A definitive unitage of the 1st WHO International Standard for HPV Type 16 DNA Nucleic Acid Amplification Techniques has been assigned a unitage of $5 \times 10^7$ International Units (IU) per ampoule.

**Traceability statement:**

It was proposed at a WHO meeting in January 2008 (WHO Meeting Report, 2008) that the instructions for use of the International Standard for HPV-16 DNA include the calculations and assumptions used in determining the theoretical HPV-16 genome equivalents (GEq) of the bulk material used in formulating the International Standard, thus demonstrating that 1 IU is equivalent to 1 GEq for HPV-16 DNA. The definitive unitage of the 1st WHO International Standard for HPV-16 DNA therefore remains as IU while the traceability statement would allow users to equate IU with GEq.

Assays for DNA concentration of the recombinant HPV-16 plasmid stock preparation were performed in Dr Cosette Wheeler’s laboratory, University of New Mexico (UNM). DNA concentrations were determined by absorbance at 260 nm as well as spectrophotometrically using the Picogreen assay (Invitrogen Corporation, USA). A correlation coefficient of 0.96 or higher was obtained between the two DNA measurements. 10 ng HPV-16 plasmid DNA/μl was supplied to NIBSC for formulating the bulk material for subsequent freeze-drying. The UNM laboratory also provided NIBSC with a statement indicating that 1.0 $\times 10^{-11}$ GEq/ml for HPV-16 is equal to 1.17 ng/μl. 10 ng HPV-16 plasmid DNA/μl plasmid stock preparation is therefore equivalent to $8.547 \times 10^7$ HPV-16 GEq/ml. NIBSC used this data in formulating the 1st International Standard for HPV Type 16 DNA.

Formulation of bulk material for the 1st International Standard for HPV Type 16 DNA (NIBSC code 06/202):

At NIBSC, the bulk HPV-16 plasmid DNA material was prepared according to the formula:

$\text{HPV GEq/ml of bulk material} = \left( \frac{\text{GEq/ml of plasmid stock} \times \text{volume plasmid stock}}{\text{volume bulk material}} \right)$

Therefore,

HPV-16 GEq/ml of bulk material $= (8.547 \times 10^{11} \text{ HPV-16 GEq/ml plasmid stock}) \times (0.02223 \text{ ml HPV-16 plasmid stock}) / 1900 \text{ ml HPV-16 bulk material}

$= 1.0 \times 10^7 \text{ HPV-16 GEq/ml bulk material}$

The HPV-16 DNA bulk material was subsequently freeze-dried in 0.5 ml aliquots.

Certain assumptions are required for equating IU to GEq for the 1st International Standard for HPV-16 DNA: 1) $1.0 \times 10^7$ GEq/ml for HPV-16 is equal to 1.17 ng/μl. 2) There is no loss in activity of the HPV-16 DNA upon lyophilization. 3) The recombinant HPV-16 plasmid DNA accurately mimics the activity of HPV-16 viral DNA in biological samples.

Independent calculation of GEq/ml for recombinant HPV-16 plasmid DNA.

NIBSC also independently calculated the genome equivalence of the HPV-16 plasmid stock preparation and bulk preparation in which the molecular weights of the full-length HPV-16 genome and pBR322 DNA were based on sequence content using BioEdit Sequence Alignment Editor v7.0.5.3 (Tom Hall, Isis Pharmaceuticals Inc., USA). The sequences used for determining the molecular weights are GenBank Accession number J01749.1 for pBR322 and the reference sequence for HPV-16 (Accession K02718).

**BioEdit data**

DNA molecule: HPV16 Accession K02718

Length $= 7904$ base pairs

MW= 4786756.00 Daltons, double stranded

DNA molecule: cloning vector pBR322

Length $= 4361$ base pairs

MW= 2653867.00 Daltons, double stranded

**Formulae**

GEq/ml of the HPV plasmid stock was calculated according to the formula: $\text{GEq/ml of the HPV plasmid stock} = (\text{DNA concentration of HPV plasmid stock} \times (\text{MW of HPV DNA} + \text{MW of pBR322})) \times (\text{Avogadro’s Number})$ where Avogadro’s Number $= 6.022 \times 10^{23}$ molecules/mol

GEq/ml of the bulk HPV DNA materials was calculated according to the formula:

$\text{HPV GEq/ml of bulk material} = (\text{HPV GEq/ml of plasmid stock} \times \text{volume plasmid stock}) / \text{volume bulk material}$

**Calculation**

The recombinant HPV-16 plasmid stock preparation was supplied to NIBSC at a concentration of 10 ng/μl. Using the MW determinations shown above, the GEq/ml of the HPV-16 plasmid stock is:

$= (10 \times 10^{-9} \text{ g/μl}) \times (\text{mol/(7440623 g)}) \times (6.022 \times 10^{23} \text{ molecules/mol})$

$= 8.093 \times 10^{11} \text{ molecules/μl}$

$= 8.093 \times 10^{11} \text{ HPV-16 GEq/ml}$

22.23μl of the recombinant HPV-16 plasmid stock was diluted to a final volume of 1900ml, therefore,

HPV-16 GEq/ml of bulk material $= (8.093 \times 10^{11} \text{ HPV-16 GEq/ml plasmid stock}) \times (0.02223 \text{ ml HPV-16 plasmid stock}) / 1900 \text{ ml HPV-16 bulk material}$

$= 0.947 \times 10^7 \text{ HPV-16 GEq/ml bulk material}$
4. CONTENTS
Country of origin of biological material: United Kingdom.
Each ampoule contains the lyophilized equivalent of 0.5 ml HPV-16 plasmid DNA in 10mM Tris buffer pH 7.4 containing 1mM EDTA, 5 mg/ml trehalose and -1 x 10^7 human GEq/ml derived from C33a cells.

5. STORAGE
The ampoule should be stored at -20 °C or below on receipt.
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.

The 1st International Standard for HPV-16 DNA contains high copy number template. There is a high risk of HPV-16 plasmid DNA contamination via aerosolization upon opening of the glass ampoule. The material must be opened and handled in a separate laboratory environment, away from other pre-amplification components such as reagents, labware and samples.
The material is supplied lyophilized and, before use, should be reconstituted in 0.5 ml sterile nuclease-free water. Ensure that the inside surface of the ampoule is wetted with the added water so that any particles of freeze-dried material adhering to the glass are reconstituted. The reconstituted material has a final concentration of 1 X 10^7 IU/ml. The reconstituted material is suitable for calibration of in-house or working standards for the amplification and detection of HPV-16 DNA. The material is not suitable for calibrating or assessing extraction, precipitation or centrifugation procedures. The material has NOT been calibrated for human DNA nucleic acid amplification techniques.

8. STABILITY
Reference materials are held at NIBSC within assured, temperature-controlled storage facilities. The 1st International Standard for HPV-16 DNA should be stored at -20 °C or below on receipt. Degradation studies on 06/202 and 06/206 indicate that the freeze-dried material is extremely stable and suitable for long-term storage (Wilkinson et al., 2010). Users should determine the stability of the reconstituted material according to their own method of preparation, storage and use.
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. This project was funded in part by the World Health Organization and the Bill and Melinda Gates Foundation.

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>
<th>Toxicological properties</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Physical appearance:</strong> Lyophilized powder</td>
<td><strong>Corrosive:</strong> No</td>
</tr>
<tr>
<td><strong>Stable:</strong> Yes</td>
<td><strong>Oxidising:</strong> No</td>
</tr>
<tr>
<td><strong>Hygroscopic:</strong> No</td>
<td><strong>Inflamable:</strong> No</td>
</tr>
<tr>
<td><strong>Flammable:</strong> No</td>
<td><strong>Handling:</strong> See caution, Section 2</td>
</tr>
<tr>
<td><strong>Other (specify):</strong></td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Inhalation:</th>
<th>Seek medical advice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingestion:</td>
<td>Seek medical advice</td>
</tr>
<tr>
<td>Contact with eyes:</td>
<td>Wash with copious amounts of water. Seek medical advice</td>
</tr>
<tr>
<td>Contact with skin:</td>
<td>Wash thoroughly with water.</td>
</tr>
</tbody>
</table>

Action on Spillage and Method of Disposal

Spillage of 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

<table>
<thead>
<tr>
<th>Country of origin for customs purposes*:</th>
<th>United Kingdom</th>
</tr>
</thead>
<tbody>
<tr>
<td>* 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.</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Net weight:</th>
<th>0.5 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toxicity Statement:</td>
<td>Non-toxic</td>
</tr>
<tr>
<td>Veterinary certificate or other statement if applicable:</td>
<td>Attached: No</td>
</tr>
</tbody>
</table>

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_Intertimedstandardsrev2004.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.