



**World Health
Organization**

**WHO/BS/2011.2178
ENGLISH ONLY**

EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION
Geneva, 17 to 21 October 2011

**International Collaborative Study to Establish the 3rd WHO International Standard for
HIV-1 NAT Assays**

C L Morris¹ and A B Heath²

Divisions of Retrovirology¹ and Biostatistics², National Institute for Biological Standards and
Control (NIBSC), Blanche Lane, South Mimms, Potters Bar, Herts EN6 3QG, UK.

© World Health Organization 2011

All rights reserved. Publications of the World Health Organization are available on the WHO web site (www.who.int) or can be purchased from WHO Press, World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland (tel.: +41 22 791 3264; fax: +41 22 791 4857; e-mail: bookorders@who.int).

Requests for permission to reproduce or translate WHO publications – whether for sale or for noncommercial distribution – should be addressed to WHO Press through the WHO web site: (http://www.who.int/about/licensing/copyright_form/en/index.html).

The designations employed and the presentation of the material in this publication do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement.

The mention of specific companies or of certain manufacturers' products does not imply that they are endorsed or recommended by the World Health Organization in preference to others of a similar nature that are not mentioned. Errors and omissions excepted, the names of proprietary products are distinguished by initial capital letters.

All reasonable precautions have been taken by the World Health Organization to verify the information contained in this publication. However, the published material is being distributed without warranty of any kind, either expressed or implied. The responsibility for the interpretation and use of the material lies with the reader. In no event shall the World Health Organization be liable for damages arising from its use. The named authors alone are responsible for the views expressed in this publication.

Summary

Stocks of the 2nd International Standard for HIV-1 NAT assays is running low. A replacement lyophilised standard comprising heat inactivated genotype B virus diluted in citrated human plasma has been evaluated in an international collaborative study in conjunction with the current 2nd HIV-1 NAT International Standard. The candidate standard was represented in duplicate in the study thus giving a total of three samples being evaluated. Samples were coded A, B or C. Fifteen laboratories from eleven different countries returned seventeen data sets from ten different assays systems, six of which were in a quantitative format and four as qualitative data. Six different commercial assays were used. The results showed excellent correlation between the assays used. Based on the results of this study, it is proposed that candidate material 10/152 is suitable as a replacement standard and should be established as the 3rd International Standard for HIV-1 NAT assays and be assigned a unitage of 185,000 IU/ml (5.27 Log₁₀ IU/ml).

Introduction

The 1st International Standard for HIV-1 NAT assays, NIBSC code 97/656, was established by the ECBS in 1999. Following its successful use for 6 years it was replaced by the 2nd standard, NIBSC code 97/650 in 2005 due to low stocks, this has since been extensively used for assay validation and calibration and to calibrate secondary standards and working reagents [1]. Previously the second international standard (IS) was prepared from a live preparation of a subtype B viral isolate and spiked into human plasma before being freeze dried as an infectious standard, the 2nd standard was prepared at the same time as the 1st international standard and was originally evaluated together in the study to establish the 1st IS. During this study it was observed that either candidate would be suitable for establishment as the 1st IS, therefore when replacement of the 1st IS became necessary the second candidate was re evaluated and shown to be suitable [2]. In 2009 it was identified that stocks of the 2nd IS were sufficient for up to two further years of supply and therefore a proposal was presented to and accepted by the WHO ECBS meeting held in Geneva in October 2009 to commence replacement of this standard.

Following a meeting of WHO Collaborating Centres representatives from the National Institute for Biological Standards and Control (NIBSC), the Paul Ehrlich Institute (PEI), and the Centre for Biologicals Evaluation and Research (CBER) at the Food and Drug Administration, USA (FDA) in Washington DC, 29-30 January 2007, Dr Indira Hewlett, (CBER) informed the meeting that infectious HIV cultures could be inactivated by treatment with heat at 60C for 1 hour without substantially reducing the ability of the viral RNA to react in nucleic acid-based techniques (NAT). It was therefore recommended that for future HIV International standards heat inactivation should be considered. Heat inactivation would have the advantage of (i) being safer to handle during aliquoting and freeze-drying and (ii) would make shipments easier and cheaper as the material would not have to be treated as infectious. Such a method has since been used for the successful establishment of the 1st International Standard for HIV-2 NAT [3]. The candidate 3rd HIV-1 IS was formulated from the same genotype B viral isolate as was used in the 2nd IS, viral stocks of this genotype were heat inactivated at 60⁰C for 1 hour and an appropriate dilution made into human plasma prior to freeze drying. Approximately 3000 vials were prepared each containing, when reconstituted, a volume of 1ml. This material (labelled candidate A and C) was evaluated in an international collaborative study along with the 2nd IS; recoded candidate B for the purpose of the study. All three candidates gave results that were tightly grouped, with little difference between the results from different laboratories or from the use of different assays. It is proposed that this candidate is established as the 3rd HIV-1 NAT International Standard with an assigned unitage of 5.27 Log₁₀ IU/ml.

The objectives of this collaborative study were to establish a replacement WHO International Standard for HIV- NAT assays, to determine the stability of the product using accelerated degradation studies and to establish the relative potency of the candidate replacement standard (10/152) against the WHO 2nd International Standard (97/650) to ensure continuity of the IU. The project was endorsed by the WHO ECBS in October 2009 and at the WHO Collaborating Centres (NIBSC, PEI, and CBER/FDA) meeting in PEI, Langen, Frankfurt, 17-18 February 2009 [4].

The WHO's Expert Committee on Biological Standardization (ECBS) establishes reference standards for biological substances used in the prevention, treatment or diagnosis of human disease. WHO International Standards are recognized as the highest order of reference for biological substances, and are arbitrarily assigned a potency in International Units (IU). Their primary purpose is to calibrate secondary references used in routine laboratory assays, in terms of the IU, thereby providing a uniform result reporting system, and traceability of measurements, independent of the method used [5].

Materials

Candidate standards.

The candidate standard consists of a genotype B HIV-1 RNA preparation in the form of heat-treated virus culture supernatant diluted in pooled citrated human plasma. Based on experience of developing standards for other blood transmitted viruses such as HIV-1, HCV and HBV, a whole microorganism diluted in pooled human plasma is a reasonably close homologue to the samples that are routinely used in NAT (human plasma) [6].

Selection of virus strains

The choice of subtype for inclusion in the 1st International Standard was discussed at a meeting of the WHO-sponsored SoGAT working group meeting held in 1998 and in view of the broad prevalence and geographical spread of subtype B along with the availability of suitable candidate materials, it was decided that it should be based on a subtype B virus.

The chosen virus strain was a genotype B (env V3, gag) field isolate of HIV-1 provided by Dr P Simmonds, University of Edinburgh, UK. Whilst it is recognised that genotype C may now be the main causative genotype of the HIV pandemic, in order to ensure continuity of the International Standard it was agreed at the WHO ECBS meeting held in Geneva in October 2009 that this replacement standard would continue to be formulated using a genotype B strain. There are currently two HIV-1 genotype panels undergoing development, the first one is a replacement panel for the previously approved 01/446 panel and contains genotypes A- H and a representative from groups N and O. The second panel will contain a variety of circulating recombinant forms of HIV containing different genotypes within one genome. Both of these panels will contain a genotype C from different regions and overall the panel will be vital in assessing the ability of both commercial and in house assays to detect different genotypes.

Source.

The virus was isolated post-mortem from a patient that had died from an AIDS-defining illness, patient 4 in [7]. The virus was supplied as a low-passage PBMC culture and a stock of cell-free culture supernatant stored under vapour phase liquid nitrogen.

Heat inactivation of virus.

1ml aliquots of virus were placed in a water bath adjusted to 60°C for a period of 1 hour. The heat-treated virus was pooled, re-aliquoted and stored at $\leq 80^{\circ}\text{C}$ in a laboratory where infectious material is not propagated to avoid any contamination, viruses were then tested by cell culture to confirm inactivation. For the latter, 1ml samples of the inactivated virus were inoculated onto peripheral blood mononuclear cells (PBMC's) and allowed to incubate for at least 1 hour at 37C. Ten-fold dilutions of a positive control culture (non-heat inactivated virus) was also set up in the same way and un-inoculated negative control cultures were included. Following incubation, the cells were washed, overlaid with fresh medium and the cells checked every 2-4 days for CPE, samples taken for p24 antigen detection and the cells fed with fresh medium for a period of 21 days. Samples were tested for p24 using the Innogenetics Innostest HIV p24 antigen EIA kit.

The results showed that both the viral stock readily infected the PBMC cultures producing p24 antigen within 4 days, whereas the heat-treated virus, along with the un-inoculated negative control cultures, remained p24-negative throughout the 21 day observation period.

To assess any loss of viral RNA titre following the heat-treatment, pre- and post-heat treated virus was titrated in a real time PCR assay. The mean RNA copy number as determined by the Roche HIV-1 Taqman assay combined with the High Pure extraction method is shown in table 1. In both cases the mean is derived from four replicates tested in one assay.

Table 1.

| Reagent Status | RNA copy number log ₁₀ |
|------------------------|-----------------------------------|
| Pre heat inactivation | 5.26 |
| Post heat inactivation | 5.19 |

Following heat inactivation the results show there was an observed drop in viral load of 0.07, however this is within the expected variation of all assays.

Freeze-drying.

This was undertaken at the Centre for Biological Reference Materials (CBRM), NIBSC, Potters Bar, UK, during autumn 2010. The filled material consisted of heat-inactivated HIV-1 genotype B primary viral isolate diluted in citrated pooled human plasma that had been tested and found negative for HBsAg, HIV antibody and HCV RNA by PCR. The following candidate preparation was freeze-dried:

- Candidate HIV-1 3rd International Standard: NIBSC Code 10/152

The filling was performed in a Metall and Plastic GmbH (Radolfzell, Germany) negative pressure isolator that contains the entire filling line and is interfaced with the freeze dryer (CS150 12 m², Serail, Argenteuil, France) through a 'pizza door' arrangement to maintain integrity of the operation. The bulk material was kept at 4 °C throughout the filling process, and stirred constantly using a magnetic stirrer. The bulk was dispensed into 5 mL screw cap glass

vials in 1 mL volumes, using a Bausch & Strobel (Ilshofen, Germany) filling machine FVF5060. The homogeneity of the fill was determined by on-line check-weighing of the wet weight, and vials outside the defined specification were discarded. Filled vials were partially stoppered with halobutyl 14 mm diameter cruciform closures and lyophilized in a CS150 freeze dryer. Vials were loaded onto the shelves at -50 °C and held at this temperature for 4 hrs. A vacuum was applied to 270 µb over 1 hr, followed by ramping to 30 µb over 1 hr. The temperature was then raised to -40 °C, and the vacuum maintained at this temperature for 42.5 hrs. The shelves were ramped to 25 °C over 15 hrs before releasing the vacuum and back-filling the vials with nitrogen. The vials were then stoppered in the dryer, removed and capped in the isolator, and the isolator decontaminated with formaldehyde before removal of the product. The sealed vials are stored at -20 °C at NIBSC under continuous temperature monitoring for the lifetime of the product (NIBSC to act as custodian and worldwide distributor). 2910 vials were prepared, any residual bulk material was returned to the scientist. The CV of fill mass and mean residual moisture was within WHO acceptable limits, details can be found in table 2. The freeze-dried preparation was shown by NAT to be free of HBV DNA and HCV RNA.

Post-fill testing

Assessments of residual moisture and oxygen content, as an indicator of vial integrity after sealing, were determined for twelve vials of the freeze-dried product. Residual moisture was determined by non-invasive near-infrared (NIR) spectroscopy (MCT 600P, Process Sensors, Corby, UK). NIR results were then correlated to Karl Fischer (using calibration samples of the same excipient, measured using both NIR and Karl Fischer methods) to give % w/w moisture readings. Oxygen content was measured using a Lighthouse Infra-Red Analyzer (FMS-750, Lighthouse Instruments, Charlottesville, USA).

Table 2.

| | |
|------------------------|---------|
| | 10/152 |
| No vials filled | 2910 |
| Mean fill mass | 1.0081g |
| CV of fill mass | 0.207 |
| Mean residual oxygen | 0.81% |
| Mean residual moisture | 0.35% |
| | |

Stability studies on the product in the final container.

Accelerated degradation studies are underway at NIBSC. To date, the results of accelerated degradation studies at 4 and 8 months are available, shown in table 3. Samples were incubated at the temperatures and times indicated and were evaluated using a real time PCR assay.

Subsequent testing will take place at a further 12, 24 and 36 months.

Table 3.

| | Candidate 10/152 (Log 10/IU) | | | | |
|----------|------------------------------|-------|-------|-------|-------|
| | -20 | 4 | 20 | 37 | 45 |
| 4 months | 5.102 | 5.127 | 5.066 | 4.855 | 4.683 |
| 8 months | 5.27 | 5.26 | 5.25 | 5.02 | 4.47 |

The results show that the freeze-dried virus preparation was stable at -20°C, +4°C and +20°C at the 8 month time point. Samples that had been stored at +37°C and +45°C proved difficult to reconstitute, this has been observed in previous studies where samples are based on pooled human plasma. Rather than actual loss of RNA activity, the lower viral copy number seen at elevated temperatures (+37 and +45°C) may reflect an inability to fully reconstitute the freeze-dried pellet. However, in line with other studies it is expected that this material will show good long term stability [2].

Real time stability studies are also carried out. The standard is included in NAT assays used on site at NIBSC, it is also used in the batch release of CE marked materials. Graphical representation of each titre obtained is used to monitor performance.

Design of collaborative study.

Fifteen laboratories in eleven countries were invited to take part in the collaborative study. Each laboratory received six heat-inactivated, lyophilised coded samples of the candidate material, three labelled A and three labelled C, the intentional duplication of the candidate standard was included to facilitate the evaluation of intra and inter lab variability. The current infectious International Standard was also included and relabelled as sample B; each laboratory received three vials of this standard. Labs were informed that all samples should be treated as infectious thus avoiding giving any indication which sample was the heat inactivated candidate. It was requested that all vials were frozen at -20°C on receipt and participants were requested to return an acknowledgement form sent with the package to report the safe receipt of the samples. All samples were lyophilised preparation in a 2 ml glass vial, samples A and C had a screw cap lid, whilst sample B had a crimp sealed top. Participants were asked to reconstitute each vial with 1ml of deionised, nuclease-free, molecular biology-grade water and left for a minimum of 20 minutes with occasional agitation before use; a visual check should be made to ensure all vials were fully reconstituted after this time. An IFU for both materials was included in the shipment. The IFU for this candidate preparation can be seen in appendix 2.

Participants were requested to perform serial dilutions of the reagents in 3 independent assays. A fresh vial of each reagent was used in each assay. It was requested that all dilutions were carried out in the diluents normally used in the laboratory's assay system and this was recorded on the results form.

Initially, participants were requested to assay tenfold dilutions of each preparation in order to determine either the HIV-1 end point or in the case of quantitative assays, to estimate the RNA concentration at each dilution. It was suggested that they use the neat material and four tenfold dilutions (neat to 10⁻⁴) to determine the end points of the preparations. Using the end point information determined in assay one, at least two (and preferably three) further assays were carried out. For qualitative assays, participants were requested to assay a minimum of two half

log dilutions either side of the pre-determined end point; for quantitative assays participants were requested to select a dilution close to the end point and the four half-log dilutions before the end point.

Statistical Methods.

Quantitative Assays: Quantitative assays provide estimates in “copies/ml” based on the calibration of the particular assay kit or method. To obtain a single estimate of copies/ml per sample for each laboratory, a single estimate for each assay was obtained by taking the mean of the log₁₀ estimates across replicates and dilutions (after correcting for dilution factor). A mean of the assay means was then calculated to give a single laboratory mean value of log₁₀ copies/ml. The linearity of the assay response across the dilutions range was looked at, and in some cases, estimates that fell outside the apparent linear range were excluded from subsequent calculations.

Laboratories using quantitative assays reported the number of ‘detectable units per ml’ for each dilution tested. For each laboratory and assay method, the linearity of these estimates was checked by plotting the log₁₀ estimate against the log₁₀ dilution. Ideally, a straight line with a slope of 1.0 should be observed and deviations from linearity are readily observed. Results that were clearly aberrant or deviated markedly from linearity were discounted. A single estimate of copies per ml for the undiluted material was obtained by correcting each individual estimate by the dilution factor, and calculating an overall arithmetic mean of the log estimates.

Qualitative Assays: Qualitative assays were analysed by determining an end-point from the dilution series of number positive out of number tested, pooled across assays. After correction for the equivalent volume of sample amplified, the result is given in “PCR detectable units/ml” (or NAT detectable units) and expressed as a log₁₀ value. These were treated as a dilution series and used to provide a single estimate of ‘detectable units per ml’ in the undiluted sample using the method of maximum likelihood for ‘dilution assays’ [8]. This model assumes that the probability of a positive result at a given dilution follows a Poisson distribution (with mean given by the expected number of “copies” in the sample tested), and that a single “copy” will lead to a positive result. Calculations were performed using the statistical package GLIM [9]. When a single value is obtained for each laboratory and sample no assessment of within laboratory, i.e between assay variability can be made.

The units from qualitative and quantitative assays are not directly comparable, and overall means of qualitative and quantitative assay results are not therefore appropriate. Potencies of the candidate 10/152 were expressed relative to the current IS, 97/650, using the mean results from the duplicate samples A & C for the candidate. After expressing results as potencies relative to the IS, the units for both qualitative and quantitative assays are in log₁₀ IU/ml, and so results from both assay types can be combined.

Results.

Overall fifteen laboratories reported results some laboratories reported data using more than one assay system, in total seventeen data sets were returned. Ten different NAT systems were used, six (60%) of which were from quantitative assays and four (40%) from qualitative assays. Six different commercial kits were used, five of which were of a quantitative format and one in a qualitative format. Two labs reported results using the Roche COBAS Taqman assay version 1 using the high pure extraction method, four labs used the Roche Ampliprep version 2, one lab used the Roche Ampliprep version 1 and one lab reported data using the Ampliprep version 1.5, whilst three labs reported using the Abbott Real time assay. The qualitative Chiron Procleix

Ultrio assay (Ultrio) was used by one lab. Three laboratories reported results from in-house assays in qualitative formats and one lab reported data from an in house quantitative test. Of the qualitative assays, different extraction volumes and methods were used, this was taken into account during data analysis and calculations of potency \log_{10} IU/ml.

The laboratory mean estimates for the candidate 10/152 (study samples A & C), and the current IS 97/650 (study sample B) are shown in figures 1-3 The overall means for the different assay methods and overall for qualitative and quantitative assays, are shown in tables 4 and 5.

The laboratory mean estimates (\log_{10} copies/ml) for this study given in table 4 are based on the geometric mean estimates of copies/ml across dilutions and across assays for the quantitative assays, or calculated from the series of number positive out of number tested for the qualitative assays. Table 6 gives the relative potencies for the current study. Figure 4 shows a histogram of the overall relative potency of the 3rd candidate replacement standard to the 2nd International Standard in IU.

Discussion.

All assays used in this study detected all candidate materials. From the histograms and tables it is clear that there is more variation between laboratories using qualitative assays. However, although the results from some of these laboratories are low, this is not the case for all qualitative assays. For example, the In-House assay from lab 14 gives results that are higher, i.e more sensitive, than many of the quantitative assays The method of determining an estimate from qualitative assays, based on a dilution-series end-point, is intrinsically more variable than quantitative assays, which may contribute to the between lab variability observed. For example, compare the relative values of results from labs 9 & 10, using the Ultrio assay, for the duplicate samples A & C.

The estimates from the one laboratory using the Roche Amplicor v1.5 assay were lower (0.5 – 0.8 log) than those labs using Ampliprep v2, this may be due to the improved technology and efficiency of the Ampliprep v2 whereby an automated extraction platform is used with an amplification protocol targeting both the gag and LTR genome regions. The Amplicor v1.5 relies on the use of a manual extraction method and detection using a 96 well microtitre plate, amplification is targeted solely to the gag region.

The overall mean copies/ml (\log_{10}) from the quantitative assays for the current IS 97/650 was 5.47 \log_{10} copies/ml. It can be seen from figure 4 that expressing results for the candidate (samples A & C) relative to the current IS reduces the variability between laboratories. In particular, it brings the results of the qualitative assays and the quantitative Roche Amplicor v1.5 into closer agreement with the other quantitative assays. The quantitative assays other than the Amplicor v1.5 were already in close agreement, so little improvement is noted for these.

Results reported by Lab 12 using the Roche Amplicor v1.5 appear to be non-linear across the dilution range, with results appearing relatively higher at higher dilutions. However, the pattern was similar for all samples and so will not impact on the calibration of the candidate against the current IS. Whilst such an assay has been used in previous studies [1,2] the effect was less marked by the comparison with similar manual methods. The current level of assay automation highlights the possible inefficiencies with manual methods such as pipetting variation and operator technique.

The data sets showed no apparent differences between any of the reported assays in the estimates of the candidate relative to the current IS, this is demonstrated in figure 4.

Commutability of this candidate standard relative to clinical HIV samples has not been specifically assessed in this study. Commutability is determined by a range of factors including the matrix and molecular variants of the virus.

The overall mean for the candidate standard 10/152 is 5.27 log₁₀ IU/ml based on all seventeen data sets, or 5.23 log₁₀ IU/ml based on the twelve quantitative data sets. The range across laboratories was 4.88 to 5.52 log₁₀ IU/ml.

Conclusions.

Based on the results of this collaborative study, it is apparent that this heat inactivated lyophilised preparation (10/152) is suitable to be established as the 3rd International Standard for HIV-1 NAT assays. This standard is intended for use in both quantitative and qualitative assays and therefore it would be appropriate to assign a unit incorporating both data sets. From the data seen in this study a unitage of 185,000 IU/vial (1ml) (5.27 log₁₀ IU/vial) should be assigned.

Proposal.

It is proposed that a value of 185,000 IU/ml (5.27 log₁₀ IU/ml) is assigned to the 3rd International Standard for HIV-1 NAT (NIBSC code 10/152).

Comments from Participants

This report has been circulated to all participants, two of the fifteen participants returned comments; these were minor editorial changes and have been incorporated into the text. No participant commented on the proposal to assign the described unitage. There were no proposals suggesting this material is not suitable for establishment as the 3rd HIV-1 NAT IS.

References.

1. Holmes, H., Davis, C., Heath, A., Hewlett, I., Lelie, N. 2001. An International collaborative study to establish the 1st international standard for HIV-1 RNA for use in nucleic acid-based techniques. *J Virol Methods*, 92, 141-150.
2. Davis C, Berry N, Heath A, Holmes H. 2008. An international collaborative study to establish a replacement World Health Organization International Standard for human immunodeficiency virus 1 RNA nucleic acid assays. *Vox Sang*. 95: 218-25.
3. Morris C, Berry N, Heath A, Holmes H. Preparation and evaluation of a proposed 1st International Standard for HIV-2 RNA
WHO ECBS Report - 09.2118 Rev.1
4. Development of WHO Biological Reference Preparations for Blood Safety-related in vitro Diagnostic Tests .
Report of the 2nd meeting with the WHO Collaborating Centres for Biological Standards and Standardization, 17-18 February 2009
5. Recommendations for the preparation, characterization and establishment of international and other biological reference standards (revised 2004). WHO Technical Report Series 2007. Geneva, Switzerland:WHO 2007;932,73-131:
http://www.who.int/bloodproducts/publications/TRS932Annex2_2004.pdf
6. WHO Consultation on Global Measurement Standards and their use in the in vitro Biological Diagnostic Field, Switzerland, Geneva, Switzerland, 7-8 June 2004:
<http://www.who.int/bloodproducts/publications/en/Minutes-220804.pdf>
7. Collet, D., 1991. Modelling Binary Data, Chapman Hall, London.
8. Donaldson, Y.K., Bell, J.E., Holmes, E.C., Hughes, E.S., Brown, H.K., Simmonds, P., 1994. In vivo distribution and cytopathology of variants of human immunodeficiency virus type 1 showing restricted sequence variability in the V3 loop. *J. Virol.* 68, 5991-6005.
9. Francis, B., Green, M., Payne, C. (Eds), 1993. The GLIM System Release 4 Manual. Oxford Science Publications, Clarendon Press, Oxford.

Table 4: Overall Mean Estimated PCR Detectable Units/ml or Copies/ml (log₁₀) per assay type

| Sample | | Assay | N | Mean | Min | Max | SD | Range |
|-------------|--------------|------------------------------|---|------|------|------|------|-------|
| A 10/152 | Qualitative | In-House | 3 | 4.75 | 4.32 | 5.49 | 0.64 | 1.17 |
| | | Ultrio | 2 | 4.89 | 4.51 | 5.26 | 0.53 | 0.75 |
| | Quantitative | Abbott Real Time | 3 | 5.07 | 4.98 | 5.22 | 0.13 | 0.24 |
| | | COBAS Taqman 48/High pure v1 | 2 | 5.15 | 5.03 | 5.27 | 0.17 | 0.24 |
| | | In-House | 1 | 5.23 | - | - | - | - |
| | | Roche Amplicor v1.5 | 1 | 4.62 | - | - | - | - |
| | | Roche Ampliprep v2 | 4 | 5.28 | 5.17 | 5.47 | 0.13 | 0.30 |
| | | Roche Ampliprep v1 | 1 | 5.06 | - | - | - | - |
| B 97/650 | Qualitative | In-House | 3 | 4.98 | 4.61 | 5.68 | 0.61 | 1.07 |
| | | Ultrio | 2 | 5.06 | 4.86 | 5.26 | 0.28 | 0.40 |
| | Quantitative | Abbott Real Time | 3 | 5.38 | 5.23 | 5.64 | 0.22 | 0.41 |
| | | COBAS Taqman 48/High pure v1 | 2 | 5.45 | 5.30 | 5.60 | 0.22 | 0.30 |
| | | In-House | 1 | 5.52 | - | - | - | - |
| | | Roche Amplicor v1.5 | 1 | 4.83 | - | - | - | - |
| | | Roche Ampliprep v2 | 4 | 5.64 | 5.37 | 6.00 | 0.23 | 0.63 |
| | | Roche Ampliprep v1 | 1 | 5.37 | - | - | - | - |
| C 10/152 | Qualitative | In-House | 3 | 4.75 | 4.34 | 5.24 | 0.46 | 0.90 |
| | | Ultrio | 2 | 4.94 | 4.74 | 5.14 | 0.29 | 0.40 |
| | Quantitative | Abbott Real Time | 3 | 5.07 | 4.96 | 5.20 | 0.13 | 0.24 |
| | | COBAS Taqman 48/High pure v1 | 2 | 5.11 | 5.06 | 5.15 | 0.07 | 0.09 |
| | | In-House | 1 | 5.19 | - | - | - | - |
| | | Roche Amplicor v1.5 | 1 | 4.75 | - | - | - | - |
| | | Roche Ampliprep v2 | 4 | 5.23 | 5.01 | 5.46 | 0.16 | 0.45 |
| | | Roche Ampliprep v1 | 1 | 5.00 | - | - | - | - |

Table 5: Mean quantitative and qualitative comparisons

| Sample | | N | Mean | Min | Max | SD | Range |
|-------------|--------------|----|------|------|------|------|-------|
| A 10/152 | Qualitative | 5 | 4.81 | 4.32 | 5.49 | 0.53 | 1.17 |
| | Quantitative | 12 | 5.15 | 4.62 | 5.47 | 0.22 | 0.85 |
| B 97/650 | Qualitative | 5 | 5.01 | 4.61 | 5.68 | 0.46 | 1.07 |
| | Quantitative | 12 | 5.47 | 4.83 | 6.00 | 0.30 | 1.17 |
| C | Qualitative | 5 | 4.82 | 4.34 | 5.24 | 0.37 | 0.90 |

| | | | | | | | |
|--------|--------------|----|------|------|------|------|------|
| 10/152 | Quantitative | 12 | 5.12 | 4.75 | 5.46 | 0.18 | 0.71 |
|--------|--------------|----|------|------|------|------|------|

Table 6: Potency of 10/152 relative to current IS 97/650 (\log_{10} IU/ml)

| Sample | | Assay | N | Mean | Min | Max | SD | Range |
|--------|--------------|------------------------------|---|------|------|------|------|-------|
| 10/152 | Qualitative | In-House | 3 | 5.33 | 5.24 | 5.44 | 0.10 | 0.20 |
| | | Ultrio | 2 | 5.41 | 5.30 | 5.52 | 0.16 | 0.22 |
| | Quantitative | Abbott Real Time | 3 | 5.25 | 5.13 | 5.31 | 0.10 | 0.18 |
| | | COBAS Taqman 48/High pure v1 | 2 | 5.24 | 5.17 | 5.31 | 0.09 | 0.14 |
| | | In-House | 1 | 5.25 | - | - | - | - |
| | | Roche Amplicor v1.5 | 1 | 5.42 | - | - | - | - |
| | | Roche Ampliprep v2 | 4 | 5.18 | 4.88 | 5.29 | 0.17 | 0.41 |
| | | Roche Ampliprep v1 | 1 | 5.23 | - | - | - | - |

Table 7: Overall mean data of candidate 10/152

| Sample | | N | Mean | Min | Max | SD | Range |
|--------|--------------|----|------|------|------|------|-------|
| 10/152 | Qualitative | 5 | 5.36 | 5.24 | 5.52 | 0.12 | 0.28 |
| | Quantitative | 12 | 5.23 | 4.88 | 5.42 | 0.13 | 0.54 |
| | Overall | 17 | 5.27 | 4.88 | 5.52 | 0.14 | 0.64 |

Appendix 1: Participants

| Participant/Organisation | Country |
|---|----------------|
| M Tatsumi, National Institute of Infectious Diseases, Tokyo | Japan |
| M Stolz, Blood Transfusion Service, Bern | Switzerland |
| Yi Chen Yang/Dr. Der-Yuan Wang, Food and Drugs Administration, Taipei | Taiwan |
| D Candotti, NHS Blood and Transplant, Cambridge | UK |
| C Gschwentner, Baxter, Vienna | Austria |
| H Cuypers, Sanquin Diagnostics. Amsterdam | Netherlands |
| M Nuebling, Paul Ehrlich Institute, Langan | Germany |
| M Naukkarinen, Finnish Red Cross, Helsinki | Finland |
| S Saulda, Banc de Sang I Teixits, Barcelona | Spain |
| A Zucchini – Kedrion, Lucca | Italy |
| I Hewlett, FDA/CBER, Washington DC | USA |
| C Boor – Grifols S.A | Spain |
| M Lin – Roche, California | USA |
| C Morris, NIBSC, Potters Bar | UK |
| K Cristiano, ISS, Rome | Italy |

Appendix 2: IFU

**WHO International Standard
Candidate 3rd HIV-1 International Standard
NIBSC code: 10/152
Instructions for use
(Version 1.00, Dated 05/07/2011)**

1. INTENDED USE

For the purposes of this collaborative study to evaluate this material as a suitable candidate for establishment as the 3rd HIV-1 IS, the enclosed protocol should be followed.

2. CAUTION

This preparation is not for administration to humans.

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

This reagent is currently being evaluated, a unitage will be established following this collaborative study.

4. CONTENTS

Country of origin of biological material: United Kingdom.
Each vial contains 1ml freeze dried human plasma that has been screened and found negative for antibodies to HIV-1/2, anti HCV and HBsAg. The plasma has been spiked with a genotype B HIV-1 heat inactivated virus.

5. STORAGE

Vials can be shipped at ambient temperatures, they should then be stored at -20C on receipt.

6. DIRECTIONS FOR OPENING

Vials have a screw cap; an internal stopper may also be present. The cap should be removed by turning anti-clockwise. Care should be taken to prevent loss of the contents. Please note: If a stopper is present on removal of the cap, the stopper should remain in the vial or be removed with the cap.

7. USE OF MATERIAL

No attempt should be made to weigh out any portion of the freeze-dried material prior to reconstitution

Each vial should be reconstituted using 1ml of molecular grade water. For the purposes of this collaborative study the enclosed protocol should be followed.

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. This is a candidate material and stability studies are currently on going.

9. REFERENCES

National Institute for Biological Standards and Control - Assuring the quality of biological medicines
Blanche Lane South Mimms Potters Bar Hertfordshire EN6 3QG United Kingdom
Tel +44 (0)1707 641000 Fax +44 (0)1707 641050 www.nibsc.ac.uk
A World Health Organization Laboratory for Biological Standards

**10. ACKNOWLEDGEMENTS****11. FURTHER INFORMATION**

Further information can be obtained as follows:

This material:

enquiries@nibsc.hpa.org.uk

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.who.int/biologicals/reference_preparations/en/

Ordering standards from NIBSC:

Http://www.nibsc.ac.uk/products/ordering_information/frequently_asked_questions.aspx

NIBSC Terms & Conditions:

Http://www.nibsc.ac.uk/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.hpa.org.uk

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

| Physical and Chemical properties | |
|---|---|
| Physical appearance: Freeze dried | Corrosive: No |
| Stable: Yes | Oxidising: No |
| Hygroscopic: No | Irritant: No |
| Flammable: No | Handling: See caution, Section 2 |
| Other (specify): none | |
| 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

Information provided by the Institute is given after the exercise of all reasonable care and skill in its compilation, preparation and issue, but it is provided without liability to the Recipient in its application and use. It is the responsibility of the Recipient to determine the appropriateness of the standards or reference materials supplied by the Institute to the Recipient ("the Goods") for the proposed application and ensure that it has the necessary technical skills to determine that they are appropriate. Results obtained from the Goods are likely to be dependant on conditions of use by the Recipient and the variability of materials beyond the control of the Institute.

All warranties are excluded to the fullest extent permitted by law, including without limitation that the Goods are free from infectious agents or that the supply of Goods will not infringe any rights of any third party.

The Institute shall not be liable to the Recipient for any economic loss whether direct or indirect, which arise in connection with this agreement.

The total liability of the Institute in connection with this agreement, whether for negligence or breach of contract or otherwise, shall in no event exceed 120% of any price paid or payable by the Recipient for the supply of the Goods.

If any of the Goods supplied by the Institute should prove not to meet their specification when stored and used correctly (and provided that the Recipient has returned the Goods to the Institute together with written notification of such alleged defect within seven days of the time when the Recipient discovers or ought to have discovered the defect), the Institute shall either replace the Goods or, at its sole option, refund the handling charge provided that performance of either one of the above options shall constitute an entire discharge of the Institute's liability under this Condition.

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: 1g |
| Toxicity Statement: Toxicity not assessed |
| Veterinary certificate or other statement if applicable. |
| Attached: No |