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Establishment of the WHO International Standard and Reference Panel for anti-SARS-CoV-2 antibody

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NOTE:
This document has been prepared for the purpose of inviting comments and suggestions on the proposals contained therein, which will then be considered by the Expert Committee on Biological Standardization (ECBS). **Comments MUST be received by 3rd December 2020** and

should be addressed to the World Health Organization, 1211 Geneva 27, Switzerland, attention: Technologies, Standards and Specifications (TSS). Comments may also be submitted electronically to the Responsible Officer: **Dr Ivana Knezevic** at email: knezevici@who.int.

Summary

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is a zoonotic disease that was first reported in Wuhan, China in December 2019. The virus has spread worldwide and was reported a Public Health Emergency of International Concern by the WHO on January 30th, 2020. Vaccines and treatments are rapidly being developed and reliable assays are needed for their evaluation. The availability of an International Standard (IS) for antibodies would facilitate the standardisation of SARS-CoV-2 serological methods and allow for comparison and harmonisation of datasets across laboratories. This will help determine the antibody levels that are needed for efficacious vaccines and therapeutics, and improve our understanding of virus epidemiology. In this collaborative study, a pool of plasma from 11 SARS-CoV-2 convalescent patients, was evaluated for its suitability as an IS for anti-SARS-CoV-2 antibody by 51 laboratories across 125 methods including ELISAs, neutralisation assays, flow cytometry-based assays, lateral flow immunoassays, inhibitory assays and one Double Antigen Binding Assay. The candidate preparation, sample G, NIBSC code 20/136, was assessed as part of a blinded samples panel, which included plasma and serum from SARS-CoV-2 convalescent patients, to

assess commutability and harmonisation of the results. Four pools of convalescent plasma were included in the study and segregated into high, medium and low antibody titre sample. Together with a negative plasma from healthy individual collected before 2019, these five samples constitute a candidate WHO Reference Panel for the assessment of serological assay for the detection of SARS-CoV-2 antibodies. Also included was a plasma sample from an individual donor that has been distributed through the NIBSC catalogue (NIBSC code 20/130) since April 2020, as research reagent. The Reference Panel was found fit for purpose in all the assays, with few participants failing to detect the lowest titre samples; there was good concordance in the ranking of the samples in the Reference Panel for both neutralising and binding antibodies, and therefore it is proposed that the WHO Reference Panel will be distributed without an unitage, but with the following ranking: sample F (20/150) High titre, J (20/148) Mid titre, E (20/144) low anti-S, relative high anti-N protein antibodies, sample I (20/140) Low titre and sample H (20/142), negative. Expressing SARS-CoV-2 antibody titres of the collaborative study samples as relative to the candidate IS allows for better comparability of the results with a reduction in the inter-laboratory variability. The potencies of the samples expressed in International Unit per millilitre (IU/mL) for binding antibodies and for neutralising antibodies were similar; however, the proportion of binding *versus* neutralising antibodies varies between samples. Therefore, it is proposed that sample G, NIBSC code 20/136 serves as the IS for different methods and an arbitrary assigned unitage of 250 IU/ampoule has been assigned for neutralising activity and the same 250 IU/ampoule for binding activity. Secondary reagents should be calibrated to the IS in the type of assay they are used and may have different potencies of neutralising and binding antibody. The Reference Panel was found fit for purpose in all the assays, with few participants failing to detected the lowest titre samples; there was good concordance in the ranking of the samples in the Reference Panel for both neutralising and binding antibodies as follow: sample F (20/150) High titre, J (20/148) Mid titre, E (20/144) low anti-S, relative high anti-N protein antibodies, sample I (20/140) Low titre and sample H (20/142), negative.

Introduction

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) previously known as novel coronavirus 2019 (nCOV-2019) is the aetiological agent of the **Co**rona**vi**rus **D**isease 20**19** (COVID-19). It causes mild symptoms in the majority of cases, however ~10% of cases require medical intervention, with a small percentage progressing to severe pneumonia and death. The World Health Organization (WHO) declared COVID-19 a Public Health Emergency of International Concern on 30th January 2020, and a Pandemic on 11th March 2020. On 25th October 2020, over 42 million confirmed cases and 1.1 million deaths had been recorded globally [1]. Urgent and rapid vaccine development is underway; as of the 19th October 2020, there are 44 vaccine candidates in clinical evaluation, with 10 in phase 3 clinical trials, and a further 154 candidate vaccines at the preclinical stage [2]. Other therapeutics are also in clinical trials including cocktails of monoclonal antibodies [3] and convalescent plasma [4,5]. Development and harmonisation of serological assays for COVID-19 antibodies are important to evaluate the vaccine and treatment responses and to compare the multiple candidates. Furthermore, reliable serological assays are needed to understand the real impact of COVID-19 through sero-epidemiological studies, as most of the cases are asymptomatic and those with mild symptoms are mostly undetected [6]. The scientific and clinical community urgently requires a

COVID-19 antibody Standard to support serological assay development and allow the calibration of assays to a common unitage. This will both assist evaluation of vaccine efficacy and aid comparison of data collected as part of epidemiological and immunological surveillance studies. Plasma or serum from convalescent patients is the preferred candidate material as these are the most commutable samples that closely represent clinical samples analysed in the assays.

In this report, we have evaluated a candidate preparation to serve as the WHO International Standard for anti-SARS-CoV-2 antibodies and a Reference Panel. The Reference Panel can be used to facilitate the characterisation of factors which may contribute to assay variability. The collaborative study was organised by the National Institute for Biological Standards and Control (NIBSC) in collaboration with WHO, and has been facilitated by the Coalition for Epidemic Preparedness Innovations (CEPI) which sponsored the sourcing and formulation of the candidate material.

International Standards (IS) are recognised as the highest order of reference materials for biological substances and they are assigned potencies in International Units (IU). International Standards are used to quantify the amount of biological activity present in a sample in terms of the IU, allowing assays from different laboratories to be compared and the results rendered comparable. This makes it possible to better define both analytical parameters such as the sensitivity of tests and the clinical parameters such as protective levels of antibody. The availability of an IS for anti-SARS-CoV-2 antibodies would facilitate the standardisation of serological assays used for detection of exposure to SARS-CoV-2 and in vaccinology studies to measure antibodies elicited by human vaccination.

The aims of this WHO International collaborative study are to:

- Assess the suitability of the candidate to serve as the International Standard for anti-SARS-CoV-2 antibody with an assigned unitage per ampoule for use in the harmonisation of SARS-CoV-2 serology assays.
- Identify candidate COVID-19 antibody preparations for inclusion in a WHO Reference Panel for anti-SARS-CoV-2 antibodies.
- Characterise the antibody preparations in terms of reactivity/specificity in different assay systems.
- Assess each preparation’s potency i.e. readout in a range of typical assays performed in different laboratories.
- Assess commutability i.e. to establish the extent to which each preparation is suitable to serve as a standard for the variety of different samples and assay types.
- Recommend to the WHO ECBS the antibody preparation found to be suitable to serve as the International Standard and propose an assigned unit
- Advise WHO ECBS on the establishment of the WHO Reference Panel for anti-SARS-CoV-2 antibody.

Materials and Methods

Ethical statement

Convalescent plasma and serum from PCR-confirmed SARS-CoV-2-infected patients was kindly donated by ISARIC4C consortium through the University of Liverpool, UK; Papworth Hospital, Cambridge, UK; NHS Blood and Transplant, UK; and Oslo University Hospital, Norway. All patient donors gave informed consent for the use of their plasma or serum, and samples were anonymised. For material provided by ISARIC4C, ethical approval was given by the South Central-Oxford C Research Ethics Committee in England (reference 13/SC/0149), and by the Scotland A Research Ethics Committee (reference 20/SS/0028). The study was registered at https://www.isrctn.com/ISRCTN66726260. The NIBSC Human Material Advisory Committee (project 16/005MP) approved this project.

Study samples

Candidate WHO International Standard for anti-SARS-CoV-2 antibody

The candidate International Standard for anti-SARS-CoV-2 antibody (NIBSC code 20/136) is a freeze-dried preparation of a pool of plasma from 11 SARS-CoV-2 recovered patients from England, UK. Material was collected more than 28 days after the onset of symptoms. Donations from each patient were processed at NIBSC using a solvent-detergent treatment to minimise the risk of the presence of enveloped viruses [7]. Additionally, they were tested for known blood borne virus markers (HIV antibodies, HBsAg and HCV RNA) and found to be negative. Approximately 3500 2.5mL glass DIN ampoules containing 0.25 mL pooled plasma were lyophilised on 11th June 2020. Freezing was performed in a CS15 freeze drier to -50˚C for 4 hours. Primary drying was performed at -35˚C for 40 hours at 100µbar vacuum followed by a ramp to 25˚C over 10 hours then 30 hours secondary drying at 25°C and 30µbar vacuum. Vials were back filled with dry nitrogen to atmospheric pressure and sealed.

Candidate WHO Reference Panel for anti-SARS-CoV-2 antibody

The five candidate panel members comprise freeze-dried preparations of four pools of convalescent plasma from SARS-CoV-2 positive patients, and a negative control plasma collected from healthy UK donors before 2019. All convalescent samples were collected at least 28 days post onset of symptoms; all samples were solvent-detergent treated at NIBSC and confirmed negative for known blood borne virus markers (HIV ab, HBsAg and HCV RNA). Pooling of the convalescent plasma was decided based on antibody titre obtained by ELISA targeting the receptor binding domain (RBD), full spike or subunit 1 (S1) proteins and nucleoprotein (N), as well as by live virus and pseudotyped-based neutralisation assays performed at NIBSC. The reference panel was composed of a high titre sample, NIBSC code 20/150; mid-titre sample, 20/148 and two low antibody titre preparations, one with a higher anti-N antibody titre, NIBSC code 20/144; and the other with low anti-S1 and anti-N antibody, 20/140. The negative plasma was also freeze-dried, under NIBSC code 20/142. Approximately 2500 2.5mL glass DIN ampoules containing 0.25 mL pooled plasma for each panel member were lyophilised between 18th -26th June 2020 using the same 4-day cycle as per the candidate International Standard described above.

Additional samples included in the collaborative study

Further clinical samples were included in the study to help evaluate the level of harmonisation achieved by the candidate International Standard, therefore providing a limited assessment of commutability. This included two pools of serum samples, each from three COVID-19 recovered individuals. The sera were pooled as a higher titre convalescent serum sample (CS-high) or lower titre sample (CS-low) based on SARS-CoV-2 antibody titre obtained in ELISA and PV-based neutralisation assay at NIBSC. Convalescent plasma from one individual with very low antibody titre (CP-low) was also included to provide a sample that would challenge assay sensitivity. Each was provided as a liquid preparation, frozen and filled in 0.2mL aliquots in screw cap tubes, to assess harmonisation of the results obtained for the candidate International Standard.

Finally, the research reagent NIBSC code 20/130 (Appendix 2) was also included as an additional sample in the collaborative study. This is convalescent plasma from one recovered individual with a relatively high antibody titre and was made available globally at the end of April 2020 for the development of SARS-CoV-2 serology assays and its inclusion in this study will allow assignment of a unitage relative to the International Standard. This will enable users to back-calibrate their assays where the reagent has been used.

Coded study samples

Table 1 lists the collaborative study samples, provided coded and blinded to the participants. For each method in use in their laboratory, participants received 3 sets of the 10 samples, plus one additional set as a spare. Samples were labelled “COVID-19 Ab CS678 Sample X”, where X was one of the following letters A, B, C, D, E, F, G, H, I or J. Samples were shipped on dry ice under NIBSC reference CS678.

Participants

Fifty-one laboratories agreed to participate in the study; however three participants based in mainland China could not receive the collaborative study samples due to the time required to issue the import permit. Four laboratories did not return results in time as to be included in the collaborative study report. The forty-four participants providing included results were from 15 countries covering 6 continents: Australia (4), Brazil (1), Canada (1), China (1), Germany (3), India (1), Italy (1), Japan (1), Kenya (1) the Netherlands (2), Saudi Arabia (1), Singapore (1), South Korea (3), United Kingdom (11) and United States of America (12). All laboratories are referred to by a code number randomly allocated and not reflected in the order presented in Appendix 1. Participating organisations included vaccine developers, national control/reference laboratories, diagnostics laboratories, kit manufacturers, non-profit vaccine research organisations and academic laboratories.

Study design

The collaborative study protocol is given in Appendix 3. The study took place between July and October 2020 and was conducted within an accelerated timeframe in order to support the global response to the pandemic. Participants were requested to test the study samples using their established method(s) for the detection of antibodies against SARS-CoV-2. Participants were asked to perform three independent assays and for each assay, at least two independent serial dilutions of the study samples. For those commercial kits where a dilution of test samples was

not recommended by the manufacturer, the participants were asked to report the value obtained with the neat sample. A results reporting sheet was provided for participants to record all essential information including the raw data from each assay. Participants were asked to return results within 6 weeks of receipt of materials.

Assays methods

The two main methods used by the participants were neutralisation assays (Table 2) and ELISAs (Table 3); other methods used are summarised in Table 4. Where laboratories performed multiple assay methods or multiple targets, laboratory codes are followed by a letter indicating the different methods e.g. laboratory 1a, 1b.

The neutralisation assays were either live virus (*n*=15) or pseudotyped virus assays (*n*=12), with the latter including non-replicative vesicular stomatitis virus (VSV)-based pseudotypes and Human immunodeficiency virus (HIV)-based pseudotyped virus. The Spike protein from the Wuhan-Hu-1 isolate (Genbank accession number MN908947.3) was used predominantly in the pseudotyped-neutralisation assays. Nine different SARS-CoV-2 isolates were used in the live neutralisation assays.

Results were returned for fifty-four in-house ELISA methods and fourteen different commercial assays as listed in Table 3. The majority of the ELISA methods were specific for human IgG; nine assays were specific for IgA and eight for IgM. Recombinant Spike protein (S) and the receptor-binding domain (RBD) were the most common antigen, followed by the subunit 1 of S (S1) and nucleoprotein (N); three methods targeted the membrane (M) and envelope (E) proteins, and only one used the subunit 2 of S (S2) alone, not associated with S1.

Additionally, there were three laboratories using flow cytometry-based assays for the detection of binding antibodies, two inhibition assays, one double antigen bridging assay (DABA) and two lateral flow immunoassays.

Stability study of the candidate International Standard

Stability of the lyophilised ampoules of the candidate International Standard sample G, NIBSC code 20/136, was assessed in an accelerated degradation study. Fifteen ampoules of sample 20/136 were stored at each of the following temperatures -20, +4, +20, +37 and +45°C. Three ampoules for each temperature were retrieved at the following time points: 2 weeks, one month, and three months. The final six ampoules for each temperature will be retrieved at the 6 month (21st December 2020) and one year (21st June 2021) time points. The potency of the preparations relative to the baseline, -20°C sample, were assessed by in house ELISA. Briefly, purified recombinant SARS-CoV-2 S1 protein kindly provided by Dr Peter Cherepanov, Francis Crick Institute, London UK (CFAR cat. no. 100979) was coated onto a 96-well maxisorp plate at a concentration of 1 µg/mL adding 50 µL per well and incubating overnight at 4˚C. The day after, wells were washed with 0.05% (v/v) Tween-20 in PBS (wash buffer) three times. Plates were blocked with 5% (w/v) skimmed milk (Marvel) in wash buffer for 1 hour at ambient temperature. Three-fold serial dilutions of the candidate International Standard 20/136 accelerated degradation samples were added in duplicate to the plate for 1 hour at ambient temperature. Wells were washed with 0.05% (v/v) Tween-20 in PBS three times. Horseradish peroxidase-conjugated anti-human IgG Fc (SIGMA, A0170) was added to each well at a dilution of 1:3000 in blocking buffer for 1 hour at ambient temperature. After three washes, the substrate Enhanced K-Blue

(TMB, Neogen cat no. 308177) was added to each well. Reactions were stopped by adding an equivalent volume of sulphuric acid 1M. Absorbance measurements were acquired on a FluorStar Omega plate reader (BMG Labtech). Relative potencies were calculated by parallel line analysis using European Directorate for the Quality of Medicines & Healthcare (EDQM) software CombiStats™ [8].

Due to the accelerated timeframe of the study, and the increased workload due to the concurrent pandemic, the predicted stability of the individual members of the candidate Reference Panel is inferred from the stability of the candidate International Standard.

Statistical methods

For the neturalisation assays, the geometric mean (GM) of the potency of each sample was calculated from the endpoint titres or 50% reduction neutralisation titres (NT_{50}) provided by the participants.

Quantitative ELISA data were analysed using a sigmoidal curve model or parallel line analysis with log transformed responses. Calculations were performed using the software CombiStats™ . Model fit was assessed visually, and non-parallelism was assessed by calculation of the ratio of fitted slopes for the test and reference samples under consideration. The samples were concluded to be non-parallel when the slope ratio was outside of the range 0.80 – 1.25. Relative potency estimates from all valid assays were combined to generate an unweighted GM for each laboratory and assay type, with these laboratory means being used to calculate overall unweighted geometric means for each analyte.

Variability between laboratories has been expressed using geometric coefficients of variation (GCV = [10s-1] ×100% where s is the standard deviation of the $\log_{10}$ transformed estimates) and the ratio of the upper quartile to lower quartile of the estimates. Variability was also analysed by calculating the percentage of laboratory estimates within 2-fold of the overall sample median.

Results

Production of the candidate WHO IS and Reference panel

In June 2020, NIBSC filled and freeze-dried the candidate International Standard, NIBSC code 20/136 and the Reference Panel members (NIBSC codes 20/140, 20/142, 20/144, 20/146, 20/148, 20/150) using documented procedures. The product summary for the candidate WHO IS, sample G, is shown in Table 5, and for the Reference Panel in Table 6. The mean residual moisture of the candidate IS, the low titer (20/140) and negative (20/142) panel member were higher than the ideal 1%, but a higher moisture content can be acceptable if the final product is proven stable [9]. The residual oxygen content of the candidate IS fell within the NIBSC working limit of 1.1%. Currently there are approximately 3000 ampoules of 20/136 and 2500 ampoules of each of the Reference Panel members available for distribution.

Collaborative study data received

The collaborative study under NIBSC reference CS678 started on 2nd July 2020. The first set of results were received on 5th August 2020 and the last data on 16th October 2020. Forty-four participants returned results from 125 methods (Table 2, 3 and 4). These methods comprised 78

ELISAs, 27 neutralisation assays, 16 flow cytometry-based assays, 2 lateral flow immunoassay, 2 inhibitory assays.

Neutralising antibodies in the candidate reference material were assessed by live or pseudotyped-based virus neutralisation assays (Table 2). Fifteen laboratories used the live neutralisation assay; three laboratories used the Australia/VIC01/2020 isolate (Lab 6, 18 and 42), four the USA-WA1/2020 isolate (Lab 12b, 14a, 25, 37b), and two the European isolate Germany/BavPat1/2020 (Lab 31 and 36b). The other six laboratories each employed a different isolate, including one (lab33d) which used a clinical isolate at a low passage number (Table 2). Eight of the fifteen methods detected the virus by plaque or foci staining and reported the results as 50% neutralisation titre. Five laboratories reported infection by detecting cytophatic effect (CPE) on the cell monolayer; the results were provided as the inverse of the dilution factor preventing 50% CPE (Lab 7, 18, 33d and 42). Lab 28b reported the data as inverse of the highest dilution factor preventing 100% CPE. Lab 25 and 37b performed the live neutralisation assay as a microneutralisation assay, detecting absorbance (optical density, O.D.) following immunostaining for SARS-CoV-2 proteins; results were reported at 50% neutralisation titre. For the pseudotyped virus (PV)-based neutralisation, two main vectors were used: single round, replication incompetent HIV (Lab 2, 10a, 11b, 13b, 16, 32d, 44a) or a delta glycoprotein (ΔG) non-replicative VSV virus (Lab 14b, 29d, 32e, 44b); in all cases, a luciferase reporter gene was used and results reported as 50% neutralisation titre based on the reduction of relative luminescence units of the PV only control. Lab 16 was the only Lab that reported the data as the reciprocal of the area under the curve. Lab 14 reported the data from both the live and PV neutralisation assay. Lab 44 and Lab 32 used two PV neutralisation methods, HIV and VSV-based. Lab 40b has not confirmed which pseudotyped virus was used in their neutralisation assay.

Participants returned seventy-eight data sets from ELISA methods (Table 3). Twenty-one laboratories analysed the collaborative study samples using an in house ELISA, providing forty-three individual sets of results. Thirteen laboratories used one in house ELISA (Lab 3, 11a, 12a, 13a, 20a, 21a, 26, 27, 28a, 36a, 37a, 38 and 43a) all detecting IgG against one of the following SARS-CoV-2 antigen: RBD, S1 or full spike. Lab 9 used two ELISAs against full Spike protein detecting IgG or IgA. Lab 19 had two in house indirect ELISA methods against the same target S1, but detecting either IgG or IgM, and a Double Antigen Bridging Assay (DABA) for total immunoglobulins against RBD. Lab 22 provided data from fifteen ELISA methods, against five targets (RBD, S, M, E and N) and detecting IgG, IgA and IgM. Lab 24 returned data from five immunoassays against IgG anti-RBD, S1, S2, N and spike protein (S1+S2ECD). Lab 6, 29 and Lab 32 used three ELISA methods against RBD, S and N, all detecting IgG. Lab 30a-g returned data from seven ELISA methods using coated antigen peptides from the S (30a, 30b, 30c, 30e and 30f) or N protein (30d and 30g). Their assays were also able to distinguish between 2 sub-classes of IgG (IgG1 and IgG3). Nine participants used commercial kits for the detection of anti-SARS-CoV-2 antibodies. Lab 5, 8, 20c and 40c used one commercial assay. Lab 15 used an assay which was not commercially available at the time of the collaborative study. The assay simultaneously measures antibody against multiple targets and has been reported in Table 3 as three different assays (15a, 15b and 15c) based on the target. Lab 4 returned data from three commercial assays measuring IgG and IgA against S1 and a commercially available surrogate virus neutralisation assay. Lab 23 used four ELISA kits directed against S1 (IgG and IgA) or N (IgG and IgM). Lab 33 and Lab 34 returned results from three and five, respectively, different

manufacturers' ELISA kits. Overall thirteen commercial assays were used in this collaborative study, generating eighteen data sets.

Seven participants returned results from other types of assays (Table 4). Lab 17 and 39 returned qualitative data from two different commercially available rapid tests based on lateral flow with a colorimetric result. Both tests detected IgG and IgM against S and N proteins. Lab 1, 30 and 41 measured binding antibody by flow cytometry. Lab 1 returned IgG, IgA and IgM titres against seven antigens (M, E, N, S1, S2, extracellular domain of S1 and S2, and RBD). Lab 30h-m returned the data from one target antigen (S), but detecting different classes and sub-classes of immunoglobulins (total IgG, IgM, IgG1, IgG2, IgG3, IgG4). Lab 41 returned two sets of data for binding IgG against either S or RBD, and results from a flow cytometry-based surrogate neutralisation assay (Lab 41c). Finally, Lab 10 used a novel cell-cell fusion inhibition assay, detecting antibodies which prevents fusion, and Lab 43b detected total antibodies against spike which inhibits binding to its human receptor ACE2.

Neutralisation assays

Table 7 shows the geometric means of the neutralisation results as provided by the participants. The neutralisation assays are divided based on whether a live virus or a non-replicative pseudotyped virus was used. All the laboratories correctly identified sample H as negative. Lab 21b was the only participant able to identify the pool of very low titre convalescent sera sample C above cutoff of their assay. Neutralising antibodies were detected by sixteen out of twenty-seven methods (59%) in Sample D (very low titre convalescent plasma from one COVID-19 recovered individual). Neutralising antibodies were detected by nine out of fifteen methods using live virus (60%) and eleven out of twelve methods using pseudotyped virus (92%) in Sample I (pool of convalescent plasma with low titre antibody) from the reference panel. Lab 33d was the only method that did not detect neutralizing antibodies in Sample E, from the Reference Panel with low anti-S, and relatively high anti-N protein antibodies. The same laboratory did not detect neutralising antibody in the reference panel member with mid-titre antibody, sample J, suggesting a low sensitivity of the assay. The candidate IS, sample G generated one of the top three highest titres in all the neutralisation assay methods. The expected ranking of the Reference Panel members was also consistent between laboratories F-high>J-mid>E-lowS,highN>I-low, and H-RP neg was negative in all assays (Figure 1). The only exceptions were Lab 14a which scored sample J-RP mid lower than the two low samples E and I, and Lab 35 which ranked sample I higher than sample E. The geometric mean (GM) of the neutralising antibody titre reported by the participants for every sample was lower for the live virus-based neutralisation assay than the PV-based assay. Laboratory 28b was excluded from the calculation of the GM as they reported 100% neutralisation; Lab 16 was also excluded as it was the only Lab reporting the sample potency as area under the curve.

Neutralising antibody titres expressed as relative to candidate International Standard

The neutralisation titres for each sample were expressed as relative to the sample G, IS (Table 8). The difference in the titres reported from the participants was over 350-fold (Table 7, sample F), while reporting as relative to sample G reduced the difference to less than 50-fold at the most (Table 8, sample D). The reduction in the inter-laboratory variation by expressing the titres as relative to the candidate International Standard is also summarised in Table 9. Both sample F, the reference panel member with the highest antibody titre, and sample G, the candidate

International Standard, are pools of convalescent plasma with high anti-SARS-CoV-2 antibodies. Sample G is a pool of eleven donors, while sample F is a pool of four donors. We assume that pooling many donors will increase the antibody repertoire and serve better as an candidate International Standard. Looking at the reduction in the inter-laboratory variation, comparing the data reported by the participants against the data as relative to sample G or sample F and analysing 1) the percentage of the coefficient of variation (%GCV; the lower the percentage, the smaller the difference between laboratories); 2) the percentage of laboratories with a GM within 2-fold of the median (Lab GM:Median<2, the higher the percentage, the greater the agreement between labs); 3) the ratio of upper and lower quantile which represents the inter-quartile fold range of potencies (UQ/UL, values closer to 1 represent a smaller range of potencies and better agreement between labs). For all the three parameters for every sample, expressing the data relative to sample G achieved a greater harmonisation than using sample F as the calibrator. In comparison with the data reported by the participants, there is a reduction in %GCV for all the positive samples except for the two lowest samples: sample C (n/a, not enough data), and sample D, which has a slight increase in the %GCV when expressed as relative to either sample G or F, but there is a small increase in GM:Median<2 and decrease in UQ/LQ. On the other side, for sample I, RP low there is a slight decrease in %GCV, but a small decrease in the GM:Median<2 and increase in UQ/LQ. The reduction in the spread of the titres between laboratories when reported as relative to sample G, the candidate International Standard, is also visualised in Figure 2. To ease the comparison in the figure the candidate International Standard was arbitrary assigned a unitage of 1000 International units/mL (IU/mL) to be able to plot the data using the same y-axis scale.

Binding antibody immunoassays

The geometric means of the binding total antibodies or IgG titres as reported by the participants from ELISA methods are summarised in Table 10 for in-house assays and Table 11 for commercial kits. For the in-house ELISAs, the majority of the assays targeted the full Spike protein (17/43), followed by RBD (11/43) and S1 (4/43). Lab 22 also measured antibody against M and E protein, and Lab 24 against S2 and the extracellular domain of S1+S2. The commercial assays used by the participants of this collaborative study were mainly based on N protein (5/18) ans S1 (4/18) followed by full spike (3/18) and RBD full spike (2/18, each). Lab 34d used the extracellular domain of S1+S2 and Lab 33c total cell lysate from SARS-CoV-2 infected cells. Two labs, 4c and 20c, used a surrogate neutralisation assay.

Sample A-CP high (20/130), F-RP high, and G, candidate IS were scored positive in every ELISA, both in-house and commercial assays. One laboratory, Lab 24, reported for sample F and G against S2, and for sample G against N protein that the results did not meet the assay's acceptance criteria. The raw values returned from the participant suggested that these samples have saturated the assay. Lab 24 performed the assay once as one single dilution series. Sample H was scored as a false positive by Lab 22 for antigen N, M & E, Lab 24 for antigen RBD and Lab 27 for antigen Spike, respectively All the commercial kits correctly identified sample H as negative. The expected ranking of the Reference Panel members was also consistent between laboratories, and between antigens F-high > J-mid > E-low S, High N ≥ I-low (Figure 3). The only exceptions were Lab 22a (RBD), 33a and 40b (S1) which scored the sample I higher than E; also, Lab 15c (N) and 33c (cell lysate) score sample E, RP mid higher than sample J-RP mid. Lab 24e (S2) scored sample J-RP mid lower than both low samples E and I. Lab 22d was the

only laboratory which scored the samples for detecting antibodies against E protein in an order (E-low S, high N>H-neg>J-mid>I-low>F-high) that was different from those against RBD or Spike.

Binding antibody titres expressed as relative to candidate International Standard

SARS-CoV-2 antibody titre from the ELISA methods were expressed relative to the sample G, IS (Table 12 and Table 13). Some laboratories did not perform enough steps in the serial dilutions of the sample and the calculation of the relative potency by parallel analysis was not possible; in this case the ratio between the value provided by the participant for the sample over sample G was calculated (Lab 12a, 37a, 38, 5, 33a, 33b, 33c, 4c, 20c). A small number of cases were excluded for non-parallelism (10%) using the criteria described in the Statistical Methods for the analysis of this study. Approximately 95% of estimates for samples B, F and J met the acceptance criteria for parallelism and a slightly lower percentage, around 86%, of estimates met the acceptance criteria for samples A, D, E, H and I. Sample C had the lowest proportion of valid estimates with around 71% meeting the acceptance criteria. Table 14 shows the reduced inter-laboratory variation for each sample as %GCV, Lab GM:Med <2 and UQ/LQ for the in-house and commercial ELISA. Lab 22c detecting anti-M antibodies, Lab 22d detecting anti-E antibodies and Lab 30 were considered outliers and their data have not been included in these calculations. There is a pronounced harmonisation of the data. This is not surprising as the laboratories expressed their data with different outputs (*e.g.* arbitrary unit, ng/mL, inverse of the dilution factor). There is a difference of over 10,000-fold between the values reported. Expressing the data as relative to sample G reduced the difference to less than 200-fold. The highest %GCV (sample F in the commercial assays) was reduced from 2637% to 33%. Even the samples which in the neutralisation assay did not show much improvement, once the ELISA are expressed as relative to sample G, IS, it has a clear reduction in the inter-laboratory variation; for instance, sample D in the in-house assay had a reduced %GCV from 385% down to 154% and sample I from 596% down to 114%. This also corresponded to an increased proportion from 55% to 89% of labs with Lab GM:Med<2 for sample D and from 39% to 82% for sample I as well as having a smaller UQ/LQ reducing from 3.03 to 1.58 for sample D and 8.9 to 1.38 for sample I.

Reactivities to different SARS-CoV-2 antigens

The geometric mean of the SARS-CoV-2 antibody titre for each sample against the different antigens has been calculated for RBD, S1, Spike and N (Figure 4). The values as reported by the participants (Figure 4A) are biased by the type of assay used; for RBD and Spike, the majority of the assays were in-house and results reported as the inverse of the highest dilution above cutoff or arbitrary units, which numerically were in the order of hundreds or thousands. Instead, S1 and N were the most used antigens in the commercial assays; a high proportion of them reported the results as ratio against an internal control with smaller numerical value results. Once reported as relative to the candidate IS, sample G (Figure 4B) the antibody titre for each sample were similar between the different antigens. In figure 4B, we arbitrarily assigned a unitage of 1000 IU/mL to sample G to be able to plot the data using a similar y-axis scale to Figure 4A.

Determination of immunoglobulins A (IgA) and M(IgM) in the collaborative study samples

Five laboratories, Lab 4b, 9b, 23a, 22k-p and 40c used assays for the detection of other classes of immunoglobulins as well as IgG (Table 15). Lab 22k-p and 9b used in-house assays; Lab 4b, 23a and 40c used the same commercially available kit and returned almost identical results. IgA against RBD, S1, Spike and N were detected by all labs in sample A-CP high (20/130), F-RP high and G-IS. Anti-S1, Spike and N IgA were also detected at a lower level for sample B-CS high and sample J-RP mid. In the low antibody titre samples E-RP low S, high N and I-RP low IgA against spike and N protein were also detectable. Three laboratories also tested the collaborative study samples for presence of anti-SARS-CoV-2 IgM (Table 16). Lab 19b and 22g-j used their in-house assay, while Lab 23d used a commercially available kit. No IgM antibody was detected in the negative or low antibody titer samples H, C, D and I. None of the samples were positive for anti-E IgM. Lab 19b scored positive for anti-S1 IgM sample A- CP high, sample B-CS high, the candidate IS, sample G and the Reference Panel members F-high, J-mid and E-lowS, high N. Lab 22f was able to identify anti-RBD IgM in all the same samples, except for RP sample E. The same laboratory also scored positive for IgM against M and N, the Reference Panel member high, sample F, and the candidate IS, sample G; however, the commercial kit used by Lab 23d could not detect anti-N protein IgM in sample G.

Due to the limited number of laboratories performing these assays, it was not possible to statistically analyse the data to show improvement of the agreement between the participants' results by expressing the titres as relative to the candidate IS, sample G.

Other assays

Seven participants tested the collaborative study samples in other types of assay (Table 4). While a statistical analysis of the results is not possible due to the limited number of datasets, the results are very informative. Lab 17 and 39 returned qualitative data from two commercial lateral flow tests (Table 17) for IgG and IgM against a mix of SARS-CoV-2 antigens, S and N. The two assays reported the same reactivities for all the samples; both assays scored negative the lowest SARS-CoV-2 positive samples, C-CS low and D-CP low. Although the test is defined as qualitative, Lab 39 reported values based on the colorimetric intensity of the bands; the Reference Panel samples were ranked in the same order as by neutralisation assays and ELISA methods; sample G, candidate IS and sample A, CP high were identified as the most potent samples. The antibody high and mid titre samples (A, F, G and J) were also scored positive in both assays for IgM.

Labs 1, 41 and 30h-m measured the binding antibody titres of the collaborative study samples by flow cytometry analysis (Table 18). The candidate IS, sample G, the high titre Reference Panel, sample F and sample A, CP high (20/130) were scored positive in all the assays. The ranking of the members of the Reference Panel for IgG was the same as per neutralisation assays and ELISA methods with a few exceptions; sample E, RP low S, high N was scored with a lower potency than sample I-RP low by Lab 1g and 41a against RBD and Lab 41b against Spike. Also, sample J-RP mid was scored with a lower potency than the two low RP samples E and I against S1 by lab 1e.

Finally, two assays detected antibodies by investigating the inhibition of cell fusion (Lab 10b) or blocking of binding to the SARS-CoV-2 receptor, human ACE2 (Lab 43b). The geometric mean of three independent experiments as reported by the participants and relative to the candidate IS, sample G are presented in Table 19. The candidate International Standard was detected as one of the highest samples in both assays. Lab10b could detect inhibition of fusion in all the positive

convalescent plasma samples, including the very low antibody titer sample D; however, the convalescent serum samples were scored negative. It is likely that this result is due to the type of sample (serum instead of plasma). Lab 43b was able to detect anti-SARS-CoV-2 antibodies blocking ACE2 binding in the high and mid titer samples only (Sample A, B, F, G and J).

Stability study of the candidate WHO IS

The stability of the candidate International Standard is being assessed by accelerated thermal degradation study. Ampoules of the candidate WHO IS, NIBSC code 20/136 (sample G) were stored at different temperatures -20 (baseline), +4, +20, +37 and +45°C for 2 weeks, 1 month, 3 months, 6 months and 12 months. The freeze-dried preparations retrieved at 2 weeks, 1 month and 3 months were reconstituted as per instruction for use (appendix 4) and tested concurrently in triplicate by in-house ELISA against SARS-CoV-2 S1 protein as described in the Materials and Methods. Relative potencies to the -20°C baseline and 95% confidence limits were calculated by parallel line analysis using the software CombiStats™ (Figure 5). Real time data on the degradation samples are reported as relative to the baseline -20 °C, and showed minimal loss of potency up to two weeks, even at elevated tempatures (Figure 5). The long-term stability of the candidate WHO IS was estimated by the Arrhenius model (Table 20). The predicted loss in potency for 20/136, when stored at -20°C, was 0.288 % per year. The results obtained from both the real time data and the predicted stability suggested that the preparation 20/136 is adequately stable to serve as WHO IS for SARS-CoV-2 antibody, and can be shipped at ambient temperature.

Stability studies were not conducted for each individual member of the Reference Panel due to the accelerated timeframe of the study, and the increased workload due to the concurrent pandemic. The convalescent plasma pooled to generate the Reference Panel samples were processed similar to the candidate International Standard, and each panel member formulated using the same freeze-dry conditions. This is also supported by similar product reviews (Table 5 and 6).

Discussion and conclusion

The purpose of this collaborative study was to evaluate a pool of convalescent plasma from COVID-19 recovered patients as a candidate International Standard. The aim was to assess whether the candidate material is able to harmonise the results from serological assays detecting anti-SARS-CoV-2 antibodies. Also, as part of the study, a candidate International Reference Panel for anti-SARS-CoV-2 antibody was characterised; the panel will facilitate the development and evaluation of serological assays. Forty-four participants from fifteen countries took part in the study. Several other laboratories volunteered to join the study; however, due to the accelerated timeframe for the establishment of the International Standard in response to the pandemic concurrent with this study, no more participants were accepted after the study commenced on 2nd July 2020. Despite the restrictions and increased workload due to the pandemic, the main issue encountered was obtaining import permits for the dispatch of the collaborative study material, indeed three participants have still not received the collaborative study samples (as of 11th November 2020).

The candidate International Standard was evaluated in parallel with other preparations including two pools of convalescent sera. There has not been any evidence of a difference in the performance of the assays between serum or plasma, with only one exception when using a cell fusion inhibition assay (Lab 10b, Table 19); this assay could detect the low antibody titre plasma samples, but not the high titre serum pool. Overall the collaborative study samples were evaluated in 125 assays, including live and pseudotyped virus neutralisation assays (Table 2), ELISA (Table 3), lateral flow immunoassays, flow-cytometry based assays and inhibition assays (Table 4). The majority of the ELISA methods detected total immunoglobulins or IgG, but five laboratories used IgA-specific assays (Table 15) and three laboratories IgM-specific assays (Table 16). Antibody reactivities to the main SARS-CoV-2 antigens were also evaluated.

The candidate International Standard, sample G, was detected as one of the top three highest titre samples in every assay, with a few exceptions; it was scored as negative in ELISA for anti-M IgG (Lab 1a; Table 18) borderline for IgM against M protein and negative for E protein-specific IgM (Lab 22h,i; Table 16).

Expressing the neutralising antibody titres relative to the candidate International Standard reduced inter-laboratory variation for almost all the positive samples as measured by a reduction in %GCV, narrowing of the inter-quartile range and an increased percentage of laboratories within a two-fold range of the sample median (Table 9, Figure 2). For the two samples with the lowest detectable neutralising antibody titres, D-CP low and I-RP low, expressing the titre relative to the candidate International Standard did not change significantly the %GCV or the variability of the results between laboratories (Figure 2). A possible explanation is that because the titres for these samples are towards the limit of detection of the assays, which is similar between all the assays, there is much less spread in the results, making it difficult to further reduce the variability; indeed, both samples have the lowest reported %GCV, the highest percentage of laboratories within a two-fold range of sample median and narrowest inter-quartile range (Table 9). Reduction of the inter-laboratory variation by expressing the ELISA antibody titres relative to the candidate International Standard was greater than the one observed for neutralisation assay (Table 14). The main reason is the variety of units used to report the antibody titre from the different types of assays, with potency values reported between laboratories for the same sample varying by over 10000-fold (Table 10 and 11). The use of a common unitage greatly improves comparability between assays (Table 12 and 13). Most of the ELISAs detected total antibody or IgG against various SARS-CoV-2; in-house assays were directed mainly against RBD and full Spike protein, whilst the majority of the commercial assays in this study were specific for S1 or N protein. In every case, the candidate International Standard was detected and harmonised the results between laboratories for a specific antigen (Figure 4). Two laboratories, Lab 1 and 22, detected antibodies against M and E. Lab1a did not detect anti-M immunoglobulins in the candidate IS, sample G, only in the lower samples C-CS-low (Table 18). Instead lab22c detected anti IgG against M for every study sample, including the negative sample H (Table 12), and anti-M IgM for sample F-RP high and the candidate IS, G was scored as borderline (Table 16). For anti-E antibodies, for IgM lab22h scored all the samples negative, while lab1b detected anti-E IgM in all the samples; the inverse was observed between these two lab for anti-E IgG. As these are results from only two laboratories it is impossible to make any conclusion.

A few laboratories tested the samples in assays specific for IgA and IgM. The candidate International Standard and at least the RP high titre, sample F contained IgA and IgM responses

to SARS-CoV-2 RBD, S1, spike and N. Due to the limited number of datasets, harmonisation of the IgA and IgM responses by the candidate material could not be assessed statistically; nevertheless, sample G can still be used to confirm assay performance for those classes of immunoglobulin against SARS-CoV-2 antigens.

In all the methods used in this collaborative study, the members of the candidate International Reference Panel were ranked similarly with very few exception and these are mostly restricted to data from a single lab; sample F-RP high (NIBSC code 20/150) has the highest antibody titer in every assay, followed by sample J-RP mid (NIBSC code 20/148). Sample E-RP lowS, high N (NIBSC code 20/144) was either higher or equally potent to sample I-RP low (NIBSC code 20/140) and sample H-RP neg (NIBSC code 20/142) was negative in almost all the assays.

The geometric mean of the collaborative study samples expressed in IU/mL, calibrated using sample G with an arbitrary value of 1000 IU/mL, is similar for the low antibody titre samples E, D, I and J measured using either ELISA or neutralisation assays, but it is two-fold higher for samples with high titre A, B and F when measured by ELISA compared with neutralisation assays. This could be due to sample G having a higher content of binding antibody than neutralising, in comparison with those samples. As the the difference is approximately 2-fold, it is likely still more useful to have the same unitage for the IS, but with a distinction between neutralising antibody activity and binding antibody activity.

Sample A-CP high (NIBSC code 20/130) is a research reagent which was made available for the development of serological assay for SARS-CoV-2 at the end of April 2020. It has been included in this collaborative study to permit accurate calibration against the candidate International Standard thus allowing laboratories to back calibrate data obtained using 20/130 as an internal control and report their results in IU/mL. The potency of 20/130 was calculated as the geometric mean antibody titre across all the neutralisation assays and the IgG-based ELISAs expressed as relative to the candidate International Standard sample G with an arbitrary assigned unitage of 1000 IU/mL: it is calculated to be 1299 IU/mL (95% confidence limits 981-1719) for neutralising antibody activity and 557 IU/mL (95% confidence limits 446-697) for binding antibody activity.

Proposal

It is proposed that the pool of convalescent plasma from 11 SARS-CoV-2 positive patients, sample G, NIBSC code 20/136, is established as the WHO International Standard for SARS-CoV-2 antibody. In the initial draft of this report sent to the participants we proposed a common arbitrary assigned unitage of 250 IU/ ampoule for the quantification of both neutralising and binding antibodies against SARS-CoV-2 antigens. Following comments from participants and further discussions, it is now proposed a unitage for the WHO Internation Standard of 250 IU/ampoule for neutralising antibody activity and 250 IU/ampoule for binding antibody activity. Instructions for Use of the proposed WHO International Standard are presented in Appendix 4. Approximately 3000 ampoules (0.25mL/ ampoule) are available for distribution. Based on the stability study results, we proposed that the International Standard is kept at -20˚C for long term storage, but can be shipped at ambient temperature.

It is proposed that a panel of freeze-dried pools of convalescent plasma from COVID-19 recovered patients consisting of sample code 20/150 (sample F, high), 20/148 (sample J, mid),

20/144 (sample E, low S, high N), 20/140 (sample I-low) and 20/142 (sample H, neg) are established as the WHO International Reference Panel for anti-SARS-CoV-2 antibodies. No unitage in IU/mL will be proposed for the Reference Panel members, however, in the Instructions for Use of the proposed WHO International Reference Panel the geometric mean of the antibody titres from this collaborative study will be included as representative data to provide guidance in the use of the panel.
Approximately 2500 reference panels (0.25mL/ ampoule) are available for distribution. Similar to the International Standard, we proposed that the Reference Panel is kept at -20˚C for long term storage, but can be shipped at ambient temperature.

Comments from participants:

The draft report was sent to participants to confirm the correct interpretation of their data and for comments on the Discussion and Proposal of this study. Thirty-six participants replied. The following participants accepted the report and returned no further comments, just minor corrections on the text, their information or their results' interpretation : Lab 2, 3, 4, 5, 6, 7, 8, 10, 11, 13, 14, 16, 17, 19, 20, 21, 23, 24, 27, 30, 31, 32, 34, 36, 37, 38, 40, 42, 44.

Comments from the following participants:

Lab 1: We find the pattern (IgGs to various antigens) *[as analysed relative to sample G by the lab and included in Figure 6]* very close to Figure 2 by neutralizing assays.

Lab 9: I reviewed the documents and there are some minor typos but otherwise it looks good. Are you going to perform any additional analysis with the dataset?

Lab 24: I would strongly suggest to have this final report submitted to a reputable scientific journal, since establishing the 1st international reference standard is very critical and more than ever for harmonizing antibody detection and vaccine development for combatting the COVID-19 pandemic.

Lab 25: Sample G contains a higher level of binding antibodies (compared to Sample A); however, a lower level of neutralizing antibodies. Assignment of an IU/mL value for Sample A is based on both neut and ELISA results as they relate to Sample G. The assignment of 739 IU/mL for Sample A potentially overestimates the binding antibodies but underestimates the neut antibodies. If both Sample A and Sample G are used to calibrate results to IU/mL; the comparison may not be consistent for both neut and ELISA methods. Support the use of Sample G (pool of plasma from 11 donors) as WHO International Standard to calibrate results to IU/mL values; however, use of Sample A (plasma from a single donor) to calibrate results to IU/mL could be problematic**.**

Lab 35: Sample G gave us a lot of trouble with a non-specific background in a fluorescent-based assay, it was quite a sticky sample, but it seemed to cause this odd background. The background titrates out before the antibody effect, so we still see it as a positive with a good titre. It would not be an issue for a non-fluorescent assay, if we try it in our manual assay which uses HRP staining there is no problem.

Lab 41: After review the report, we totally agree with the choices of the 1st WHO International Standard for SARS-CoV-2 antibody and the 1st WHO International Reference Panel for SARS-CoV-2 antibodies. We sincerely hope there is a way forward for assigning a quantitative unit, e.g. microgram/ampoule, to the 1st WHO international standard instead of the arbitrary unit of 1000 IU/mL.

Lab 43: the low sensitivity of the ACE2 binding inhibition assay may reflect the extremely high affinity of the ACE2-spike interaction. Low titer samples may tend to contain antibodies with lower net avidity, and thus may compete poorly despite containing antibodies that have similar specificities. The assay is undergoing further improvements to increase sensitivity at the low end of the assay.

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Tables

Table 1. Collaborative study samples

Samples were shipped under NIBSC dispatch reference CS678

Sample	Description	formulation/vol (mL)
A-CP high (20/130)	20/130, Convalescent plasma from one patient, positive	liquid 0.1
B-CS high	Convalescent sera pool, positive	liquid 0.2
C-CS low	Convalescent sera pool, very weak positive	liquid 0.2
D-CP low	Convalescent plasma from one donor, weak positive	liquid 0.2
E-RP low S, high N	20/144, Reference Panel member, weak S, high N	f/d 0.25
F-RP high	20/150, Reference Panel member, high	f/d 0.25
G-IS	20/136, Candidate WHO IS	f/d 0.25
H-RP neg	20/142, Reference Panel member, negative	f/d 0.25
I-RP low	20/140, Reference Panel member, low	f/d 0.25
J-RP Mid	20/148, Reference Panel member, mid	f/d 0.25

CP: convalescent plasma, CS: convalescent serum; RP: reference panel; IS: International Standard; f/d: freeze-dried

Table 2. Neutralisation assay methods

Lab	type of assay	Isolate or PV platform	Readout	Output

2	PV	HIV-Luc (Wuhan-Hu-1)	RLU	NT_{50}
6d	Live	Australia/VIC01/2020	Plaques	NT_{50}
7	Live	JPN/TY/Wk-521	CPE	Inverse of the highest dilution preventing 50% CPE
10a	PV	HIV-Luc (Wuhan-Hu-1)	RLU	NT_{50}
11b	PV	HIV-Luc (Wuhan-Hu-1)	CPM	NT_{50}
12b	Live	USA-WA1/2020	Plaques	NT_{50}
13b	PV	HIV-Luc	RLU	NT_{50}
14a	Live	USA-WA1/2020	Plaques	NT_{50}
14b	PV	VSV-Luc	RLU	NT_{50}
16	PV	HIV-Luc (Wuhan-Hu-1)	RLU	AUC
18	Live	Australia/VIC01/2020	CPE	Inverse of the highest dilution preventing 50% CPE
20b	Live	Hong Kong/VM20001061/2020	Plaques	NT_{50}
21b	Live	Korea/KCDC03/2020	Foci	NT_{5o}
25	Live	USA-WA1/2020	O.D.	NT_{5o}
28b	Live	2019 n-CoV/ITALY/INMI	CPE	Inverse of the highest dilution preventing 100% CPE
29d	PV	VSV-Luc	RLU	NT_{5o}
31	Live	BavPat1/2020	Foci	NT_{5o}
32d	PV	HIV-Luc (Wuhan-Hu-1)	RLU	NT_{5o}
32e	PV	VSV-Luc (Wuhan-Hu-1)	RLU	NT_{5o}
33d	Live	clinical isolate (low passage)	live cells	Inverse of the highest dilution preventing 50% death
35	Live	England/2/2020	Plaques	NT_{5o}
36b	Live	BavPat1/2020	Plaques	NT_{5o}
37b	Live	USA-WA1/2020	O.D.	NT_{5o}
40b	PV	Not disclosed	RLU	NT_{5o}
42	Live	Australia/VIC01/2020	CPE	Inverse of the highest dilution preventing 50% CPE
44a	PV	HIV-Luc (Wuhan-Hu-1)	RLU	NT_{5o}
44b	PV	VSV-Luc (Wuhan-Hu-1)	RLU	NT_{5o}

PV: pseudotyped virus; HIV: human immunodeficiency virus; VSV: vesicular stomatitis virus; Luc: luciferase; RLU: relative luminescence units; CPM: count per minute; CPE: cytophatic effect; O.D.= optical density; NT_{50}= 50% neutralisation titre; AUC: reciprocal area under the curve.

Table 3. Immunoassay methods

Lab	In-house/commercial	class Ig	antigen	readout	Output
3	In-house	total	RBD	O.D.	highest dilution above cutoff
4a	EUROIMMUN Anti-SARS-CoV-2 ELISA (IgG)	IgG	S1	O.D.	O.D. value at 1:100
4b	EUROIMMUN Anti-SARS-CoV-2 ELISA (IgA)	IgA	S1	O.D.	O.D. value at 1:100
4c	GENSCRIPT SARS-CoV-2 Surrogate Virus Neutralization Test Kit	total	S	O.D.	% neut at 1:100 dilution
5	VITROS® Anti-SARS-COV-2 Total Ig assay	total	S1	O.D.	ratio S/CO
6a	In-house	IgG	Spike	O.D.	highest dilution above cutoff
6b	In-house	IgG	RBD	O.D.	highest dilution above cutoff
6c	In-house	IgG	N	O.D.	highest dilution above cutoff
8	Simoa Quantitative SARS-CoV-2 IgG Antibody Test	IgG	S	O.D.	ng/mL calibrated to internal standard
9a	In-house	IgG	S	O.D.	ng/mL calibrated to internal standard
9b	In-house	IgA	S	O.D.	ng/mL calibrated to internal standard
11a	In-house	IgG	S1	O.D.	highest dilution above cutoff
12a	In-house	IgG	RBD	O.D.	ratio S/CO
13a	In-house	IgG	S	O.D.	ng/mL calibrated to internal standard
15a	Meso Scale Discovery immunoassay	IgG	RBD	RLU	AU
15b	Meso Scale Discovery immunoassay	IgG	S	RLU	AU
15c	Meso Scale Discovery immunoassay	IgG	N	RLU	AU
19a	In-house	IgG	S1	O.D.	binding ratio (S/neg control)
19b	In-house	IgM	S1	O.D.	binding ratio (S/neg control)

19c	Double Antigen Bridging Assay	total	RBD	O.D.	binding ratio
20a	In-house	IgG	RBD	O.D.	highest dilution above cutoff
20c	GENSCRIPT SARS-CoV-2 Surrogate Virus Neutralization Test Kit	total	S	O.D.	% neut at 1:100 dilution
21a	In-house	IgG	S	O.D.	AU
22a	In-house	IgG	RBD	O.D.	highest dilution above cutoff
22b	In-house	IgG	S	O.D.	highest dilution above cutoff
22c	In-house	IgG	M	O.D.	highest dilution above cutoff
22d	In-house	IgG	E	O.D.	highest dilution above cutoff
22e	In-house	IgM	N	O.D.	highest dilution above cutoff
22f	In-house	IgM	RBD	O.D.	highest dilution above cutoff
22g	In-house	IgM	S	O.D.	highest dilution above cutoff
22h	In-house	IgM	M	O.D.	highest dilution above cutoff
22i	In-house	IgM	E	O.D.	highest dilution above cutoff
22j	In-house	IgM	N	O.D.	highest dilution above cutoff
22k	In-house	IgA	RBD	O.D.	highest dilution above cutoff
22m	In-house	IgA	S	O.D.	highest dilution above cutoff
22n	In-house	IgA	M	O.D.	highest dilution above cutoff
22o	In-house	IgA	E	O.D.	highest dilution above cutoff
22p	In-house	IgA	N	O.D.	highest dilution above cutoff
23a	EUROIMMUN Anti-SARS-CoV-2 ELISA (IgA)	IgA	S1	O.D.	ratio S/CO at 1:100

23b	EUROIMMUN Anti-SARS-CoV-2 ELISA (IgG)	IgG	S1	O.D.	ratio S/CO at 1:100
23c	EUROIMMUN Anti-SARS-CoV-2 NCP ELISA (IgG)	IgG	N	O.D.	Ratio S/CO at 1:100
23d	EUROIMMUN Anti-SARS-CoV-2 NCP ELISA (IgM)	IgM	N	O.D.	ratio S/CO at 1:100
24a	In-house	IgG	S1	MFI	AU
24b	In-house	IgG	S1+S2ECD	MFI	AU
24c	In-house	IgG	N	MFI	AU
24d	In-house	IgG	RBD	MFI	AU
24e	In-house	IgG	S2	MFI	AU
26	In-house	IgG	S	O.D.	highest dilution above cutoff
27	In-house	IgG	S	O.D.	AU
28a	In-house	IgG	RBD	O.D.	highest dilution above cutoff
29a	In-house	IgG	S	O.D.	AU
29b	In-house	IgG	N	O.D.	AU
29c	In-house	IgG	RBD	O.D.	AU
30a	In-house	IgG	peptides (S)	O.D.	pos/neg
30b	In-house	IgG	peptides (S)	O.D.	pos/neg
30c	In-house	IgG	peptides (S)	O.D.	pos/neg
30d	In-house	IgG	peptides (N)	O.D.	pos/neg
30e	In-house	IgG3	peptides (S)	O.D.	pos/neg
30f	In-house	IgG1	peptides (S)	O.D.	pos/neg
30g	In-house	IgG1	peptides (N)	O.D.	pos/neg
32a	In-house	IgG	N	O.D.	highest dilution above cutoff
32b	In-house	IgG	S	O.D.	highest dilution above cutoff

32c	In-house	IgG	RBD	O.D.	highest dilution above cutoff
33a	EUROIMMUN Anti-SARS-CoV-2 ELISA (IgG)	IgG	S1	O.D.	ratio S/CO at 1:100
33b	Abbott Architect SARS-CoV-2 IgG	IgG	N	RLU	ratio S/CO neat
33c	Diesse Chorus SARS-CoV-2 IgG ELISA	IgG	total lysate		ratio S/CO
34a	Beijing WANTAI SARS-CoV-2 Ab ELISA	total	RBD	O.D.	highest dilution above cutoff
34b	Roche - Elecsys Anti-SARS-CoV-2	total	N	COI	highest dilution above cutoff
34c	Abbott - Architect SARS-CoV-2 IgG	IgG	N	RLU	highest dilution above cutoff
34d	DiaSorin - LIAISON® SARS-CoV-2 S1/S2 IgG	IgG	S1/S2	AU/mL	highest dilution above cutoff
34e	Abbott-Architect SARS-CoV-2 IgG Quant Assay (RUO version)	IgG	Spike	AU*/ mL	highest dilution above cutoff
36a	In-house	IgG	RBD	O.D.	highest dilution above cutoff
37a	In-house	IgG	S	O.D.	AU
38	In-house	IgG	S	O.D.	ratio S/CO
40a	In-house	IgG	S1	O.D.	highest dilution above cutoff
40c	EUROIMMUN Anti-SARS-CoV-2 ELISA (IgA)	IgA	S1	O.D.	ratio S/CO at 1:100
43a	In-house	IgG	S	O.D.	AU

RBD: receptor binding domain; S: spike protein; S1: subunit 1 of the S protein; S2: subunit 2 of the S protein; N: nucleoprotein; E: envelope; M: membrane protein: ECD: extra-cellular domain; O.D.:optical density; RLU: relative luminescence units; MFI: mean fluorescence intensity; COI: cut-off index; AU: arbitrary units;AU*:Abbott units; ratio S/CO: ratio between value of the sample (S) and value of the control or cut-off (CO).

Table 4. Other assays methods

Lab	type of assay	class Ig	antigen	Readout	Output
1a	Flow cytometry (binding Ab)	IgG, IgM and IgA	M	MFI	inverse dilution factor
1b	Flow cytometry (binding Ab)	IgG, IgM and IgA l	E	MFI	inverse dilution factor

1c	Flow cytometry (binding Ab)	IgG, IgM and IgA	N	MFI	inverse dilution factor
1d	Flow cytometry (binding Ab)	IgG, IgM and IgA	S1	MFI	inverse dilution factor
1e	Flow cytometry (binding Ab)	IgG, IgM and IgA	S2	MFI	inverse dilution factor
1f	Flow cytometry (binding Ab)	IgG, IgM and IgA	S1+S2 ECD	MFI	inverse dilution factor
1g	Flow cytometry (binding Ab)	IgG, IgM and IgA	RBD	MFI	inverse dilution factor
10b	Fusion inhibition assay	total	S	RLU	IC_{50}
17	Imuno-Rápido COVID-19 IgG/IgM – WAMA Diagnóstica.	IgG and IgM	N+S	Colometric	pos/neg
30h	Flow cytometry (binding Ab)	IgG	S	%pos	pos/neg
30i	Flow cytometry (binding Ab)	IgM	S	%pos	pos/neg
30j	Flow cytometry (binding Ab)	IgG1	S	%pos	pos/neg
30k	Flow cytometry (binding Ab)	IgG2	S	%pos	pos/neg
30l	Flow cytometry (binding Ab)	IgG3	S	%pos	pos/neg
30m	Flow cytometry (binding Ab)	IgG4	S	%pos	pos/neg
39	SGTi-flex COVID-19 IgM/IgG-Sugentech, Inc.	IgG and IgM	N and S	Colometric	pos/neg
41a	Flow cytometry (binding Ab)	IgG	RBD	MFI	µg/mL
41b	Flow cytometry (binding Ab)	IgG	S	MFI	µg/mL
41c	Flow cytometry (surrogate neut)	total		MFI	NT_{50}
43b	ACE2 binding inhibition	total	S	O.D.	IC_{50}

RBD: receptor binding domain; S: spike protein; S1: subunit 1 of the S protein; S2: subunit 2 of the S protein; N: nucleoprotein; E: envelope; M: membrane protein: ECD: extra-cellular domain; O.D.:optical density; RLU: relative luminescence units; MFI: mean fluorescence intensity; NT_{50}: 50% neutralisation titre; IC_{50}: 50% inhibitory concentration.

Table 5. Candidate International Standard for anti-SARS-CoV-2 antibody formulation review

Microbiological test for bacterial and mould/yeast colony count returned negative

Sample name	G, candidate IS
Product code	20/136
No. containers filled	3361
Mean fill mass (g)	0.2686 (n=143)
CV of fill mass (%)	0.9686
Mean residual moisture (%)	2.9163 (n=12)
CV of residual moisture (%)	54.54
Mean oxygen head space (%)	0.25 (n=12)
CV of oxygen space (%)	36.68

n = number of samples tested

Table 6. Candidate Reference Panel members for anti-SARS-CoV-2 antibody formulation review

Microbiological test for bacterial and mould/yeast colony count returned negative

Sample name	F-RP High	J-RP Mid	E-RP low S, high N	I-RP low	H- RP neg
Product code	20/150	20/148	20/144	20/140	20/142
No. containers filled	2930	2836	2842	2701	2891
Mean fill mass (g)	0.2688 (n=105)	0.2681 (n=108)	0.2686 (n=105)	0.2688 (n=99)	0.2686 (n=113)
CV of fill mass (%)	0.6166	0.8705	0.76	0.4179	0.80
Mean residual moisture (%)	0.8740 (n=12)	1.0650 (n=12)	0.74 (n=12)	1.7935 (n=12)	1.5437 (n=12)

CV of residual moisture (%)	53.09	35.41	23.1	30.35	19.03
Mean oxygen head space (%)	0.35 (n=12)	0.15 (n=12)	0.44 (n=12)	0.48 (n=12)	0.44 (n=12)
CV of oxygen space (%)	53.09	72.95	24.74	19.75	30.74

n = number of samples tested

Table 7. Geometric mean of SARS-CoV-2 neutralising antibodies, as reported by the participants

Type	Lab	Sample									
		A-CP high (20/130)	**B- CS high**	**C-CS low**	**D-CP, low**	**E- RP low S, high N**	**F- RP high**	**G- IS**	**H- RP neg**	**I-RP low**	**J-RP mid**
Live-PRNT	6d	1751	727	<20	54	526	3462	3020	<20	280	683
Live-PRNT	12b	5313	301	<20	66	249	3143	2911	<20	96	733
Live-PRNT	14a	375	172	<20	91	165	886	446	<20	125	105
Live-PRNT	20b	320	40	<10	<10	80	640	640	<10	10	160
Live-PRNT	35	640	63	<20	22	32	373	403	<20	40	173
Live-PRNT	36b	2032	80	<20	20	34	1280	871	<20	<20	160
Live-FRNT	21b	8127	570	28	80	403	7241	4064	<20	202	718
Live-FRNT	31	>1024	43	<8	17	12	698	290	<8	<8	70
Live-CPE	7	127	18	<5	<5	13	113	113	<5	7	25
Live-CPE	18	120	35	<20	<20	23	148	109	<20	<20	55
Live-CPE	28b	403	25	<10	<10	13	63	202	<10	<10	32
Live-CPE	33d	1280	80	<40	<40	<40	905	640	<40	<40	<40
Live-CPE	42	3225	160	<20	32	127	4064	1140	<20	57	202
Live-MN	25	1489	43	<10	<10	17	617	431	<10	<10	120
Live-MN	37b	>2560	226	<20	<20	160	>2560	1280	<20	43	453
GM (Live)#		**1079**	**103**	**28***	**40**	**70**	**957**	**686**	**-**	**55**	**178**
PV-LVV	2	4104	393	<40	88	197	4659	2239	<40	91	586

PV-LVV	10a	1016	85	<10	12	90	1076	830	<10	50	151
PV-LVV	11b	4094	290	<50	<50	212	23212	5939	<50	93	723
PV-LVV	13b	2378	460	<10	26	495	7191	9319	<10	<10	292
PV-LVV	16	7	1	<1	<1	1	11	10	<1	1	2
PV-LVV	32d	3711	854	<50	<50	660	7990	4794	<50	116	1324
PV-LVV	40b	7186	227	<10	15	130	5711	3902	<10	43	386
PV-LVV	44a	1867	262	<20	30	229	6090	2766	<20	72	472
PV-VSV	14b	355	200	<20	102	324	490	254	<20	114	335
PV-VSV	29d	5232	163	<10	19	99	2623	1382	<10	25	246
PV-VSV	32e	>12150	330	<50	<50	320	7594	3440	<50	129	677
PV-VSV	44b	4304	206	<20	119	124	3653	2729	<20	65	249
GM (PV)#		**3028**	**267**	**-**	**36**	**215**	**4256**	**2431**	**-**	**72**	**415**
GM (combined)#		**1699**	**157**	**28***	**38**	**117**	**1920**	**1197**	**-**	**63**	**262**

*titre from one participant; #Lab 28b, and 16 were excluded from calculation of the geometric mean (GM); PRNT: plaque reduction neutralisation assay; FRNT: foci reduction neutralisation assay; CPE: cytophatic effect detection assay; MN: microneutralization assay; PV: pseudotyped virus-based neutralisation assay; LVV: lentiviral (HIV) vector; VSV: vesicular stomatitis virus.

Table 8. Geometric mean of SARS-CoV-2 neutralising antibodies expressed relative to the candidate IS, Sample G

Type	Lab	Sample								
		A-CP high (20/130)	**B- CS high**	**C-CS low**	**D-CP, low**	**E- RP low S, high N**	**F- RP high**	**H- RP neg**	**I-RP low**	**J-RP mid**
Live-PRNT	6d	0.580	0.272	-	0.019	0.200	1.146	-	0.094	0.246
Live-PRNT	12b	1.825	0.103	-	0.023	0.086	1.381	-	0.033	0.252
Live-PRNT	14a	0.840	0.386	-	0.214	0.363	1.984	-	0.289	0.236
Live-PRNT	20b	0.500	0.063	-	-	0.125	1.000	-	0.016	0.250
Live-PRNT	35	2.000	0.157	-	0.050	0.070	0.926	-	0.088	0.429
Live-PRNT	36b	2.333	0.092	-	0.023	0.039	1.470	-	-	0.184
Live-FRNT	21b	2.000	0.140	-	0.020	0.099	1.782	-	0.050	0.177
Live-FRNT	31	-	0.150	-	0.058	0.040	2.412	-	-	0.242

Live-CPE	7	1.122	0.157	-	-	0.111	1.000	-	0.053	0.223
Live-CPE	18	1.099	0.320	-	-	0.214	1.360	-	-	0.509
Live-CPE	28b	2.000	0.125	-	-	0.063	0.315	-	-	0.157
Live-CPE	33d	2.000	0.125	-	-	-	1.414	-	-	-
Live-CPE	42	2.828	0.140	-	0.028	0.111	3.564	-	0.050	0.177
Live-MN	25	3.453	0.100	-	-	0.039	1.431	-	-	0.279
Live-MN	37b	-	0.177	-	-	0.125	-	-	0.035	0.354
GM (Live)		**1.503**	**0.149**	**-**	**0.037**	**0.097**	**1.334**	**-**	**0.056**	**0.251**
PV-LVV	2	1.833	0.175	-	0.039	0.088	2.081	-	0.041	0.262
PV-LVV	10a	1.414	0.074	-	0.014	0.105	1.297	-	0.096	0.210
PV-LVV	11b	0.689	0.049	-	-	0.036	3.908	-	0.016	0.122
PV-LVV	13b	0.247	0.029	-	0.003	0.084	0.602	-	-	0.031
PV-LVV	16	0.747	0.114	-	-	0.104	1.131	-	0.057	0.159
PV-LVV	32d	0.774	0.178	-	-	0.138	1.667	-	0.024	0.276
PV-LVV	40b	1.841	0.058	-	0.004	0.033	1.463	-	0.011	0.099
PV-LVV	44a	0.675	0.095	-	0.010	0.083	2.202	-	0.026	0.171
PV-VSV	14b	1.396	0.785	-	0.389	1.274	1.928	-	0.382	1.318
PV-VSV	29d	3.787	0.118	-	0.014	0.071	1.898	-	0.018	0.178
PV-VSV	32e	-	0.096	-	-	0.093	2.208	-	0.037	0.197
PV-VSV	44b	1.577	0.076	-	0.043	0.045	1.339	-	0.024	0.091
GM (PV)		**1.093**	**0.103**	**-**	**0.019**	**0.093**	**1.653**	**-**	**0.036**	**0.172**
GM (Combined)		**1.299**	**0.126**	**-**	**0.026**	**0.095**	**1.473**	**-**	**0.044**	**0.210**

GM: geometric mean; PRNT: plaque reduction neutralisation assay; FRNT: foci reduction neutralisation assay; CPE: cytophatic effect detection assay; MN: microneutralization assay; PV: pseudotyped virus-based neutralisation assay; LVV: lentiviral (HIV) vector; VSV: vesicular stomatitis virus.

Table 9. Inter-laboratory variation in the neutralisation assays

		A-CP high (20/130)	**B- CS high**	**C-CS low**	**D-CP, low**	**E- RP low S, high N**	**F- RP high**	**G- IS**	**H- RP neg**	**I-RP low**	**J-RP mid**

%GCV	reported	249	179	n/a	116	231	281	241	n/a	152	161
	Relative to sample G	94	95	n/a	250	119	67	-	n/a	150	93
	Relative to sample F	118	115	n/a	218	149	-	65	n/a	182	115
GM:Med<2	reported	37%	44%	n/a	50%	46%	26%	22%	n/a	70%	46%
	Relative to sample G	67%	74%	n/a	56%	65%	85%	-	n/a	55%	81%
	Relative to sample F	63%	70%	n/a	44%	58%	-	85%	n/a	50%	69%
UQ/LQ	reported	4.039	3.759	n/a	4.118	4.094	8.177	6.765	n/a	2.671	3.851
	Relative to sample G	2.607	1.781	n/a	3.148	1.888	1.666	-	n/a	2.638	1.503
	Relative to sample F	2.887	2.350	n/a	3.348	3.050	-	1.604	n/a	4.537	2.690

GCV: Inter-Lab geometric coefficient of variation; GM:Med <2: Percentage of labs with a Lab GM to median ratio less than 2-fold; UQ/LQ: Ratio of upper quartile to lower quartile; n/a: could not be calculated as less than three data points.

Table 10 . Geometric mean of SARS-CoV-2 Total/IgG In-house ELISA methods, as reported by the participants

Antigen	Lab	Sample									
		A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**
RBD	3	81	81	<1	1	6	256	256	<1	5	32
RBD	6b	6801	4284	110	134	536	7011	9915	<200	675	4284
RBD	12a	5.1	4.8	-	-	2.6	5.3	5.6	-	2.6	4.6
RBD	19c	25.8	20.7	-	3.2	10.6	24.9	25.3	-	2.5	11.2
RBD	20a	>3200	1270	<100	<100	252	>3200	>3200	<100	252	1270
RBD	22a	8063	4032	<100	100	449	10159	8063	<100	635	2540
RBD	24d	970	459	16	<Cut-off	158	1816	2154	19	108	286
RBD	28a	9130	8171	<100	182	814	12290	14268	<100	541	2800
RBD	29c	3822	2686	<50	55	392	7139	9717	<50	236	2065
RBD	32c	433	433	<100	<100	100	1539	1539	<100	100	433
RBD	36a	800	1600	<100	<100	100	1600	2540	<100	100	504
S1	11a	>3200	>3200	<100	<100	200	>3200	>3200	<100	141	800

S1	19a	21.4	9.1	-	-	1.9	20.4	21.0	-	1.6	9.9
S1	24a	476	220	<Cut-off	<Cut-off	37	803	1245	<Cut-off	26	162
S1	40a	9535	2880	2549	2799	1963	87160	13430	641	1064	3476
S2	24e	196	102	15	<Cut-off	118	invalid	Invalid	<Cut-off	79	60
S1+S2	24b	354	173	<Cut-off	<Cut-off	75	844	1065	<Cut-off	41	171
Spike	6a	6801	5398	110	169	1349	7011	9915	<200	675	2699
Spike	9a	25199	13306	387	779	4524	47381	55372	-	2567	13312
Spike	13a	5363	1387	-	-	876	6176	12004	-	484	1640
Spike	21a	4576	2479	<20	123	545	8072	10271	<20	330	2344
Spike	22b	10159	5080	<100	100	1270	12800	18102	<100	635	2851
Spike	26	10057	5846	<60	239	1745	24880	29437	<60	847	5932
Spike	27	1023	623	Neg	22	136	1371	2439	14	90	569
Spike	29a	2878	1887	-	56	373	5262	7882	-	278	1736
Spike	30a	-	-	-	-	-	+	+	-	-	-
Spike	30b	-	-	-	-	-	+	+	-	-	-
Spike	30c	+	+	-	-	-	+	+	-	+	-
Spike	30e	+	+	-	-	-	+	+	-	-	-
Spike	30f	+	+	-	-	+	+	+	-	+	+
Spike	32b	2632	1067	100	100	513	5474	5474	<100	208	1067
Spike	37a	3464	1764	-	42	510	5284	7215	-	340	1812
Spike	38	18.7	13.9	-	-	4.1	21.4	23.7	-	3.1	11.9
Spike	43a	11825	8946	<200	294	2955	29797	28104	<200	1464	7224
M	22c	1270	566	252	<100	898	2016	2016	898	252	252
E	22d	449	713	635	<100	2016	178	283	356	252	252
N	6c	14022	5394	134	<200	1071	4284	5869	<200	134	2142
N	22e	64508	12800	504	252	6400	22807	36204	224	635	8063
N	24c	1231	270	<Cut-off	<Cut-off	198	1090	Invalid	<Cut-off	31	345
N	29b	26723	5258	38	58	1246	5043	10022	-	95	2141
N	30d	+	-	-	-	-	+	+	-	-	+
N	30g	+	-	-	-	-	+	+	-	-	+
N	32a	15601	6326	144	100	1040	6326	10817	<100	144	2565

Table 11 . Geometric mean of SARS-CoV-2 Total/IgG commercial ELISA methods, as reported by the participants

Antigen	Lab	Sample									
		A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**
RBD	15a	6360	2840	<100	<100	525	7759	12743	<100	349	3560
RBD	34a	235	65	-	-	39	1089	676	-	8	47
S1	4a	6.8	6.5	-	-	1.5	9.5	9.8	-	1.3	5.5
S1	5	234	355	-	1.6	48	280	404	-	39	222
S1	23b	7	6	-	-	1.5	7.6	7.7	-	1.3	5.3
S1	33a	8	7.2	-	-	1.7	10.6	11	-	1.8	6.3
S1+S2	34d	19	9	-	-	-	41	43	-	-	8
Spike	8	9613	4598	-	-	1007	20897	27641	-	805	4686
Spike	15b	4557	2315	<100	67	824	10982	10004	<100	491	2874
Spike	34e	53	39	-	-	6.3	109	144	-	1	32
N	15c	40332	12926	<100	<100	3089	9546	22841	<100	341	2781
N	23c	6.6	2.5	-	-	2	6.3	6.3	-	-	5.4
N	33b	4.7	3.2	-	-	3.3	7	7.3	-	-	5.6
N	34c	8	5	-	-	5	33	35	-	-	10
N	34b	6	34	-	-	46	402	240	-	5	49
Total lysate	33c	2.8	>5	-	-	2.7	>5	2.8	-	1	2.2
Surrog neut	4c	88.9%	76.6%	<20%	<20%	31.9%	90.1%	96.1%	<20%	27.5%	77.4%
Surrog neut	20c	85.3%	76.9%	<20%	<20%	38.8%	85.9%	91.7%	<20%	28.5%	79.3%

Table 12 . Geometric mean of SARS-CoV-2 Total/IgG in-house ELISA nethods expressed relative to the candidate IS, Sample G

Antigen	Lab	Sample								
		A	B	C	D	E	F	H	I	J
RBD	3	0.233	0.233	-	NP	NP	NP	-	NP	NP
RBD	6b	0.414	0.341	0.015	0.008	0.042	0.798	-	0.041	0.210
RBD	12a[1]	0.907	0.844	-	-	0.462	0.938	-	0.456	0.813
RBD	19c	1.045	0.687	-	0.088	0.282	0.963	-	0.070	0.299
RBD	20a	0.405	0.199	-	-	0.044	0.706	-	0.036	0.178
RBD	22a	0.727	0.460	-	0.008	0.053	1.076	-	0.043	0.157
RBD	24d	NP	NP	0.003	-	0.037	NP	0.004	0.023	0.069
RBD	28a	0.589	0.540	-	0.008	0.056	0.840	-	0.036	0.175
RBD	29c	0.413	0.289	-	0.006	0.041	0.723	-	0.026	0.229
RBD	32c	0.365	0.267	-	-	0.051	0.589	-	0.037	0.228
RBD	36a	0.353	0.463	-	-	0.036	0.560	-	0.035	0.247
S1	11a	0.340	0.354	-	-	0.032	0.672	-	0.024	0.167
S1	19a	0.964	0.180	-	-	0.033	0.922	-	0.026	0.202
S1	24a	0.344	0.159	-	-	0.027	0.563	-	0.018	0.111
S1	40a	0.914	0.384	NP	-	-	0.759	NP	0.099	0.216
S2	24e	-	-	-	-	-	-	-	-	-
S1+S2	24b	0.275	0.136	-	-	0.058	0.755	-	0.034	0.134
Spike	6a	0.445	0.328	0.014	0.014	0.074	0.865	-	0.046	0.218
Spike	9a	0.463	0.246	0.008	0.013	0.085	0.836	-	0.047	0.230
Spike	13a	0.485	0.131	-	-	0.086	0.552	-	0.044	0.135
Spike	21a	0.370	0.209	-	0.008	0.042	0.708	-	0.027	0.194
Spike	22b	0.457	0.284	-	0.008	0.114	0.949	-	0.047	0.154
Spike	26	0.501	0.495	-	0.010	0.160	0.771	-	0.066	0.217
Spike	27 [2]	0.826	0.507	-	0.192	0.232	1.146	0.189	0.214	0.470
Spike	29a	0.380	0.252	-	0.008	0.049	0.680	-	0.038	0.236
Spike	30a	-	-	-	-	-	0.625	-	-	-

Spike	30b	-	-	-	-	-	2.967	-	-	-
Spike	30c	18.044	0.507	-	-	-	3.186	-	0.518	-
Spike	30e	1.021	0.295	-	-	-	0.613	-	-	-
Spike	30f	25.817	0.385	-	-	0.228	2.491	-	0.323	0.198
Spike	32b	0.470	0.256	NP	0.012	0.087	0.842	-	0.043	0.242
Spike	37a[1]	0.480	0.244	-	0.006	0.071	0.732	-	0.047	0.251
Spike	38[1]	0.787	0.585	-	-	0.175	0.900	-	0.131	0.503
Spike	43a	0.425	0.296	-	0.010	0.104	1.090	-	0.061	0.250
M	22c	NP	0.269	0.109	-	0.615	0.924	0.308	0.089	0.101
E	22d	1.430	2.275	2.404	-	5.282	0.661	1.464	1.442	1.008
N	6c	1.904	0.548	0.017	-	0.141	0.591	-	0.017	0.235
N	22e	1.504	0.393	0.011	0.007	0.104	0.666	0.005	0.031	0.271
N	24c	-	-	-	-	-	-	-	-	-
N	29b	2.872	0.529	0.004	0.006	0.131	0.505	-	0.010	0.221
N	30d	13.144	-	-	-	-	1.707	-	-	2.210
N	30g	NP	-	-	-	-	2.294	-	-	4.049
N	32a	1.583	0.447	0.013	0.007	0.110	0.571	-	0.015	0.226

[1]Potencies calculated using values reported by lab; [2]Parallel line model used; NP: Non-parallel as described in the Statistical methods.

Table 13 . Geometric mean of SARS-CoV-2 Total/IgG commercial ELISA methods expressed relative to the candidate IS, Sample G

Antigen	Lab	Sample								
		A	B	C	D	E	F	H	I	J
RBD	15a	0.636	0.280	-	-	0.050	0.608	-	0.025	0.277
RBD	34a[2]	0.476	0.148	-	0.004	NP	1.631	-	NP	0.089
S1	5[1]	0.580	0.878	-	0.004	0.119	0.693	-	0.099	0.548
S1	23b	0.562	0.308	-	-	0.031	0.872	-	0.025	0.213
S1	33a[1]	0.727	0.655	-	-	0.155	0.964	-	0.164	0.573
S1+S2	34d[2]	0.409	0.218	-	-	-	0.838	-	-	0.185
Spike	8	0.392	0.216	-	-	0.046	0.775	-	0.035	0.215
Spike	15b	0.472	0.237	-	0.007	0.092	1.107	-	0.052	0.304

Spike	34e[2]	0.397	0.301	-	-	0.046	0.762	-	0.037	0.226
N	23c	1.026	0.097	-	-	0.072	0.857	-	-	0.477
N	33b[1]	0.644	0.438	-	-	0.452	0.959	-	-	0.767
N	34c[2]	0.298	0.156	-	-	0.151	0.899	-	-	0.430
N	34b[2]	0.015	0.093	-	-	0.180	1.227	-	0.012	0.240
Total lysate	33c[1]	1.000	-	-	-	0.964	-	-	-	0.786
Surrog neut	4c[1]	0.925	0.797	-	-	0.332	0.937	-	0.286	0.805
Surrog neut	20c[1]	0.931	0.839	-	-	0.423	0.937	-	0.311	0.865

[1]Potencies calculated using values reported by lab; [2]Parallel line model used; NP: Non-parallel as described in the Statistical methods. Lab 4a was not included as insufficient data were provided for the calculation of the relative potencies.

Table 14. Inter-laboratory variation in the ELISA methods

		A-CP high (20/130)	**B- CS high**	**C-CS low**	**D-CP, low**	**E- RP low S, high N**	**F- RP high**	**G- IS**	**H- RP neg**	**I-RP low**	**J-RP mid**
In-house	GM (reported)	1808	980	124	89	247	2555	3104	79	128	697
	GM (relative to G)	0.586	0.328	0.009	0.011	0.077	0.758	-	0.015	0.042	0.217
	%GCV (reported)	907	765	251	385	709	887	910	354	596	720
	%GCV (relative to G)*	82	61	96	154	102	25	-	811	114	56
	Lab GM:Med <2 (reported)	22%	28%	38%	55%	25%	40%	41%	17%	44%	39%
	Lab GM: Med <2 (Relative to G)*	75%	84%	75%	89%	70%	94%	-	67%	74%	82%
	UQ/LQ (reported)	17.40	17.18	3.80	3.03	10.19	5.89	5.61	3.95	7.44	8.90
	UQ/LQ (Relative to G)*	2.20	1.95	2.27	1.58	2.65	1.33	-	7.34	1.77	1.38

Commercial	GM (reported)	46	34	n/a	6	14	84	88	n/a	9	32
	GM (relative to G)	0.509	0.308	n/a	0.005	0.137	0.874	-	n/a	0.048	0.344
	%GCV (reported)	2772	2081	n/a	723	1690	2637	3029	n/a	1712	1745
	%GCV (Relative to G)	179	111	n/a	43	168	33	-	n/a	279	97
	Lab GM:Med <2 (reported)	39%	33%	n/a	67%	24%	11%	11%	n/a	14%	39%
	Lab GM:Med <2 (relative to G)	88%	56%	n/a	100%	40%	100%	-	n/a	45%	65%
	UQ/LQ (reported)	37.42	49.11	n/a	6.47	28.24	118.79	80.37	n/a	185.56	28.00
	UQ/LQ (Relative to G)	2.26	3.24	n/a	1.37	4.08	1.24	-	n/a	5.04	2.66

*Excluding Lab 22c, 22d and 30; GM: geometric mean; GCV: Inter-laboratory geometric coefficient of variation; GM:Med <2: Percentage of labs with a Lab GM to median ratio less than 2-fold; UQ/LQ: Ratio of upper quartile to lower quartile; n/a: could not be calculated as less than three data points

Table 15. Geometric mean of SARS-CoV-2 IgA ELISA methods, as reported by the participants

Antigen	**Lab**	**Sample**									
		A-CP high (20/130)	**B- CS high**	**C-CS low**	**D-CP, low**	**E- RP low S, high N**	**F- RP high**	**G- IS**	**H- RP neg**	**I-RP low**	**J-RP mid**
S1	4b	7.5	1.4	-	-	-	7.4	5.8	-	-	2.8
Spike	9b	1397	305	-	-	673	2915	720	-	325	629
RBD	22k	200	-	-	-	-	400	400	-	-	-
Spike	22m	252	-	-	-	100	566	400	-	-	-
M	22n	-	-	-	-	-	-	-	-	-	-
E	22o	-	-	-	-	-	-	-	-	-	-
N	22p	800	100	-	-	100	400	317	-	-	178
S1	23a	7.2	1.5	-	-	-	7.3	6.4	-	-	3.1
S1	40c	5.5	1.1	-	-	-	4.3	4.1	-	1.1	1.9

Lab 4b, 23a and 40c used the same commercially available kit

Table 16. Geometric mean of SARS-CoV-2 IgM ELISA methods, as reported by the participants

Antigen	Lab	Sample									
		A-CP high (20/130)	B- CS high	C-CS low	D-CP, low	E- RP low S, high N	F- RP high	G- IS	H- RP neg	I-RP low	J-RP mid
S1	19b	22.7	1.4	-	-	1.3	21.1	21.1	-	-	4.3
RBD	22f	32254	356	-	-	-	1131	898	-	-	112
Spike	22g	1600	-	-	-	-	1796	1008	-	-	126
M	22h	-	-	-	-	-	158	Bor	-	-	-
E	22i	-	-	-	-	-	-	-	-	-	-
N	22j	-	-	-	-	-	141	119	-	-	-
N	23d	-	-	-	-	-	1.1	-	-	-	-

bor:borderline; 23d used a commercially available kit

Table 17. Lateral Flow rapid tests for the detection of anti-SARS-CoV-2 antibody.

		A-CP high (20/130)	B- CS high	C-CS low	D-CP, low	E- RP low S, high N	F- RP high	G- IS	H- RP neg	I-RP low	J-RP mid
Lab 17	IgG	+	+	-	-	+	+	+	-	+	+
	IgM	+	-	-	-	-	+	+	-	-	+
Lab 39	IgG	1993	1112	-	-	492	1457	1784	-	68	1260
	IgM	152	-	-	-	-	332	333	-	-	158

Table 18. Geometric mean of SARS-CoV-2 binding antibody by flow cytometry-based assays, as reported by the participants

Antigen (IgG)	Lab	Sample									
		A-CP high (20/130)	B- CS high	C-CS low	D-CP, low	E- RP low S, high N	F- RP high	G- IS	H- RP neg	I-RP low	J-RP mid
M	1a	-	-	100	-	-	-	-	-	-	-
E	1b	-	-	100	-	-	-	-	-	-	-
N	1c	3057014	879207	4765	1967	148129	464311	1200669	142	9158	86281
S2	1d	160779	39190	86	4140	72671	368753	383946	-	47992	41306
S1	1e	185319	143300	-	3651	43525	946185	530254	-	32270	117497
S1+S2(ECD)	1f	151167	53955	1806	4549	69687	348996	405675	1590	64014	63118
RBD	1g	28913	5269	-	123	260	69500	69656	-	306	11263
Spike	30h	72.2	66.5	-	-	57.3	77.9	76.5	-	37.4	62.6
RBD	41a	17.3	11.8	-	0.08	1.2	26.2	28.9	-	2.1	4.9
Spike	41b	7.1	4	-	0.1	1.6	15	13.2	-	1.8	2.5
Surrog neut	41c	44	26	-	0.8	9.2	92	88	-	5.9	22
Ig class (Spike)	**Lab**	**A-CP high (20/130)**	**B- CS high**	**C-CS low**	**D-CP, low**	**E- RP low S, high N**	**F- RP high**	**G- IS**	**H- RP neg**	**I-RP low**	**J-RP mid**
IgG1	30j	72.1	77.7	-	-	69.9	83.4	82.1	-	49.9	75.2
IgG2	30k	9.6	20.3	-	-	-	24.2	14.8	-	-	-
IgG3	30l	24.7	16.2	-	-	-	54.8	44.4	-	-	10.6
IgG4	30m	8.5	-	-	-	-	11.9	11.1	-	-	-

Antigen (IgA)	Lab	A-CP high (20/130)	B- CS high	C-CS low	D-CP, low	E- RP low S, high N	F- RP high	G- IS	H- RP neg	I-RP low	J-RP mid
M	1a	-	-	100	-	-	-	-	-	-	-
E	1b	-	-	100	-	-	-	-	-	-	-
N	1c	1166	-	-	350	-	1975	420	-	-	-
S2	1d	1034	-	308	-	1032	1829	1973	-	-	107
S1	1e	1813	-	-	-	-	28980	4566	-	-	-
S1+S2(ECD)	1f	24.7	16.2	-	-	-	54.8	44.4	-	-	10.6
Antigen (IgM)	**Lab**	**A-CP high (20/130)**	**B- CS high**	**C-CS low**	**D-CP, low**	**E- RP low S, high N**	**F- RP high**	**G- IS**	**H- RP neg**	**I-RP low**	**J-RP mid**
M	1a	28	-	1721	3163	-	314	-	-	-	-
E	1b	932	5053	9829	10171	1265	3031	3687	1841	2396	2657
N	1c	627	1056	4154	3851	-	3624	935	-	-	-
S2	1d	47	1838	3282	3858	1898	10645	649	291	846	1593
S1	1e	2465	-	-	-	-	6328	4765	12	-	-
S1+S2(ECD)	1f	887	1276	3402	3559	933	6899	606	-	-	1617
Spike	30i	73.8	9.3	-	-	26.0	73.3	55.9	-	10.2	19.6

Table 19. Geometric means of the antibody titers from other serological assays.

		A-CP high (20/130)	B- CS high	C-CS low	D-CP, low	E- RP low S, high N	F- RP high	G- IS	H- RP neg	I-RP low	J-RP mid
Lab 10b-fusion inhibition	**Reported**	50.4	-	-	14.1	40	80	63.5	-	25.2	31.7
	Relative to sample G	0.794	-	-	0.222	0.630	1.260		-	0.397	0.499
Lab 43b-hACE2 blocking	**Reported**	57.6	21.6	<10	<10	<10	102.0	107.0	<10	<10	28.7
	Relative to sample G	0.538	0.202	-	-	-	0.953		-	-	0.268

Table 20. Predicted stability of the candidate WHO IS for anti-SARS-CoV-2 antibody, NIBSC code 20/136.

Temp (˚C)	K	S.E.	% loss (per year)	95% UCL
-20	0.00288	0.00191	0.288	1.235
4	0.04866	0.01787	4.75	12.891
20	0.24754	0.05025	21.928	39.273
37	1.1598	0.10273	68.645	81.24

k: degradation rate constant; S.E.:standard error of k; UCL: upper confidence level

Figures

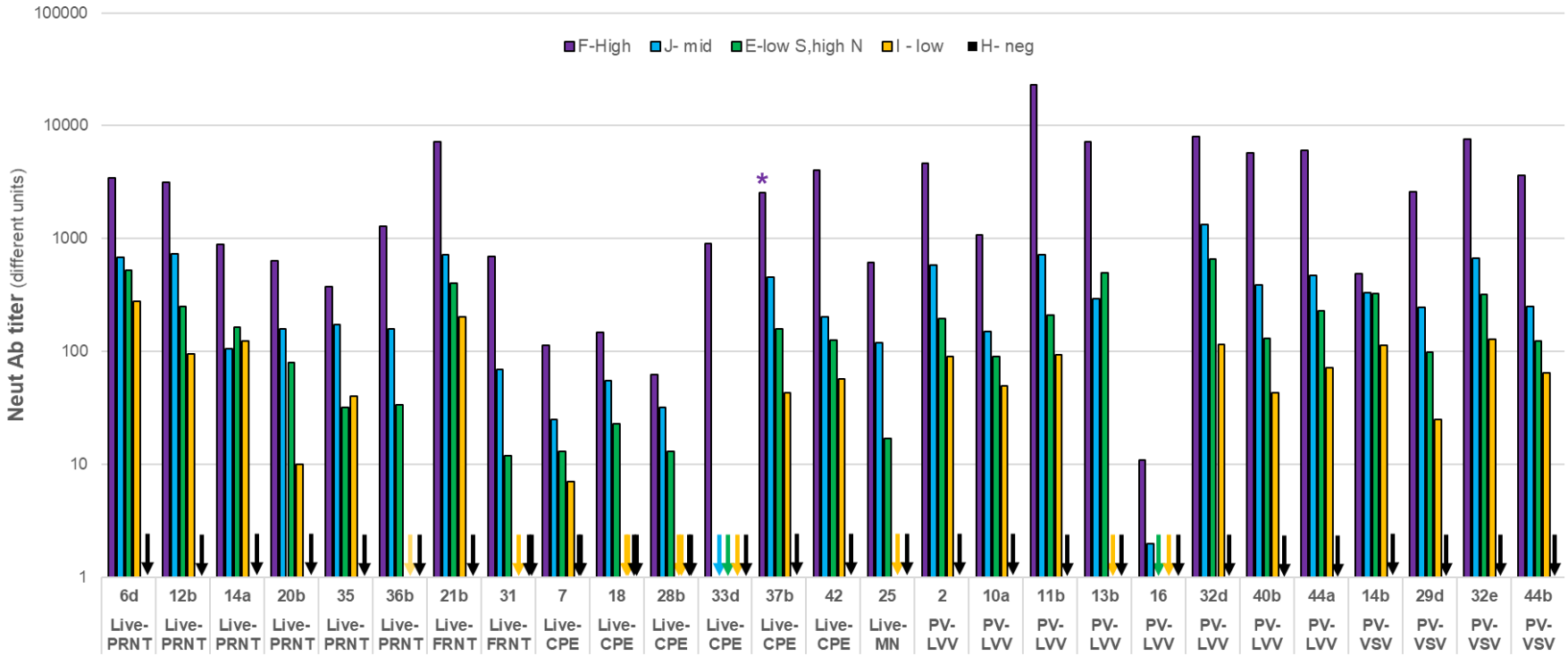


Figure 1. SARS-CoV-2 neutralising antibody titre for the Reference Panel members. Geometric mean of the antibody titre from three independent experiments, as reported by the participants. Downwards arrow indicates value below the cut-off of the assay; (*): value above the highest dilution used in the assay; PRNT: plaque reduction neutralisation assay; FRNT: foci reduction neutralisation assay; CPE: cytophatic effect detection assay; PV: pseudotyped virus-based neutralisation assay; LVV: lentiviral (HIV) vector; VSV: vesicular stomatitis virus.

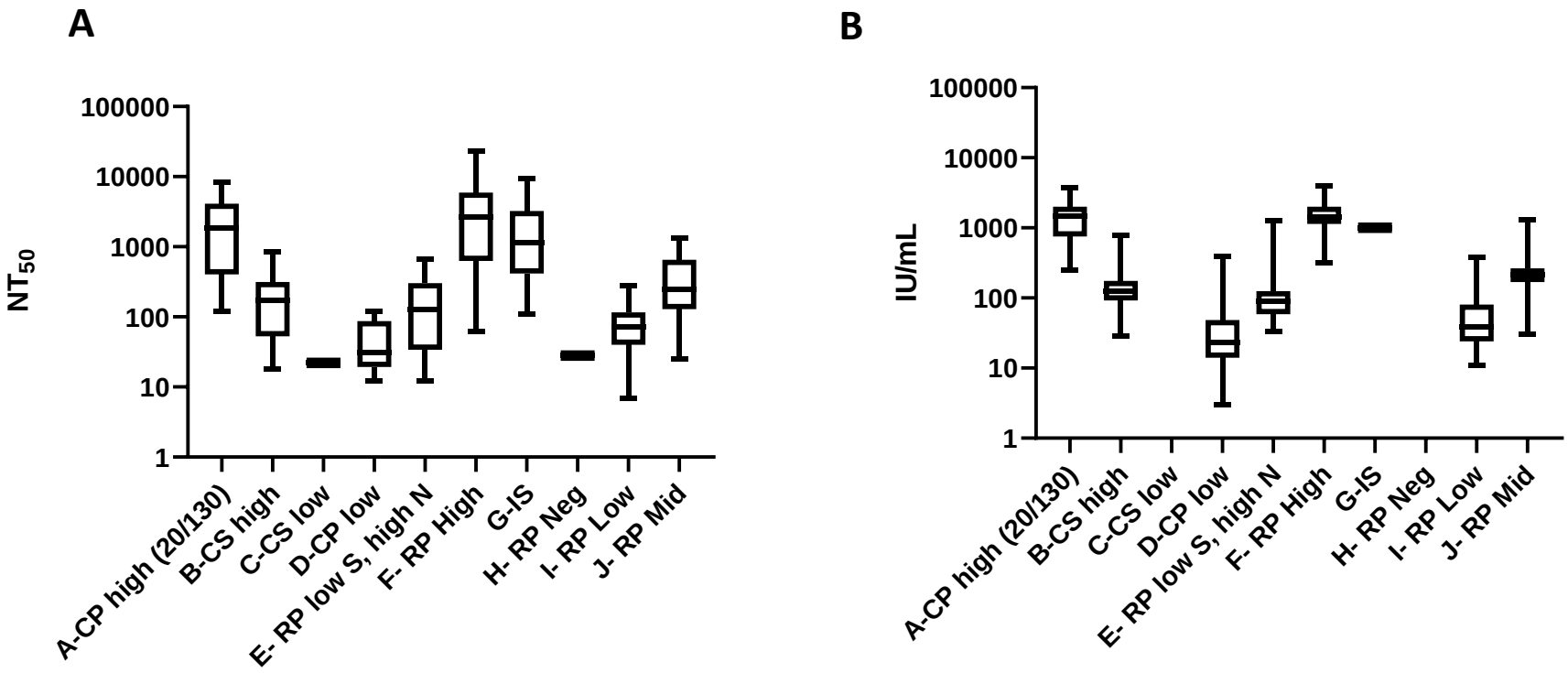


Figure 2. Harmonisation of SARS-CoV-2 antibody titres in all of the neutralisation assays when reported as relative to the candidate International Standard. A) 50% neutralisation titres reported by participants and **B)** antibody potencies expressed as relative to the candidate International Standard, sample G with an arbitrary assigned unitage of 1000 International Units per mL. The range of the values for each samples from each laboratory is represented as a box; the black line within the box marks the median; the boundary of the box indicate minimum (lower bar) and maximum (upper bar) value

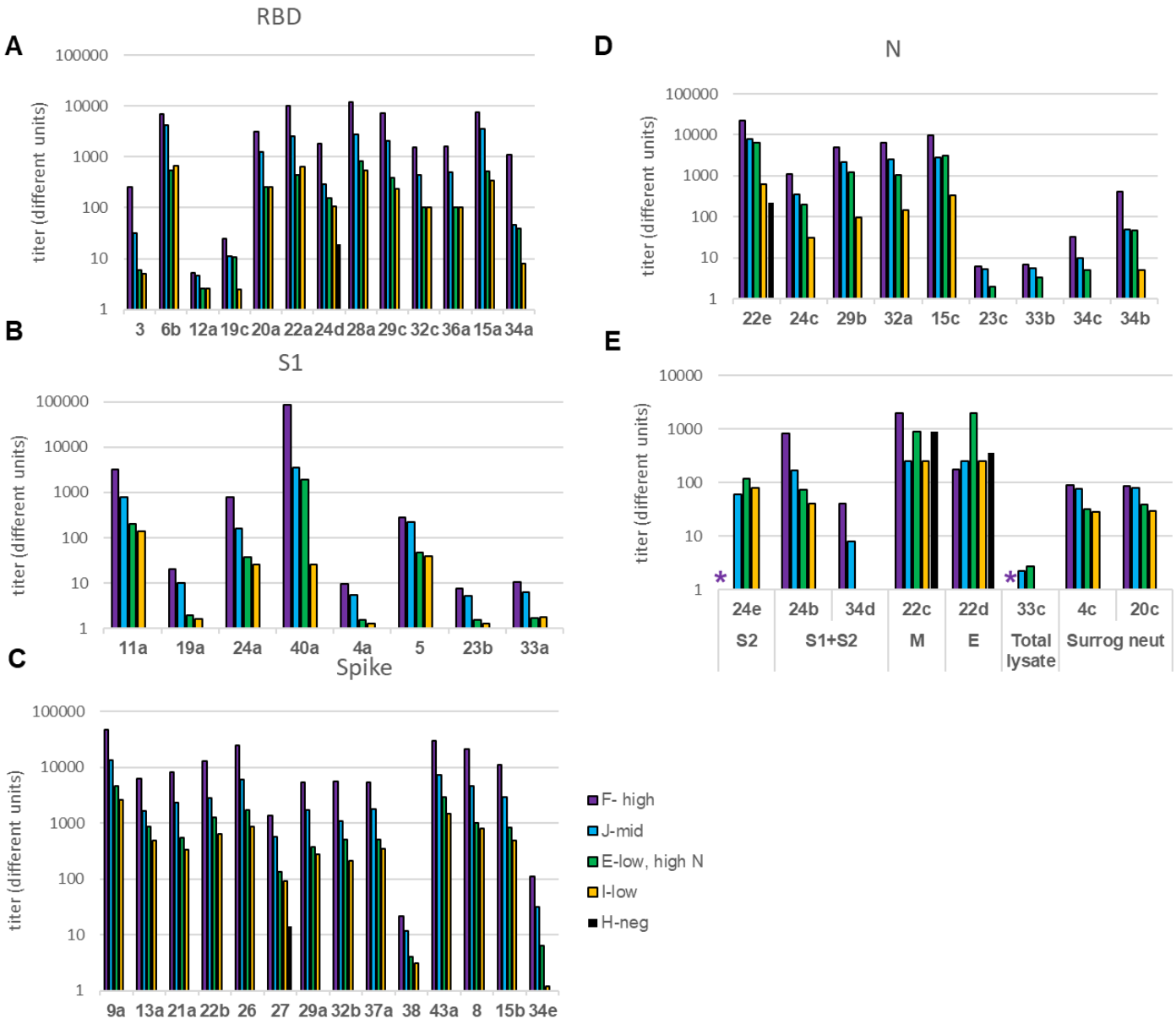
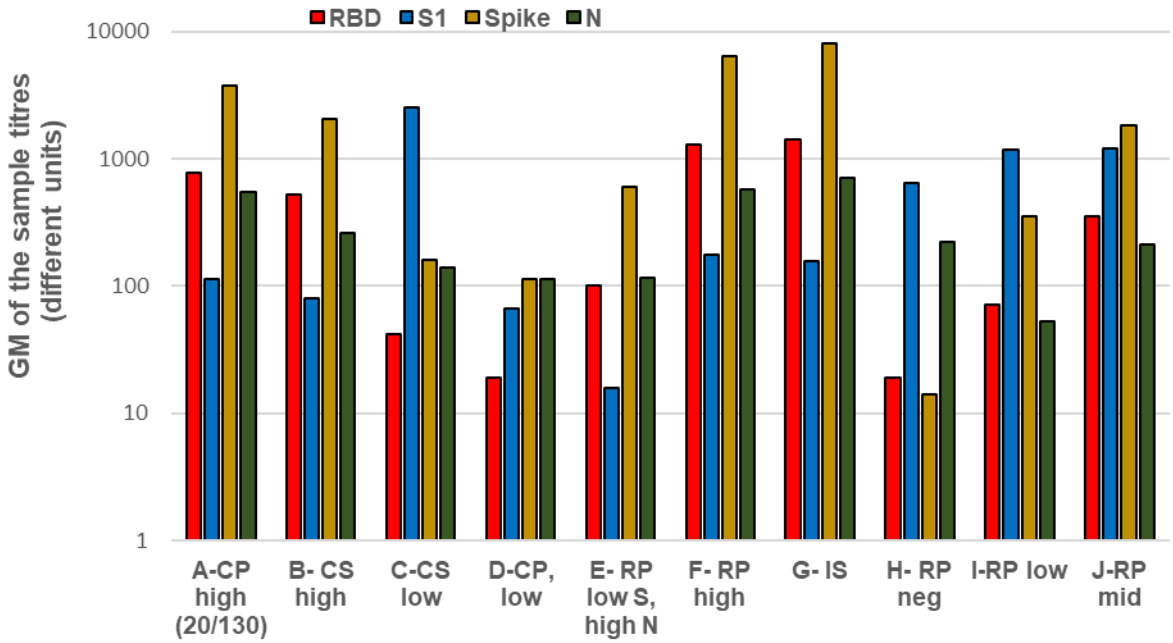


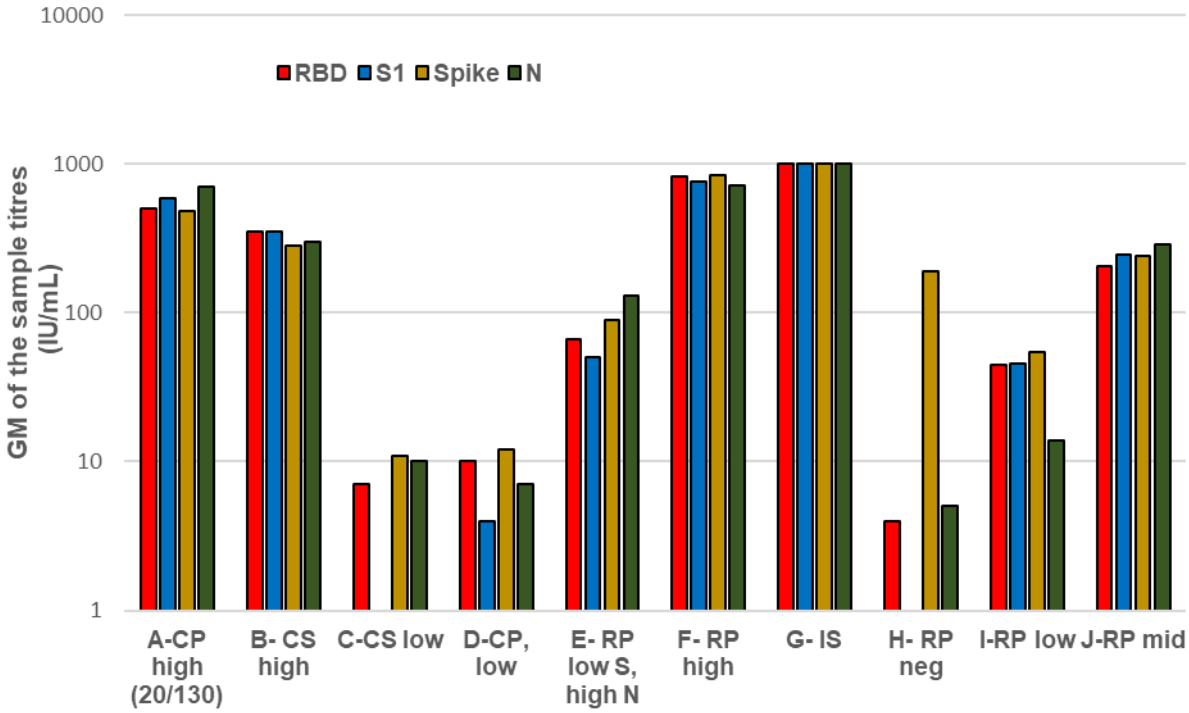
Figure 3. SARS-CoV-2 binding antibody titres for the Reference Panel members. Geometric mean of the antibody titre from three independent experiments, as reported by the participants in either an in-house or commercial ELISA. The results were divided by target antigen A) Receptor binding domain (RBD); B) subunit 1 of the spike protein (S1); C) full spike protein; D) nucleoprotein (N); E) other targets; S2: subunit 2 of the spike protein; S1+S2: extracellular domain of S1 and S2; M: membrane protein; E: envelope protein. * value higher than the limit of the assay

A



	A-CP high (20/130)	B- CS high	C-CS low	D-CP, low	E- RP low S, high N	F- RP high	G- IS	H- RP neg	I-RP low	J-RP mid
RBD	769	524	42	19	101	1283	1419	19	72	354
S1	114	81	2549	67	16	177	157	641	1186	1191
Spike	3589	1892	197	109	568	6266	7952	14	336	1788
N	501	233	140	113	125	548	657	224	87	205
GM	630	370	233	63	103	940	1039	79	224	627

B



	A-CP high (20/130)	B- CS high	C-CS low	D-CP, low	E- RP low S, high N	F- RP high	G- IS	H- RP neg	I-RP low	J-RP mid
RBD	502	350	7	10	66	817	1000	4	45	205
S1	588	353	n/a	4	50	766	1000	n/a	46	246
Spike	476	285	11	12	86	832	1000	189	53	241
N	750	301	10	7	146	713	1000	5	15	240
GM	562	321	9	8	79	783	1000	16	35	244

Figure 4. Geometric mean of binding antibody titres for each SARS-CoV-2 antigen. In-house and commercial ELISA methods were grouped based on antigen specificity; receptor binding domain (RBD), subunit 1 of spike protein (S1); spike protein; and N protein (N). The geometric mean of the antibody titre specific for each antigen was calculated for the collaborative study samples from the titres reported by the participants (**A**) or as relative to the candidate International Standard, sample G (**B**).

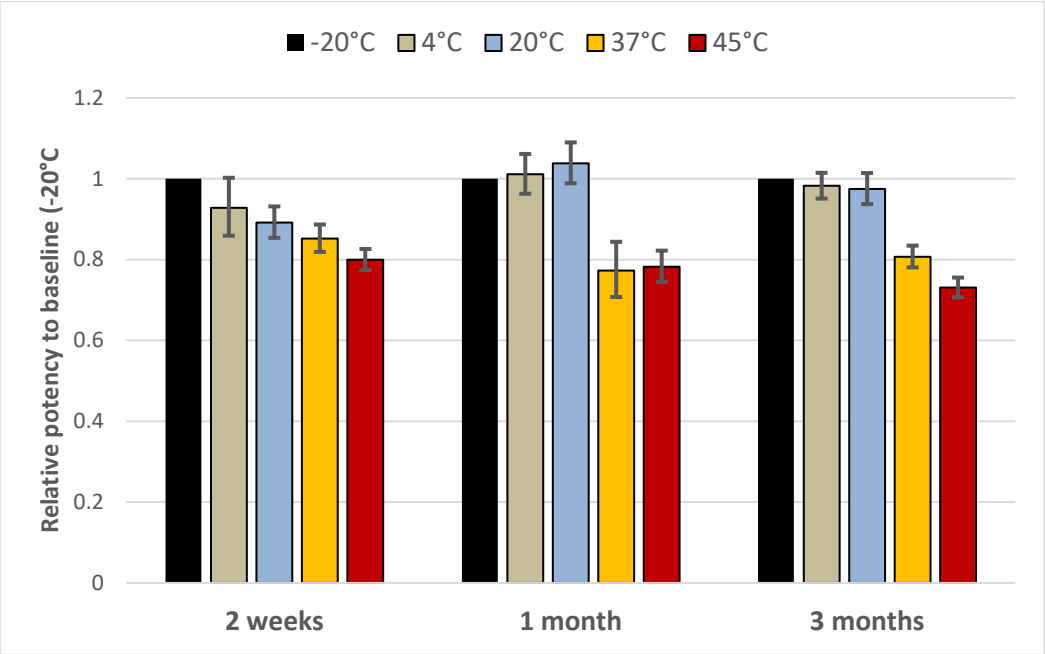


Figure 5. Thermal degradation assessment of the candidate International Standard for anti-SARS-CoV-2 antibody. Freeze-dried ampoules of sample G, NIBSC code 20/136 were stored at five different temperatures (-20, 4, 20, 37 and 45˚C). At each time point, three vials were retrieved and reconstituted according to the instruction for use. Each vial was assessed by in-house ELISA against recombinant SARS-CoV-2 S1 protein. Data are reported relative to the the baseline storage temperature of -20˚C. Error bars represent the upper and lower 95% confidence limit.

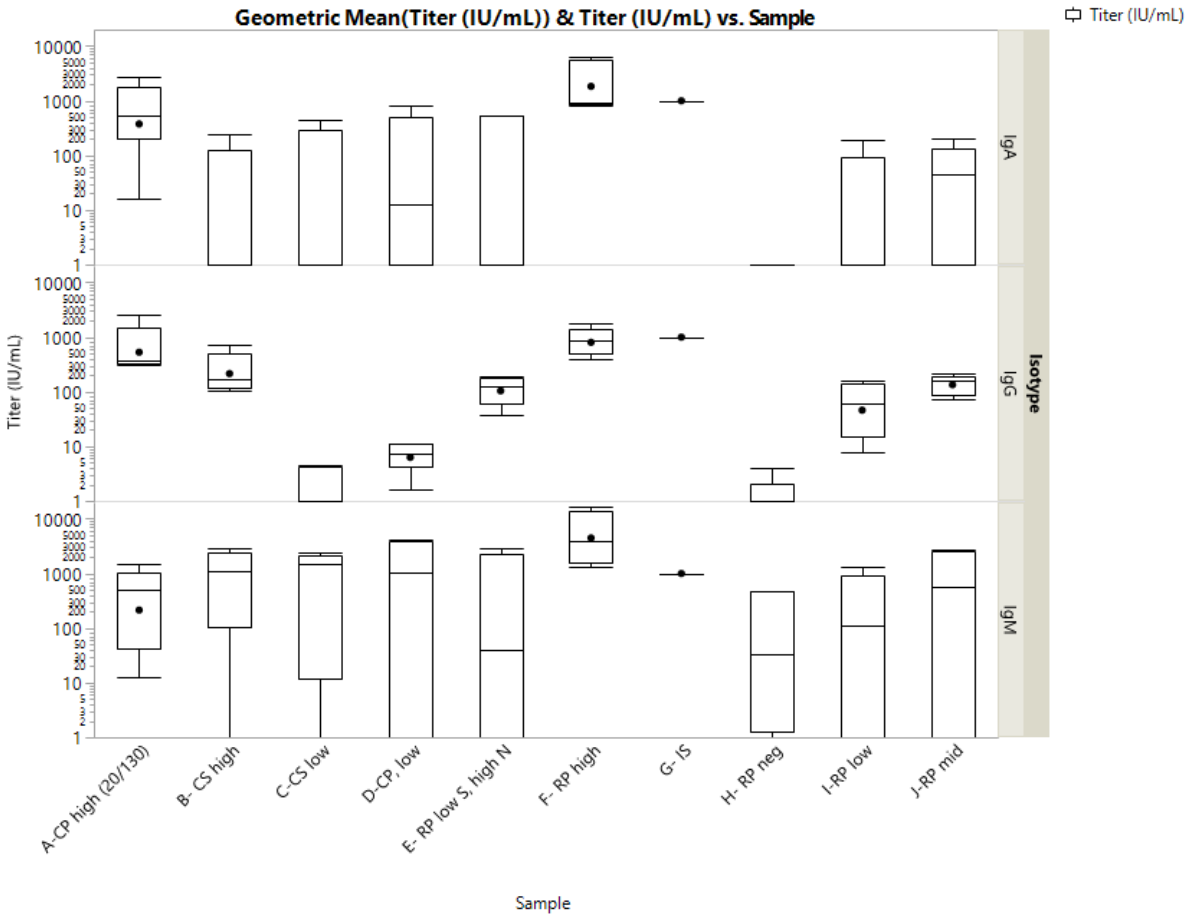


Figure 6. Antibody titres against different SARS-CoV-2 antigens from Lab 1 as relative to sample G. As feedback after consultation of the draft report, Lab 1 using a flow-cytometry based assays for the detection of IgA, IgG and IgM against SARS-CoV-2 antigens (E, M, N, RBD, S1, S2 and S1+S2ECD) provided their data expressed as relative to sample G with an arbitrary assigned unitage of 1000 IU/mL. Each box represents the mean antibody titres against all antigens tested. The results as reported by the participants are included in Table 18.

Appendix 1

Collaborative study participants

(in alphabetic order by country, and by Institution within the same country)

Participant	Organisation	Country
Melinda Pryor	360Biolabs	Australia
William Rawlinson, Zin Naing	Serology, Virology and OTDS Laboratories (SAViD), NSW Health Pathology Randwick	Australia
Kanta Subbarao, Francesca Mordant	The Peter Doherty Institute for Infection and Immunity, University of Melbourne	Australia
Theo Karapanagiotidis, Suellen Nicholson	Victorian Infectious Disease Reference Laboratory, The Peter Doherty Institute for Infection and Immunity.	Australia
Jéssica Caldeira de Lima, Ana Luísa Rubert Barcelos	WAMA Diagnóstica	Brazil
Luc Gagnon	Nexelis	Canada
Ranawaka A.P.M. Perera, Malik Peiris	School of Public Health, University of Hong Kong, Hong Kong	China
Barbara Schnierle	AIDS, New and Emerging Pathogens, Paul-Ehrlich-Institut	Germany
Katja Steinhagen, Philip Rosenstock, Konstanze Stiba	EUROIMMUN Medizinische Labordiagnostika AG	Germany
Angela Filomena	Paul-Ehrlich-Institut, Testing Laboratory for IVD	Germany
Guruprasad Medigeshi, Gagandeep Kang	Translational Health Science and Technology Institute	India
Alessandro Torelli, Alessandro Manenti, Emanuele Montomoli	Vismederi	Italy
Ken Maeda, Shuetsu Fukushi, Saya Moriyama, Yoshimasa Takahashi	National Institute of Infectious Diseases	Japan
George Warimwe, Henry Karanja	KEMRI-Wellcome Trust Research Programme	Kenya
Nisreen M.A. Okba, Bart L. Haagmans	Erasmus Medical Center	The Netherlands

Aloys Tijsma, Carel van Baalen	Viroclinics	The Netherlands
Naif Khalaf Alharbi	King Abdullah International Medical Research Centre	Saudi Arabia
Lisa FP Ng, Laurent Renia	A*STAR ID Labs, A*STAR Singapore Immunology Network, A*STAR	Singapore
Young-Joo Cha	Chug-Ang University Hospital	South Korea
Hee-Jin Huh	Dongguk University Ilsan Hospital	South Korea
Yuji Jeong, Young-Shin Park, Hoe Won Jeong, Taewoo Kim, Sang Hwan Seo, Jae-Ouk Kim, Manki Song	International Vaccine Institute	South Korea
Ruth Harvey	WHO Collaborating Centre for Reference and Research on Influenza, The Francis Crick Institute	United Kingdom
David Goldblatt	Great Ormond Street Hospital for Children, University College London	United Kingdom
Jessica O'Hara, Leon McFarlane, Hannah Cheeseman	Imperial College London	United Kingdom
Richard Tedder	Imperial College London	United Kingdom
Brian Willett, Nicola Logan, Ellen Hughes, Agnieszka Szemiel	MRC University of Glasgow Centre for Virus Research	United Kingdom
Emma Bentley, Stephanie Routley	National Institute for Biological Standards and Control	United Kingdom
Karen Buttigieg, Sue Charlton, Stephanie Longet, Bassam Hallis, Kevin Bewley, Miles Carroll	Public Health England	United Kingdom
Mustapha Bittaye, Theresa Lambe	The Jenner Institute	United Kingdom
Nazia Thakur, Carina Conceicao, Matthew Tully, Dalan Bailey	The Pirbright Institute	United Kingdom
Edward Wright, Mariliza Derveni	University of Sussex	United Kingdom

Nigel Temperton, Cecilia Di Genova	Viral Pseudotype Unit (VPU) at University of Kent	United Kingdom
Joseph Agnes, Kate Broderick, Jean Boyer	Inovio Pharmaceuticals	CA, USA
Norbert Staimer, Robert Bailer	Q2 solutions	CA, USA
Clara Di Germanio, Michael Busch	Vitalant Research Institute	CA, USA
Jian Zheng/Stanley Perlman	University of Iowa	IA,USA
Hannah Suib, Andrew Ball, David Wilson	Quanterix Corporation	MA,USA
Hang Xie Martina Kosikova, Peter Radvak, Hyung Joon Kwon, Uriel Ortega-Rodriguez,	Food and Drug Administration/CBER	MD, USA
Zhaohua Zhou & Steven Kozlowski	Food and Drug Administration/CDER	MD, USA
Linhua Tian, Elzafir Elsheikh, Paul Patrone, Anthony Kearsley, Adolfas Gaigalas, Sarah Inwood, Sheng Lin-Gibson, and Lili Wang	National Institute of Standards and Technology	MD, USA
Joyce S Plested, MingZhu Zhu, Shane Cloney-Clark,	Novavax	MD, USA
Jennifer Garver, Chris Cirimotich	Battelle Biomedical Research Center	OH, USA
Katherine Fries, Lingyi Zheng and James Huleatt	Sanofi Pasteur	PA, USA
Pedro A Piedra	Baylor College of Medicine	TX, USA

Appendix 2

Data Sheet
Research reagent for anti-SARS-CoV-2 Ab NIBSC code 20/130
(Version 1, Dated 30/04/2020)

INTENDED USE

The research reagent for anti-SARS-CoV-2 antibody is intended to be used for the development and evaluation of serological assays for the detection of antibodies against SARS-CoV-2, as a positive control.
This material is for research use only, and it has only been characterised in-house.

CONTENTS

Each vial contains 0.1 mL of frozen human plasma from a donor recovered from COVID-19. The material has been solvent-detergent treated, to inactivate any envelope virus present, using a method validated at NIBSC [1,2].

CAUTION

This preparation is not for administration to humans or animals in the human food chain.
The preparation contains material of human origin. It has been tested and found negative for HBsAg, anti-HIV and HCV RNA. As with all materials of biological origin, this preparation should be regarded as potentially hazardous to health. It should be used and discarded according to your own laboratory's safety procedures. Such safety procedures should include the wearing of protective gloves and avoiding the generation of aerosols. Care should be exercised in opening ampoules or vials, to avoid cuts.

DESCRIPTION

The material was obtained by plasmapheresis from a COVID-19 PCR positive-confirmed patient, at least 4 weeks after symptoms and recovery. No information was provided on the severity of the symptoms. The project was approved by the National Institute for Biologicals Standards and Control (NIBSC) Human Material Advisory Committee (project 16/005MP). Plasma was donated to NIBSC anonymised by the UK National Health Service Blood and Transplant (NHSBT). The donor patient signed an informed consent for the use of their plasma.

STORAGE

Vials should be stored at -20°C upon receipt or below. Avoid freeze/thaw cycles. No stability studies have been conducted on this material yet.

USE OF THE MATERIAL

Thaw the research reagent at ambient temperature. The research reagent should be processed according to the end user's method. In our in-house assays, the material can be diluted over 500-fold and have a positive signal by ELISA and neutralisation methods.

REPRESENTATIVE DATA

The research reagent 20/130 has only been characterised in-house. The following results are for information only, it is the end user's responsibility to assess performance of the research reagent 20/130 in their assays.

1) Neutralisation assay

Potencies are reported as the reciprocal of the endpoint titer dilution. Experiments were run once in quadruplicate.

	Titer
Live virus Neutralisation by CPE	1280

VSV-pseudotyped Virus Neutralisation	2240
$PRNT_{50}$	853

CPE: cytopathic effect; $PRNT_{50}$: 50% Plaque Reduction Neutralisation Assay

2) ELISA

The research reagent 20/130 was tested once in both a commercial and in-house ELISA. For the in-house assays, endpoint titers were calculated using GraphPad v.8.

EuroImmun IgG*	pos (7.77)
EuroImmunIgA*	pos (9.74)
In-house IgG S1**	5388
In-house IgG N**	17197
In-house IgG sSpike	2707
In-house IgM	+

*results are based on a ratio calculated against the kit calibrator; ** S1 and N proteins kindly provided by Dr P. Cherepanov (The Francis Crick Institute, London, UK) deposited in CFAR cat. no. 100979; ***stabilised Spike protein produced in house from plasmid kindly donated by Dr B. Graham (NIAID/NIH, Bethesda, MD, USA).

ACKNOWLEDGEMENTS

We would like to wholeheartedly thank the anonymous donor of the plasma sample for their consent which has allowed this reagent to be prepared. We would like to express our gratitude to Dr H. Harvala Simmonds and colleagues at the UK National Health Service Blood and Transplant (NHSBT) for the collection of the plasma sample.
This work has been funded and facilitated by the Coalition for Epidemic Preparedness Innovations (CEPI).

REFERENCES

[1] Dichtelmuller, H.O., et al., Robustness of solvent/detergent treatment of plasma derivatives: a data collection from Plasma Protein Therapeutics Association member companies. Transfusion, 2009. 49(9): p. 1931-43.

[2] Wilkinson, D.H., et al., WHO collaborative study to assess the suitability of the 1st International Standard and the 1st International Reference Panel for antibodies to Ebola virus. 2017.

CUSTOMER FEEDBACK

Customer are encouraged to provide feedback on the suitability or use of the research reagent 20/130. Please send any comments to Covid19_reagents@nibsc.org.

LIABILITY AND LOSS

In the event that this document is translated into another language, the English language version shall prevail in the event of any inconsistencies between the documents. Unless expressly stated otherwise by NIBSC, NIBSC‟s Standard Terms and Conditions for the Supply of Materials (available at http://www.nibsc.org/About_Us/Terms_and_Conditions.aspx or upon request by the Recipient) (“Conditions”) apply to the exclusion of all other terms and are hereby incorporated into this document by reference. The Recipient's attention is drawn in particular to the provisions of clause 11 of the Conditions.

CITATION

In any publication making reference to the materials, the acknowledgment should read: “The research reagent for SARS-CoV-2 RNA (NIBSC 20/130) was obtained from the National Institute for Biological Standards and Control,UK”.

MATERIAL SAFETY SHEET

Physical properties (at room temperature)			
Physical appearance	Pale yellow, frozen liquid		
Fire hazard	None		
Chemical properties			
Stable	Yes	Corrosive:	No
Hygroscopic	No	Oxidising:	No
Flammable	No	Irritant:	No
Other: Contains material of human origin			
Handling:	See caution section		
Toxicological properties			
Effects of inhalation:	Not established, avoid inhalation		
Effects of ingestion:	Not established, avoid ingestion		
Effects of skin absorption:	Not established, avoid contact with skin		
Suggested First Aid			
Inhalation	Seek medical advice		
Ingestion	Seek medical advice		
Contact with eyes	Wash with copious amounts of water. Seek medical advice.		
Contact with skin	Wash thoroughly with water.		
Action on Spillage and Method of Disposal			
Spillage of ampoule or vial contents should be taken up with absorbent material wetted with a virucidal agent. Rinse area with a virucidal agent followed by water. Absorbent materials used to treat spillage should be treated as biologically hazardous waste.			

Appendix 3 WHO Collaborative Study Protocol

Protocol for the WHO collaborative study to establish the 1st International Standard for anti-SARS-CoV-2 antibody and Reference Panel

This multi-centre International collaborative study aims to evaluate candidate preparations to serve as 1st WHO International Standard for anti-SARS-CoV-2 antibodies and a Reference Panel organized by NIBSC in collaboration with the World Health Organization (WHO). The study has been facilitated by the Coalition for Epidemic Preparedness Innovations (CEPI) which sponsored the sourcing and formulation of the candidate material.

International Standards (IS) are recognized as the highest order of reference materials for biological substances and they are assigned potencies in International Units (IU). International Standards are used to quantify the amount of biological activity present in a sample in terms of the IU, making assays from different laboratories comparable. This makes it possible to better define parameters such as the analytical sensitivity of tests or clinical parameters such as protective levels of antibody. The availability of an IS for anti-SARS-CoV-2 antibodies will facilitate the standardisation of COVID19 serological assays used for detection of anti-SARS-CoV-2 antibodies to establish infection, epidemiology and vaccine responses. The establishment of such a standard will follow published WHO guidelines and be submitted for formal endorsement by the WHO Expert Committee on Biological Standardization (ECBS) [1].

Aims

The aims of this WHO international collaborative study are to

- assess the suitability of different antibody preparations to serve as the International Standard with an assigned unitage per ampoule for use in the harmonisation of COVID-19 serology assays.
- characterise the antibody preparations in terms of reactivity/specificity in different assay systems.
- assess each preparation's potency i.e. readout in a range of typical assays performed in different laboratories.
- assess commutability i.e. to establish the extent to which each preparation is suitable to serve as a standard for the variety of different samples and assay types.
- recommend to the WHO ECBS, the antibody preparation(s) found to be suitable to serve as the standard and propose an assigned unit.

Materials

Coded study samples

The study samples should be stored at -20°C or below. The study samples shall not be administered to humans or animals in the human food chain.

All samples will be provided coded and blinded. The samples are labelled "COVID-19 Ab CS678 Sample xx" where xx is A, B, C, D, E, F, G, H, I, J. The coded samples may include negative samples as well as reactive samples. Laboratories will receive at least 4 sets of study samples which should allow for 3 independent assays (plus 1 spare) by one method. Laboratories with more than one method or which

require more than 0.1 mL of material per method will receive additional sample sets to allow 3 independent tests per method. **For multiple ELISA with different target antigens, run in parallel, we recommend use of the same sample, if possible.**

Plasma/serum obtained from convalescent patients

The source materials are plasma or serum obtained from patients tested positive for SARS-CoV-2, collected at least 28 days post onset of symptoms.
Source material was kindly donated by ISARIC4C consortium through the University of Liverpool; Papworth Hospital, Cambridge; NHS Blood and Transfusion, United Kingdom and Oslo University Hospital, Norway.

The samples are:

- plasma samples from one individual each containing either high or low titre anti-SARS-CoV-2 antibody;
- pools of serum samples from three individuals each containing either high or low titre anti-SARS-CoV-2 antibody;
- pools of convalescent plasma from multiple donors containing different titres of anti-SARS-CoV-2 antibody;
- negative control plasma from multiple healthy UK donors, collected before 2019.

Preparations are either freeze-dried, filled in 0.25 mL aliquots into 2.5 mL ampoules (sample E, F, G, H, I, J) or liquid frozen and filled in 0.1 mL (sample A) or 0.2 mL (samples B, C, D) aliquots into screw cap tubes.

Assay Methods

For testing the study samples, participants are requested to use the method(s) in routine use in their laboratory for the detection of antibodies to SARS-CoV-2. Laboratories may use multiple methods to test the study materials, provided that the study design (see below) is followed for each method.

Design of study

Participants are requested to:

- Perform 3 independent tests on different days for detection of antibodies against SARS-CoV-2
- Reconstitute freeze-dried samples according to the Instructions for Use (IFU) supplied with the sample shipment. Use a freshly thawed/reconstituted sample for each independent test
- For the liquid frozen samples, use a freshly thawed aliquot for each independent test. Each sample should be thawed at room temperature or 37°C and used immediately or placed on ice until used
- For each independent test, prepare a series of dilutions from each coded sample, using the sample matrix specific to their individual assay(s) (e.g. plasma, serum, buffer, media). **The optimal dilution range should cover at least 5 to 6 points including one point beyond the endpoint dilution.** Adjust dilutions accordingly for subsequent assays if needed. Record in the Excel spreadsheet changes to the dilutions tested.
- **For some commercial kits, dilution of the test samples is not recommended unless specified in the manufacturer's instructions.** Report the value obtained with the neat sample
- Use the Excel reporting sheet to record for each dilution the raw assay readout (e.g. absorbance O.D./RLU/plaques/GFP%, etc.). Provide the result (endpoint titre/IC50 etc.) as per analysis in your laboratory. Our statistician will use the raw data readouts to perform statistical analysis.

- Include the cut-off value indicating sero-reactivity for each assay and state whether each sample dilution tested is considered positive or negative according to their criteria (it is of interest for us to know whether the samples are considered 'positive' in each assay)
- If feasible, include all study samples in each assay so that the concentration of antibodies relative to one another may be calculated. Please note in the reporting sheet, if it is not practical to test all samples concurrently, indicate which samples were tested concurrently.
- Record in the Excel reporting sheet any deviations from the assay protocol.

Results and data analysis

An Excel spreadsheet is provided so that all essential information can be recorded, including details of assay methodology and the raw data obtained from each assay. The use of the reporting spreadsheet facilitates the analysis and interpretation of results.

The confidentiality of each laboratory is assured with each participant being anonymous to the other laboratories. Analysis of the study will assess the potencies of each material relative to each other, and the sensitivity of the different assay methods.

A draft study report will be sent to participants for comment. The report will include data analysis, proposed conclusions and recommendations on the selection, use and unitage of the most appropriate antibody preparation to serve as the 1st WHO IS for anti-SARS-CoV-2 antibodies. Participants' comments will be included in the report prior to submission to the WHO ECBS. Study participants will be notified of the outcome of the study after the WHO ECBS meeting.

Participation in the feasibility study is conducted under the following conditions:

- The study samples have been prepared from materials provided by donors and therefore must be treated as proprietary. The materials must not be used for any other purpose other than for this study;
- The materials provided must not be shared with anyone outside of the study;
- The materials must not be used for application in human subjects or animals in the human food chain in any manner or form;
- There must be no attempt to reverse engineer, ascertain the chemical structure of, modify, or make derivatives of, any of the materials;
- Participants accept responsibility for safe handling and disposal of the materials provided in according to the local regulations in their organization/country.
- Data obtained through testing of the materials must not be published or cited before the formal establishment of the standard by World Health Organization, without the express permission of the NIBSC study organiser.

NIBSC, as the Collaborative Study coordinator, notes that:

- It is normal practice to acknowledge all participants as contributors of data rather than co-authors in publications;
- Data published from participating labs will be anonymised;
- Participation of this study is at the participant's discretion and does not include remuneration costs;
- Prior to the establishment of the standard, NIBSC reserves the right to disclose specific information about the use of the material(s), without acknowledgement of the study participants;
- Participants will receive a copy of the report of the study with proposed conclusions and recommendations for comment before it is further distributed.

Deadline for completed results spreadsheets is 6 weeks from receipt of study materials. If it is not practical to return results within 6 weeks, please inform Giada Mattiuzzo.

All completed results spreadsheets should be returned electronically to:

Dr Giada Mattiuzzo
Senior Scientist
Emerging Viruses Group
Division of Virology
National Institute for Biological Standards and Control
Blanche Lane
South Mimms
Hertfordshire
EN6 3QG
UK

Tel. +44(0)1707 641283
Giada.Mattiuzzo@nibsc.org

References:

[1] WHO, Recommendations for the preparation, characterization and establishment of international and other biological reference standards. WHO Technical Report Series, No. 932., in Expert Committee on Biological Standardization. 2006.

Appendix 4. Proposed IFU for WHO International Standard for anti-SARS-CoV-2 antibody

Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

WHO International Standard
WHO International Standard for SARS-CoV-2 antibody (human)
NIBSC code: 20/136
Instructions for use
(Version 1.00, Dated)

1. INTENDED USE

The WHO International Standard for anti-SARS-CoV-2 antibody is the freeze-dried equivalent of 0.25 mL of pooled plasma obtained from eleven individuals recovered from SARS-CoV-2 infection. The preparation has been evaluated in a WHO International Collaborative study (1). The intended use of the International Standard is for the calibration and harmonisation of serological assays detecting anti-SARS-CoV-2 neutralising and binding antibody. The material has the same assigned unitage for neutralising and binding antibody. Secondary reagents should be calibrated to the International Standard in the type of assay required. The preparation has been solvent-detergent treated to minimise the risk of the presence of enveloped viruses (2).

2. CAUTION

This preparation is not for administration to humans or animals in the human food chain.

The preparation contains material of human origin, and either the final product or the source materials, from which it is derived, have been tested and found negative for HBsAg, anti-HIV and HCV RNA. As with all materials of biological origin, this preparation should be regarded as potentially hazardous to health. It should be used and discarded according to your own laboratory's safety procedures. Such safety procedures should include the wearing of protective gloves and avoiding the generation of aerosols. Care should be exercised in opening ampoules or vials, to avoid cuts.

3. UNITAGE

The assigned potency of the WHO International Standard for SARS-CoV-2 antibody is 250 IU/ampoule for neutralising activity and 250 IU/ampoule for binding activity. After reconstitution in 0.25 mL of distilled water, the final concentration of the preparation is 1000 IU/mL for neutralising and binding antibody.

4. CONTENTS

Country of origin of biological material: United Kingdom.
Each ampoule contains the freeze-dried equivalent of 0.25 mL pooled human plasma.

5. STORAGE

The International Standard 20/136 should be stored at -20°C or below upon receipt.
Please note: because of the inherent stability of lyophilized material, NIBSC may ship these materials at ambient temperature.

6. DIRECTIONS FOR OPENING

DIN ampoules have an 'easy-open' coloured stress point, where the narrow ampoule stem joins the wider ampoule body. Various types of ampoule breaker are available commercially. To open the ampoule, tap the ampoule gently to collect material at the bottom (labelled) end and follow manufactures instructions provided with the ampoule breaker.

7. USE OF MATERIAL

No attempt should be made to weigh out any portion of the freeze-dried material prior to reconstitution
This material should be reconstituted in 0.25 mL sterile distilled water. Following addition of water, the ampoule may be left at ambient temperature for approximately 30 minutes until dissolved and then mixed thoroughly, avoiding the generation of excessive foam.

8. STABILITY

Reference materials are held at NIBSC within assured, temperature-controlled storage facilities. Reference Materials should be stored on receipt as indicated on the label.

NIBSC follows the policy of WHO with respect to its reference materials.

9. REFERENCES

(1) Mattiuzzo et al. Establishment of the WHO International Standard and Reference Panel for anti-SARS-CoV-2 antibody. 2020, WHO Expert Committee on Biological Standardization. WHO/BS/2020.xxxx
(2) Dichtelmüller et al. Robustness of solvent/detergent treatment of plasma derivatives: a data collection from Plasma Protein Therapeutics Association member companies. Transfusion. 2009;49:1931–43.

10. ACKNOWLEDGEMENTS

We would like to wholeheartedly thank the anonymous donors of the plasma samples for their consent which has allowed this material to be prepared; we would like to express our gratitude to those groups and individuals who have coordinated the collection of the convalescent plasma: Malcom Semple (Univeristy of Liverpool, UK), Lance Turtle (University of Liverpool, UK), Peter Openshaw (Imperial College London, UK) and Kenneth Baillie (University of Edinburgh) on behalf of the ISARIC4C Investigators; Heli Harvala Simmonds and David Roberts (National Health Service Blood and Transplant, UK). We would also like to thank NIBSC Standards Production and Development staff for the formulation and distribution of materials. The International Standard for SARS-CoV-2 antibody wouldn't have been possible without the Coalition for Epidemic Preparedness Innovations (CEPI) sponsored the sourcing and formulation of the candidate material.

11. FURTHER INFORMATION

Further information can be obtained as follows;
This material: enquiries@nibsc.org
WHO Biological Standards:
http://www.who.int/biologicals/en/
JCTLM Higher order reference materials:
http://www.bipm.org/en/committees/jc/jctlm/
Derivation of International Units:
http://www.nibsc.org/standardisation/international_standards.aspx
Ordering standards from NIBSC:
http://www.nibsc.org/products/ordering.aspx
NIBSC Terms & Conditions:
http://www.nibsc.org/terms_and_conditions.aspx

12. CUSTOMER FEEDBACK

Customers are encouraged to provide feedback on the suitability or use of the material provided or other aspects of our service. Please send any comments to enquiries@nibsc.org

13. CITATION

In all publications, including data sheets, in which this material is referenced, it is important that the preparation's title, its status, the NIBSC code number, and the name and address of NIBSC are cited and cited correctly.

14. MATERIAL SAFETY SHEET

Classification in accordance with Directive 2000/54/EC, Regulation (EC) No 1272/2008: Not applicable or not classified

Physical and Chemical properties		
Physical appearance: freeze-dried	Corrosive:	No

National Institute for Biological Standards and Control,
Potters Bar, Hertfordshire, EN6 3QG. T +44 (0)1707 641000, nibsc.org
WHO International Laboratory for Biological Standards,
UK Official Medicines Control Laboratory

World Health Organization

Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

Stable:	Yes	Oxidising:	No
Hygroscopic:	No	Irritant:	No
Flammable:	No	Handling:See caution, Section 2	
Other (specify):	material of human origin.		

Toxicological properties	
Effects of inhalation:	Not established, avoid inhalation
Effects of ingestion:	Not established, avoid ingestion
Effects of skin absorption:	Not established, avoid contact with skin

Suggested First Aid	
Inhalation:	Seek medical advice
Ingestion:	Seek medical advice
Contact with eyes:	Wash with copious amounts of water. Seek medical advice
Contact with skin:	Wash thoroughly with water.

Action on Spillage and Method of Disposal

Spillage of ampoule contents should be taken up with absorbent material wetted with an appropriate disinfectant. Rinse area with an appropriate disinfectant followed by water.
Absorbent materials used to treat spillage should be treated as biological waste.

15. LIABILITY AND LOSS

In the event that this document is translated into another language, the English language version shall prevail in the event of any inconsistencies between the documents.
Unless expressly stated otherwise by NIBSC, NIBSC's Standard Terms and Conditions for the Supply of Materials (available at http://www.nibsc.org/About_Us/Terms_and_Conditions.aspx or upon request by the Recipient) ("Conditions") apply to the exclusion of all other terms and are hereby incorporated into this document by reference. The Recipient's attention is drawn in particular to the provisions of clause 11 of the Conditions.

16. INFORMATION FOR CUSTOMS USE ONLY

Country of origin for customs purposes*: United Kingdom * Defined as the country where the goods have been produced and/or sufficiently processed to be classed as originating from the country of supply, for example a change of state such as freeze-drying.
Net weight: 0.25 g
Toxicity Statement: Non-toxic
Veterinary certificate or other statement if applicable. **Attached:** No

17. CERTIFICATE OF ANALYSIS

NIBSC does not provide a Certificate of Analysis for WHO Biological Reference Materials because they are internationally recognised primary reference materials fully described in the instructions for use. The reference materials are established according to the WHO Recommendations for the preparation, characterization and establishment of international and other biological reference standards http://www.who.int/bloodproducts/publications/TRS932Annex2_Inter_biolefstandardsrev2004.pdf (revised 2004). They are officially endorsed by the WHO Expert Committee on Biological Standardization (ECBS) based on the report of the international collaborative study which established their suitability for the intended use.