1. INTENDED USE
The second WHO International Standard for anti-SARS-CoV-2 immunoglobulin is the freeze-dried equivalent of 0.25 mL of pooled plasma obtained from seven individuals recovered from SARS-CoV-2 in the United Kingdom between May-August 2020. The source material for the pooling was selected based on antibody titre to resemble similar levels to that of the 1st WHO International Standard 20/136 and solvent-detergent treated to minimise the risk of the presence of enveloped viruses [1]. The preparation has been evaluated in an International Collaborative study [2].

The intended use of the International Standard is for the calibration and harmonisation of neutralisation assays using SARS-CoV-2 early isolates (2020). For the calibration of secondary reagents used in neutralisation assay against variants of concern the 1st WHO International Standard for anti-SARS-CoV-2 variants of concern immunoglobulin, NIBSC code 21/338, should be used.

The preparation can also be used as an internal reference reagent for the harmonisation of binding antibody assays.

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 2nd WHO International Standard for SARS-CoV-2 immunoglobulin is 356 IU/ampoule for neutralising antibody activity. After reconstitution in 0.25 mL of distilled water, the final concentration of the preparation is 1424 IU/mL.

The preparation has been assessed for neutralising activity against variants of concern and for binding antibody in the collaborative study [2]. The potencies reported as geometric mean of the values relative to the first WHO International Standard for anti-SARS-CoV-2 immunoglobulin, 20/136, are listed below. These values are based on the results from the assays used in the collaborative study and are intended for guidance only.

<table>
<thead>
<tr>
<th>Method</th>
<th>Isolate/viral target</th>
<th>Potency</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutralisation</td>
<td>Alpha</td>
<td>1414</td>
<td>IU/mL</td>
</tr>
<tr>
<td></td>
<td>Beta</td>
<td>843</td>
<td>IU/mL</td>
</tr>
<tr>
<td></td>
<td>Gamma</td>
<td>680</td>
<td>IU/mL</td>
</tr>
<tr>
<td></td>
<td>Delta</td>
<td>1663</td>
<td>IU/mL</td>
</tr>
<tr>
<td></td>
<td>Omicron (BA.1:BA.2)</td>
<td>1882</td>
<td>IU/mL</td>
</tr>
<tr>
<td>Binding assay</td>
<td>Spike</td>
<td>652</td>
<td>BAU/mL</td>
</tr>
<tr>
<td>Receptor binding domain</td>
<td>Subunit 1 of Spike</td>
<td>723</td>
<td>BAU/mL</td>
</tr>
<tr>
<td></td>
<td>Nucleoprotein</td>
<td>563</td>
<td>BAU/mL</td>
</tr>
</tbody>
</table>

IU: international unit; BAU: binding antibody unit
For binding assays, the viral antigens were derived from an early 2020 isolates, and the assays were IgG-specific.

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
International Standard 21/340 should be stored at -20°C or below upon receipt.

6. DIRECTIONS FOR OPENING
Open 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

10. ACKNOWLEDGEMENTS
We would like to wholeheartedly thank the anonymous donors of the plasma and serum samples for their consent and Heli Harvala and Hatica Baklan from NHS Blood and Transplant (United Kingdom) for the provision of the material.

We would also like to thank MHRA Standards Production team for the formulation of the standards; Graham Prescott and Katrina Temmouth for the blood virology testing and Corinne McDonough for the distribution of study samples.

The project has been funded by the Coalition for Epidemic Preparedness Innovations.

11. FURTHER INFORMATION
Further information can be obtained as follows;
This material: enquiries@nibsc.org
WHO Biological Standards: http://www.who.int/biologicals/en/
Derivation of International Units: http://www.nibsc.org/standardisation/international_standards.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:

Physical and Chemical properties

| Physical appearance: freeze-dried | Corrosive: No |
| Stable: Yes | Oxidising: No |
| Hygroscopic: No | Irritant: No |
| Flammable: No |

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_Instruction_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.