WHO International Standard
4th WHO International Standard for Hepatitis C Virus for Nucleic Acid Amplification Techniques
NIBSC code: 06/102
Instructions for use
(Version 3.0, Dated 09/10/2014)

1. INTENDED USE
The 4th WHO International Standard for hepatitis C virus (HCV), NIBSC code 06/102, is intended to be used in the standardization of nucleic acid amplification technology (NAT)-based assays for HCV. The standard comprises genotype 1a HCV antibody-negative, HCV RNA-positive plasma, diluted in pooled human plasma. The virus stock was tested and found negative for HIV-1 RNA, HBV DNA, HAV RNA and parvovirus B19 DNA. The pooled human plasma diluent was sourced from blood donations and had been tested and found negative for HIV antibody, HCV antibody, HBsAg, syphilis antibody, HTLV antibody, as well as HIV and HCV RNA. The standard has been lyophilized in 0.5 mL aliquots and stored at -20 °C. The material has been calibrated in International Units (IU), in parallel with the 2nd and 3rd WHO International Standards for HCV [1,2].

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 and HIV antibody. 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
This material has been assigned a unitage of 260,000 IU/mL (~5.41 log10 IU/mL) when reconstituted in 0.5 mL of nucleic-acid-free water.

Uncertainty: the assigned unitage does not carry an uncertainty associated with its calibration. The uncertainty may therefore be considered to be the variance of the vial content and was determined to be +/- 1.5%.

4. CONTENTS
Country of origin of biological material: United Kingdom.
Each vial contains 0.5 mL of lyophilized plasma containing infectious HCV.

5. STORAGE
Vials of 06/102 are shipped on dry ice and must be stored at -20 °C.

6. DIRECTIONS FOR OPENING
Vials have a ‘flip-up’ circular cap. Either on the cap or the collar of the vial, there is an indication of the point at which to lever off the cap. This exposes an area of the stopper through which reconstitution and withdrawal of the preparation can be made using a hypodermic needle and syringe. If use of a pipette is preferred, then fully remove the metal collar using, for example, forceps, taking care to avoid cuts by wearing appropriate gloves. Remove the stopper for access. Care should be taken to prevent loss of the contents.

7. USE OF MATERIAL
No attempt should be made to weigh out any portion of the freeze-dried material prior to reconstitution
The material should be reconstituted with 0.5 mL of deionized, nucleic-acid-free molecular-grade water and left for a minimum of 20 minutes with occasional agitation before use. The reconstituted material has a final concentration of 260,000 IU/mL.

The International Standard should be used to calibrate secondary reference materials, for example, by determining the equivalent concentration of secondary reference reagent being calibrated, against the International Standard, in parallel. The secondary reference reagent can then be assigned a concentration in IU. Once reconstituted, the International Standard should be diluted in the matrix appropriate to the material being calibrated, and should be extracted prior to HCV RNA measurement.

8. STABILITY
Reference materials are held at NIBSC within assured, temperature-controlled storage facilities.
06/102 was assessed originally in the 2007 collaborative study evaluating the 3rd WHO International Standard for HCV (NIBSC code: 06/100) [1]. A more recent collaborative study evaluation of 06/102 demonstrated a drop in potency of ~ 0.2 log10 for 06/102 when compared to the 2007 study. Further investigations into the stability of 06/102 provided evidence that the loss of potency for 06/102 occurs at ambient temperature during shipping, rather than over time during storage at -20 °C [2]. The suitability of the candidate as the replacement 4th WHO International Standard for HCV is given provided that the product is maintained at -20 °C. Therefore 06/102 must be shipped on dry ice and stored at -20 °C immediately upon receipt. The potency of 06/102 as determined in the 2007 collaborative study (260,000 IU/mL) should be applied when reconstituted in 0.5 mL of nucleic-acid-free water [1]. The stability of 06/102 when reconstituted has not been specifically determined. Therefore, it is recommended that the standard is for single use only.

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

9. REFERENCES

10. ACKNOWLEDGEMENTS
We gratefully acknowledge the important contributions of the collaborative study participants.

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>
<td>Corrosive:</td>
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<tr>
<td>Stable:</td>
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<tr>
<td>Oxidising:</td>
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<tr>
<td>Hygroscopic:</td>
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<td>Irritant:</td>
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<td>Flammable:</td>
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<tr>
<td>Handling:</td>
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<tr>
<td>Other (specify):</td>
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<table>
<thead>
<tr>
<th>Toxicological properties</th>
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<tbody>
<tr>
<td>Effects of inhalation:</td>
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<td>Effects of ingestion:</td>
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<td>Effects of skin absorption:</td>
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<table>
<thead>
<tr>
<th>Suggested First Aid</th>
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</thead>
<tbody>
<tr>
<td>Inhalation:</td>
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<tr>
<td>Ingestion:</td>
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<tr>
<td>Contact with eyes:</td>
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<tr>
<td>Contact with skin:</td>
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<tr>
<th>Action on Spillage and Method of Disposal</th>
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<tr>
<td>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.</td>
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</tbody>
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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: 0.5 g

Toxicity Statement: Non-toxic

Veterinary certificate or other statement if applicable. Attached: No