Scoring Variants by their Reduction of Pseudovirus Neutralizing Antibody Titer Sensitivity Compared to the Vaccine Strain (Reference) for the Vaccines Studied in the US Government’s First Generation COVID-19 Vaccine Efficacy Trials

For each trial unit included in trial-level evaluation of circulating-strain matched 50% neutralizing antibody titer (nAb-ID50) measured at the ~peak time point after the one- or two-dose primary vaccination as a surrogate endpoint for COVID-19, this document describes how variants are scored for the calculation. The last section describes how this peak circulating-strain matched nAb-ID50 titer marker is computed for each individual.

**P3001 Moderna COVE Trial**

The lineages circulating in the Moderna COVE trial are described in Pajon et al. (2021, Nature Medicine) and Heng et al. (2025, *Statistics in Medicine*), which are listed in Table 1. For each variant, the variant-neutralization resistance score was assigned based on 50% neutralization titers of Day 36 (7 days after dose 2) mRNA-1273 vaccine recipient sera to that variant compared to against the Reference/D614G vaccine-insert strain, using a recombinant vesicular stomatitis virus–based pseudovirus neutralization assay was used to measure pseudovirus neutralization and a phase 1 vaccine trial (Choi et al., 2021, *Journal of Virology*). No data in the neutralization resistance scoring experiment were available for Zeta. Therefore, Zeta was assumed to have the same neutralization resistance level as Gamma, due to sequence similarity between these lineages.

Table 1. [Moderna trial] Neutralizing 50% Geometric Mean Titer (GMT) ratio of each variant strain circulating in Moderna trial compared to the Reference strain for serum antibodies elicited by mRNA-1273 against SARS-CoV-2 Spike pseudotyped virus variants (Choi et al., 2021).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Reference | Epsilon | Gamma | Zeta |
| GMT | 1874 | 891 | 588 | 588 |
| Fold-Change Over Reference | 1.0 | 2.1 | 3.2 | 3.2 |

**P3002 AstraZeneca Trial**

The lineages circulating in the AstraZeneca AZD1222 trial are described in Sobieszczyk et al. (2022, *Journal of Clinical Investigation*) and listed in Table 2. For each variant, the variant-neutralization resistance score was assigned the same as in the P3003 ENSEMBLE trial described below (Table 3), given the lack of available published data for the P3002 vaccine’s neutralization against variants and the expected similar abrogation in neutralization for the two vaccines given they are both adenovirus-vector based.

Table 2. [AstraZeneca trial] Neutralizing 50% Geometric Mean Titer (GMT) ratio of each variant strain circulating in AstraZeneca trial compared to the Reference strain for serum antibodies elicited by the ChAdOx1 nCoV-19 AZD1222 vaccine against SARS-CoV-2 Spike pseudotyped virus variants, based on data for the ENSEMBLE P3003 trial.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Reference | Alpha | Delta, Epsilon, Lambda | Gamma |
| GMT | 246 | 266 | 158 | 72 |
| Fold-Change Over Reference | 1.0 | 0.9 | 1.55 | 3.4 |

**P3003 Janssen ENSEMBLE Trial**

The lineages circulating in the ENSEMBLE trial are described in Sadoff et al. (2022, *NEJM*) and Magaret et al. (2024, *Nature Communications*), which are listed in Table 3. For each variant, the variant-neutralization resistance score was assigned based on 50% neutralization titers of Day 71 ENSEMBLE vaccine recipient sera to that variant compared to against the Reference/D614G vaccine-insert strain, using a Janssen-internal pseudotyped lentivirus neutralization assay (psVNA) described in Magaret et al. (2024). Eight serum samples were selected based on high D614G psVNA titers, which were then run in internal psVNAs for different variants including Alpha, Beta, Delta, Gamma and Zeta (Table 3, also reported in Supplementary Table 4 of Magaret et al. (2024, Nature Communications). Two technical replicates were performed for each serum sample, i.e. heat‐inactivated serum samples were two‐fold serial diluted in duplicates over 10 columns.

For each variant, the score is defined as log10 fold-change of the geometric mean ID50 titer to the variant vs. to the reference strain for per-protocol baseline seronegative vaccine recipients in a phase one trial of the Ad26.COV2.S vaccine. Variants causing COVID-19 endpoints in ENSEMBLE that were not included in the psVNA measurements were assigned the score of a scored variant that was shown in other studies to have similar neutralization sensitivity.

No data in the neutralization resistance scoring experiment were available for Epsilon, Iota, Lambda, or Mu, which were assumed to have the same neutralization resistance level as Delta, Alpha, Delta, and Beta, respectively, due to sequence similarity between these lineages and support from the COV2001 study that measured neutralization levels to all of these variants.

Table 3. [Janssen trial] Neutralizing 50% Geometric Mean Titer (GMT) ratio of each variant strain circulating in the ENSMBLE trial compared to the Reference strain for serum antibodies elicited by Ad26.COV2.S against SARS-CoV-2 Spike pseudotyped virus variants.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Reference | Alpha, Iota | Beta, Mu | Delta, Epsilon, Lambda | Gamma | Zeta |
| GMT | 246 | 266 | 68 | 158 | 72 | 115 |
| Fold-Change Over Reference | 1.0 | 0.9 | 3.6 | 1.55 | 3.4 | 2.2 |

**P3004 Novavax PREVENT-19 Trial**

The lineages circulating in the Novavax PREVENT\_19 trial are described in Dunkle et al. (2022, *NEJM*), which are listed in Table 4. The literature did not seem to contain data on NVX-1273 serum neutralization against the variants listed in Table 4. Accordingly, we assumed that the geometric mean titer for each variant vs. Reference equaled that seen for mRNA-1273 in Choi et al. (2021, *Journal of Virology*).

Table 4. [Novavax trial] Neutralizing 50% Geometric Mean Titer (GMT) ratio of each variant strain circulating in the PREVENT-19 trial compared to the Reference strain for serum antibodies elicited by NVX-CoV2373 against SARS-CoV-2 Spike pseudotyped virus variants, calculated based on the data for mRNA-1273 measured in Choi et al. (2021).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Reference | Alpha | Beta | Epsilon | Gamma | Iota |
| GMT | NA | NA | NA | NA | NA | NA |
| Fold-Change Over Reference | 1.0 | 1.2 | 7.4 | 2.1 | 3.2 | 2.3 |

**P3005 Sanofi VAT00008 trial**

This Phase 3 study is divided into four efficacy trials:

Stage 1 for SARS-CoV-2 naïve participants, Stage 1 for SARS-CoV-2 non-naïve participants, Stage 2 for SARS-CoV-2 naïve participants, Stage 2 for SARS-CoV-2 non-naïve participants,

as defined in the protocol (Dayan et al., 2023, The Lancet Respiratory Medicine; Dayan et al., 2023, *eClinicalMedicine*). The circulating lineages were Delta, Omicron/B.1.1.529, BA.1, BA.2, and BA.4/5. Table 5 shows the GMT ratios based on the Random Immunogenicity Subsets for the Phase 3 study population itself.

Note that the Sanofi Stage 2 naïve cohort had rather small COVID-19 endpoint counts, such that the trial-level validity surrogate analysis can consider excluding this trial unit.

Table 5. [Sanofi trial] Neutralizing 50% Geometric Mean Titer (GMT) ratio of each variant strain circulating in the VAT00008 trial compared to the Reference strain for serum antibodies elicited by the Sanofi recombinant protein vaccine against SARS-CoV-2 Spike pseudotyped virus variants, calculated based on the VAT00008 data (Gilbert et al., in preparation). The calculations are done separately for the six Random Immunogenicity Subset cohorts: Stage 1 naïve vaccine, Stage 1 non-naïve vaccine, Stage 1 non-naïve placebo, Stage 2 naïve vaccine, Stage 2 non-naïve vaccine, Stage 2 non-naïve placebo.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Study |  | Reference | Delta\* | Omicron/B.1.1.529\* | BA.1 | BA.2 | BA.4/5 |
| Stage 1 Naïve | GMT | 146.3 | N/A | N/A | 29.8 | 43.8 | 36.9 |
|  | Fold-Change Over Reference | 1.0 | 2.0 | 4.2 | 4.9 | 3.3 | 4.0 |
|  |  |  |  |  |  |  |  |
| Stage 1  Non-Naïve  Vaccine | GMT  Fold-Change | 10828.2 | N/A | N/A | 1488.5 | 2502.8 | 1653.2 |
|  | Over Reference | 1.0 | 0.59 | 4.7 | 7.3 | 4.3 | 6.5 |
|  |  |  |  |  |  |  |  |
| Stage 1  Non-Naïve  Placebo | GMT  Fold-Change | 653.5 | N/A | N/A | 55.2 | 72.0 | 57.1 |
|  | Over Reference | 1.0 | 0.43 | 5.2 | 11.8 | 9.1 | 11.4 |
|  |  |  |  |  |  |  |  |
| Stage 2 Naïve | GMT | 444.6 | N/A | N/A | 70.4 | 76.9 | 48.7 |
|  | Fold-Change Over Reference | 1.0 | 1.4 | 3.3 | 6.3 | 5.8 | 9.1 |
|  |  |  |  |  |  |  |  |
| Stage 2  Non-Naïve | GMT | 8748.5 | N/A | N/A | 1138.1 | 1687.5 | 1329.0 |
| Vaccine | Fold-Change Over Reference | 1.0 | 1.1 | 4.0 | 7.7 | 5.2 | 6.6 |
|  |  |  |  |  |  |  |  |
| Stage 2  Non-Naïve | GMT | 460 | N/A | N/A | 100.1 | 125.0 | 107.6 |
| Placebo | Fold-Change Over Reference | 1.0 | 1.1 | 4.4 | 4.6 | 3.7 | 4.3 |

\*The Sanofi VAT00008 data did not include neutralization data for the two antigens Omicron/B.1.1.529 and Delta. Therefore, the estimated fold-change in geometric mean concentration for the bAb-IgG Spike assay (for which data were available) was used to generate a proxy estimate for the fold-change.

**Calculation of the 50% neutralizing antibody titer (nAb-ID50) marker matched to circulating strains for an individual in a given trial**

Calculation for vaccine recipients:

For a given trial, suppose a vaccine recipient has a Peak time point nAb-ID50 Reference titer value of x-ref IU50/ml. Thus, their Reference marker value is log10(x-ref). Their circulating-strain matched marker value is the weighted average of this value log10(x-ref) together with all of the log10(x-vk) values across all of the variants x-vk circulating in the given trial as indicated by Tables 1 through 5. A participant’s weight assigned to each variant is the estimated proportion of viruses circulating in their local context (during their calendar period of at-risk for COVID-19 for correlates evaluation in their country), as estimated in GISIAD. Those proportions were calculated externally for all trial participants included in the correlates analysis.

Calculation for placebo recipients:

For all placebo recipients in P3001-P3004, and for all placebo recipients in the Naïve cohort analyses for P3005, all participants have their nAb ID50 values assigned the assay lower detection limit divided by 2. These numbers are reported in Rosin et al. (2025) for P3001-P3004. For P3005, the limit of detection is 2.612 IU50/ml, such that for naïve participants the assigned half-detection limit value is 1.31 IU50/ml. Following Rosin et al. (2025), then the maximum lower detection limit

For P3005 non-naïve participants, for placebo recipients the marker values are computed the same as for vaccine recipients, using the fold-change values in Table 5.