** WHO International Standard  
1st WHO International Genetic Reference Panel for quantitation of BCR-ABL translocation by RQ-PCR  
NIBSC code: 09/138  
Instructions for use  
(Version 4.0, Dated 13/12/2012)  

1. INTENDED USE  
The panel comprises four individually coded ampoules, each containing freeze-dried cells. Each ampoule has a different defined value for %BCR-ABL/control gene and they are intended to be used as a primary standard for calibrating secondary standards by RQ-PCR. This panel was established in 2009 by the Expert Committee on Biological Standardization (ECBS) of the World Health Organization (WHO) as the 1st WHO International Genetic Reference Panel for the Quantitation of BCR-ABL Translocation, NIBSC code 09/138 [1].  
These materials should not be put to any other use. Please note that the materials have been validated only for BCR-ABL detection in the range 0.01% to 10% on the International Scale. They have not been validated for other applications, e.g. International Scale measurements >10% or for determining assay sensitivity.

2. CAUTION  
**This preparation is not for administration to humans or animals in the human food chain**  
The preparations contain material of human origin. They have been tested for HIV, HBV, HCV, CMV, EBV, HTLV-I/II, HHV-8 and mycoplasma by PCR and none were detected.  
Routine microbiology testing of the freeze-dried materials showed contamination with Staphylococcus haemolyticus, which is classified in Hazard Group 2. Experiments carried out at NIBSC showed that the organism was completely killed after exposure to Trizol (30-60% Phenol) which is the first step of the RNA extraction process. 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  
The panel was tested in an international collaborative study involving 10 laboratories and the following mean %BCR-ABL / control gene values were obtained following conversion to the international scale (IS):

<table>
<thead>
<tr>
<th>Ampoule Code no.</th>
<th>%BCR-ABL / ABL</th>
<th>%BCR-ABL / BCR</th>
<th>%BCR-ABL / GUSB</th>
</tr>
</thead>
<tbody>
<tr>
<td>08/192</td>
<td>0.0118</td>
<td>0.0195</td>
<td>0.0071</td>
</tr>
<tr>
<td>08/194</td>
<td>0.1112</td>
<td>0.1753</td>
<td>0.0749</td>
</tr>
<tr>
<td>08/196</td>
<td>1.1672</td>
<td>1.6627</td>
<td>0.8295</td>
</tr>
<tr>
<td>08/198</td>
<td>10.7469</td>
<td>16.3129</td>
<td>10.1235</td>
</tr>
</tbody>
</table>

4. CONTENTS  
Country of origin of biological material: Germany & United Kingdom.  
The ampoules contain freeze-dried K562 cells (expressing the BCR-ABL translocation b3a2) and HL60 cells (BCR-ABL negative) in varying proportions. The total number of cells per ampoule is 1.5 x 10^6. The cells were suspended in 2x PBS before freeze-drying.

5. STORAGE  
Store all 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.  
a. Open ampoules as described in section 6. above.  
b. Reconstitute freeze-dried material at room temperature with 1mL Trizol or 600 µL RLT buffer.  
c. Ensure all cells are lysed by repeated aspiration with a pipette tip or a needle.  
d. Transfer the entire contents to nuclease-free tubes.  
e. Using your local method, extract RNA from the 4 ampoules of freeze-dried material and the secondary standards to be calibrated at the same time.  
f. Primary and secondary material should be analysed in the same assay to assign value to the secondary standard. The dose response curves of the WHO panel and secondary standard should be parallel to each other when log %BCR-ABL is plotted against log dilution.

If you require further information on how to use these materials please contact NIBSC.

8. STABILITY  
Reference materials are held at NIBSC within assured, temperature controlled storage facilities and they should be stored on receipt as indicated on the label. It is the policy of WHO not to assign an expiry date to their international reference materials. Accelerated degradation studies have indicated that this material is suitably stable, when stored at -20°C or below, for the assigned values to remain valid until the material is withdrawn or replaced. These studies have also shown that the material is suitably stable for shipment at ambient temperature without any effect on the assigned values. Users who have data supporting any deterioration in the characteristics of any reference preparation are encouraged to contact NIBSC.

9. REFERENCES  
1. WHO document WHO/BS/09.210  

10. ACKNOWLEDGEMENTS  
We would like to thank the German Collection of Microorganisms and Cell Cultures (DSMZ), the Hammersmith Hospital, London and the UK National Genetics Reference Laboratory (Wessex) for supplying materials and assistance with the collaborative study.

11. FURTHER INFORMATION  
Further information can be obtained as follows;  
This material: enquiries@nibsc.org
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></th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical appearance:</td>
<td>Freeze-dried solid</td>
</tr>
<tr>
<td>Stable:</td>
<td>Yes</td>
</tr>
<tr>
<td>Hygroscopic:</td>
<td>Yes</td>
</tr>
<tr>
<td>Flammable:</td>
<td>No</td>
</tr>
<tr>
<td>Other (specify):</td>
<td>Contains material of human origin</td>
</tr>
<tr>
<td>Corrosive:</td>
<td>No</td>
</tr>
<tr>
<td>Oxidising:</td>
<td>No</td>
</tr>
<tr>
<td>Irritant:</td>
<td>No</td>
</tr>
<tr>
<td>Handling:</td>
<td>See caution, Section 2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Toxicological properties</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Effects of inhalation</td>
<td>Not established, avoid inhalation</td>
</tr>
<tr>
<td>Effects of ingestion</td>
<td>Not established, avoid ingestion</td>
</tr>
<tr>
<td>Effects of skin absorption</td>
<td>Not established, avoid contact with skin</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Suggested First Aid</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhalation:</td>
<td>Seek medical advice</td>
</tr>
<tr>
<td>Ingestion:</td>
<td>Seek medical advice</td>
</tr>
</tbody>
</table>

**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 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
   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: 0.0114g per ampoule
   Toxicity Statement: Non-toxic
   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_refstandardsrev2004.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.
Suggested method for calibration of secondary standards
This method is based on the publication Branford et al [Blood 2008;112(8):3330-8] but uses Grubbs’ test for the identification of outliers and regression analysis to determine if there is a trend in bias.
The method will allow you to assign International Scale (IS) values to your materials. Either of the control genes BCR, ABL or GUSB can be used for this calculation and the corresponding assigned IS values for each of the four panel members are shown below:

| BCR-ABL  | 08/192 | 0.0118 | 0.0195 | 0.0071 |
| BCR-ABL  | 08/194 | 0.1112 | 0.1753 | 0.0749 |
| BCR-ABL  | 08/196 | 1.1672 | 1.6627 | 0.8295 |
| BCR-ABL  | 08/198 | 10.7469 | 16.3129 | 10.1235 |

1. Order 5 panels of materials (i.e. 20 ampoules).
2. On 5 different days, take one complete panel of 4 materials, reconstitute them according to the Instructions For Use supplied and extract the RNA.
3. Using these samples and your own secondary reagents, make two batches of cDNA on different days and test these in duplicate/triplicate by RQ-PCR on separate days i.e. testing is over 10 days.
4. You should now have 40 data points from the primary standard. Exclude all failed reactions, but if you have less than 6 data points for each level of BCR-ABL then start again from step 3.
5. Determine the conversion factor using the following procedure and using the example on the following page as a guide;
   a) Convert IS and testing lab % BCR-ABL/control gene values to log_{10}
   b) For each sample tested, calculate the mean of the assigned IS value and the testing lab result:
      \[ m = \frac{1}{2} \left( \log_{10} \text{assigned IS value} + \log_{10} \text{testing lab result} \right) \]
   c) For each sample tested, calculate the difference (d) between the assigned log 10 IS value and the testing lab log 10 value:
      \[ d = \log_{10} \text{assigned IS value} - \log_{10} \text{testing lab result} \]
   d) Calculate the mean (M_d) and the standard deviation (S_d) of the differences (d).
   e) For each sample tested, calculate the outlier test statistic:
      \[ z = \frac{|M_d - d|}{S_d} \]
   f) If the maximum value of z exceeds the relevant value in the table of critical values for Grubbs’ outlier test (overleaf), delete all data for the corresponding sample and repeat steps d) and e).
   g) Perform regression analysis of differences (d) against mean values (m). Assignment of IS values to your materials will be valid ONLY if no significant trend is observed (p>0.05).
   h) The correction factor (analogous to a laboratory-specific conversion factor) is defined as the anti-log of the mean difference i.e. antilog (M_d).
6. Multiply results obtained with your secondary standard by the correction factor to obtain results on the International Scale.
**SUMMARY OUTPUT**

- **df**: 38
- **SS**: 0.0677
- **MS**: 0.0001
- **F**: 0.0039
- **Significance**: 0.0001

- **ANCOVA Statistics**
  - **Multiple R**: 0.0034
  - **R Square**: 0.0001
  - **Adjusted R Square**: -0.0028
  - **Standard Error**: 0.0039
  - **Observations**: 40

**Coefficients**

<table>
<thead>
<tr>
<th>IS</th>
<th>Ampoule</th>
<th>BCR-ABL/ABL %</th>
<th>Log$_{10}$ BCR-ABL/ABL %</th>
<th>Mean (m)</th>
<th>Difference (d)</th>
<th>OUTLIER test</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.0118</td>
<td>0.0231</td>
<td>-1.9281</td>
<td>-1.5820</td>
<td>-0.3486</td>
<td>1.42</td>
</tr>
<tr>
<td>2</td>
<td>0.0118</td>
<td>0.0261</td>
<td>-1.9281</td>
<td>-1.5836</td>
<td>-0.3466</td>
<td>1.41</td>
</tr>
<tr>
<td>3</td>
<td>0.0118</td>
<td>0.0350</td>
<td>-1.9281</td>
<td>-1.5391</td>
<td>-0.3990</td>
<td>1.38</td>
</tr>
<tr>
<td>4</td>
<td>0.0118</td>
<td>0.0315</td>
<td>-1.9281</td>
<td>-1.7502</td>
<td>-2.0171</td>
<td>0.32</td>
</tr>
<tr>
<td>5</td>
<td>0.0118</td>
<td>0.0161</td>
<td>-1.9281</td>
<td>-1.7650</td>
<td>-2.0017</td>
<td>0.32</td>
</tr>
<tr>
<td>6</td>
<td>0.0118</td>
<td>0.0184</td>
<td>-1.9281</td>
<td>-1.7850</td>
<td>-2.0217</td>
<td>0.32</td>
</tr>
</tbody>
</table>

**Critical values for Grubbs' outlier test**

<table>
<thead>
<tr>
<th>N</th>
<th>Critical value (M;):</th>
<th>Critical value (S;):</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>3.49</td>
<td>2.23</td>
</tr>
<tr>
<td>4</td>
<td>3.69</td>
<td>2.56</td>
</tr>
<tr>
<td>5</td>
<td>3.93</td>
<td>2.87</td>
</tr>
<tr>
<td>6</td>
<td>4.16</td>
<td>3.20</td>
</tr>
</tbody>
</table>

**Bias plot**

- Mean difference (M): -0.2278
- Standard deviation (S): 0.0629
- N: 40

**Note**: 40 is the exclude sample if I > 3.04.