In vitro data on neutralising monoclonal antibodies for COVID-19: methods framework

1.1 Background

1.1.1 NICE has published a suite of guidelines on COVID-19. It has also developed a multiple technology appraisal on casirivimab plus imdevimab, nirmatrelvir plus ritonavir, sotrovimab and tocilizumab for treating COVID-19, and a single technology appraisal on tixagevimab plus cilgavimab for preventing COVID-19 is in development.

1.1.2 The virus, SARS-CoV-2, that causes COVID-19 evolves over time, resulting in new variants and subvariants. Clinical-effectiveness evidence for neutralising monoclonal antibodies (nMABs) is from clinical trials done before the Omicron variant became the predominant variant. Also, because SARS-COV-2 is evolving rapidly, it is difficult to do clinical trials in real time. This means that clinical trials on new variants will not be completed in time to help understanding about how effective nMABs are against those variants before the virus evolves again. It is also unlikely that findings from observational studies will be reported in the timeframe needed to inform decision making. So, NICE needs to develop methodology to help understanding about whether nMABs developed for a previous variant can be used for people infected, or at risk of infection, with a newer variant.

1.1.3 With little clinical trial and observational data on the efficacy of nMABs against newer variants, policy makers are using in vitro data. This data is generated from laboratory studies outside of a living body and usually involves cell culture. For these reasons, in vitro studies are not thought to fully replicate the conditions seen in humans, and the evidence type and aims of the studies differ from clinical trial evidence. In vitro data on nMABs is from laboratory

studies investigating their neutralisation effect on cells infected with the SARS-CoV-2 variant of interest.

1.1.4 In general, some in vitro data suggests that some nMABs may have reduced neutralisation against some of the more recent variants in circulation, such as the Omicron variant and subvariants. Timely decisions need making on whether these nMABs should be recommended for pre-exposure prophylaxis and treatment of COVID-19. But the clinical-effectiveness and in vitro data covers different situations because clinical-effectiveness data was obtained when previous SARS-CoV-2 variants were dominant and in vitro data has been generated from newer circulating variants. The fundamental challenge for decision making is around how in vitro data translates into clinical and health economic outcomes in the absence of clinical studies in people infected, or at risk of infection, with new SARS-CoV-2 variants.

1.1.5 This document outlines a framework to assist technology appraisal and guideline committees in making decisions based on in vitro data. The framework does not include consideration of real-world data as the use of real-world data within NICE guidance is covered by the NICE real-world evidence framework. See below for the in vitro data framework overview.

1.2 Scope of this framework

1.2.1 This framework applies to in vitro data on nMABs for pre-exposure prophylaxis or treatment of COVID-19 only. Although there has been some suggestion that antivirals (for example, Paxlovid) could work differently against different variants, this has not transpired to date, so the principles outlined here do not cover those treatments.

1.3 How this framework was developed

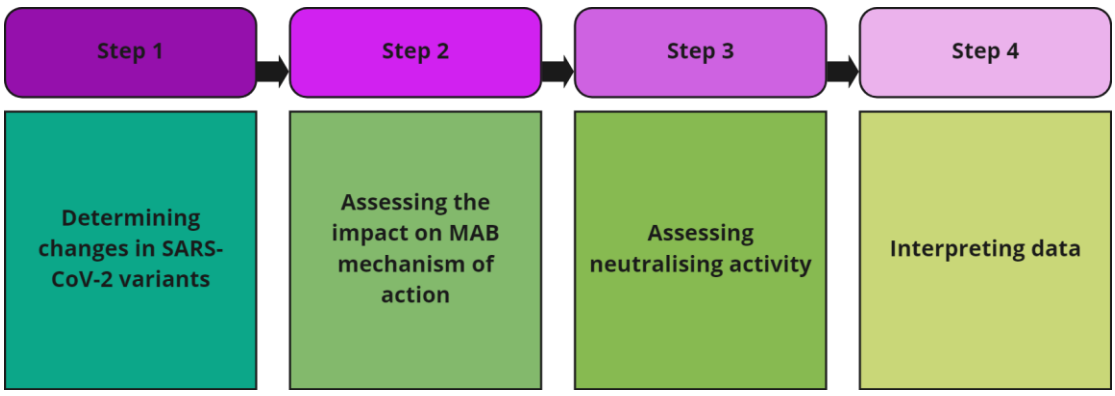
1.3.1 In December 2022, NICE established an in vitro data expert advisory group (In Vitro Advisory Group [IVAG], see appendix 2). This included people with expertise in using and understanding

COVID-19 in vitro data, or making clinical and health economic decisions in the setting of uncertainty. The main aims of this group were to advise on translating in vitro evidence on the neutralising activity of nMABs into clinical and health economic outcomes to help decision making for NICE guidance. This was to determine when nMABs were likely to be less effective or ineffective in the event of a new variant emerging, and to describe the uncertainty around those decisions. The group also advised on the type of data needed to inform decision rules and how to use the data. The group met times during December 2022, and the discussions were used to generate this interim framework and decision rules.

1.3.2 This is a living framework and will be updated as new information emerges.

1.4 Framework overview

Summary of key considerations for using in vitro data on the effectiveness of nMABs against new variants



Step 1: Determining changes in SARS-CoV-2variants

Anticipated future trajectory of circulating variants

1.4.1 The IVAG acknowledged the uncertainty around predicting the incidence of future variants, with reduced COVID-19 testing in the UK adding to this uncertainty. But, reflecting on the patterns and

emergence of previous variants, the IVAG anticipated that these principles will apply:

- It is certain that new SARS-CoV-2 variants will emerge with significantly different antigenic properties. It is also possible but less likely that new variants will have different properties in terms of transmissibility, cell tropism and disease severity. It is expected that there will continue to be 2 types of evolution of the virus:
  - frequent incremental changes leading to small changes in antigenicity
  - infrequent antigenic shifts leading to selective sweep of a new fit variant.
- There is a certain level of standing genetic diversity that can fluctuate over time and ‘changes’ to viral genotype are a continuous process. Historically, there has been a major sweep approximately every 6 months. What constitutes a major sweep of a new lineage is somewhat subjective. Less dramatic changes are a continuous process. At any given time, some lineages will be growing and slowly replacing other lineages. Antigenically similar previous variants are unlikely to re-emerge because of population immunity but cannot be ruled out. It is possible that a new lineage could emerge that is partially or completely ancestral to a previous lineage like Delta, but this would likely be antigenically distinct.
- A future variant could be neutralised by a given nMAB when this has not been seen for previous variants.

1.4.2 Based on the above assumptions, the IVAG supports steps for regular monitoring of the emergence of variants and determining whether further action is needed.

Surveillance and identification of new emergent variants

1.4.3 The UK Health Security Agency (UKHSA) has a surveillance system in place for monitoring the emergence of changes to SARS-CoV-2 variants. This intelligence will be shared with NICE.

1.4.4 Also, the World Health Organization (WHO) defines variants of concern as those meeting the following criteria:

- increase in transmissibility or detrimental change in COVID-19 epidemiology, or
- increase in virulence or change in clinical disease presentation, or
- decrease in effectiveness of public health and social measures or available diagnostics, vaccines and therapeutics.

1.4.5 The WHO also has a list of variants that it monitors. NICE will also use this information as a source of intelligence. But it is recognised that the WHO's information is not always relevant to the UK because there have been previous variants of concern recognised by WHO (for example, Beta) that have been important globally but have never become dominant in the UK.

Monitoring increasing prevalence of a variant (or subvariant)

1.4.6 Variants of interest are typically antigenically different from previous variants and generally exhibit 'immune escape', that is, the person's immune system is no longer able to recognise and eliminate the virus. For this reason, the variants tend to quickly

increase in prevalence across a population over a period of weeks to months.

Threshold for determining a new 'dominant' variant (or subvariant)

1.4.7 Predicting when a variant will become dominant is a complex task and depends on expert interpretation of evidence about the relative growth rates of cocirculating variants and interpretation of functional mutations in novel variants. There is also a distinction between genetic difference (such as a genetic shift away from a predominant variant) and immune escape, which links to the ability of a subvariant to increase in prevalence and replace other variants. The IVAG indicated that it is usually clear if a variant will replace others once it has reached about 10% sample frequency and has a logistic growth rate of over 25% per week. Intelligence from the UKHSA and the WHO should indicate which variants are emerging and increasing in prevalence, and should be used as a trigger to move to the next step in this framework.

1.4.8 Actions in this step of the framework:

- UKHSA shares surveillance intelligence on emerging variants that it anticipates will increase in prevalence or become dominant in the UK.
- NICE considers the UKHSA data in addition to the WHO's information on variants of concern.
- NICE, with input from the UKHSA, will decide whether there has been a step-change in variants from those that informed the decisions when the guideline recommendations were developed.

Decision point: If a new variant is becoming dominant, NICE will move to the next step on assessing impact on nMAB mechanism of action.

Step 2: Assessing impact on MAB mechanism of action

MAB and mechanism of action

1.4.9 MABs have different mechanisms of action in terms of which proteins they bind to, meaning they can neutralise the SARS-CoV-2 virus in different ways. This is important when considering the MAB of interest. Some treatments include a combination of 2 antibodies and it is possible that one but not the other may retain activity against a variant. NICE is evaluating the clinical and cost effectiveness of 3 nMABs; these have the following reported mechanism of action against the SARS-CoV-2 virus:

- **Casirivimab plus imdevimab (Ronapreve)** is a combination of 2 non-competing recombinant human IgG1 MABs. This combination targets 2 distinct epitopes (the part of the virus to which the nMABs attach) binding simultaneously to the S protein receptor-binding domain. Casirivimab plus imdevimab block the virus's interaction with the angiotensin-converting enzyme 2 (ACE2) receptor that is used by the virus to enter host cells.
- **Sotrovimab (VIR-7831)** is a dual-action, engineered human IgG1 MAB that binds to a conserved epitope on the spike protein receptor-binding domain of SARS-CoV-2. Amino acid substitutions in the Fc region result in a median half-life of 49 days while retaining the ability of the antibody to recruit effector functions.
- **Tixagevimab and cilgavimab (Evusheld)** is a combination of 2 recombinant human IgG1 MABs, with amino acid substitutions in the Fc regions that extend antibody half-life. Tixagevimab plus cilgavimab have longer half-lives of 87.9 and 82.9 days respectively. Tixagevimab and cilgavimab can simultaneously bind to non-overlapping regions of the spike protein receptor-binding domain of SARS-CoV-2.

1.4.10 The IVAG noted that the nMABs exhibit dose-linear and proportional pharmacokinetics across the range of doses at which they have been studied. What this generally means in practice is that, if the dose is doubled, the concentrations in serum are doubled and, if the dose is halved, the concentration in serum is halved.

1.4.11 Most available nMABs were developed in the context of early SARS-CoV-2 variants. Some in vitro data has shown that many of them may be less effective at neutralising newer variants, resulting in a perception that they may work less well in people infected with or exposed to new variants.

1.4.12 Considering the mechanism of action of nMABs in relation to new variants, NICE sought advice from the IVAG to determine whether it is likely that nMABs could retain neutralising activity. For example, if a specific nMAB target epitope is lost in a new variant, this could be a potential trigger for considering whether neutralisation activity is reduced or lost.

1.4.13 Based on its experience, the IVAG indicated that:

- Neutralisation activity of combination treatments may be more resilient to changes in variants because they tend to have a broader mechanism of action.
- Drug-selected resistance has been seen during use against susceptible variants (up to Omicron BA.1).
- Marked reductions in neutralisation have been reported since Omicron BA.2 and subsequent sublineages emerged.
- Neutralisation can also be compromised when mutations occur outside of the specific epitope because of the overall effect on protein structure.

1.4.14 Actions in this step of the framework:

- Determine whether the nMABs' mechanism of action is still effective against the new variant:
  - The main impact is expected when a variant has a mutation eliminating the target epitope of the nMAB or a mutation outside of the specific epitope that compromises neutralisation.
  - Assessment of impact will need a combination of evidence on mechanism of action and expert input.

Decision point: If there is a potential impact on the effectiveness of the nMAbs' mechanism of action move to next step of assessing neutralising activity.

Step 3: Assessing neutralising activity

Determining the evidence base

1.4.15 NICE needs in vitro data to inform discussions on whether the nMABs included in NICE guidance still have neutralising activity against the new dominant variants. NICE's search strategy for identifying published evidence is outlined in appendix 3. NICE may get additional data from the UKHSA, regulators and companies.

Relationship between in vitro neutralisation data and clinical effectiveness

1.4.16 Neutralisation assays are considered the gold standard for determining antibody efficacy against viruses. The results of these in vitro ELISA assays, usually reported as the 50% and 90% effective concentrations (EC50 and EC90), show the concentration of drug needed to neutralise 50% or 90% of the virus. The goal of neutralisation is not necessarily to neutralise the virus completely, but to reduce the growth rate of the virus to below a self-sustainable level. The IVAG indicated that different nMABs may remain effective despite having reduced neutralising activity against a different variant than that prevalent when the clinical trial

which led to marketing authorisation was done. This may occur if the concentration of the treatment used in clinical practice is, for example, 100-fold higher than that needed to reduce the viral level. In this example, the nMABs may have a similar effect on viral growth rate even if there is a 100-fold reduction in neutralising activity against a new viral variant compared with original studies against older variants. In an attempt to maximise a positive outcome in clinical trials, some companies have used the highest dose possible initially followed by lower doses. For example, a clinical trial on casirivimab plus imdevimab used doses of 8.0 g, 2.4 g and 1.2 g (O'Brien et al. 2021). This is important to note when considering the neutralising activity of the nMABs.

1.4.17 The gold standard for assessing the clinical effectiveness of medicines is blinded randomised controlled trials (RCTs). In the absence of RCTs on the effectiveness of nMABs against new SARS-CoV-2 variants, whether there could be a plausible link between in vitro neutralisation data and clinical and health economic outcomes needs to be established. While there is no consensus on the exact relationship between in vitro neutralisation data and clinical outcomes for COVID-19 (such as reducing hospitalisation rates or mortality), the IVAG concluded that it is plausible that an association exists. The main reason for this conclusion is because scientists have consistently used in vitro neutralisation data to select antibodies and doses for further testing in RCTs for several decades of antiviral pharmacological research. The IVAG noted, however, that a link between in vitro data showing a fold change in neutralisation activity against newer variants and clinical outcomes is difficult to establish because of how a new variant may affect disease severity.

1.4.18 One of the key methodological steps in the usual process of reviewing evidence of clinical effectiveness is to appraise the clinical trials to critically to assess quality and robustness, risk of

bias and generalisability. There is no validated tool for appraising in vitro neutralisation data. So, the IVAG discussed key components of quality for studies on in vitro neutralisation and identified important characteristics to consider when assessing studies. The IVAG was also aware of the ongoing work of the Department of Health and Social Care Antivirals and Therapeutics Taskforce, which aims to standardise aspects of in vitro neutralisation studies.

Key components of in vitro neutralisation studies

Virus and cell lines

1.4.19 In vitro neutralisation studies typically use either pseudovirus or live virus. Pseudoviruses do not replicate and have their surface envelope proteins replaced with those of SARS-CoV-2. The IVAG agreed that it preferred studies using live SARS-CoV-2 virus but acknowledged that both types of virus were associated with uncertainty. The IVAG agreed that in vitro data from pseudovirus generally agrees with in vitro data from live virus, and the advantage is that results from pseudovirus are generated quicker.

1.4.20 The IVAG noted it is also important that the cell line used for viral culture has been clonally selected and that the batch of virus has been sequenced, characterised and reported in the studies. This would enable NICE to assess the consistency across studies.

Reproducibility of assays

1.4.21 The IVAG agreed that in vitro neutralisation assays should be reproducible, so studies should clearly detail the methods used.

1.4.22 Different manufacturers of nMABs assume different degrees of tissue penetration, and some, but not all, companies also include a margin of error (up to 10-fold) in their assays. According to the IVAG, few companies use EC50 because inhibiting only 50% of replication is not a recognised basis for efficacy of medicines to

prevent or treat viral illnesses, and EC90 is at least 9-fold higher than EC50.

1.4.23 The IVAG concluded that EC50 values would be acceptable to initially assess whether an nMAB has lost efficacy against new variants relative to older variants. But, when detailed pharmacokinetic and pharmacodynamic (PK/PD) assessments are needed, EC90 should be used.

Repeatability of results

1.4.24 When new SARS-CoV-2 variants emerge, it is likely that numerous groups of scientists will generate and publish in vitro data. The IVAG considered it important that results are broadly consistent across studies. The IVAG noted, however, that fold-differences in neutralisation between different variants have generally been more reproducible than the absolute concentrations of nMAB needed for neutralisation.

Comparator

1.4.25 The IVAG discussed that in vitro neutralisation studies should report fold change in EC50 against the new variants relative to the ancestral or reference variants.

Measuring uncertainty in the results

1.4.26 The IVAG discussed that using 95% confidence intervals (95% CIs) when reporting EC50 and EC90 point estimates would be helpful for measuring uncertainty in the results. For example, comparing 2 absolute EC50 values without a 95% CI could be misleading.

However, the IVAG acknowledged that 95% CIs are not always reported in the literature.

1.4.27 Actions in this step of the framework:

- Search for in vitro data to determine if there are any studies that report neutralisation data for nMABs against new variants of interest.
- Determine the quality and reproducibility of the data using the appraisal approach outlined in appendix 4.

Decision point: If there is in vitro data available that is of sufficient quality and reproducible, move to next step of interpreting the data.

Step 4: Interpreting changes to in vitro neutralisation by monoclonal antibodies

In vitro data presentation

1.4.28 There are generally 2 presentation types for in vitro data used in the published literature:

- heat maps (for example, as shown in Wang et al. 2022)
- concentration dose–response curves (for example, as shown in Planas et al. 2022).

1.4.29 These present the concentration of nMABs needed to neutralise the variant in vitro to a stated degree (for example, EC50). Heat maps show the nMABs drugs in columns, and the variants in rows (see figure 2). A red colour represents a loss of neutralising activity while no colour reflects maintained neutralising activity. A dose–response curve plots drug concentration on the x axis as a function of percent viral inhibition on the y axis. With separate plots per treatment, each neutralisation curve reflects neutralisation activity of therapeutic monoclonal antibodies against variants of interest. Although the IVAG acknowledged that heat maps provide a good summary of a lot of data, the IVAG concluded that it preferred

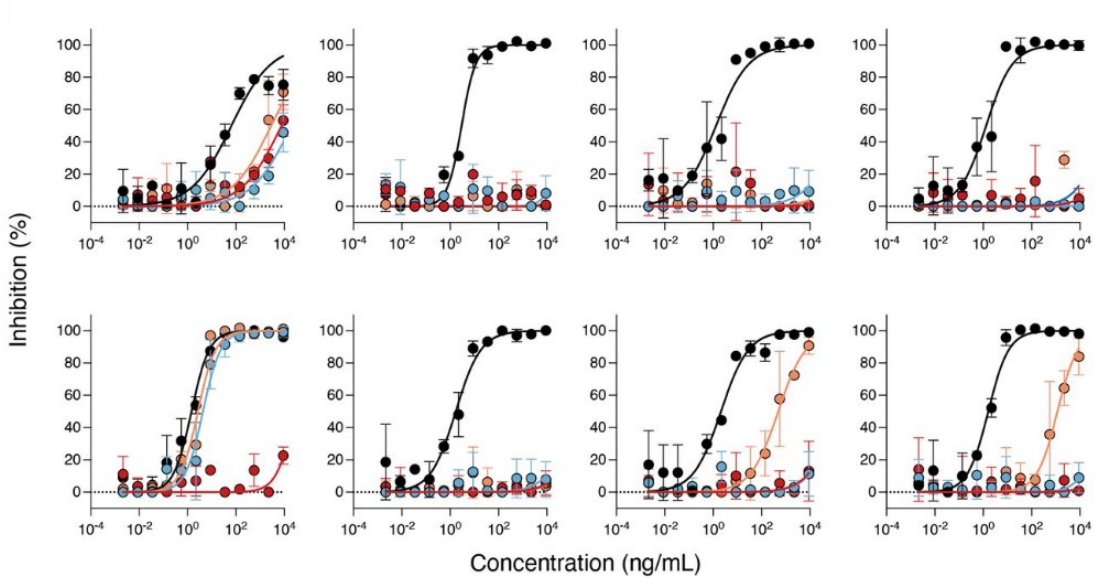
dose–response curves (see figure 3) because they provide more information. Specifically, they enable assessment of whether the slope of the concentration response curve changes between variants. If the slope changes (showing that higher concentrations of nMAbs are needed to retain neutralisation), the EC90 moves even further away from the EC50 and, in some cases, the nMAB cannot achieve EC90.

Figure 2 Example heatmap from Wang et al. 2022

IC$_{50}$ (μg/ml)	NTD	NTD-SD2	SD1	RBD Class 1				RBD Class 2				RBD Class 3											RBD Class 4	Evusheld
	C1520	C1717	S3H3	S2K146	Omi-3	Omi-18	BD-515	XGv051	XGv347	ZCB11	COV2-2196	LY-CoV1404	XGv289	XGv264	S309	P2G3	SP1-77	BD55-5840	XGv282	BD-804	35B5	COV2-2130	10-40	
D614G	0.002	0.125	0.022	0.004	0.004	0.012	0.010	0.001	0.002	0.002	0.002	0.002	0.002	0.001	0.023	0.001	0.003	0.002	0.001	0.011	0.014	0.007	0.049	0.003
BA.4/5	0.001	0.209	0.014	0.090	0.023	0.013	0.010	0.050	3.450	4.868	>10	0.001	0.038	0.002	0.514	0.002	0.005	0.009	0.001	0.019	>10	0.021	2.414	0.035
BQ.1	0.001	0.666	0.019	0.585	0.860	0.131	0.343	0.159	2.830	>10	>10	>10	0.425	0.494	0.600	1.608	>10	0.034	0.020	>10	>10	>10	>10	>10
BQ.1.1	0.003	1.117	0.025	0.527	0.804	0.170	0.377	0.191	3.311	>10	>10	>10	1.013	>10	2.140	>10	>10	>10	0.093	>10	>10	>10	>10	>10
BA.4/5-R346T	0.002	0.141	0.009	0.081	0.019	0.009	0.006	0.042	2.166	2.560	>10	0.001	0.045	0.003	1.726	0.041	>10	1.447	0.001	>10	>10	>10	5.069	>10
BA.4/5-K444T	0.002	0.116	0.009	0.104	0.016	0.010	0.006	0.040	4.766	3.731	>10	>10	0.161	0.273	0.552	1.245	4.007	0.038	0.006	>10	>10	>10	6.976	>10
BA.4/5-N460K	0.002	1.166	0.016	0.542	1.279	0.186	0.431	0.152	3.046	>10	>10	0.002	0.353	0.003	0.934	0.003	0.009	0.012	0.002	0.122	>10	0.030	>10	0.063
BA.2	0.002	0.561	0.016	0.028	0.015	0.005	0.012	0.001	0.003	0.012	1.924	0.001	0.067	0.003	0.833	0.002	0.006	0.014	0.001	0.036	0.827	0.009	8.770	0.019
XBB	>10	0.836	0.016	0.223	1.181	0.468	0.555	>10	>10	>10	>10	>10	>10	>10	0.343	>10	>10	>10	>10	>10	>10	>10	>10	>10
XBB.1	>10	0.693	0.019	0.190	1.705	0.605	0.803	>10	>10	>10	>10	>10	>10	>10	0.405	>10	>10	>10	>10	>10	>10	>10	>10	>10
BA.2-V83A	0.001	0.354	0.015	0.036	0.019	0.007	0.015	0.002	0.003	0.013	3.039	0.001	0.070	0.002	0.641	0.002	0.007	0.019	0.001	0.045	1.274	0.011	>10	0.025
BA.2-Del144	0.002	0.501	0.011	0.026	0.016	0.004	0.011	0.002	0.002	0.008	4.134	0.001	0.063	0.002	0.455	0.002	0.005	0.014	0.001	0.031	0.341	0.010	8.766	0.021
BA.2-H146Q	0.001	0.356	0.011	0.032	0.011	0.004	0.009	0.002	0.002	0.010	2.924	0.002	0.055	0.002	0.641	0.003	0.007	0.019	0.001	0.044	1.107	0.009	9.106	0.019
BA.2-Q183E	0.002	0.307	0.019	0.034	0.018	0.006	0.014	0.002	0.003	0.013	3.098	0.001	0.067	0.003	0.649	0.002	0.008	0.020	0.002	0.028	1.019	0.011	9.251	0.022
BA.2-V213E	0.002	0.406	0.013	0.030	0.014	0.004	0.010	0.002	0.002	0.006	2.177	0.001	0.047	0.003	0.720	0.002	0.006	0.014	0.001	0.026	1.247	0.009	8.198	0.018
BA.2-G252V	0.001	0.577	0.013	0.030	0.012	0.004	0.008	0.002	0.003	0.008	2.258	0.001	0.048	0.002	0.564	0.002	0.005	0.012	0.001	0.032	0.939	0.011	>10	0.026
BA.2-G339H	0.001	0.485	0.017	0.034	0.020	0.006	0.012	0.002	0.002	0.010	3.876	0.002	0.114	0.002	0.302	0.002	0.007	0.040	0.002	0.050	0.661	0.012	8.575	0.023
BA.2-R346T	0.003	0.372	0.012	0.017	0.010	0.003	0.007	0.001	0.002	0.007	2.109	0.002	0.048	0.004	1.433	0.007	>10	1.442	0.001	0.112	>10	>10	7.767	1.486
BA.2-L368I	0.003	0.453	0.019	0.027	0.010	0.004	0.010	0.002	0.001	0.006	2.603	0.001	0.030	0.002	0.605	0.002	0.005	0.021	0.001	0.026	0.324	0.008	3.202	0.018
BA.2-V445P	0.001	0.433	0.019	0.026	0.009	0.004	0.009	0.002	0.002	0.008	2.313	>10	>10	1.141	0.428	>10	0.007	0.144	>10	1.582	0.486	>10	6.311	3.135
BA.2-G446S	0.002	0.367	0.012	0.021	0.009	0.004	0.009	0.001	0.003	0.008	2.614	0.002	0.026	0.004	0.686	0.002	0.004	0.014	0.022	0.026	0.965	0.017	5.774	0.029
BA.2-N460K	0.002	1.323	0.012	0.132	0.764	0.013	0.358	0.007	0.004	0.073	1.756	0.001	0.355	0.003	0.878	0.002	0.011	0.017	0.001	0.058	1.957	0.013	>10	0.025
BA.2-F486S	0.002	0.677	0.008	>10	0.583	0.011	0.017	>10	>10	>10	>10	0.001	0.049	0.003	0.581	0.002	0.006	0.009	0.002	0.060	2.264	0.011	>10	0.023
BA.2-F490S	0.001	0.428	0.014	0.022	0.033	0.004	0.008	0.001	0.004	0.012	1.105	0.001	0.030	0.002	0.564	0.002	0.006	0.011	>10	0.048	>10	0.013	5.337	0.016
BA.2-R493Q	0.003	0.338	0.024	0.005	0.006	0.006	0.006	0.001	0.001	0.002	0.034	0.001	0.045	0.002	1.109	0.002	0.007	0.022	0.000	0.010	1.175	0.010	3.419	0.008

>10	1-10	0.1-1	0.01-0.1	<0.01

Figure 3 Example concentration dose–response curves from Planas et al. 2022



In vitro neutralisation activity interpretation

1.4.30 The IVAG discussed different scenarios (see table 1) of changes in neutralising activity against variants compared to the reference strains. It concluded that some scenarios had a clear interpretation that could inform recommendations made by technology appraisal or guidelines committees. These scenarios are when there can be no plausible argument for continuing efficacy for the antibodies against a new variant (see table 1). But there will also be scenarios in which the fold change in neutralising activity, particularly at higher concentrations of drugs, will be harder to interpret without further information. The IVAG indicated that, if the in vitro data shows a fold change but in vitro neutralisation is still achieved at concentrations that could be achieved in serum, then the nMAB may still be effective at a higher dose. But the IVAG considered that this may need higher dosages than licensed and acknowledged that NICE must make recommendations based on the licensed dose only.

Table 1 Scenarios for changes in the in vitro neutralising activity relative to the reference variant (either ancestral variant or predominant variant in pivotal RCT) - applicable to prophylaxis and treatment

Scenario	Agreed action	Rationale
No or minimal fold change in neutralising activity relative to the reference variant.	Use existing randomised controlled trial (RCT) evidence for decision making.	We are confident that the neutralising activity has been minimally impacted therefore the conclusions from the RCT hold.
No or minimal neutralising activity at very high concentrations.	Move to decision to not recommend a nMAB.	These concentrations could not be achieved in the body. Clear in vitro evidence that nMABs will not be clinically

		effective (or by extension cost effective).
Some neutralisation at higher concentration, but substantial fold change compared with the reference variant.	Insufficient information to make a decision.	If there is a substantial fold change, PK/PD data is needed to attempt linking of the data to clinical outcomes.

Visualising the scenarios

Figure 4 Example showing no or minimal neutralising activity at very high concentrations for the variants in blue and red compared with the black reference variant (Planas et al. 2022)

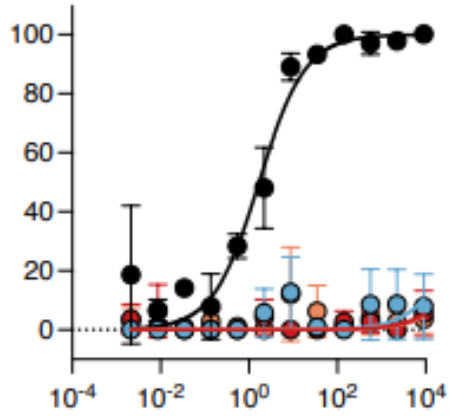
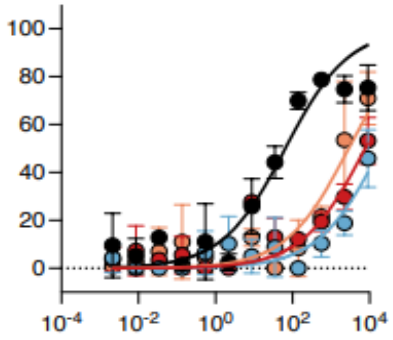


Figure 5 Example showing some neutralisation at higher concentrations (Planas et al. 2022)



Pharmacokinetic and pharmacodynamic (PK/PD) data

1.4.31 The IVAG stated that simply interpretating the fold-difference in the ability of an nMAB to neutralise a variant without considering the

compartmental pharmacokinetics, including how the drug interacts in different bodily compartments, does not give a complete picture.

1.4.32 In general terms, the plausibility of continued efficacy of a nMAB against new viral variants needs consideration of the plausibility of the antibody still achieving sufficient neutralisation activity in patients, and this needs an understanding of the pharmacokinetics. The nMABs exhibit dose-linear and proportional pharmacokinetics. What this means in practice is that if the dose is doubled, the concentrations in serum are doubled, and if the dose is halved then the concentration in serum is halved. The IVAG indicated that there is an important step in understanding the compartmental pharmacokinetics that correspond to the clinical-effectiveness measures achieved in RCTs. This includes the doses of nMABs needed to neutralise and how a double dose that doubles the concentration in serum, for example, might overcome an expected fold reduction of neutralisation in vitro.

1.4.33 The IVAG concluded PK/PD data is needed to try to link in vitro neutralisation data to clinical outcomes when there is a substantial fold change, but some neutralisation is retained in vitro. Without this data, it is not possible to determine how this fold change may be associated with clinical outcomes.

1.4.34 The IVAG considered it essential to know the minimum concentration needed to neutralise the ancestral (or reference) viral strain and whether this differs from the licensed dose of a nMAB treatment. If this dose was substantially above the minimum concentration, then there is potentially still a tolerance to accommodate a large fold reduction in neutralisation in vitro. If the neutralisation activity achieved by the dose was close to the minimum needed for effectiveness in the ancestral (or reference) viral strain, then there is a high possibility that even a small fold

change in neutralisation would render the nMAB clinically ineffective.

1.4.35 The IVAG agreed that clinical trials reporting failed doses provide important information. Although it did note that the more data points presented, the more confidence this adds to the dose–clinical response relationship. From this data, the concentration of drug or level of neutralisation of virus the investigators found to be clinically ineffective is known. Unfortunately, for most nMABs, IVAG acknowledged that this PK/PD data is not available. It suggested that the regulators and NICE should encourage companies to collect this data in registrational trials to allow rapid assessment based on in vitro data.

Differences between the monoclonal antibodies

1.4.36 The IVAG noted that there is some in vitro data showing that tixagevimab and cilgavimab for pre-exposure prophylaxis of COVID-19 does not neutralise newer dominant variants of the virus. According to the IVAG, sotrovimab shows some neutralisation if the concentration used in vitro is increased. But the higher concentrations of sotrovimab needed to inhibit some variants in vitro were much larger than the drug dosages used in published RCTs. Also, the IVAG indicated that the mechanism of sotrovimab differs from other nMAbs and that it may have additional beneficial effects beyond neutralisation through 'effector functions'. The IVAG acknowledged that this may be an additional benefit, but is hard to quantify. Overall, the IVAG concluded that evidence of in vitro neutralisation is a necessary requirement, and evidence of an effector function effect alone is insufficient to conclude clinical benefit.

1.4.37 Actions in this step of the framework:

- Use the appraised in vitro data to determine which scenarios from table 1 apply.

- Use the scenarios outlined in table 1 to determine the appropriate action.
- Seek expert advice on interpreting in vitro data and the proposed action.

Decision point: There are 3 outcomes in this step of the framework:

- No or minimal fold change in neutralising activity of a drug against a viral variant relative to the ancestral variant: no action is needed, continue to monitor.
- No or minimal neutralising activity at very high concentrations: determine whether there is a need to update recommendation.
- Some neutralisation at higher concentrations, but substantial fold change compared with ancestral variant: there is insufficient information to make a decision, seek expert input and ask companies for dose-failure data.

Appendix 2: IVAG members

Amanda Adler (Chair)
Director, Diabetes Trials Unit, University of Oxford

David Bauer
Group Leader and Head, RNA Virus Replication Laboratory. The Francis Crick Institute

Rupert Beale
Clinician Scientist Group Leader, Consultant Nephrologist, The Francis Crick Institute, UCL Division of Medicine

Sanjay Bhangani
Consultant Physician and Honorary Associate Professor, Royal Free Hospital and University College London

Neil Ferguson
Director, MRC Centre for Global Infectious Disease Analysis, Imperial College London

Neil Hawkins
Professor of Health Technology Assessment, University of Glasgow

Mark Jit
Professor of Vaccine Epidemiology, London School of Hygiene and Tropical Medicine

Saye Khoo
Professor in Pharmacology, Honorary Consultant Physician in Infectious Diseases, University of Liverpool

David Lalloo
Director, Liverpool Tropical School of Medicine

Siraj Misbah
Consultant Clinical Immunologist, Oxford University NHS Foundation Trust

Andrew Owen

Professor of Pharmacology, University of Liverpool

Derek Smith

Professor of Infectious Disease Informatics, Zoology Department at Cambridge University

David Stuart

MRC Professor of Structural Biology, University of Oxford

Mark Sutton

Scientific Leader at Healthcare Biotechnology, and Professor for Antimicrobial Therapy, UKHSA and King's College London

Laurie Tomlinson

NIHR Research Professor, Honorary Consultant Nephrologist, London School of Hygiene and Tropical Medicine

Erik Volz

Reader in Population Biology of Infectious Diseases, Faculty of Medicine, School of Public Health, Imperial College London

Appendix 3: Search strategy

Targeted searches are conducted to identify published and preprint in vitro data on the nMABs included in NICE guidelines. For information on how NICE identifies clinical trial and observational data see NICE guideline 191 COVID-19 rapid guideline: managing COVID-19.

The search is run regularly to identify journal articles, letters, editorials, preprints and grey literature.

The following databases are searched to identify records in all relevant formats:

- Embase (via Ovid)
- MEDLINE ALL (via Ovid)

The following source is searched to identify preprints:

- Europe PMC (via https://europepmc.org)

The contents of the Medicines Daily Alerts provided by the Specialist Pharmacy Service to NICE are reviewed each week.

As no validated search filters for in vitro studies could be identified, a broad approach to the search strategy should be adopted. The structure of the strategies may be reviewed as appropriate, according to the volume of results and the relevant records that are identified.

Appropriate search limits may be applied in agreement with the technical team.

The Information Services team at NICE will quality assure the principal MEDLINE search strategy and peer review the strategies for the other sources. The principal strategy will be adapted, as appropriate, for use in the other sources listed, taking into account their size, search functionality and subject coverage.

The search strategies should be reviewed and updated on a regular basis. Appropriate terms should be included if additional nMABs are to be covered. The strategies will not cover the ancestral strains prior to Omicron (i.e. Delta and earlier) unless these variants re-emerge. The strategies should be reviewed if a new variant is becoming dominant and NICE is moving to the next step on assessing impact on the mechanism of action of nMABs. Useful resources for identifying search terms are the European Centre for Disease Prevention and Control, the UK Health Security Agency and the World Health Organization.

The search results will be managed in EPPI Reviewer version 5 for deduplication and processing. Duplicates are removed in EPPI R5 using a 2-step process. First, automated deduplication is done using a high-value algorithm. Then, manual deduplication is used to assess low-probability matches.

Appendix 4: Appraisal of the evidence

The risk of bias assessment is to be completed using the adapted toxicological data reliability assessment tool (TOXRTOOL). The 23 questions in table 1 are allocated a score of 0 or 1.

Table 1 Risk of bias assessment questions

Number	Criteria	Score
1	**Test substance identification (monoclonal antibody [MAB]):** 1. Was the monoclonal antibody named/described in the study? 2. Is information on the source or origin of the MAB given? Generally, only authentic product provided by the manufacturer should be accepted for interpretation of the findings. This should include manufacturer name. 3. Does the test substance accurately reflect MABs used in clinical practice?	**0**
2	**Test system characterisation (neutralisation assay):** 4. Is the test system described? At a fundamental level, comparison of in vitro data across laboratories is hampered by the use of different cell lines that may be infected by SARS-CoV-2 variants to different extents. Emerging evidence suggests that MABs binding outside the receptor-binding domain may be sensitive to angiotensin-converting enzyme 2 expression levels and this should be considered. 5. Was the neutralisation assay appropriate? It is expected that all neutralisation assays would be ELISA assays done in at least 2 independent experiments. 6. Is information given on the source or origin of the test system, and is there data available on the validity of that test system? This could include: • laboratory or scientist providing cell lines • commercial provider of test systems • a description of how the reactivity of the nMAB was validated • origin of tissues and primary cells. 7. Are necessary information on test system properties, and on conditions of cultivation and maintenance given? (Type of assay, type of virus, type of cell line, type of media)	**0**

	There is broad agreement that in vitro methodology should employ authentic SARS-CoV-2 isolates, and that routine sequencing of virus stocks is needed because cell culture adaptation and mutations can occur and can change replication of virus in cells. It is currently unclear whether variants isolated from different countries will behave the same in cell culture since a large study comparison has not been reported. There is evidence that some methods to propagate the virus have led to additional mutations. Pseudovirus assays present several advantages over live virus, which include the speed at which data can be generated after emergence of a new variant, and the lack of reliance upon BSL-3 facilities, and the controlled evaluation of the effect of specific mutations. But limitations are also evident because the pseudovirus may not contain the full suite of mutations or may not function like an authentic virus in every way. So, it is suggested that data from pseudovirus assays should be considered based on a clear understanding of the inherent benefits and limitations of the data. Widely available cell lines should be used such as VeroE6 and VeroE6-TMPRSS2, Calu-3 cells and A549 cells. 8. Has sufficient detail been reported on the methods to replicate the study? 9. Does the study confirm that an appropriate cell line has been used? Investigators may use cell lines which have been shown to be inappropriate for assaying certain classes of monoclonal antibodies.	
3	**Study design description** 10. Are doses administered or concentrations of test substances analysed given? 11. Are frequency and duration of exposure as well as time-points of observations explained? (duration of incubation with virus, duration of assay) Timing of assay readouts should be validated. 12. Have a range of antibody concentrations been tested that are relevant to those needed for neutralisation in serum? A limitation of many in vitro studies is the range of antibody concentrations tested, which are often lower than the average maximum serum concentrations. 13. Were negative controls included? 14. Were positive controls included? 15. Is the number of replicates (or complete repetitions of experiment) given?	**0**

	16. Is the study methodology likely to produce reliable comparison data? For example, have the study investigators utilised an assay calibrated with the WHO International Standard for anti-SARS-CoV-2 immunoglobulin and reporting of neutralisation titres in International Units – an assay useful for standardised comparisons of different monoclonal antibodies against various variants. Testing should be done on an ancestral strain of the virus or reference strain used in a randomised controlled trial in parallel to the variant under investigation.	
4	**Study results documentation** 17. Are the study endpoint(s) and their method(s) of determination clearly described? A 4-paramater, variable slope dose–response analysis has been proposed as the most effective way to determine EC50 and EC90 parameters. Luciferase endpoints for pseudovirus assays and nucleocapsid measurements (anti-N with high content imaging) for authentic live virus have been highlighted as providing reliable readouts. Cytopathic effect (for example, measured by cell titer glo) has been reported to be heterogeneous between different variants studied to date. qPCR readouts have an excellent signal to noise ratio but may not be applicable to pseudovirus assays. 18. Is the description of the study results for all endpoints investigated transparent and complete? 19. Are the outcomes appropriate, and clearly and transparently reported? EC50 and EC90 values should be generated as outcomes from the in vitro testing. 20. Were the study outcomes determined prior to analysis? 21. Are the statistical methods for data analysis given and applied in a transparent manner? 22. Are confidential intervals included? Confidence intervals are important in evaluating the uncertainty of any possible changes in neutralisation; particularly when considering IC90 values, which lie close to the plateau of the dose–response curve and are inherently noisy.	**0**
5	**Plausibility of study design and data** 23. Are the quantitative study results reliable?	**0**
-	Total score	**0**

Based on the total score, studies are allocated to category 1, 2 or 3, as indicated in table 2. Category 1 is assigned if the total score is 20 or more, category 2 is assigned for scores of 16 or more, and category 3 is assigned for scores of 15 or less.

Table 2 Study allocation based on score

Category	Definition
Reliable without restrictions	"Studies or data from the literature or reports which were carried out or generated according to generally valid and/or internationally accepted testing guidelines (preferably performed according to good laboratory practice (GLP)) or in which the test parameters documented are based on a specific (national) testing guideline (preferably performed according to GLP) or in which all parameters described are closely related/comparable to a guideline method."
Reliable with restrictions	"Studies or data from the literature, reports (mostly not performed according to GLP), in which the test parameters documented do not totally comply with the specific testing guideline, but are sufficient to accept the data or in which investigations are described which cannot be subsumed under a testing guideline, but which are nevertheless well documented and scientifically acceptable."
Not reliable	"Studies or data from the literature/reports in which there were interferences between the measuring system and the test substance or in which organisms/test systems were used which are not relevant in relation to the exposure (e.g., unphysiological pathways of application) or which were carried out or generated according to a method which is not acceptable, the documentation of which is not sufficient for assessment and which is not convincing for an expert judgment."

NICE would like to acknowledge the National Australian COVID 19 Clinical Evidence Taskforce who shared an initially adapted version of the TOXRTOOL and devised the categorisation of studies.

Appendix 5: Glossary of terms used

Ancestral: the original strain of SARS-CoV-2 identified in Wuhan.

Cell line: a defined population of cells that can be maintained in culture for an extended period of time and can be used for in vitro experiments.

Conserved epitope: an epitope retained by multiple strains of virus as a key target of a broadly neutralising antibody.

EC50: concentration needed to neutralise 50% of the virus population leaving the remaining 50% of the virus to be able to replicate.

EC90: concentration needed to neutralise 90% of the virus population, with concentration at least 9-fold higher compared with EC50.

Effector functions: when antibodies induce innate and adaptive immune responses beyond neutralisation, including antibody-dependent cellular cytotoxicity.

Epitope: a structure on the surface of an antigen that is recognised by and can bind to a specific antibody.

Immune escape: when the immune system of a host is unable to respond to an infectious agent, such as a virus.

In vitro*:* tests and experiments that researchers perform outside of a living organism in a controlled environment, for example, a test tube or petri dish.

Neutralising monoclonal antibodies: monoclonal antibodies that bind to and neutralise SARS-CoV-2.

Neutralisation curves: graphs in which the y axis is percentage inhibition and the x axis is concentration of drug, with different curves for different variants including ‘ancestral’ line (for example, Delta) and different graphs for each drug.

PK/PD data: a pharmacokinetic and pharmacodynamic model that describes exposure response in vivo.

Receptor-binding domain: a part of the SARS-CoV-2 virus located on its ‘spike’ protein that allows it to dock to body receptors to gain entry into cells and cause infection.