WHO 1st International Standard for HIV-1 RNA for Nucleic Acid-Based Techniques (NAT)
NIBSC code: 97/656

Instructions for Use (Version 3 - 14th May 2003)
Changes from previous version are shown in Red, deletions are shown as ^

1. INTRODUCTION

The WHO International Working Group on Standardisation of Gene Amplification Techniques for the Virological Safety Testing of Blood and Blood Products (SoGAT) recommended that the establishment of a WHO-approved International Standard for HIV-1 RNA based on a genotype B virus be given a high priority. Such a standard would be given a defined unitage and could be used to calibrate the range of working reagents currently in use. This would facilitate inter-laboratory comparisons and help reduce variations between different commercial and 'in-house' assays and between different laboratories. In response to this, we organised an international collaborative study involving 25 laboratories in 10 countries to evaluate three candidate standards. A report on the collaborative study was submitted to WHO Expert Committee on Biological Standardisation (ECBS) and in November 1999 the Committee established the preparation coded 97/656 as the 1st International Standard for HIV-1 RNA. It was prepared from an HIV-1 PCR-positive, p24 antigen-positive, antibody-negative plasmapheresis donation diluted in defibrinated plasma, that had been pre-screened for HBsAg and for antibodies to HCV and HIV-1/2 and found to be negative, and freeze-dried. Sequencing of the V3 region of env confirmed that this was within genotype B.

It has recently been demonstrated that as well as containing HIV-1 RNA, this reagent also contains HBV DNA. An ‘in-house’ qualitative PCR assay has demonstrated the presence of HBV DNA and this has been confirmed in a number of independent laboratories. Quantitative assay have shown that it contains approximately $3.5 \times 10^4$ amplifiable copies per ml (Alison Hardie, University of Edinburgh, personal communication). Whilst the presence of HBV DNA should not affect the use of this reagent in single HIV-1 NAT assays, the presence of HBV DNA should be taken into account when using assays that detect several markers including HBV.
2. UNITAGE

The ECBS has assigned an activity of 100,000 IU per vial to this material.

Uncertainty: the International unit of 97/656 is assigned without uncertainty. The uncertainty of the ampoule content of 97/656 may be considered to be the coefficient of variation, which was determined to be 0.587%.

3. CONTENTS

The WHO 1st International Standard for HIV-1 RNA for NAT consists of freeze-dried HIV-positive, human plasma in a 2ml glass vial with a tear-off crimp seal.

4. CAUTION

4.1 THIS PREPARATION IS NOT FOR ADMINISTRATION TO HUMANS.

4.2 This reagent contains infectious HIV-1 and material of human origin and must only be handled in appropriate containment facilities by fully trained and competent staff and in accordance with the local national safety guidelines (such as the UK "Protection against blood-borne infections in the workplace: HIV and hepatitis", Advisory Committee on Dangerous Pathogens, HMSO, London). Care should be exercised in opening vials to avoid injury. 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 probably will include the wearing of protective gloves and avoiding the generation of aerosols. Care should be exercised in opening ampoules or vials, to avoid cuts. A ‘Safety Data Sheet’ is included as the last page of these instructions.

5. DIRECTIONS FOR OPENING THE AMPOULE

5.1 Ensure the lyophilised material is collected at the bottom of the vial.

5.2 Using a pair of tweezers tear of the metal crimped seal.

5.3 Take care when removing the rubber stopper, occasionally lyophilised material will be pushed up from the base of the vials when air first enters the vacuum.
5.4 Care should be taken at all times to avoid cuts.

6. USE OF AMPOULED MATERIAL

6.1 The vial should be stored at -20°C on receipt; each vial is intended to be used only once. The ampoule should be opened as directed in 5 and should then be reconstituted in 1.0ml of de-ionised, RNAase-free water immediately before use. The vials should be gently agitated over a minimum period of 20 minutes to fully dissolve the contents. The contents should be transferred to a screw-capped plastic vial and vortexed at full speed for 10 seconds before assaying.

7. STABILITY

7.1 It is the policy of WHO not to assign an expiry date to their international reference materials. They remain valid with the assigned potency and status until withdrawn or amended.

7.2 Reference materials are held at NIBSC within assured, temperature-controlled storage facilities. Reference Materials should be stored on receipt as indicated on the label. For information specific to a particular biological standard, contact the Technical Information Officer or, where known, the appropriate NIBSC scientist.

7.3 In addition, once reconstituted, diluted or aliquoted, users should determine the stability of the material according to their own method of preparation, storage and use.

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

7.5 Users who have data supporting any deterioration in the characteristics of any reference preparation are encouraged to contact NIBSC.
8. CITATION

In all publications (or data sheets for immunoassay kits) in which this preparation is used as an assay calibrant, it is important that the title of the preparation, ampoule code and the name and address of NIBSC are cited and cited correctly.

9. PRODUCT LIABILITY

9.1 Information emanating from NIBSC is given after the exercise of all reasonable care and skill in its compilation, preparation and issue, but is provided without liability in its application and use.

9.2 This product is intended for use as a standard or reference material in laboratory work in relation to biological research, manufacturing or quality control testing of biological products or in the field of *in vitro* diagnostics. It is the responsibility of the user to ensure that he/she has the necessary technical skills to determine the appropriateness of this product for the proposed application. Results obtained from this product are likely to be dependent on conditions of use and the variability of materials beyond the control of NIBSC.

9.3 NIBSC accepts no liability whatsoever for any loss or damage arising from the use of this product, whether loss of profits, or indirect or consequential loss or otherwise, including, but not limited to, personal injury other than as caused by the negligence of NIBSC. In particular, NIBSC accepts no liability whatsoever for:-

(i) results obtained from this product; and/or
(ii) non-delivery of goods or for damages in transit.

9.4 In the event of any replacement of goods following loss or damage a customer accepts as a condition of receipt of a replacement product, acceptance of the fact that the replacement is not to be construed as an admission of liability on NIBSC's behalf.
MATERIAL SAFETY SHEET

<table>
<thead>
<tr>
<th>Physical properties (at room temperature)</th>
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<tr>
<td>Physical appearance</td>
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<td>Fire hazard</td>
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<table>
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<tr>
<th>Chemical properties</th>
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<tbody>
<tr>
<td>Stable</td>
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<tr>
<td>Hygroscopic</td>
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<tr>
<td>Flammable</td>
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<tr>
<td>Other (specify)</td>
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</tbody>
</table>

Handling: See precautions in section 4.2

Toxicological properties

Effects of inhalation:

Effects of ingestion:

Effects of skin absorption:

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 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.

Compiled by: Retrovirology    Date: 14th May 2003

The Institute's UKAS accreditation relates only to our programme of testing for biological medicines. Full details can be found on our Schedule of Accreditation, which is available on request.