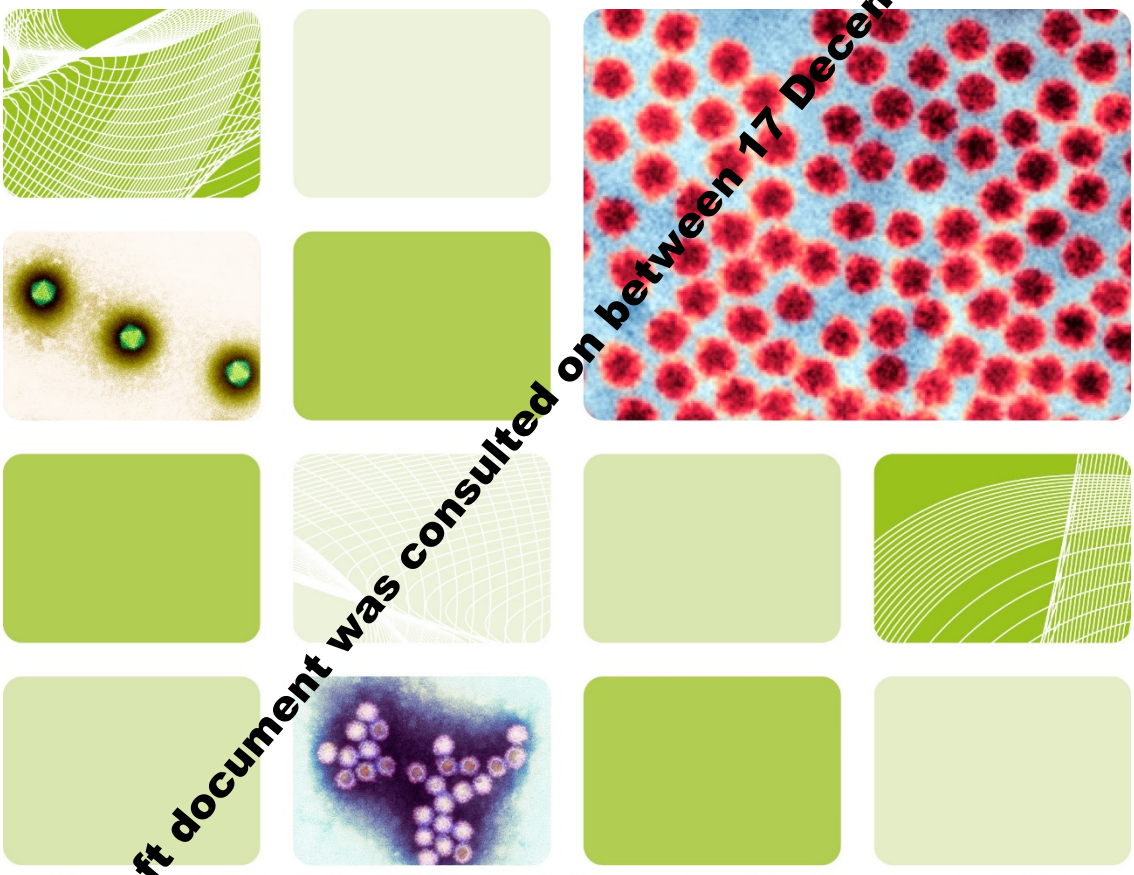
UK Health Security Agency

UK Standards for Microbiology Investigations

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



National Institute for Health and Care Excellence (NICE) has renewed accreditation of the process used by the **UK Health Security Agency** to produce **UK Standards for Microbiology Investigations (UK SMIs)**. The renewed accreditation is valid until **30 June 2026** and applies to guidance produced using the processes described in '**UK Standards for Microbiology Investigations Development Process' (2021)**. The original accreditation term began on 1 July 2011.

Issued by the Standards Unit, Specialised Microbiology and Laboratories, UKHSA

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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 (see the Steering Committee page on GOV.UK).

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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@phe.gov.uk.

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

Amendment number/date	
Issue number discarded	
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Anticipated next review date*	dd.mm.yy
Section(s) involved	**Amendment**

*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 (2). See COVID-19: the green book, chapter 14a for 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 neutralising antibodies. Vaccines targeting the S protein elicits an immune response in vaccinated individuals.

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.

Since September 2021 testing for anti-spike antibodies is integral to the clinical commissioning pathway for casirivimab and imdevimab in the treatment of COVID-19 in hospitalised patients (8).

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 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 will differentiate 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 (9). While there is an increase in evidence to suggest memory T cells develop post SARS-CoV-2 infection correlates of immunity are not yet well defined (10). 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.

Since September 2021 testing for anti-spike antibodies is integral to the clinical commissioning pathway for the neutralising monoclonal antibody (nMAB) combination casirivimab and imdevimab in the treatment of COVID-19 in hospitalised patients (8). The monoclonal antibody combination bids specifically to two different sites on the spike protein of the SARS-CoV-2 virus particle, blocking its entry into the host cell and consequently inhibiting virus replication.

The RECOVERY trial announced findings that the neutralising monoclonal antibody (nMAB) combination reduced the relative risk of mortality by 20% in hospitalised patients with COVID-19 who had not mounted an antibody response of their own to the virus (11).

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 (12, 13).

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 (14). 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 (15) (see Figure 2).

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.

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

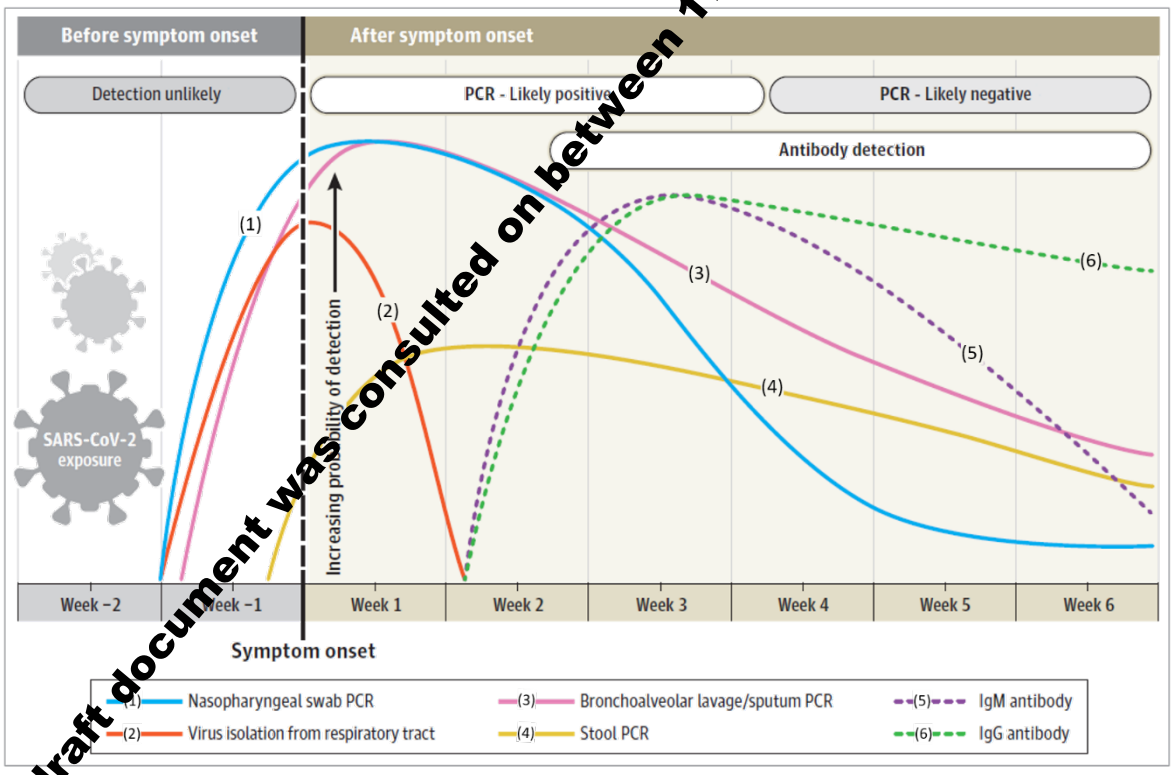
immunodominant, consisting of 2 subunits: the S1 protein containing the receptor binding domain (RBD); and the S2 protein which mediates fusion of the virus particle to the cell membrane (17). To date, SARS-CoV-2 vaccines in the UK are based on the spike (S) protein thus spike (S) antibody confirms past 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 (18). SARS-CoV-2 is similar to SARS-CoV-1, showing sequence homology of 90% in the nucleocapsid and 76% in the spike protein (19).

Commercially available serological assays can detect IgG alone, or both IgG and IgM (total antibody) (20). See evaluation of commercial kits, 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 (21).

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.

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



Note: there may be differences depending on the dominant variant circulating at the time.

A number of different serological assays have been developed for use in detecting antibodies. These assays usually detect the anti-spike (S) protein or anti-nucleoprotein (N) antibody responses in those with COVID-19, because the two proteins are highly immunogenic.

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.

5 Safety considerations

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

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.

Refer to guidance on COVID-19: safe handling and processing for samples in laboratories and Annex 2 of The approved list of biological agents (2021) (23).

6 Specimen processing and procedure

6.1 Specimen type

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

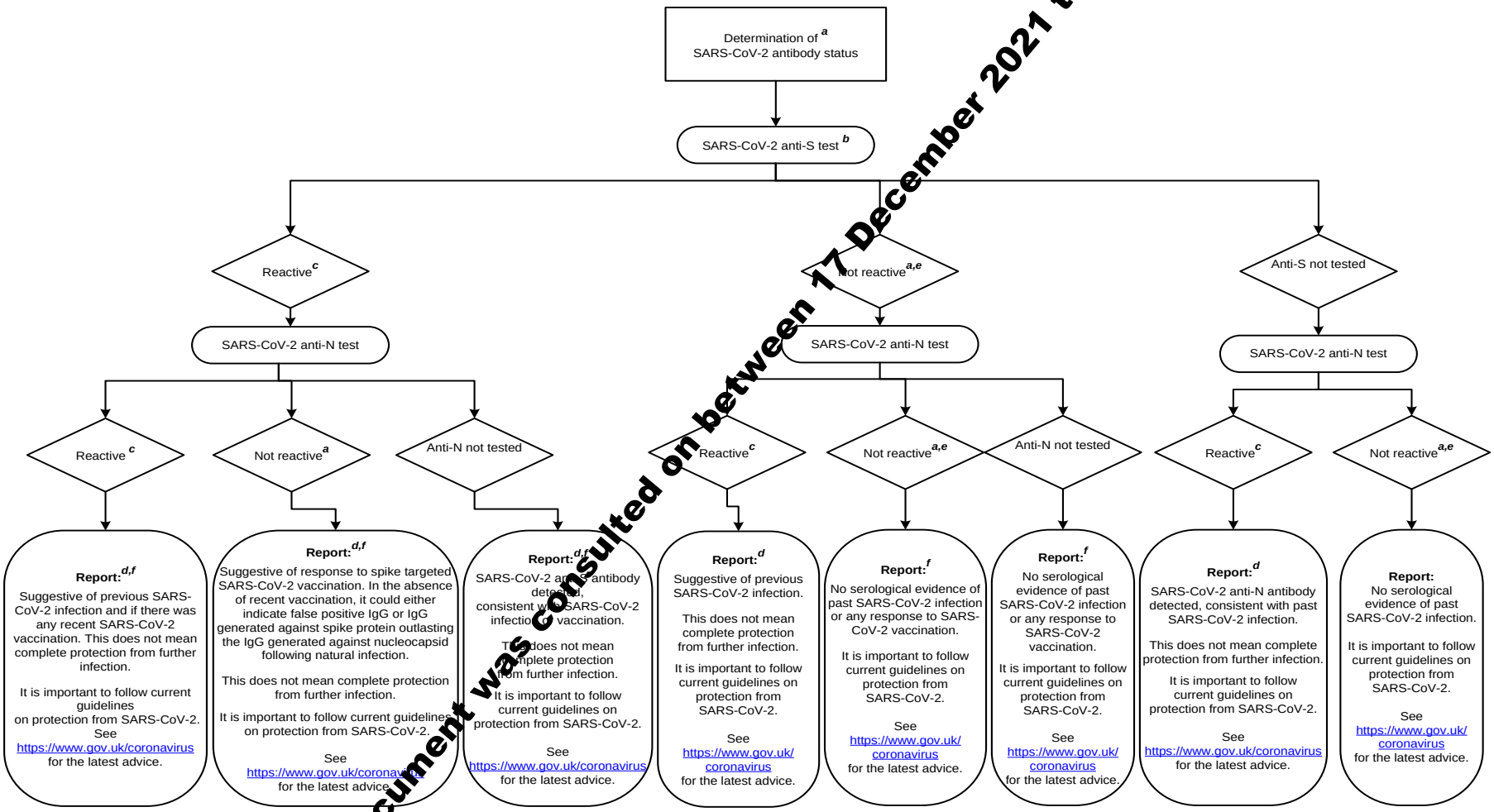
6.2 Specimen transport and storage conditions

Specimens should be collected in appropriate CE 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(42).
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

An accessible text description of this flowchart is provided with this document



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

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

b) Anti-spike testing is used to guide monoclonal antibody treatment in patients with COVID-19. Patients may be tested for anti-spike antibodies using any validated quantitative or qualitative anti-S assay that measures either IgG or total antibody levels. In immunocompromised groups, very low "positive" levels of anti-S antibody on a quantitative assay (within the bottom 10% of the assays positive range) should be interpreted in the context of clinical decision making and laboratory advice and a decision to treat may still be made by the multidisciplinary team (MDT) on a case by case basis (8).

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

d) Data is not currently available to support the use of a reactive result to exclude the possibility of re-infection and 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 or that they cannot transmit the virus to others.

e) This result does not exclude recently acquired infection (7 to 14 days after symptom onset). Please send an appropriate respiratory sample for SARS-CoV-2 PCR if symptomatic.

f) The interpretation of vaccination only applies to UK approved vaccines which currently target Spike protein only.

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

8 Interpreting and reporting laboratory results

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

	Anti-S	Anti-N	Interpretative Comment
1	Detected	Detected	Suggestive of previous SARS-CoV-2 infection and if there was any recent SARS-CoV-2 vaccination. 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. If your patient has received immunoglobulin (intravenous or subcutaneous) within the last 4 weeks passive transmission of antibodies should be considered. Refer to latest guidance criteria for monoclonal antibodies.
2	Detected	Not Detected	Suggestive of response to spike targeted SARS-CoV-2 vaccination. 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.
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 guidelines on protection from SARS-CoV-2 for the latest advice. If your patient has received immunoglobulin (intravenous or subcutaneous) within the last 4 weeks passive transmission of antibodies should be considered. Refer to latest guidance criteria for monoclonal antibodies.

4	Not Detected	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.
5	Not Detected	Not Detected	No serological evidence of past SARS-CoV-2 infection or any response to SARS-CoV-2 vaccination. It is important to follow current guidelines on protection from SARS-CoV-2.
6	Not detected	Not tested	No serological evidence of past SARS-CoV-2 infection or any response to SARS-CoV-2 vaccination. It is important to follow current guidelines on protection from SARS-CoV-2 for the latest advice.
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 guidelines on protection from SARS-CoV-2 for the latest advice. If your patient has received immunoglobulin (intravenous or subcutaneous) within the last 4 weeks passive transmission of antibodies should be considered. Refer to latest guidance criteria for monoclonal antibodies.
8	Not tested	Not detected	No serological evidence of past SARS-CoV-2 infection. It is important to follow current guidelines on protection from SARS-CoV-2 for the latest advice.

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An explanation of the reference assessment used is available in the scientific information section on the UK SMI website.

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