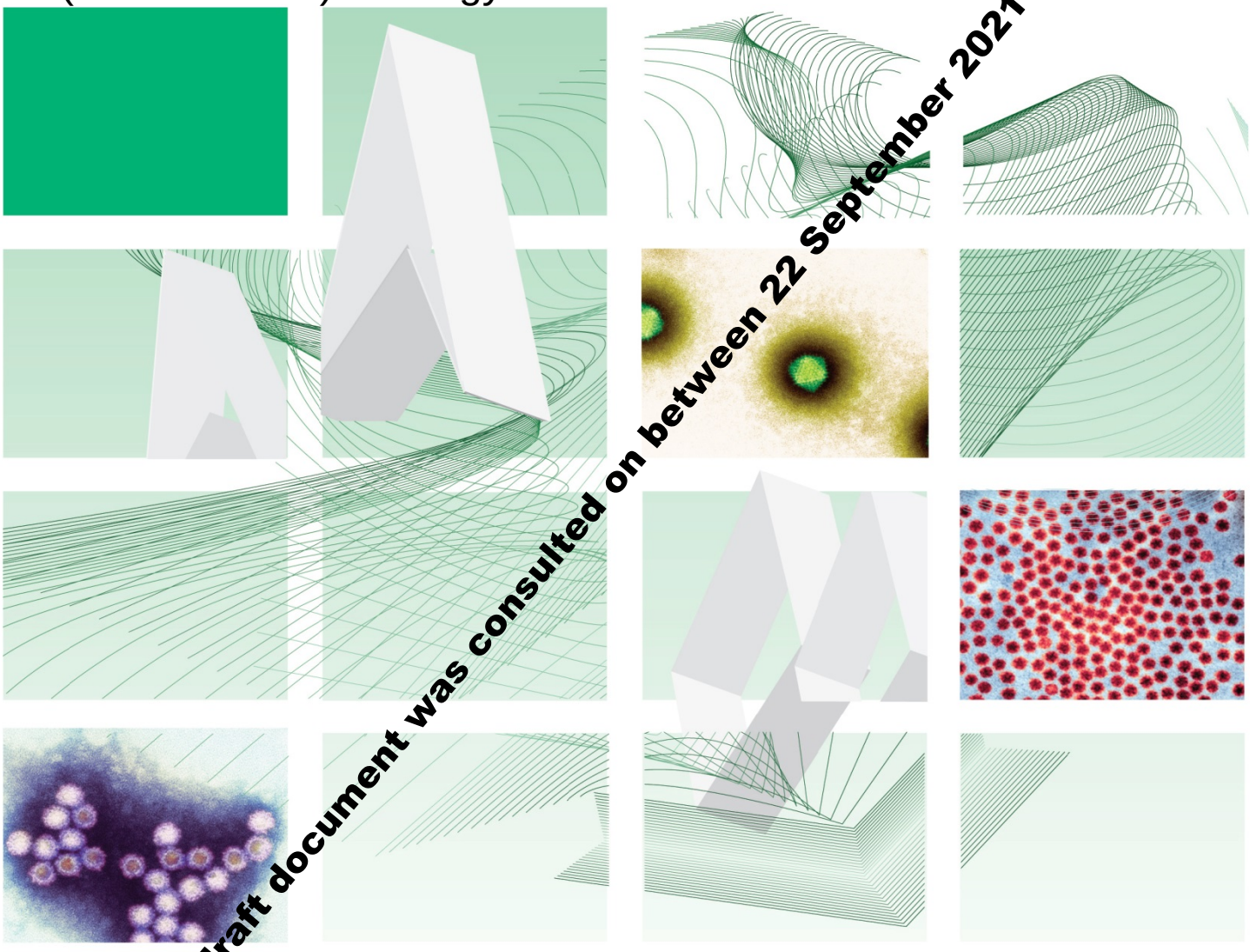
Public Health England

UK Standards for Microbiology Investigations

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



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Amendment table

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

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

Amendment number / date	
Issue number discarded	
Insert issue number	
Anticipated next review date*	
Section(s) involved	Amendment

*Reviews can be extended up to 5 years subject to resources available.

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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 special 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 (2).

Seroprevalence testing programmes have been rolled out across all 4 nations of the UK with different approaches for testing certain key workers or patients or both. These antibody testing programmes have aimed to provide information on the prevalence of COVID-19 in different regions of the country (3), how the disease spreads amongst symptomatic and asymptomatic individuals (4), the protective immunity against reinfection(5), the persistence of antibodies (6), trends in natural infection transmission and vaccine induced immunity (7). The programmes have worked alongside PCR testing which confirms whether someone currently has the virus.

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

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

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

4. Background

Serological assays for SARS-CoV-2 detect the antibody-based immune response induced by the SARS-CoV-2 virus and/or SARS-CoV-2 vaccination. Unlike methods which detect the genetic material (and thus the presence) of the virus, antibody tests help to determine that an individual has been exposed to the virus immunologically regardless of symptom presentation. Therefore, serological tests provide information on whether an individual has encountered SARS-CoV-2 natural infection or vaccination. The serological differentiation between different viral targets such as nucleocapsid or spike antigen might help in differentiating vaccine response from natural exposure as long as the vaccine target remains solely the spike protein.

A longitudinal study has reported that patients who recovered from mild COVID-19 infection developed SARS-CoV-2-specific IgG antibodies, neutralising plasma, and memory B and memory T cells that persisted for at least 3 months (8). While there is

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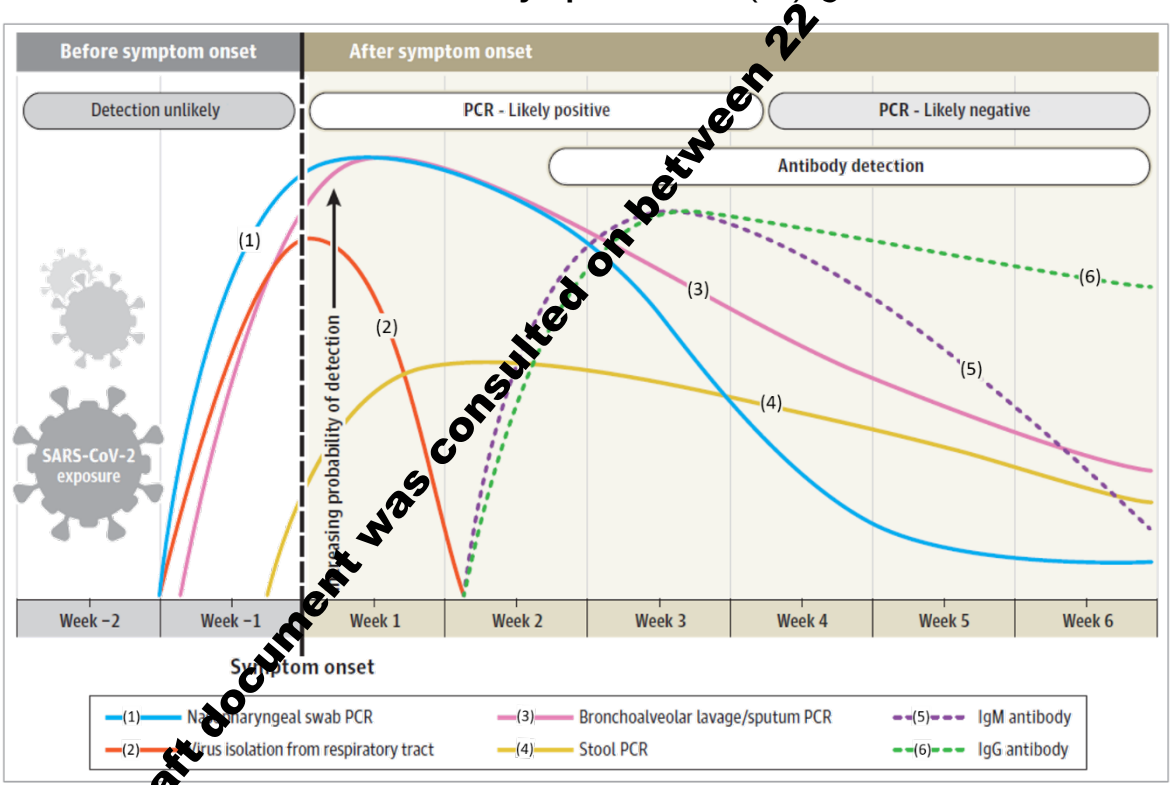
an increase in evidence to suggest memory T cells develop post SARS-CoV-2 infection correlates of immunity are not yet well defined(9). Therefore, at present, positive serological assays cannot be used to infer protective immunity against SARS-CoV-2 or as a sole method for the diagnosis of COVID-19 disease.

Thus, the role of serology is currently limited to indicating whether someone has SARS-CoV-2 antibodies either due to past natural infection or vaccination or both. 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 be useful to determine reinfection and how the virus spreads 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 are currently participating 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 (10, 11).

Figure 1: Estimated variation over time in diagnostic tests for detection of SARS-CoV-2 infection relative to symptom onset (12).



In symptomatic, immunocompetent individuals, SARS-CoV-2 will normally elicit the development of IgM and IgG antibodies. Early in SARS-CoV-2 infection (first 7 days) the adaptive immune response begins to develop, and antibodies may not yet be detectable. IgG and IgM antibodies are increasingly likely to be detected from 7 days after the onset of symptoms. The majority of individuals will have a detectable antibody response (13). IgM levels then begin to decline, reaching lower levels by week 5 and almost disappearing by week 7, while IgG levels persist beyond 7 weeks (12) (see Figure 1).

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Asymptomatic and immunocompromised individuals may show a delayed or absent antibody response to SARS-CoV-2 infection (14). As more data becomes available, understanding of the antibody response will increase.

Antibody testing in the UK

Coronaviruses have 4 structural proteins: the spike protein, nucleocapsid, envelope protein and membrane protein. Since the start of the COVID-19 pandemic several antibody tests have been developed. Some tests target the nucleocapsid N protein found within the viral genome and others target the spike S protein 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 (15). To date, SARS-CoV-2 vaccines in the UK are based on the spike (S) protein thus spike (S) antibody confirms past infection or past vaccination or both. Sequence homology of the nucleocapsid and spike proteins of SARS-CoV-1 to other *Betacoronaviruses* is 33 to 47% and 29% respectively(16). SARS-CoV-2 is similar to SARS-CoV-1, showing sequence homology of 90% in the nucleocapsid and 76% in the spike protein (17).

Commercially available serological assays can detect IgG alone, or both IgG and IgM (total antibody) (18). Evaluation of commercial kits by PHE, using serum samples from PCR-positive individuals, has shown no substantive differences in sensitivity of assays whether they test for IgG or total antibodies.

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 (19).

Impact of variant strains on serology tests is not understood just yet, but likely to be limited in commercial test kits and assays which are looking for broad antibody response with diverse antibody repertoire.

5. Safety considerations

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.

For safe handling and processing for COVID-19 related samples in laboratories please refer to PHE guidance and Annex 2 of The approved list of biological agents (2021)(20).

6. Specimen processing and procedure

6.1 Specimen type

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

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6.2 Specimen transport and storage conditions

Specimens should be collected in appropriate CE 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 (21).

Samples should be retained in accordance with The Royal College of Pathologists guidelines 'The retention and storage of pathological records and specimens' (22).

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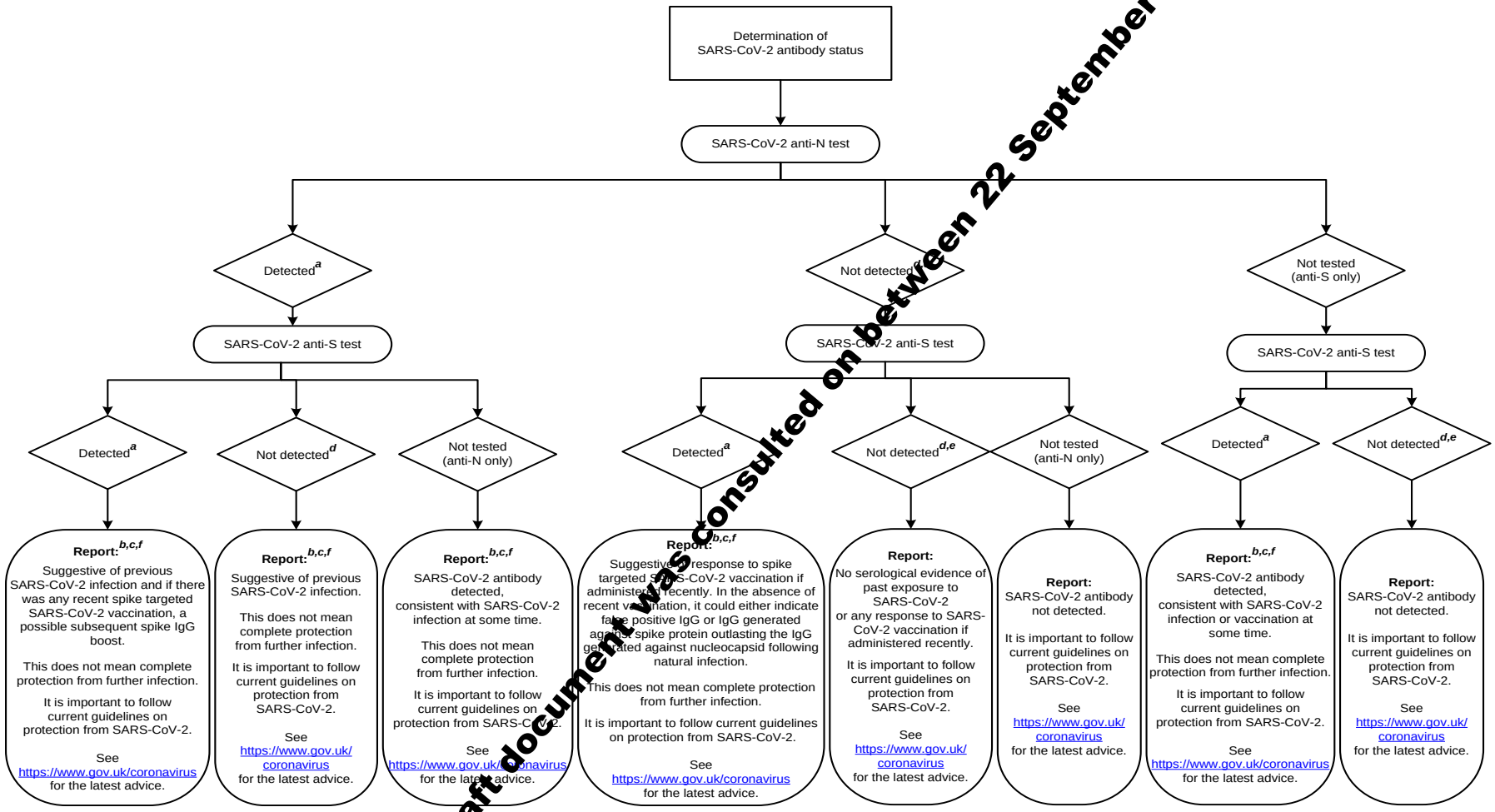
7. Investigation detection of SARS-CoV-2 antibodies

[An accessible text description of this flowchart is provided with this document.]



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

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

b) Data not currently available to support the use of a reactive result to exclude the possibility of re-infection.

c) Data not currently available on how IgG correlates to functional immunity, therefore a reactive result cannot be interpreted to mean that the patient is immune, or that they are not currently infected and/or that they cannot transmit the virus to others.

d) Immunocompromised individuals may not mount a detectable antibody response or may present a delayed response.

e) This result does not exclude recently acquired infection (7 to 21 days after symptom onset). Please send an appropriate respiratory sample for SARS-CoV-2 PCR if symptomatic.

f) Every report should include the assay manufacturer, the antibody class(es) and the target antigen(s).

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8. Interpreting and reporting laboratory results

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

	Anti N	Anti S	Interpretative Comment
1	Detected	Detected	Suggestive of previous SARS-CoV-2 infection and if there was any recent spike targeted SARS-CoV-2 vaccination, a possible subsequent spike IgG boost. This does not mean complete protection from further infection. It is important to follow current guidelines on protection from SARS-CoV-2 for the latest advice.
2	Detected	Not Detected	Suggestive of previous SARS-CoV-2 infection. This does not mean complete protection from further infection. It is important to follow current guidelines on protection from SARS-CoV-2 for the latest advice.
3	Not Detected	Detected	Suggestive of response to spike targeted SARS-CoV-2 vaccination if administered recently. In the absence of recent vaccination, it could either indicate false positive IgG or IgG generated against spike protein outlasting the IgG generated against nucleocapsid following natural infection. This does not mean complete protection from further infection. It is important to follow current guidelines on protection from SARS-CoV-2 for the latest advice.
4	Not Detected	Not Detected	No serological evidence of past exposure to SARS-CoV-2 or any response to SARS-CoV-2 vaccination if administered recently. It is important to follow current guidelines on protection from SARS-CoV-2.
5	Not tested (anti S only)	Detected	SARS-CoV-2 antibody detected, consistent with SARS-CoV-2 infection or vaccination at some time. This does not mean complete protection from further infection. It is important to follow current guidelines on protection from SARS-CoV-2 for the latest advice.
6	Not tested (anti S only)	Not detected	SARS-CoV-2 antibody not detected. It is important to follow current guidelines on protection from SARS-CoV-2 for the latest advice.
7	Detected	Not tested (anti N only)	SARS-CoV-2 antibody detected, consistent with SARS-CoV-2 infection at some time.

	Anti N	Anti S	Interpretative Comment
			This does not mean complete protection from further infection. It is important to follow current guidelines on protection from SARS-CoV-2 for the latest advice.
8	Not detected	Not tested (anti N only)	SARS-CoV-2 antibody not detected. It is important to follow current guidelines on protection from SARS-CoV-2 for the latest advice.

Note: Interpretation and use of equivocal results will depend on manufacturer instructions and on local validation data.

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9. References

For the information on the reference assessment grades given, refer to the scientific information section on the UK SMI website.

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