1. INTENDED USE
Preparation 07/202 is intended to be used to standardise immunoassays for the measurement of serum transferrin receptor (sTfR).

Cellular uptake of iron bound to its carrier protein transferrin (Tf) is mediated by the transferrin receptor (TIR) [1-3]. A truncated, soluble form of the receptor is present in serum (sTfR) [4], formed as a result of protease action. The sTIR circulates as a complex with transferrin which is present at approximately 250 times the concentration of the sTIR in molar terms. TIR density is upregulated when there is increased erythropoiesis and in iron deficiency. As the sTIR concentration correlates with the total TfR content, a raised sTIR concentration is therefore a marker of iron deficiency. The joint WHO/CDC Technical Consultation on Assessment of Iron Status at Population level (Geneva, April 6-8, 2004) concluded that measurement of both serum ferritin and the serum transferrin receptor (sTIR) provides the best approach for estimating the iron status of populations [5].

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
21.7 mg/L or 303 nmol/L (when reconstituted with 0.50 mL distilled or deionised water). These values apply to free sTfR monomer.

4. CONTENTS
Country of origin of biological material: United Kingdom.

sTIR was prepared from the portion of the human Tf R gene encoding residues 121-760 (the C-terminal amino acid of wild-type TfR) by Caltech (CA, USA) [8]. The choice of the N-terminal start site for sTIR had been based on studies of a previously characterised soluble fragment produced by trypsin digestion of placental TIR [7]. In common with sTIR, the trypsin fragment and sTIR have been reported to form a stable dimer binding 2 molecules of transferrin [8, 6]. Mass spectrometry of sTIR revealed a major peak of 78,336 Da. Analysis of peptides cleaved from an SDS-PAGE-derived sTIR spot using MALDI-TOF confirmed mass identity with equivalent theoretical peptides from published TIR sequence data [9, 10].

The sTIR was shown to be glycosylated by a mobility shift of approximately 7,000 Da upon SDS-PAGE of sTIR treated withPNGase F to remove N-linked glycans compared to untreated sTIR.

The concentration of sTIR was determined from the A280nm and using the adjusted theoretical extinction coefficient and molecular weight calculated from its published sequence [6, 9, 10; molecular weight = 71,725; extinction coefficient = 93,790; 1 mg/mL solution therefore has an absorbance of 1.308]. The sTIR was diluted to 21.7 mg/L in transferrin-receptor-depleted human serum that had been tested and found negative for anti-HIV I and II, HBsAg and anti-HCV (SCIPAC, Sittingbourne, Kent, UK; sTIR was not detected in the depleted serum in immunoassays kindly performed by Dade Behring), dispensed in glass ampoules at 4°C (~0.5 mL/ampoule), and lyophilised. The mean weight of the dispersed solution in 82 ampoules was 0.5062 g. The imprecision of the filling (CV) was 0.24%, the oxygen head space was 0.97%, and the residual moisture was 0.54%.

5. STORAGE
Store unopened ampoules at -20°C or below. 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. Tap the ampoule gently to collect the material at the bottom (labeled) end. Ensure that the disposable ampoule safety breaker provided is pushed down on the stem of the ampoule and against the shoulder of the ampoule body. Hold the body of the ampoule in one hand and the disposable ampoule breaker covering the ampoule stem between the thumb and first finger of the other hand. Apply a bending force to open the ampoule at the coloured stress point, primarily using the hand holding the plastic collar. Care should be taken to avoid cuts and projectile glass fragments that might enter the eyes, for example, by the use of suitable gloves and an eye shield. Take care that no material is lost from the ampoule and no glass falls into the ampoule. Within the ampoule is dry nitrogen gas at slightly less than atmospheric pressure. A new disposable ampoule breaker is provided with each DIN ampoule.

7. USE OF MATERIAL
No attempt should be made to weigh out any portion of the freeze-dried material prior to reconstitution.

Reconstitute the ampoules contents with 0.50 mL distilled or deionised water. The reconstituted material should be used to standardise assays for the serum transferrin receptor (sTIR).

Preparation 07/202 was subjected to an international collaborative study involving 6 commercial immunoassay kits which showed that measurement of the sTIR content of three serum samples relative to 07/202, rather than against kit calibrators, markedly improved agreement between different assay methods.

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. Accelerated degradation studies on 07/202 are ongoing, but the estimated % loss per year for an earlier trial fill of sTIR in sTIR-depleted serum is 0.05%, which represents very good stability.

9. REFERENCES
6. Lebron, J.A.; Bennett, M.J.; Vaughan, D.E.; Chinino, A.J.; Snow, P.M.; Mintier, G.A.; Feder, J.N.; Bjorkman, P.J. Crystal structure of the...
hemochromatosis protein HFE and characterization of its interaction with transferrin receptor. Cell, 1998, 93, 111.  
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  

<table>
<thead>
<tr>
<th>Physical and Chemical Properties</th>
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<tbody>
<tr>
<td>Physical appearance:</td>
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<tr>
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<td>Yes</td>
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<tr>
<td>Hygroscopic:</td>
<td>No</td>
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<tr>
<td>Flammable:</td>
<td>No</td>
</tr>
<tr>
<td>Other (specify):</td>
<td>Contains human serum</td>
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</tbody>
</table>

| 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 |

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: 0.04g  
Toxicity Statement: Toxicity not assessed  
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_biol_esstandardsrev2004.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.