Correlates of Protection, Thresholds of Protection, and Immunobridging among Persons with SARS-CoV-2 Infection

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Several studies have shown that neutralizing antibody levels correlate with immune protection from COVID-19 and have estimated the relationship between neutralizing antibodies and protection. However, results of these studies vary in terms of estimates of the level of neutralizing antibodies required for protection. By normalizing antibody titers, we found that study results converge on a consistent relationship between antibody levels and protection from COVID-19. This finding can be useful for planning future vaccine use, determining population immunity, and reducing the global effects of the COVID-19 pandemic

Determining the relationship between immune response and protection from symptomatic SARS-CoV-2 infection (i.e., COVID-19) is useful for predicting the future effectiveness of vaccines. That relationship should enable immunobridging (i.e., predicting the efficacy of candidate vaccines) that can help with approval of new or updated vaccines based on immunogenicity data, without the need for large phase 3 trials (*1*). Immunobridging is used for approval of seasonal influenza vaccines in the European Union and the United States and reduces the costs and time required to develop vaccines. In addition, defining levels of immunity required for protection from novel SARS-CoV-2 variants will be useful for predicting population-level immunity to infection and guiding public health policy on vaccination and boosting.

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DOI: https://doi.org/10.3201/eid2902.221422

Several studies have shown that higher levels of neutralizing antibody are associated with immune protection from symptomatic SARS-CoV-2 infection during short-term follow-up after vaccination (*2*–*6*). Three of those studies also tried to estimate the level of protection associated with particular antibody levels by using 2 approaches to estimate the relationship between neutralizing antibody levels and vaccine efficacy (*2*–*4*) (protection curve; Table; Figure 1). Although those studies reported threshold antibody levels required for 50% or 70% protection, all found that protection changes gradually with neutralization titer and, thus, there is not a strict threshold below which persons are not protected or above which protection is achieved.

The study of immune correlates by Khoury et al. used a vaccine-comparison approach, which estimated the relationship between mean neutralizing antibody levels (in phase 1/2 trials) and vaccine efficacy (in phase 3 trials) across 7 vaccines and convalescing persons (after first normalizing neutralization titers to convalescing persons in each study) (*2*) (Table; Figure 1, panels A–C). That study estimated that the neutralizing antibody level associated with 50% protection from COVID-19 was ≈20% of the mean titer for persons in the convalescent phase (or 54 IU/mL) (*2*). More recently, 2 studies compared neutralizing antibody titers from persons vaccinated with mRNA 1273 (Moderna, https://www.modernatx.com) or ChAdOx1 (AstraZeneca, https://www.astrazeneca.com) with or without symptomatic breakthrough infection (Figure 1, panels D–F). Those studies reported 70% protective thresholds ranging from 4 to 33 IU/mL (Table), depending on the assay used, suggesting a potential role of assay differences in the discrepancies (Appendix, https://wwwnc.cdc.gov/EID/article/29/2/22-1422-App1.pdf) (*3*,*4*). The apparent discrepancies between studies pose a challenge to the use of protection curves in guiding public health

decisions. Therefore, we studied whether those results can be reconciled by accounting for the different methods used. This work was approved under the University of New South Wales Sydney Human Research Ethics Committee (approval HC200242). All data and codes are available from GitHub (https://github.com/InfectionAnalytics/ReconcilingCorrelatesOfProtection).

Reconciling the Studies on Thresholds of Protection

A major limitation for reconciling thresholds of protection (Table) is lack of a standardized assay for measuring in vitro neutralization titers. Although an international standard has been established (*7*), reported titers seem affected by the assay used, as would be expected from differences in cells, virus, and outcomes measured (*8*). For example, even when neutralization titers are measure against the same stocks of pooled convalescent-phase plasma (e.g., the World Health Organization [WHO] 20/130 standard), different assays produced geometric mean neutralization titers (GMT) that varied from 120 to >12,000 (*7*). Even after standardizing measurements from different assays into international units (Table), standardized neutralization titers across the assays still differed by up to 50-fold (*7*). This difference in neutralization titers across different assays is also evident when comparing the 3 studies quantifying the threshold of protection (Table) (*2*–*4*). For example, Gilbert et al. reported the GMT for mRNA-1273 as ≈247 IU/mL (*4*), compared with 1,057 IU/mL reported by Khoury et al. (*2*) (Appendix). A quick survey of the literature reveals 6 reported estimates of the GMT for mRNA-1273 vaccinees, ranging from 247 IU/mL (95% CI 231–264) to 1,404 (95% CI 795–2,484) IU/mL, depending on the study (Appendix Table 1). Similarly, estimates of the GMT for ChAdOx1 vaccinees ranged from 23 IU/mL (*3*) to 144 IU/mL (*2*). When the same neutralization assay is run across different laboratories, then international standards are probably a very effective mechanism for adjusting for interlaboratory variability. However, it is clear from those discrepancies that expression of titers in international units is insufficient for normalizing between different assays and comparing the thresholds of protection reported in these studies (Appendix), which most likely results from differences in the assays themselves (*8*).

Table. Glossary of terms used in study of correlates of protection for SARS-CoV-2 infection

Term	Definition
Protection curve	The relationship between the measured immune response of a vaccine in a subgroup of persons and the level of protection from symptomatic infection provided by the vaccine in that subgroup compared with placebo group (protection = vaccine efficacy).
Threshold of protection	The level of immune response required to provide a specified level of protection (vaccine efficacy) from COVID-19. The 50% protective threshold is commonly reported.
Fold-of-convalescent scale	An attempt to compare different assays by normalizing titers to that of convalescing persons in the same assay. Accurate comparison requires convalescing persons to have similar infection histories.
IU/mL	A neutralization titer (or mean neutralization titer) calibrated to a World Health Organization international standard and reported in IU/mL.

An alternative approach for normalizing neutralization titers between studies is to assume that similar groups of vaccinees should have similar titers. For example, rather than relying on conversion to the WHO international units, we can assume that the mean neutralization for the mRNA-1273 vaccinees is similar in the phase 1/2 trials (as analyzed by Khoury et al. [*2*,*10*]) and in the phase 3 trial (as analyzed by Gilbert et al. [*4*,*11*]) (Appendix). Normalization is limited because it does not account for differences in baseline characteristics of the cohort vaccinated in each study (e.g., age), which may influence neutralization titers. However, because immunobridging studies also rely on comparing vaccine titers among different groups, this approach is pragmatic for overcoming the limitations of comparing different assays.

Applying this normalization approach enabled us to compare the protection curves across different immune correlate studies (Appendix). We aligned the data by assuming that the mean titer for mRNA-1273- or ChAdOx1-vaccinated persons is the same between the phase 1/2 trials and the phase 3 trials for each vaccine (Figure 2; Appendix). Although this normalization is independent of the x-axis scale used, we plotted both curves onto a fold-of-convalescent level scale (Table) developed by Khoury et al. (*2*) for illustration. This transformation enabled a more direct comparison of the protection curve across the 3 studies. Considering the mRNA-1273 breakthrough-infection model (*4*) (Figure 2, panel A), for example, we saw good agreement with the Khoury et al. model (*2*) at the higher neutralization levels achieved with mRNA-1273 vaccination (albeit a seemingly slightly lower maximum protection level predicted in the breakthrough-infection model) but very poor agreement at low neutralization levels. This finding is easily

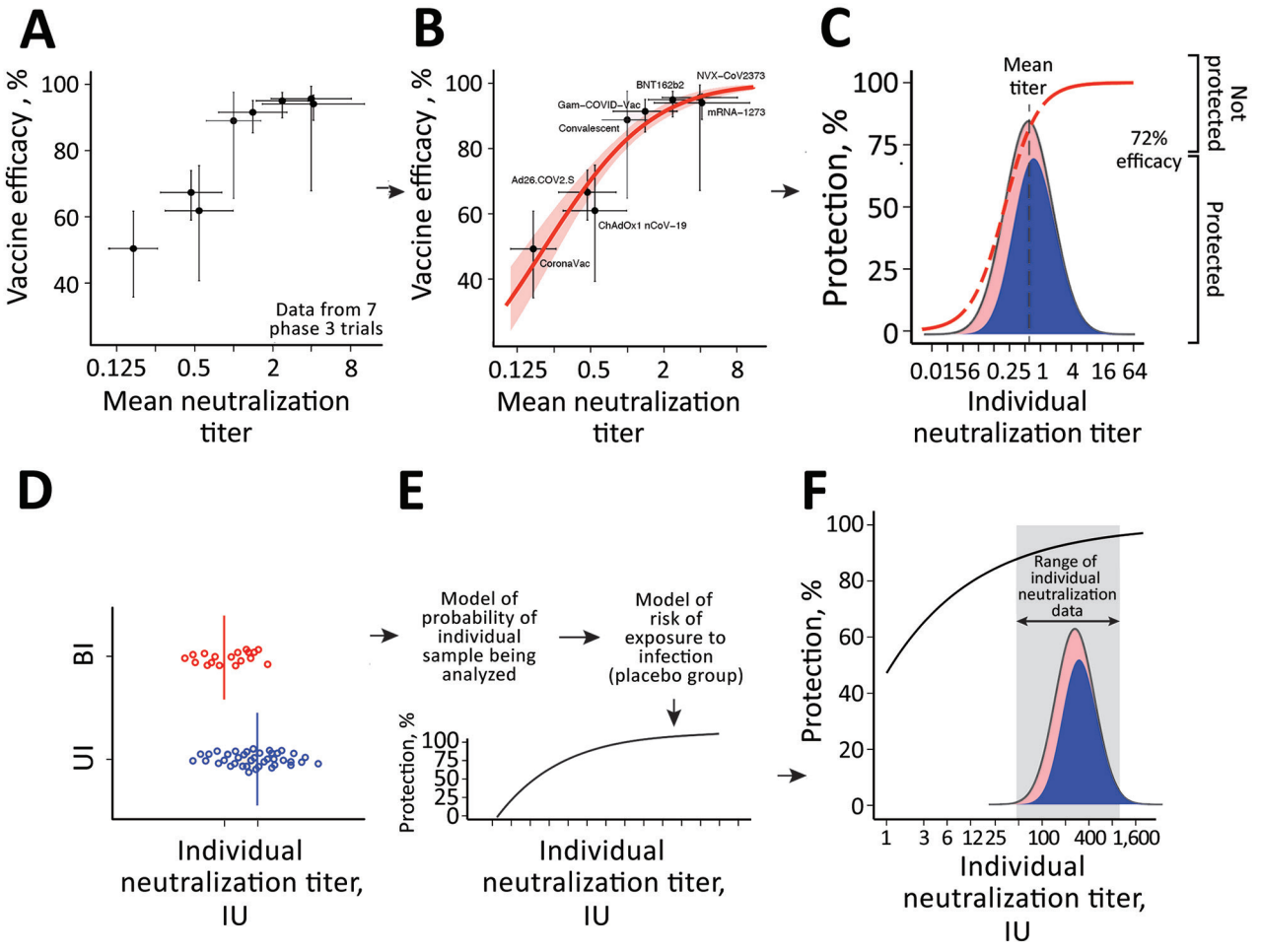

Figure 1. Predicting protection from symptomatic SARS-CoV-2 infection by using approaches to elucidate the relationship between neutralizing antibody titers and protection from COVID-19 (the protection curve): the vaccine-comparison (A–C) and breakthrough-infection (D–F) approaches. The 2 approaches are illustrated schematically: data used (A, D); model fit to data (B, E); and estimated protection (C, F) The vaccine-comparison approach used data on mean neutralization titer from phase 1/2 vaccine trials (normalized to convalescing persons in the same study; x-axis) and observed vaccine efficacy against symptomatic SARS-CoV-2 infection in phase 3 trials (y-axis; n = 7 vaccine trials plus 1 study of infection risk in convalescing persons) (A, B). Using the observed distribution in neutralization titers for a given vaccine and the protection curve, we sum over the whole population to predict the proportion of susceptible (red) or protected (blue) persons for a given vaccine and to estimate protective efficacy for different neutralizing antibody levels (C). Fitting across all vaccines and convalescent persons simultaneously derives the protection curve that best fits the neutralization and protection data (B). The breakthrough-infection model uses neutralization titers of persons with symptomatic breakthrough infections (n = 36 for mRNA-1273 [Moderna, https://www.modernatx.com] and n = 47 for ChAdOx1 [AstraZeneca, https://www.astrazeneca.com]) and uninfected persons (n = 1,005 for mRNA-1273 and n = 828 for ChAdOx1) (*3*,*4*). This method's underlying risk model adjusts for demographic risk factors and for the probability of being sampled in the study to remove these potential sources of bias (E). The protection curve reflects an estimate of the vaccine efficacy in subgroups of persons with specific neutralization titers after the 2-phase sampling design was adjusted for (F). Data and model relationship in panels A and B are from (*2*).

understandable considering the distribution of individual neutralization titers in the mRNA-1273 breakthrough-infection study, in which only ≈10% of participants had a neutralization titer less than the mean titer of early convalescent-phase participants (Figure 2, panel A). Thus, neutralization data with which to estimate protection at lower neutralization levels are sparse (hence, the wide confidence intervals in this region of the curve). Similarly, the ChAdOx1 protection curve (Figure 2, panel B) shows good agreement with the Khoury et al. analysis (2) in the region in which neutralization data are available in the breakthrough-infection study (Figure 2, panel B).

The broad CIs and divergence of the models for which neutralization data are sparse suggests the need for caution when extrapolating the relationship

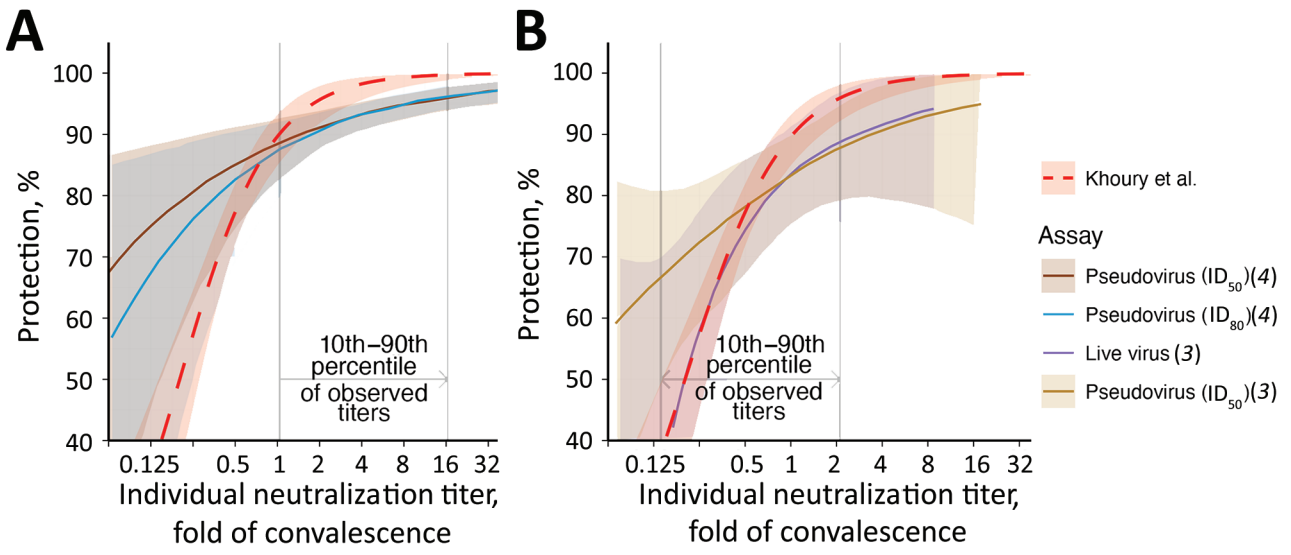

Figure 2. Comparisons of the estimated curves for protection from SARS-CoV-2 infection from 2 vaccines: A) mRNA-1273 (Moderna, https://www.modernatx.com) (*4*); B) ChAdOx1 (AstraZeneca, https://www.astrazeneca.com) (*3*). The relationships between vaccine efficacy against COVID-19 infection (y-axis) and neutralization titers (protection curve) that were estimated in each study (*2*–*4*) are shown. The protection curve derived from the vaccine-comparison model (red dashed line) is compared with the modeled protection curves estimated from breakthrough-infection studies by Gilbert et al. (*4*) (dark brown for the results from the ID_{50} and teal lines for the results from the ID_{80} neutralization titer in in vitro pseudovirus neutralization assays) (A) and Feng et al. (*3*) (purple for the results from in vitro native (live) SARS-CoV-2 virus and light brown for the pseudovirus neutralization assays) (B). Shaded areas indicate 95% CIs from each model. These curves were extracted from the cited studies (Appendix, https://wwwnc.cdc.gov/EID/article/29/2/22-1422-App1.pdf), and differences between assays were controlled for by normalizing the curve from each study by the mean neutralization titer of the uninfected vaccinees in each study. The normalized curves were then represented on a fold-of-convalescent scale by multiplying by the mean neutralization titer of vaccinees compared with convalescing persons as reported in the phase 1/2 trials (*9*,*10*). The vaccine-comparison model agrees closely with the breakthrough-infection models in the neutralization titer ranges where data were most abundant (vertical gray lines indicate 10th to 90th percentiles of the data available in each study). ID_{50}, 50% infectious dose; ID_{80}, 80% infectious dose.

between neutralization and protection beyond the ranges of data available in each study. The vaccine comparison approach has the advantage of fitting to a large span of neutralization titers (a 20-fold range in GMT between the 7 vaccines) (*2*), enabling prediction of the vaccine efficacy over a wide range of neutralization titers. Because none of the reported phase 3 studies of ancestral SARS-CoV-2 infection reported efficacy <50% or >95%, the vaccine-comparison analysis also extrapolates efficacy above and below these levels. However, studies of vaccine efficacy and effectiveness against SARS-CoV-2 variants suggests that the curve remains predictive against the Alpha, Beta, Delta, and Omicron variants, for which lower neutralization titers are observed (*12*; D.S. Khoury et al., unpub. data, https://www.medrxiv.org/content/10.1101/2021.12.13.21267748v2).

The analysis above does not allow direct visualization or comparison of the fit of the data from breakthrough infection to the data from the vaccine-comparison study. We developed a method for estimating unadjusted protection at different neutralization levels from the breakthrough-infection data (Appendix), which also enables inclusion of data from a third breakthrough-infection study of BNT162b2 (Pfizer-BioNTech, https://www.pfizer.com) vaccinees (*5*). We show data from the 3 breakthrough-infection studies compared with the vaccine-comparison approach (normalized for the mean vaccinee titer in each study) (Figure 3). Data from the breakthrough-infection studies show remarkable agreement with the vaccine-comparison model (within the neutralization ranges for which sufficient data were available for each breakthrough-infection study), despite the fundamentally different data, assays, and approaches used to estimate protection curves in each study. Furthermore, after alignment to the GMT of each vaccine group, we can use the underlying distribution in neutralizing antibody titers along with the protection curves from each of these studies to predict the overall vaccine efficacy for existing vaccines (as has been done for the Khoury et al. model [*2*]) (Appendix Methods). That approach reveals good agreement between all models and the observed data, at least in the ranges where data were available to parameterize the models (Appendix Figure 1). This approach provides cross-validation of the protection curves but also provides a lesson that all

models should be used cautiously outside the ranges of the data over which they were developed.

Using the Protection Curve

Immunobridging to Predict Vaccine Efficacy

For vaccine development, an immune correlate to predict the efficacy of a novel vaccine without the need for large and expensive phase 3 efficacy trials would greatly accelerate the approval of novel vaccines (*13*). Similarly, for incorporating novel SARS-CoV-2 variant immunogens, being able to use surrogate measures to predict vaccine efficacy would be helpful. On a public health level, information about neutralization of new variants as they arise and predicting likely population immunity to them would help with predicting future infection risk. In addition, predicting changes in vaccine efficacy with immunity waning and in cohorts with lower neutralization titers after vaccination (e.g., in elderly or immunocompromised persons) could provide information about the need for boosting and other immune protective strategies (*12*).

If a standardized neutralization assay were widely used, it would, in principle, be possible to offer a globally applicable GMT neutralization titer (threshold) associated with a given level of protection, which regulators and vaccine developers could use as a target when assessing and approving vaccines (e.g., as the hemagglutination inhibition titer provides for influenza infection). However, the lack of assay standardization means that no such threshold in international units can be determined that is broadly applicable across different neutralizing antibody assays. Alternatively, regulators have signaled that immunobridging studies, which compare the immunogenicity of new vaccines with that of existing vaccines (for which efficacy has previously

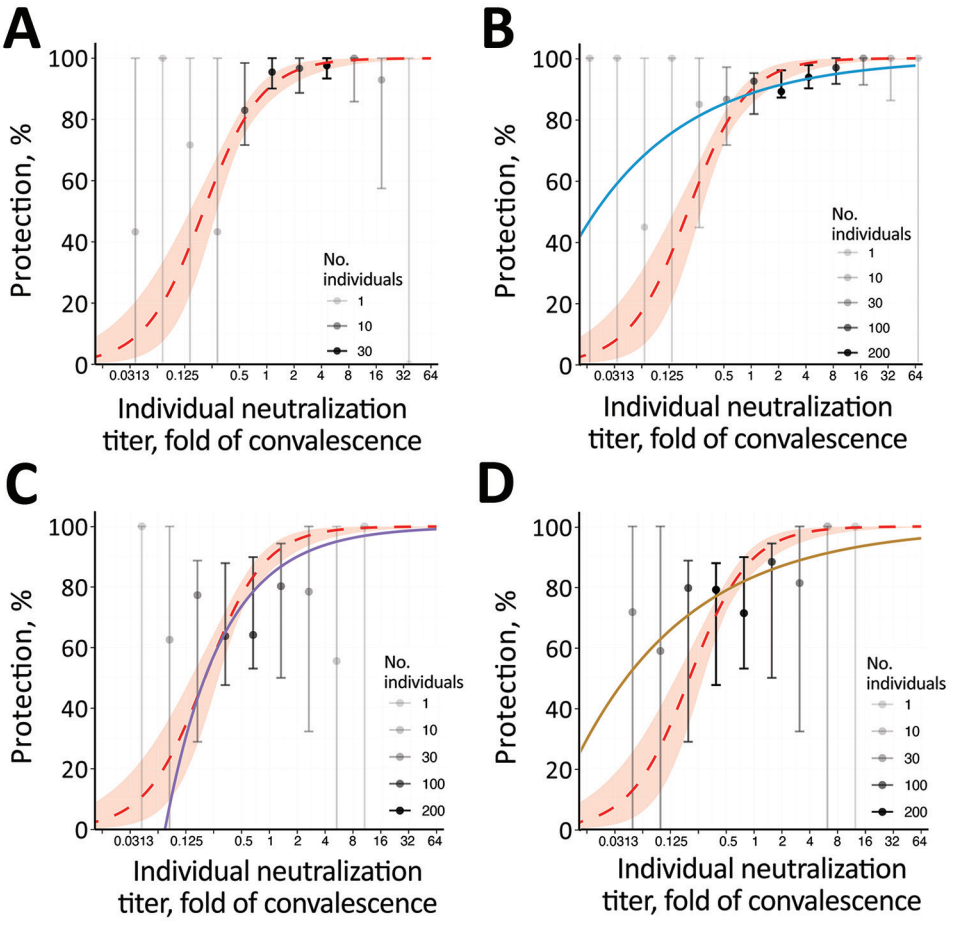

Figure 3. Breakthrough-infection data and protection from SARS-CoV-2 infection showing the association between neutralizing antibody titer and protection from symptomatic SARS-CoV-2 infection for an individual person. A) BNT162b2 (Pfizer-BioNTech, https://www.pfizer.com) (*5*); B) mRNA-1273 (Moderna, https://www.modernatx.com), pseudovirus ID_{50} (*4*); C) ChAdOx1 (AstraZeneca, https://www.astrazeneca.com), live virus (*3*); D) ChAdOx 1, pseudovirus ID_{50} (*3*). The protection curve derived from the vaccine-comparison model (red dashed line and shading 95% CIs) is compared with the observed normalized frequencies of neutralization level (calculations in Appendix, https://wwwnc.cdc.gov/EID/article/29/2/22-1422-App1.pdf) of breakthrough infections reported in 3 studies (gray/black dots). Data from 2 mRNA vaccine studies of mRNA-1273 (A) and BNT162b2 (B), and the adenoviral vector vaccine ChAdOx1 nCoV19 (C, D) are shown. Lower opacity dots indicate fewer persons with neutralization titers in that range. Also shown in each panel are modelled protection curves showing the relationship between individual neutralizing antibodies and protection estimated in each breakthrough-infection study. Note: Breakthrough-infection data of BNT162b2 vaccinees were generously supplied by the authors of reference (*5*). The data were unavailable for the other 2 studies and were extracted from the original manuscripts; extraction of data from Gilbert et al. (*4*) was conducted manually and may be less reliable than that of the other studies (Appendix). ID_{50}, 50% infectious dose; ID_{80}, 80% infectious dose.

been determined) should be conducted (*14*,*15*). That is, vaccine developers need to identify a suitable existing vaccine for comparison and determine the noninferiority or superiority margins relative to these vaccines in a randomized controlled trial (i.e., how much higher neutralization titers are required to be or how much lower titers are permitted to be compared with existing vaccines). The protection curves reported so far (*2*–*4*) can be used to define the parameters of these noninferiority or superiority trials. For example, using the vaccine-comparison model derived by Khoury et al. (*2*) (Figure 1, panel C), we can estimate the noninferiority or superiority margins to existing vaccines that would provide ≥80% efficacy against ancestral virus (Appendix Table 2, Figure 2). If mRNA-1273 or BNT162b2 are used as comparator vaccines, finding a noninferiority margin of 0.44-fold of the GMT observed in mRNA-1273 vaccinees or 0.54-fold of the GMT observed in BNT162b2 vaccinees would provide high confidence that the candidate vaccine has ≥80% efficacy (against ancestral virus). Using ChAdOx1 (with 4-week spacing of doses) as a comparator, we found that a superiority margin of 2.6-fold of GMT compared with ChAdOx1 vaccinees would provide similarly high confidence of ≥80% vaccine efficacy. Of note, those margins are in strong agreement with the lower 95% CIs predicted in the breakthrough-infection studies (Figure 2), which would predict that a candidate vaccine that induced 0.44-fold of the GMT for mRNA-1273 vaccinees would be expected to have an efficacy of ≥85% (either of the 2 neutralization assays reported in that study, on the basis of the reported lower 95% CI) and that a margin of 2.6-fold of the mean ChAdOx1 titer would predict an efficacy of ≥76% (the lower 95% CI of Feng et al. models do not reach 80% in all cases (Figure 2; Appendix) (*3*,*4*). The consensus of these 3 studies provides strong support for using noninferiority or superiority margins in future immunobridging studies.

Identifying Protective Thresholds for Individual Persons

A second goal for the study of protective thresholds is to identify a protective titer for clinical use, that is, a simple blood test for clinically relevant antibody level to indicate if a person is likely to have good protection from COVID-19. The studies that have defined the relationship between neutralization titer and vaccine efficacy have not been designed for, and are not primarily concerned with, defining such a threshold because they deal only with estimates of vaccine efficacy at a population level. Furthermore, individual predictions from population statistics can be fraught with difficulty. Unfortunately, the term "threshold" gives the impression that there might be an antibody level above which one is fully protected (and below which one is susceptible). However, the shapes of the protection curves (Figure 2) make it clear that there is a gradient of risk at different neutralization titers. Moreover, the between-run variability of assays is typically large enough that the uncertainty in the neutralization titer estimated for an individual serum sample is sufficient to lead to wide confidence intervals for the predicted protection for that person (Appendix). For example, when typical duplicate-well and 2-fold serum dilution neutralizing assay designs are used (*16*,*17*), a person with a neutralizing antibody titer at exactly the level associated with 50% protection would have 95% CIs on the estimated protection, ranging from 15% to 85% protection (Appendix), although that range will depend on the precision of a particular assay. It is worth noting that these are estimates of protection from symptomatic SARS-CoV-2 infection (the primary outcome of the studies analyzed), and protection against severe outcomes is achieved at lower neutralization titers (*2*). Together, the wide CIs when estimating individual neutralization titers and the standardization between different serologic assays are major limitations for ability to accurately assess individual neutralizing antibody titers and predict individual protection.

Discussion

Predicting vaccine efficacy or a clinically useful threshold of protection against COVID-19 would be a major advance. The in vitro neutralization titer has been demonstrated by multiple studies to be well correlated with vaccine efficacy and with a person's protection from symptomatic SARS-CoV-2 infection (*2*–*6*,*12*; D.H. Khoury et al., unpub. data). The 4 studies that found significant relationships between neutralization titers and vaccine efficacy used different methods (*2*–*5*), and data from different clinical trials with neutralization data assessed across a range of neutralization assays. Those factors may all contribute to apparent discrepancies between the relationships reported in each study. However, we show that after centering the data from each study on the GMT of the vaccine used in each study, the 4 studies converge on a common prediction of the relationship between neutralization and protection against infection (within the bounds of data available within each study). The agreement of these studies strongly supports the use of neutralizing antibody titers to predict the efficacy of new vaccines or vaccine efficacy against new variants (assuming the fold drop in neutralization titer for the variant can be estimated). Although neutralizing antibody levels are a clear correlate of protection, identifying a protective threshold applicable to a serologic test is more challenging, in part because no such threshold exists, but instead, there is a

gradient of vaccine efficacy that increases with neutralization. Furthermore, significant challenges to defining a particular threshold at which a person's neutralization titer might be deemed to provide high protection from COVID-19 include the diversity of assays used to measure neutralization, the difficulty in translating neutralization levels between assays, the constant emergence of new and more escaped variants, and the uncertainties of estimating individual neutralization titers.

An additional major challenge is adapting assays (and protection curves) to deal with neutralization of current and future SARS-CoV-2 variants. The studies discussed in this analysis primarily deal with neutralization of and protection from the ancestral SARS-CoV-2 strain because the breakthrough-infection data and vaccine efficacy data in most studies was from phase 3 clinical trials (*2*–*4*), which studied infection within the first few months after vaccination and which were mainly conducted before variants of concern had a major foothold, except for the Bergwerk et al. study (*5*), which was conducted during in the Alpha-dominant period. It would be ideal to be able to adapt each model of immune correlates to test its ability to predict protection against variants of concern. However, until recently, only the vaccine-comparison model has been extended to analyze protection against SARS-CoV-2 variants (*12*,*18*,*19*; D. Cromer et al., unpub. data, https://www.medrxiv.org/content/10.1101/2022.06.09.22275942v1), although a recent study has begun to explore this question by using a breakthrough-infection approach (*20*). The work on the vaccine-comparison model approach has so far shown that this model, which was originally calibrated on data for ancestral SARS-CoV-2 infections, can also be used to predict vaccine effectiveness against SARS-CoV-2 variants and after boosting, as long as one adjusts for the drop in neutralization titers to the variants and rise in neutralization after boosting (*12*,*18*,*19*; D. Cromer et al., unpub. data). However, the need to standardize neutralization assays for SARS-CoV-2 variants presents an ongoing challenge.

In vitro neutralizing antibody titers against SARS-CoV-2 present a clear correlate of protection from symptomatic SARS-CoV-2 infection. Studies of passive administration of neutralizing monoclonal antibodies in animals and humans support that neutralizing antibody titers are a mechanistic correlate of protection (*21*–*23*). Indeed, a recent study comparing protective titers in prophylactic and therapeutic studies suggests that the protective titers may be very similar (E. Stadler et al., unpub. data, https://www.medrxiv.org/content/10.1101/2022.03.21.22272672v2). Neutralizing antibody levels are also correlated with protection from severe SARS-CoV-2 infection (*2*).

In conclusion, our findings show that the different COVID-19 correlate of protection studies, which seemingly report different thresholds of protection, have strong agreement. However, other immune responses may also play a substantial role in protection against progression from symptomatic to severe SARS-CoV-2 infection. The agreement across multiple studies of the relationship between neutralizing antibodies and efficacy against COVID-19 can be useful for planning future vaccine use, determining population immunity, and reducing the global effects of the COVID-19 pandemic.

Acknowledgments

We thank Moriah Bergwerk, Tal Gonen, Gili Regev-Yoshay, and colleagues for supplying the raw data from their study of neutralization titers in BNT162b2-vaccinated healthcare workers (*5*).

This work is supported by Australian government Medical Research Future Fund awards GNT2002073 (M.P.D. and S.J.K.), MRF2005544 (S.J.K. and M.P.D.), MRF2005760 (M.P.D.), and MRF2007221 (J.A.T. and M.S.); a National Health and Medical Research Council (NHMRC) program grant GNT1149990 (S.J.K. and M.P.D.); and the Victorian Government (S.J.K.). D.S.K. and S.J.K. are supported by NHMRC fellowships. D.C. and M.P.D. are supported by NHMRC Investigator grants. The Melbourne WHO Collaborating Centre for Reference and Research on Influenza is supported by the Australian Government Department of Health.

The funding sources had no role in the writing of the manuscript or the decision to submit it for publication or in data collection, analysis, or interpretation or in any aspect pertinent to the study. Authors were not precluded from accessing data in the study, and they accept responsibility to submit for publication

D.S.K., D.C., M.S., K.S., S.J.K., J.A.T., and M.P.D. contributed to the conceptual development and data collection. D.S.K., T.E.S., D.C., Y.F., P.B.G., and M.P.D. contributed to the methods, formal analysis, and visualization. All authors contributed to the writing and reviewed and approved the final report. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

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Article DOI: https://doi.org/10.3201/eid2902.221422

Correlates of Protection, Thresholds of Protection, and Immunobridging among Persons with SARS-CoV-2 Infection

Appendix

Supplementary Methods and Analysis

Conversion of Khoury et al., fold-convalescence scale to WHO international units (IU)

The geometric mean neutralization titer in convalescent subjects at ≈30 day post symptom onset in a cohort of 56 individuals described in (*1*) (and used in ref [*2*]) was measured (considering censoring [*1*]) as 55.7. In the same laboratory, the same microneutralization assay was performed on the international standard 20/130, reported to equate to 1300 IU/ml (95% CI: 981-1719) (*3*). The assay was performed on the standard 29 times, with a geometric mean neutralization titer of 272 (95% CI: 234-316). Therefore, the geometric mean neutralization titer of convalescent subjects is given by $55.7 \times 1300/272 = 266$ IU/ml, and the 50% protective titer calculated in (*1*) is $20.11\% \times 266/100 = 53.5$ IU/ml.

In Khoury et al. (*1*), using data from Phase 1/2 trials, the mRNA-1273 and ChAdOx1 nCoV-19 vaccines were estimated to have geometric neutralization titers of 4.1-fold and 0.54-fold of the geometric mean titers of convalescent individuals, respectively. Therefore, in WHO international units these geometric mean neutralization titers for each vaccine equate to $266 \times 4.1 = 1099$ IU/ml for mRNA-1273 and $266 \times 0.54 = 144$ IU/mL for ChAdOx1 nCoV-19.

The role of differing neutralization titers assays in the different threshold of protection reported in the literature

Three of these studies have estimated the level of protection associated with particular antibody levels and reported a ‘threshold’ antibody level required for 50% or 70% protection from symptomatic SARS-CoV-2 infection (*1*,*4*,*5*). There was not agreement between all of these studies, with some of the three studies yielding quite different estimates of the protection curves

and thresholds of protection from symptomatic infection, especially when different assays were used to assess neutralizing antibody titers. This is a critical issue for the field to reconcile in order to move forward with a defined correlate of protection. Khoury et al. used a meta-analysis of Phase 1/2 and Phase 3 vaccine trials (vaccine comparison approach, main article Figure 1) and estimated that 50% vaccine efficacy was achieved with a neutralization titer of 54 IU/ml (in a live virus neutralization assay). More recently, two studies used data from subjects with 'breakthrough infection' after antibody responses were measured following two doses of mRNA 1273 (n= 36) or ChAdOx1 nCoV19 (n= 47) vaccination to model the protection curve (main article Figure 1, panels D-F). Across these studies both pseudovirus and live SARS-CoV-2 neutralization assays were used to measure neutralizing antibodies. Interestingly, the estimated curves using data from the same pseudoviral neutralization IC50 assay yielded similar 70% protective threshold of 4 IU/ml and 8 IU/ml, respectively (*4*,*5*) (Appendix Figure 3). However, Feng et al. used both a pseudovirus neutralization assay and a live SARS-CoV-2 neutralization assay, and showed that the latter yielded a 70% protective titer that was >4-fold higher than the pseudovirus assay (in international units) (*4*). Given the large variance in neutralizing antibody reports for notionally similar groups of vaccinated individuals when different assays are employed, even after conversion to IU, it is perhaps unsurprising that similar assays yield more similar results once converted to IU, but it would appear that the different assays contribute directly to different estimated titers required for the same level of protection (even within the same study using the same breakthrough infections).

Normalizing neutralization titers between studies.

To normalize neutralization titers between the vaccine comparison study (*1*), and the breakthrough infection studies (*4–6*) we assumed that the geometric mean neutralization titer (all references to "mean of neutralization titers" in this text refer to the geometric mean) of individuals vaccinated with a particular vaccine should be equivalent across studies. That is,

$$\hat{n}_v^s = \frac{n^s}{\bar{n}_v^s}$$

where n^s is a given neutralization titer or geometric mean neutralization titer reported in a study s, $\bar{n}_v^s$ is the geometric mean neutralization titer of a vaccine v reported in the study s and

$\hat{n}_v^s$ is the corresponding normalized neutralization titer or geometric mean neutralization titer, after normalization to the geometric mean vaccine titer for vaccine v from study s.

For example, when comparing neutralization data from the Khoury et al. and Gilbert et al. studies, we noted that in the Gilbert et al. study only mRNA-1273 vaccinees were considered, and in the uninfected mRNA-1273 vaccinees the mean neutralization titer reported for the ID50 assay was 247 IU/ml. Similarly, in the Khoury et al. study the mean neutralization titer for mRNA-1273 vaccinees was taken from (*7*) and was estimated as 4.1-fold of the mean of convalescent individuals analyzed in the same study. Further, we assume that neutralization titers across the different assays in the different studies will vary colinearly. Thus, to align all ID50 measurements and curves in the Gilbert et al. and Khoury et al. studies we divided all the titers by the GMT of 247 IU/ml (for Gilbert et al.) and 4.1-fold (for Khoury et al.). In addition, for visualization, we place the ID50 data from the Gilbert et al. study on the fold-of-convalescence scale reported in Khoury et al., by multiplying these aligned curves by 4.1. In the case of Feng et al. and Bergwerk et al., the same approach was used but using the geometric mean neutralization titer for ChAdOx1 nCoV-19 and BNT162b2, respectively (Feng et al. reported the median neutralization titer which was used in place of the geometric mean).

It is important to note that the above normalization assumes that the mean neutralization titer in the Phase 1/2 studies of each of these vaccines (*7–9*) (used in the Khoury et al.) is approximately equivalent to the mean neutralization titer for the corresponding vaccine in the breakthrough-infection studies (*4–6*). Of note is that vaccination schedules were not equivalent for ChAdOx1 nCoV-19 vaccinees in the Phase 1/2 trial (4 week schedule) and the Feng et al. study (a mixture of prime-boost schedules of between <6 week and >12 week), where dose spacing was altered from the intended schedule due to supply issues (*10*). The mixture of dosing schedules is likely to make the mean neutralization titers differ between the studies. We assessed the impact of this difference using data reported in the meta-analysis of these neutralization results from a large number of the Phase 3 trial participants (*11*). Performing a weighted average of the neutralization titers of the whole population from this meta-analysis (which resembles the cohort in the Feng et al. study) we found the mean neutralization titer was only 1.40-fold higher than the average of vaccinees with doses spaced by <6 weeks (*11*) (i.e. the <6 week spacing is comparable with the Phase 1/2 trial cohort dosing regimen [*9*]). This demonstrated that differences in dosing schedules will only have a minor impact on the overall normalization of the

Feng et al. study with the Khoury et al. study. Thus, in the main text we use the raw mean neutralization titers from the Phase 1/2 and Phase 3 trials for simplicity.

Extracting the model relating neutralization and protection

In the Gilbert et al. (*5*) and Feng et al. (*4*) studies a mathematical model was used to estimate the relationship between neutralization titer and protection. This relationship was modelled for two different neutralization assays per study and the final model and confidence intervals were extracted from Figure 4 in (*4*) and Figures 4 and S23 in (*5*) using the WebPlotDigitizer online application (https://automeris.io/WebPlotDigitizer). These extracted models were normalized to a fold-of-convalescence scale (as described above) and plotted in main article Figure 2 against the fitted model reported in Khoury et al (*1*).

Data on breakthrough infection

Data was requested from the authors of (*4–6*). Raw data were provided by the authors for (*6*). Data were unavailable for the other two studies, and therefore were extracted from the published work. Data were extracted using Adobe Illustrator (by saving figures in an SVG format and using a text editor to extract coordinates of datapoints from the vector graphic images contained in the publication) from Extended data Figure 2 in (*4*), and the WebPlotDigitizer online application (https://automeris.io/WebPlotDigitizer/) from figure S10 in (*5*). The neutralization titers of the uninfected vaccinated groups and the symptomatic breakthrough infections groups were extracted. Neutralization data on control and breakthrough infections from (*6*) were provided by the authors.

Calculating the unadjusted protection curve from breakthrough infection data to compare with the fitted models

In the three breakthrough infection studies reported (*4–6*), two groups of individuals are considered, vaccinated individuals with breakthrough infection and vaccinated individuals without breakthrough infection. Importantly, their uninfected status is not necessarily due to vaccine protection but in many cases will reflect simply that those individuals were not exposed to COVID-19. Hence the Gilbert et al., and Feng et al., studies used an additional risk model to characterize and account for the unvaccinated placebo arm's rate of symptomatic SARS-CoV-2 infection. A challenge with the breakthrough infection studies is visualizing the data and the risk of symptomatic infection at different neutralization titers. Here we use the neutralization data reported in these three breakthrough infection studies to visualize an unadjusted protection curve

(main article Figure 3). These unadjusted protection curves were calculated by assuming the following:

1. Control data on the neutralization titers of individuals who received the vaccine but were not infected, provides a reasonable approximation of the distribution of neutralization titers in individuals who were vaccinated (independent of breakthrough infections). Despite control populations inherently excluding individuals who experienced breakthrough infections, as the proportion of breakthrough infections is small (<7%), any bias resulting from this assumption is likely to be small.

2. Let i and e be the events of an individual being infected, and an individual being exposed to SARS-Cov-2 (in a manner that would cause infection in an unvaccinated individual) respectively. $P(i)$ and $P(e)$ denotes the probabilities of these events. Then

$$P(i) = P(e)(1 - E)$$

where E is the vaccine efficacy. Therefore,

$$\frac{P(i)}{P(e)} = 1 - E.$$

3. The probability of exposure is independent of neutralization titer, i.e.

$$P(e \cap n) = P(e)P(n)$$

Given the above, we are primarily concerned with calculating the probability that an individual becomes infected given that they are exposed and have a given neutralization titer (n). Denote an individual's risk of becoming infected given that they are exposed and have a neutralization titer (n) as, $P(i|e \cap n)$, therefore:

$$P(i|e \cap n) = \frac{P(i \cap e \cap n)}{P(e \cap n)} = \frac{P(i \cap e \cap n)}{P(e)P(n)} \quad (1)$$

We note, using conditional probability, and noting that the probability an individual was exposed if they were infected is 1, i.e. $P(e \cap i) = P(i)$:

$$P(n|e \cap i) = \frac{P(i \cap e \cap n)}{P(e \cap i)} = \frac{P(i \cap e \cap n)}{P(i)} \quad (2)$$

Combining equations 1 and 2 above, and using assumption 2, it follows that:

$$P(i|e \cap n) = \frac{P(i)P(n|e \cap i)}{P(e)P(n)} = (1 - E)\frac{P(n|i)}{P(n)} \quad (3)$$

Therefore, the probability that an individual who is exposed becomes infected, given that they have a neutralization titer (n), is equal to the ratio of the probability that a breakthrough infection (Case) has a neutralization titer (n), divided by the probability an uninfected vaccinee has a neutralization titer n, and this is multiplied by 1 minus the vaccine efficacy. Using the neutralization data reported in each of the three breakthrough infection studies, we performed binning of neutralization data into bins spaced by 2-fold titers, and for each neutralization bin (corresponding to a neutralization titer n), we calculated the fraction of individuals with each neutralization titer n who had a breakthrough infection (i.e. $P(n|i)$) and the fraction of individuals in the control vaccinee group (i.e. $P(n)$). Since the breakthrough infection data for Gilbert et al. and Feng et al., were taken from randomized placebo-controlled Phase 3 trials, the vaccine efficacy, E, within these specific populations was determined in the papers and risk model themselves (for Gilbert et al., efficacy was reported in figure 4 of the paper and for Feng et al. the overall efficacy was back-calculated using the model reported in this study using the approach described in *Immunobridging: Predicting the efficacy of another vaccine*), and for the Bergwerk et al. study an observational study of the vaccine efficacy from the same region with overlapping calendar time was used for an estimate of the vaccine efficacy in that setting (*12*) (Appendix Table 5). These unadjusted protection curves were normalized to the fold-of-convalescence scale in the same way as described earlier, to generate main article Figure 3.

The confidence intervals of these unadjusted estimates of protection were determined by parametric bootstrapping of the neutralization titers. That is, we first fitted the (extracted or provided) neutralization data for individuals with breakthrough infection ("cases", assume n_{case} number of individuals) and uninfected vaccinated individuals ("uninfected-vaccinated", assume $n_{uninfected}$ number of individuals) with a normal distribution using censoring regression (*1*). These fitted distributions of the neutralization titers for cases and uninfected-vaccinated from each study were then sampled randomly n_{case} and $n_{uninfected}$ times, respectively, and the

resulting data were used to recalculate the unadjusted protection of individuals within each 2-fold range of neutralization titers (as describe above). This was repeated 10,000 times for each study, and the 2.5th and 97.5th percentiles of the 10,000 estimates of the unadjusted protection were used to estimate the confidence intervals. Some iterations produced missing values for the vaccine efficacy estimate because by random sampling some ranges of neutralization titers had no uninfected-vaccinated individuals – in this case we did two things, we excluded the missing iterations from the calculation of the confidence intervals and we also set these missing values to an extreme estimate of 1 or 0 (i.e. 0% protection or 100% protection) and recalculated the 95% CIs. We then took the maximum of these two approaches as the Upper bound of the 95% CI, and the minimum of these two approaches as the Lower bound of the 95% CI.

Estimating the standard deviation of neutralization titers

For the Feng et al. study, raw neutralization titers could be precisely extracted and in this case censoring regression was used to fit a normal distribution to the log-transformed data to estimate the standard deviation of ($\log_{10}$) neutralization titers from both assays reported in that study (*4*). However, for the Gilbert et al. study, the raw data were not available and so the standard deviation of the neutralization titers were calculated from the confidence interval reported for the means in table 1 of (*5*). This was performed as follows:

$$SD_1 = \big(log10(M) - log10(L)\big) \times \frac{\sqrt{1005}}{1.96}$$

$$SD_2 = \big(log10(U) - log10(M)\big) \times \frac{\sqrt{1005}}{1.96}$$

$$\sigma = \frac{SD_1 + SD_2}{2}$$

where, M is the geometric mean titer of the uninfected vaccinated population, L and U are the lower and upper bounds of the 95% CI of the mean ($\log_{10}$) neutralization titer for the uninfected vaccinated population and SD_1 and SD_2 are two estimates of the SD from the lower and upper bounds of the 95% CI, respectively. Note that neutralization titers for 1005 uninfected individuals vaccinated with mRNA-1273 were reported in this study.

We found that the standard deviation of the neutralization titers were close (Appendix Table 3, range of 0.41-0.47, across the different neutralization markers measured in the different

two studies) to the standard deviation of neutralization titers estimated by Khoury et al. (*1*), when all neutralization titers from all phase 1/2 studies were pooled (0.46).

Immunobridging: Predicting the efficacy of another vaccine (generating Appendix Figure 1)

Using the models from Gilbert et al. and Feng et al., that estimate the relationship between neutralization and an individual's protection from symptomatic SARS-CoV-2 infection it is possible to predict the efficacy of another vaccine if one knows the distribution of neutralization titers induced by that vaccine. To calculate the predicted vaccine efficacy for a vaccine V, using each of the published models, one approach is based on the following formula:

$$PE(V) = \int_{m}^{M} V(n)P(n)\,dn$$

where, $V(n)$ is the empirical probability density of $\log_{10}$ neutralization titers induced by the vaccine V, and $P(n)$ is the estimated protection (i.e. equivalent to the "vaccine efficacy" or "controlled vaccine efficacy" curves as reported in Feng et al. and Gilbert et al., respectively) for a given $\log_{10}$ neutralization titer as reported in the models published in (*4*,*5*). M and m are the maximum and minimum $\log_{10}$ neutralization titers. The estimated protection (vaccine efficacy) level given a $\log_{10}$ neutralization titer (n) is given in the Gilbert et al. and Feng et al. studies by combining multiple methodologies, including inverse probability weightings, a Cox regression model (with an estimate of the baseline hazard) (curves in figure 4, S23 and figure 4 of those studies, respectively). However, under the causal assumptions of (*13*) (no unmeasured confounders and positivity), with a simplified approach that does not control for baseline covariates, the combined functional form of this model can be written as

$P(n) = 1 - c(1 - e^{-ae^{-b\,n}})$ (4).

We estimated values for *a, b* and *c* by fitting the curve in equation 4 to points extracted from Figure 4, S23 and Figure 4 of (*5*) and (*4*), respectively. Fitting was performed using a standard least squares approach, to the natural log of the extracted values (extraction described above) (Appendix Figure 4 and Appendix Table 4).

For any vaccine that induces approximately normally distributed neutralization titers (mean μ, standard deviation σ_s) we can then estimate the predicted vaccine efficacy (PE(μ)) using the Gilbert et al. and Feng et al. models of protection as:

$$PE(\mu) = \int_{-\infty}^{\infty} N(n|\mu, \sigma_s) \left(1 - c(1 - e^{-ae^{-bn}})\right) dn, (5)$$

where N is the probability density function of a normal distribution with mean μ and standard deviation σ_s. Note that the log-transformed neutralization titers in Feng et al., and Gilbert et al. appear approximately normally distributed (Appendix Figure 5).

Using equation 5 and the estimated parameters in Appendix Table 3 and 4, we calculated the efficacy of other vaccines that would be predicted by the models in the Gilbert et al. and Feng et al. studies (Appendix Figure 1).

Estimating non-inferiority or superiority margins that will give high confidence of at least 80% efficacy for a candidate vaccine

It is useful for regulators to be able to define minimum criteria for vaccine developers to meet in order to define an effective new agent based on neutralizing antibodies. However, given assay variability it is not possible to define a particular neutralizing antibody titer that should be achieved by a new vaccine in order for it to have a certain vaccine efficacy. Instead, direct comparison of a new candidate vaccine against an existing comparator vaccine in a non-inferiority or superiority trial will be a more robust approach. Here we estimate what difference in geometric mean neutralization titers between a candidate vaccine and an existing vaccine is acceptable/necessary in order for there to be a high confidence the candidate vaccine has at least 80% efficacy. This analysis can also be adjusted to report superiority / non-inferiority margins for other efficacy thresholds.

Using the model reported by Khoury et al. (*1*), we can predict vaccine efficacy for a given geometric mean neutralization titer normalized to the mean of a convalescent panel, but there are a number of sources of uncertainty. In particular, the mean neutralization titer of the comparator vaccine has uncertainty (we consider three comparator vaccines here BNT162b2, mRNA-1273 and ChAdOx1 nCoV-19), and the model itself had uncertainty (see 95% CI's in main article Figure 2). Thus, for a given reference vaccine with a mean ($\log_{10}$) neutralization titer m, and a given fold-change in the geometric mean neutralization titer of a new candidate vaccine compared with that reference vaccine (10^d), we compute the lower 95% confidence bound of the estimated vaccine efficacy for the new vaccine with, using the model reported by (*1*):

$$VE(m) = \int_{-\infty}^{\infty} N(n|m + d, \sigma) \frac{1}{1 + e^{-k(n - n_{50})}} dn$$

where, m is the $\log_{10}$ of the neutralization titer (on a fold of convalescence scale) of the reference vaccine, n_{50} is the ($\log_{10}$) neutralization titer estimated to give 50% VE, k is the slope parameter relating neutralization and vaccine efficacy, and N is the probability density function of a normal distribution representing the distribution in neutralization titers induced by a given vaccine mean ($\log_{10}$) neutralization titer of m and standard deviation of ($\log_{10}$) neutralization titers of σ. To compute the lower 95% confidence bound from this model, we use parametric bootstrapping (as in [*14*]). Briefly, we estimate the vaccine efficacy using the above model 50,000 times, after sampling the model parameters at random from a normal distribution to capture the uncertainty in these parameters. The normal distributions used for randomly sampling the parameters are assumed to have means given by the parameter estimates and standard deviations given by the standard error (and covariance matrix for jointly distributed parameters) of the estimated parameters n_{50}, $\log(k)$ and σ obtained during model fitting in the original study (*1*). Given that the mean neutralization titer of the reference vaccine (m) also contains uncertainty, we similarly, draw this parameter randomly using the standard error in m estimated in the original study (*1*) (horizontal confidence bands, in Appendix Figure 2). Of the 50,000 repeated estimates we then take the lower 5th percentile of these bootstrapped predictions to estimate the one-tailed lower 95% confidence interval of candidate vaccine efficacy given the fixed change in neutralization titer (shaded region in Appendix Figure 2). We then find the change in neutralization titer of the novel agent compared to the existing comparator vaccine that will provide a lower bound on the vaccine efficacy confidence interval of 80% (Appendix Figure 2, Appendix Table 2). Thus, as long as a candidate vaccine is shown to have a fold change neutralization titer compared to the comparator vaccine that is no less than this margin in a non-inferiority trial (or more than this margin in a superiority trial), then the vaccine efficacy of the candidate vaccine has a high confidence of being above 80%.

Estimating assay variability for predicting an individual's level of protection

The 29 replicate estimates of neutralization on the same standard (i.e. WHO international standard 20/130) allow quantification of the precision of an assay's estimate for an individual's neutralization titer. The standard deviation of the $\log_{10}$ neutralization titers measured in these 29 technical replicates of the 20/130 standard was 0.41 in the above described assay. Assuming an individual has a true neutralization titer of N, the chance of observing a neutralization titer n for that individual on repetition of the neutralization assay is described by a normally distributed

random variable with mean N and standard deviation 0.41. Therefore, the measurements of the mean of the duplicate assay for such an individual will follow a normal distribution with mean N and standard deviation $0.5 \times \sqrt{2 \times 0.41^2} = 0.29$. It follows that the observed neutralization titer of an individual with a true neutralization titer of N is expected to fall within the range $(N \times 10^{-z_{crit}}, N \times 10^{z_{crit}})$ for 95% of observations, where z_{crit} is the 2.5th percentile of the normal distribution with mean 0 and standard deviation 0.29. These data are used in the main text to determine the precision in the estimated efficacy of an individual given they are observed to have a particular neutralization titer.

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Appendix Table 1. Variation in geometric mean neutralization titers (reported in IU/mL): Summary of different studies identified that report the mean neutralization titer of a group of mRNA-1273 vaccinees using different assays, and which have all been calibrated to international units (IU/mL). The range of estimates is 247-1404 IU/mL, highlighting that even after calibrating assays to the WHO established international standards discrepancies remain between laboratories and assays

Study	Cohort size	Timing (gender)	Median Age (range)	Assay	GMT (IU/ml)	Reference
Gilbert et al.	n=1005	28 days post second dose (47% Female)	55 (18- 87)	Pseudo-neutralization (50% inhibition)	247	(*5*)
Huang et al.	N=30	28 days post second dose	NR	Pseudo-neutralization (2 methods used, Duke and Monogram) (50% inhibition)	480 or 275 (Duke and Monogram respectively)	(*15*)
Khoury et al. / Jackson et al.	n=15	14 days post second dose, (53% Female)	31 (18-55)	Live-virus neutralization	1,057	(*1*,*7*)
Garcia-Beltran et al.	n=24	within 3 months of 2nd dose (71% Female)	54 (24-72)	Pseudo-neutralization (50% inhibition)	1,362	(*16*)
Zhang et al.	n=30	Within the first 28 days post second dose (60% Female)	44 (NR)	Pseudo-neutralization (50% inhibition)	1,399	(*17*)
Kung et al.	n=20	14 days post second dose	NR (22-69)	Live-virus neutralization	1,404	(*18*)

Appendix Table 2. Estimated margins for non-inferiority (or superiority) trials which seek to compare a new vaccine candidate against an existing comparator vaccine. The model reported by Khoury et al. (*1*) predicts that as long as the difference in neutralizing antibody titer between the comparator and reference vaccine is not less than (or is greater than, in the case of a superiority trial) the margin reported here, then the efficacy of the new candidate is predicted to have a lower bound 95% CI of 80%

Comparator Vaccine	Non-inferiority or Superiority trial	Margin predicted to give at least 80% efficacy (fold-change in the GMT of candidate compared to the comparator vaccine)
BNT162b2	Non-inferiority	0.54
mRNA-1273	Non-inferiority	0.44
ChAdOx1 nCoV-19	Superiority	2.6

Appendix Table 3. Estimates of SD of neutralization data from each study. #Gilbert et al. (*5*) using the confidence intervals of the reported mean neutralization titer and Feng et al. (*4*) from censored regression

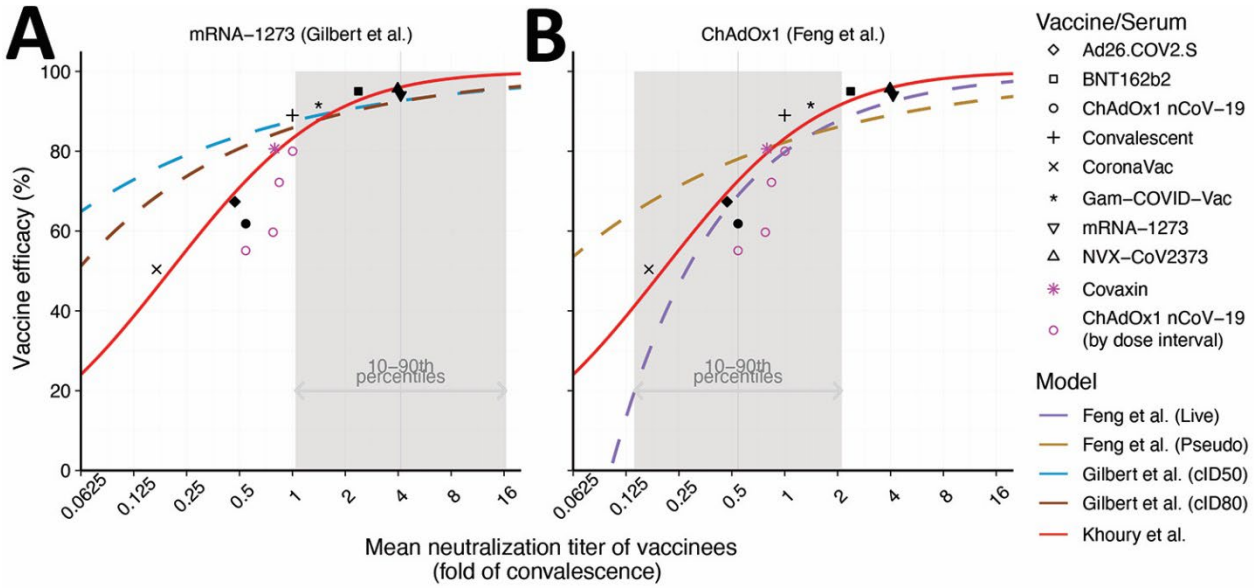
Study	Assay	Estimated standard deviation of (log_{10}) neutralization titers in uninfected vaccinees#
Gilbert et al.	ID50 (Pseudovirus)	0.47
Gilbert et al.	ID80 (Pseudovirus)	0.43
Feng et al.	ID50 (Pseudovirus)	0.46
Feng et al.	NF50 (Live virus)	0.41

Appendix Table 4. Estimated parameters for equation 4 from fitting risk model in Appendix Figure 4

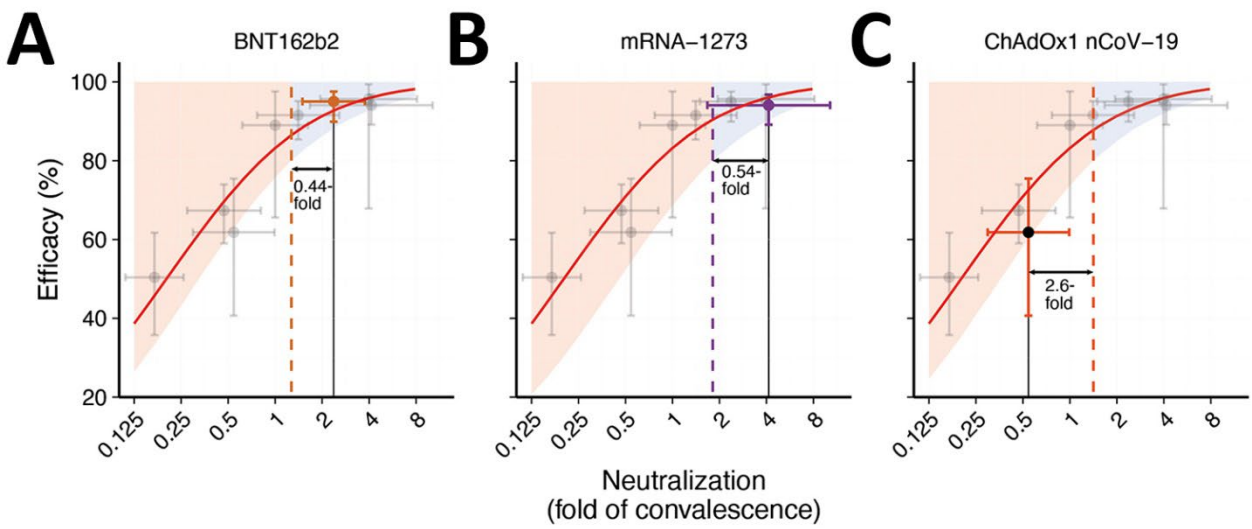
		Parameters		
Study	Assay	a	b	c
Gilbert et al.	ID50 (Pseudovirus)	3.3×10^{-3}	1.0	3.3×10^{2}
Gilbert et al.	ID80 (Pseudovirus)	2.3×10^{-3}	0.86	2.3×10^{2}
Feng et al.	ID50 (Pseudovirus)	2.5×10^{-3}	0.80	2.5×10^{2}
Feng et al.	NF50 (Live virus)	9.8×10^{-3}	1.2	9.8×10^{2}

Appendix Table 5. Efficacies used for each study to calculate unadjusted protection curves from breakthrough infection data

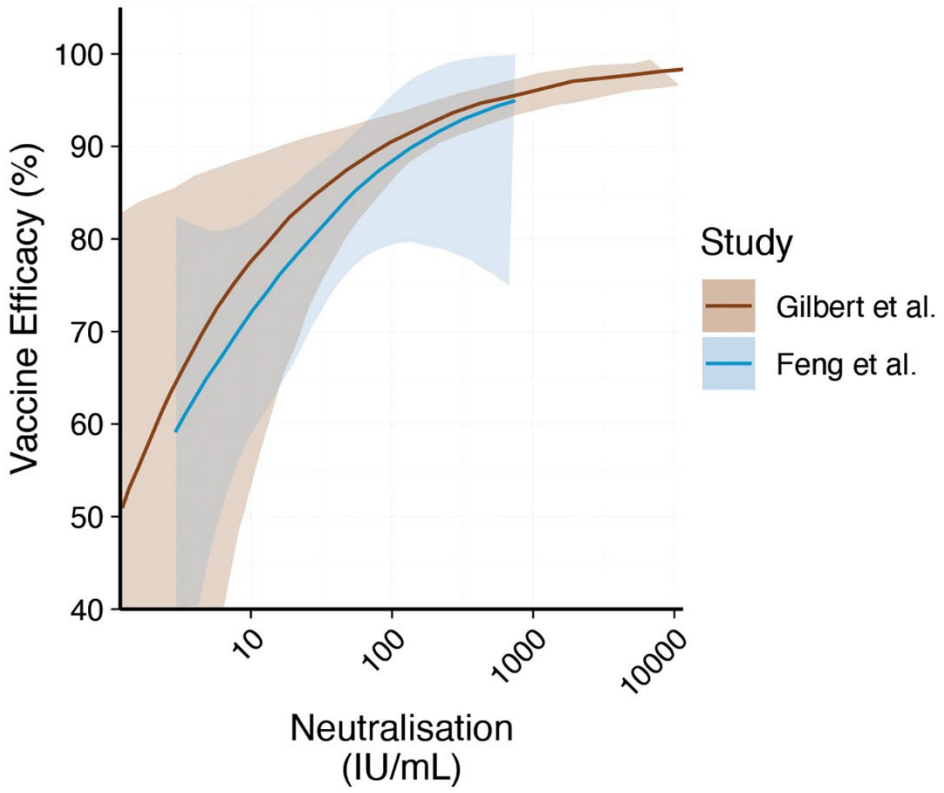
Study	Assay	Efficacy (%)	Source
Gilbert et al.	Both ID50 and ID80 (Pseudovirus)	92.8	Figure 4 of (*5*)
Feng et al.	ID50 (Pseudovirus)	78.1	Calculated using equation 5 and parameters in table S3 and S4.
	Live virus (NF50)	69.0	
Bergwerk et al.	Pseudoviral assay	94.0	(*12*)



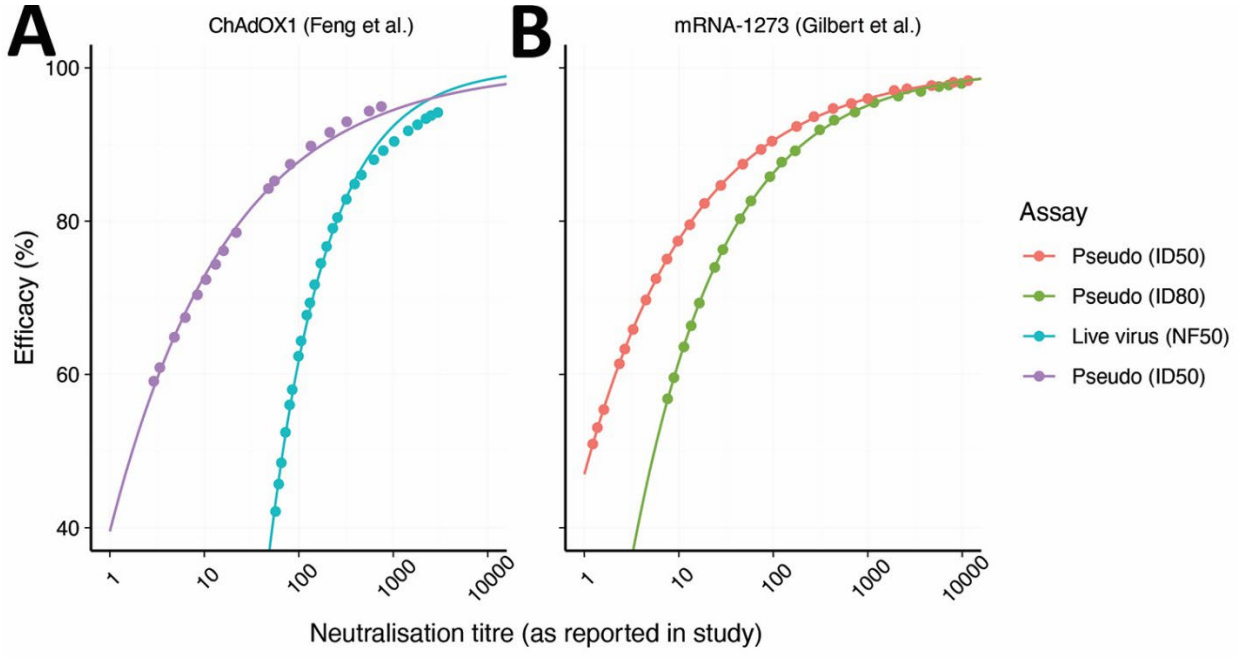
Appendix Figure 1. Predicting the efficacy of other vaccines (immunobridging): Both the breakthrough-infection (*4*,*5*) and vaccine-comparison (*1*) models can be used for 'immunobridging' to estimate the efficacy of novel vaccines (explained in supplementary material), using the protection curve and the distribution of neutralization titers of vaccinees to estimate overall vaccine efficacy. The vaccine-comparison model (red line) is fitted to the data on neutralization and protection for seven individual vaccines and convalescent subjects (black shapes). The predicted vaccine efficacy from the breakthrough infection model applied to mRNA-1273 vaccinees is shown (A). Two different protection curves were derived using either the 50% and 80% in vitro neutralization titers (labeled as ID50 and ID80, respectively) (*5*). The shaded area indicates the 10^{th} - 90^{th} percentiles of the neutralization data. (B) The predicted vaccine efficacy from study of breakthrough infection in ChAdOx1 nCoV19 vaccinees is also shown using neutralization data from either a SARS-CoV-2 (purple) or pseudovirus (light brown) neutralization assays (*4*) (colored rectangle indicates 10-90^{th} percentiles of the data). We see that the breakthrough-infection models agree closely with both the vaccine-comparison model and the reported vaccine efficacies in the ranges where data was available for each study. The pink star indicate reported efficacy of an eighth vaccine (*19*) and pink open circles indicate the results of a meta-analysis of ChAdOx1 nCoV19 at different dose intervals, showing higher efficacy and neutralization titers with wider dose intervals (*11*).



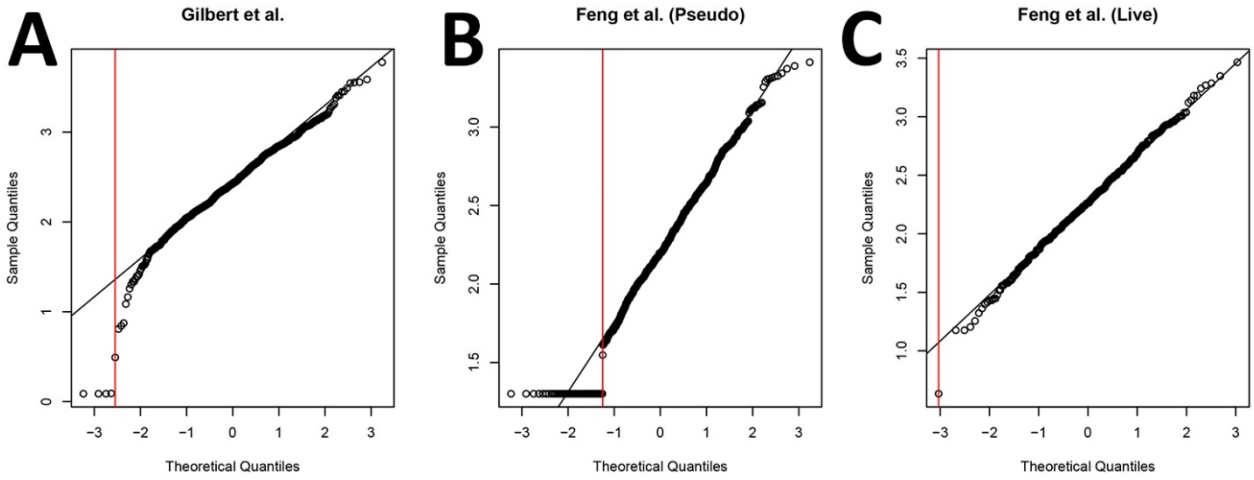
Appendix Figure 2. The model published by Khoury et al. (*1*) can be used to predict the fold drop in neutralization titer compared to a reference vaccine (either BNT162b2 or mRNA-1273) that would lead to an efficacy estimate where the lower 95% Confidence bound is 80%. The red curve is the model relating neutralizing antibodies and efficacy from Khoury et al. The shaded region indicates the upper 95% confidence region of vaccine efficacy estimates from the Khoury et al. model. The colored dots are the neutralizing antibody titer for each of the three reference vaccines as well as the reported efficacy from the original clinical trials (*7*,*9*). The grey dots are the mean neutralizing antibody titers and reported vaccine efficacies of the other vaccines used in fitting the Khoury et al model (*1*). All error bars are 95% confidence intervals. From this model, if a candidate vaccine can be demonstrated to induce a GMT that is more than 0.44-fold (i.e. 44%) of the level seen in BNT162b2 vaccinees, or more than 0.54-fold (i.e. 54%) of the level observed in mRNA-1273 vaccinees, than the Khoury et al. model would predict such a vaccine has an efficacy with a lower 95% confidence interval of 80%. Similarly, the same approach predicts that a candidate vaccine should have a GMT at least 2.6-fold higher the level seen in ChAdOx1 nCoV-19 vaccinees in order that there is high confidence that the predicted efficacy of the candidate vaccine is above 80%. Note that these non-inferiority/superiority margins are computed independently for different reference vaccines and depends on the uncertainty in the model parameters, as well as the uncertainty in the actual position of the reference vaccines (based on the Phase 1/2 clinical trial data) on the fold-convalescence scale (i.e. the horizontal error bars of the reference has been included in the margins reported for each vaccine).



Appendix Figure 3. The models from Gilbert et al. (brown) (*5*) and Feng et al. (blue) (*4*), showing the estimated relationship between neutralization titer (measured using a pseudo-virus neutralization assay with 50% neutralization endpoint calibrated to international units) and vaccine efficacy. The model and 95% confidence bands were extracted from the respective studies as described in the data extraction section. This highlights that the relationship between neutralization and vaccine efficacy is perhaps more similar when the assay used to define the relationship is more similar between studies.



Appendix Figure 4. Fitting the extracted model (dots) describing the relationship between neutralization titers and protection as reported in (*5*) and (*4*), with the functional form of this relationship (equation 4). Line represents the fitted model in each case. Note that the dots are not evenly spaced as these were extracted manually from the figures using WebPlotDigitizer online application (https://automeris.io/WebPlotDigitizer). These fits allowed the parameters a, b and c to be estimated for equation 4 (see Appendix Table 4).



Appendix Figure 5. Normality of neutralization distributions. Neutralization data from Gilbert ID50 assay was extracted from Figure S10 of the supplementary material of (*5*) using the web tool described above and is not as reliable as the data extracted from the Feng et al. study using Adobe Illustrator.