WHO international standard for SARS-CoV-2 antibodies to determine markers of protection

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Several studies have shown that neutralising antibody level is a good biomarker for the correlate of protection against SARS-CoV-2 infection.[1–3] However, results from these studies are presented using assays that have not been calibrated using a common reference standard, making it difficult to define the exact level of neutralising antibodies required for protection and to compare with current and future studies. The most recent study[4] is the only one we have identified that reports the neutralising antibody level using WHO international units by calibrating their neutralisation assays against the WHO international standard for SARS-CoV-2 immunoglobulin; the international standard was established by the WHO Expert Committee on Biological Standardization as a primary calibrant to harmonise the measurement of anti-SARS-CoV-2 antibodies and was made available in December, 2020 from the WHO Collaborative centre, the National Institute for Biological Standards and Control (NIBSC), UK.[5]

As highlighted by the study on breakthrough infections from BNT162b2 vaccinees in Israel,[1] neutralising antibody titres are typically not readily available in most studies due to the cost and time consuming nature of any cell-based virus neutralisation test, whether using the live virus or a pseudotyped virus. Most research groups are thus relying on determining levels of binding anti-spike, subunit 1, or anti-receptor binding domain antibodies as immune correlates. However, the same study showed that the correlation between neutralising antibody levels and breakthrough infections was stronger than that for IgG binding antibodies.[1]

Many surrogate neutralisation assays are entering the market.[6] These assays offer a rapid and user-friendly way to determine neutralising antibody titres; usually they are based on the competitive inhibition of the interaction between the SARS-CoV-2 spike protein and the angiotensin-converting enzyme 2 cell surface receptor. To date, only one assay detecting neutralising antibody has received US Food and Drug Administration authorisation: a surrogate virus neutralisation test[7] that was commercialised under the trade name cPass (GenScript, Singapore). Furthermore, to increase comparability of the neutralising antibody levels across different studies, cPass has been calibrated against the WHO International Standard (NIBSC code 20/136). Using data representing 21 biological replicates from three international groups (appendix), we obtained a highly reproducible calibration of cPass reading (% inhibition) to IU/mL of the WHO International Standard with a pseudo R^2 (1 – deviance/null.deviance) at 0·978. We have developed a convenient Excel-based conversion tool that is freely available online.

See Online for appendix

For the **conversion tool** see https://github.com/Lelouchzhu/cPass-to-IU_Conversion

Bergwerk and colleagues' study on neutralising antibodies as correlates of protection against infection by the alpha variant showed that the geometric mean titre of neutralising antibodies, determined using pseudotyped virus-based virus neutralisation test, from the infected group of 22 cases was about 2·76-fold (peri-infection level) to 6·76-fold (peak level) lower than that of the matched control group of 104 individuals.[1] Using the mean pseudotyped virus neutralisation test titre to IU/mL conversion from the WHO report, we can speculate that the peri-infection neutralising antibody levels are at 99 and 250 IU/mL and the peak neutralising antibody levels at 82 IU/mL and 448 IU/mL for the infected and control groups, respectively. The observation that, for the infected group, the peri-infection neutralising antibody level was slightly higher than the peak neutralising antibody level would suggest some boosting effect for some infected individuals, given that the sampling time in relation to infection was not uniform. The authors thus concluded that the peak neutralising antibody level is a better predictor of correlate for protection than the peri-infection neutralising antibody level is.[1]

For the **WHO report** see https://www.who.int/publications/m/item/WHO-BS-2020.2403

From the study on delta variant breakthrough infection in Vietnam, neutralising antibodies were measured directly with cPass for fully vaccinated health-care workers who received the AstraZeneca vaccine.[8] There are two cohorts presented in this study. Neutralising antibody levels for the first cohort were available both at 8 weeks after the first dose and during the peri-infection period. By conversion of cPass reading (% inhibition) to IU/mL as stated above, we calculated that the peri-infection neutralising antibody levels were

100 IU/mL (geometric mean [GM] 94) and 738 IU/mL (GM 530) for the infected (n=10) and control (n=30) groups, respectively. The neutralising antibody levels at 8 weeks after the first dose were 157 IU/mL (GM 167) and 757 IU/mL (GM 623), respectively. For the second larger cohort, only peri-infection neutralising antibody levels were available, which were 151 IU/mL (GM 170) and 328 IU/mL (GM 300) IU/mL for the infected (n=59) and control (n=59) groups, respectively.

Using the cPass data from a longitudinal neutralising antibody follow-up study of a COVID-19 cohort (n=164) in Singapore,[9] we observed that at 6 months post-infection (or the last time point available), the mean neutralising antibody level was 332 IU/mL (GM 53, median 44), ranging from 0 to 3000 IU/mL (maximum modelling value for IU at the cPass value of 97·57). Using the threshold of 82 IU/mL from the study in Israel, 93 individuals (57%) had a peak neutralising antibody level at this threshold or below, indicating that they might be susceptible for reinfection by the alpha variant. Similarly, using the threshold of 170 IU/mL (GM) from the study in Vietnam, 112 individuals (68%) had neutralising antibody levels below the threshold and, hence, might be potentially susceptible for reinfection by the delta variant.

It should be emphasised that, due to the small number of samples in the different studies, the threshold IU/mL defined in this analysis needs further validation with bigger cohort studies. However, the observation that the breakthrough infection of the delta variant could occur at a higher threshold than that of the alpha variant is consistent with published findings.[10]

With the global population being vaccinated at an increased pace and many individuals being shown to be susceptible to breakthrough infections, there is an urgent need to develop a harmonised approach for risk assessment among different populations using different vaccines. Surrogate neutralisation assays calibrated to the WHO international standard, such as cPass, represent a good first step towards such an international harmonisation goal. More so than ever, with the delta variant spreading rapidly across the globe, a harmonised approach for the assessment of risk and correlate of protection is highly desirable.

CWT, WNC, and L-FW are co-inventors of the surrogate virus neutralisation technology that has been commercialised under the trade name cPass by GenScript Biotech. GM provided independent expertise on the calibration of assays using the WHO IS. There is no endorsement of any individual assay, diagnostic product or vaccine by the NIBSC authors (GM and NJR). We thank Yun Sun, Wan Su, Lam Anh Nguyet, Tran Tan Thanh, Shuting Xu, and Yanfeng Li for technical assistance and coordination of different study groups.

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