1. INTENDED USE
The panel comprises four individually coded ampoules, each containing freeze-dried cells. Each primary standard/ampoule has a different defined value for %BCR-ABL1 / control gene, intended for use as primary standards in the calibration of kits, assays and secondary standards (see pages 3-7). The 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-ABL1 detection in the range 0.01% to 10% on the International Scale. They have not been validated for other direct applications e.g. International Scale measurements >10% or for directly determining assay sensitivity <0.01%.

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 free-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-ABL1 / control gene values were obtained for each of the 4 primary standards following conversion to the international scale (IS):

<table>
<thead>
<tr>
<th>Ampoule Code</th>
<th>%BCR-ABL1 / ABL1</th>
<th>%BCR-ABL1 / BCR</th>
<th>%BCR-ABL1 / 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>1.1767</td>
<td>1.6677</td>
<td>0.6205</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-ABL1 translocation b3a2) and HL60 cells (BCR-ABL1 negative) in varying proportions. The total number of cells per ampoule is approximately 1.5 x 10E6. 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 may 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 the ampoules as described in section 6. above.

b. Reconstitute each 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 needle.

d. Transfer the entire contents of each ampoule to a nuclease-free tube.

e. Extract the RNA from each of the 4 materials using your usual method.

f. Prepare cDNA for each of the 4 RNA materials using your usual method.

g. Determine the % BCR-ABL1 / control gene values for the cDNA materials using your usual method.

h. Determine the correction factor for your BCR-ABL1 assay for reporting on the IS by analysing observed vs. expected values for these materials. See pages 3-7 for suggested calibration methods.

If you are calibrating secondary standards, extract the RNA, prepare cDNA, and test this cDNA at the same time as the primary standards. This will enable determination of IS values for your secondary standards. See pages 3-7 for suggested calibration methods.

If you require further information on how to use these materials, contact jennifer.boyle@nibsc.org.

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
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>Physical appearance: Freeze-dried solid</td>
<td>Corrosive: No</td>
</tr>
<tr>
<td>Stable: Yes</td>
<td>Oxidising: No</td>
</tr>
<tr>
<td>Hygroscopic: Yes</td>
<td>Irritant: No</td>
</tr>
<tr>
<td>Flammable: No</td>
<td>Handling: See caution, Section 2</td>
</tr>
<tr>
<td>Other (specify): Contains material of human origin</td>
<td></td>
</tr>
</tbody>
</table>

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

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.

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 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_biologicalstandardsrev2004.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 methods for the assignment of International Scale values to your BCR-ABL1 assay and secondary standards using the 1st WHO International Genetic Reference Panel for the quantitation of BCR-ABL translocation.

Two suggested methods are described below which will allow you to calibrate your assay and secondary standards to the International Scale (IS).

If you have previously purchased the 1st WHO International Genetic Reference Panel for the quantitation of BCR-ABL translocation, you should be familiar with Suggested Method (A), and it is advised that you continue to use this method. Suggested Method (B) may be adopted by those using the 1st WHO International Genetic Reference Panel for the quantitation of BCR-ABL translocation for the first time, as it is mathematically simpler and likely provides a more familiar visualisation of the data. Each method generates highly similar results.

CONTINUE TO USE EITHER SUGGESTED METHOD (A) OR SUGGESTED METHOD (B); do not switch between methods. A electronic copy of these Instructions for Use may be found at: https://www.nibsc.org/documents/ifu/09-138.pdf. If you have any queries, email jennifer.boyle@nibsc.org.

Suggested Method (A): The assignment of IS values to your BCR-ABL1 assay and secondary standards using a difference plot approach.

This method is based on the publication Branford et al. [Blood. 2008 Oct 15;112(8):3330-8. doi: 10.1182/blood-2008-04-150680], but uses Grubbs’ test for the identification of outliers, and regression analysis to determine if there is a trend in bias across the range of values in your assay. It will allow you (i.) to assign IS values to your assay, and (ii.) to calibrate your secondary standards, if required, using the 1st WHO International Genetic Reference Panel for the quantitation of BCR-ABL translocation. Any of the control genes ABL1, BCR, or GUSB may be used for this calculation, with the corresponding assigned IS values for each of the 4 primary standards shown below:

<table>
<thead>
<tr>
<th>Ampoule code</th>
<th>%BCR-ABL1 / ABL1</th>
<th>%BCR-ABL1 / BCR</th>
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</tr>
</thead>
<tbody>
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<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>

1. Order 5 panels of the 1st WHO International Genetic Reference Panel for the quantitation of BCR-ABL translocation i.e. 20 ampoules.
2. On 5 different days, take 1 complete panel of the 4 primary standards, reconstitute them according to the Instructions For Use and extract the RNA. If you need to extract RNA from your secondary standards, also perform this in parallel on at least 1 of the days.
3. For each of the primary standards, make 2 batches of cDNA on different days and test these in duplicate/triplicate on separate days by your usual method i.e. testing is over 10 days. If you are evaluating secondary standards, also prepare cDNA from this RNA in parallel on at least 1 of the days, and also test these in duplicate/triplicate in the same assay(s).

i. The assignment of IS values to your BCR-ABL1 assay.
4. You should now have at least 40 data points for the primary standards. Exclude all failed reactions, but if you have less than 6 data points for each level of BCR-ABL1 then start again from step 3.
5. Determine the conversion factor for your assay based on the primary standards’ data using the following procedure and the worked example overleaf (Suggested Method (A): The assignment of IS values to your BCR-ABL1 assay and secondary standards using a difference plot approach):

5.1. Convert assigned IS (expected) and testing lab (observed) % BCR-ABL1/control gene values to log_{10}
5.2. For each sample tested, calculate the mean (m) of the log_{10} assigned IS (expected) value and the log_{10} testing lab (observed) value:
\[ m = \frac{1}{2} \left( \log_{10} \text{assigned IS (expected) value} + \log_{10} \text{testing lab (observed) value} \right) \]

5.3. For each sample tested, calculate the difference (d) between the \( \log_{10} \) assigned IS (expected) value and the \( \log_{10} \) testing lab (observed) value:

\[ d = \log_{10} \text{assigned IS (expected) value} - \log_{10} \text{testing lab (observed) value} \]

5.4. Calculate the mean (Md) and the standard deviation (Sd) of the differences (d)
5.5. For each sample tested, calculate the outlier test statistic (z) as an absolute value:

\[ z = \left| \frac{M_d - d}{S_d} \right| \]

5.6. If any sample’s z value exceeds the relevant critical value (see Table 1. Critical values for Grubbs’ outlier test, overleaf), exclude the data for these sample(s) and repeat steps 5.4 and 5.5.
5.7. Perform a regression analysis of the differences (d) against mean values (m). Assignment of IS values to your assay is valid ONLY if no significant trend is observed (\( p > 0.05 \)).

6. The correction factor for your assay (analogous to a laboratory-specific conversion factor) is defined as the anti-\( \log_{10} \) of the mean difference i.e. anti-\( \log_{10} \) (Md). This correction factor can be applied to any observed sample values falling within the range of the primary standards to derive values on the IS.

ii. The calibration of BCR-ABL1 secondary standards.
If you also need to calibrate multiple secondary standards to the IS, for example if your secondary standards will extend beyond the range of the primary standards, follow these additional steps:
7. You should have at least 2 data points for each dilution of your secondary standards. Exclude all failed reactions, but if you have less than 2 data points for each level of BCR-ABL1 then start again from step 3.
8. Confirm a parallel relationship between the primary and secondary standards in your assay using the following procedure and the worked example overleaf (Suggested Method (A): The assignment of IS values to your BCR-ABL1 assay and secondary standards using a difference plot approach):
8.1. For your secondary standards, convert expected and testing lab (observed) % BCR-ABL1/control gene values to \( \log_{10} \)
8.2. For each sample tested, calculate the mean (m) of the \( \log_{10} \) expected value and the \( \log_{10} \) testing lab (observed) value:

\[ m = \frac{1}{2} \left( \log_{10} \text{expected value} + \log_{10} \text{testing lab (observed) value} \right) \]

8.3. For each sample tested, calculate the difference (d) between the \( \log_{10} \) expected value and the \( \log_{10} \) testing lab (observed) value:

\[ d = \log_{10} \text{expected value} - \log_{10} \text{testing lab (observed) value} \]

8.4. Calculate the mean (Md) and the standard deviation (Sd) of the differences (d)
8.5. For each sample tested, calculate the outlier test statistic (z) as an absolute value:

\[ z = \left| \frac{M_d - d}{S_d} \right| \]

8.6. If any sample’s z value exceeds the relevant critical value (see Table 1. Critical values for Grubbs’ outlier test, overleaf), exclude the data for those sample(s) and repeat steps 8.4 and 8.5.
8.7. Perform a regression analysis of the differences (d) against mean values (m). Assignment of IS values to your secondary standards will be valid ONLY if no significant trend is observed (\( p > 0.05 \)).
9. Apply the correction factor calculated in step 6 to the mean testing lab (observed) value for each of your secondary standards to derive values on the IS.
Suggested Method (A): The assignment of IS values to your BCR-ABL assay and secondary standards using a difference plot approach.

**Primary Standards**

<table>
<thead>
<tr>
<th>Material / Dilution</th>
<th>BCR-ABL/ABL %</th>
<th>Testing lab (observed)</th>
<th>Mean difference (M̄) Residual Plot</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary Standards</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Secondary Standards</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Linear (Primary Standards)**

- Material / Dilution
- BCR-ABL/ABL %
- Testing lab (observed)
- Mean difference (M̄)
- Residual Plot

**Linear (Secondary Standards)**

- Material / Dilution
- BCR-ABL/ABL %
- Testing lab (observed)
- Mean difference (M̄)
- Residual Plot

**Mean (m) Residual Plot**

- Secondary Standards
- Primary Standards
- Linear (Secondary Standards)
- Linear (Primary Standards)

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A electronic copy of these Instructions for Use may be found at: [https://www.nibsc.org/documents/ifu/09-138.pdf](https://www.nibsc.org/documents/ifu/09-138.pdf)
**Suggested Method (B): The assignment of IS values to your BCR-ABL1 assay and secondary standards using a linear regression approach.**

This method uses regression analysis of observed vs. expected values for the primary standards and your secondary standards (if required) to determine if there is a trend in bias across the range of values in your assay. It will allow you (i.) to assign IS values to your assay, and (ii.) to calibrate your secondary standards, if required, using the 1st WHO International Genetic Reference Panel for the quantitation of BCR-ABL translocation. Any of the control genes ABL1, BCR, or GUSB may be used for this calculation, with the corresponding assigned IS values for each of the 4 primary standards shown below:

<table>
<thead>
<tr>
<th>Ampoule code</th>
<th>%BCR-ABL1 / ABL1</th>
<th>%BCR-ABL1 / BCR</th>
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<tr>
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<td>0.1753</td>
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<td>08/196</td>
<td>1.1672</td>
<td>1.6627</td>
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<tr>
<td>08/198</td>
<td>10.7469</td>
<td>16.3129</td>
<td>10.1235</td>
</tr>
</tbody>
</table>

1. Order 5 panels of the 1st WHO International Genetic Reference Panel for the quantitation of BCR-ABL translocation i.e. 20 ampoules.
2. On 5 different days, take complete panels of the 4 primary standards, reconstitute them according to the Instructions For Use and extract the RNA. If you need to extract RNA from your secondary standards, also perform this in parallel on at least 1 of the days.
3. For each of the primary standards, make 2 batches of cDNA on different days and test these in duplicate/triplicate on separate days by your usual method i.e. testing is over 10 days. If you are evaluating secondary standards, also prepare cDNA from this RNA in parallel on at least 1 of the days, and also test these in duplicate/triplicate in the same assay(s).

### i. The assignment of IS values to your BCR-ABL1 assay.

4. You should now have at least 40 data points for the primary standards. Exclude all failed reactions, but if you have less than 6 data points for each level of BCR-ABL1 then start again from step 3.
5. Determine the conversion factor for your assay based on the primary standards’ data using the following procedure and the worked example overleaf (Suggested Method (B): The assignment of IS values to your BCR-ABL1 assay and secondary standards using a linear regression approach):
   5.1 Convert assigned IS (expected) and testing lab (observed) % BCR-ABL1/control gene values to log_{10}
   5.2 Perform a linear regression analysis of the log_{10} testing lab (observed) % BCR-ABL1/control gene values against the log_{10} assigned IS (expected) values. Your analysis should provide assurance that there is a linear relationship between observed and expected values over the range of the primary standards, for example requiring that the R Square value is greater than 0.95. Similarly, your analysis should provide assurance that the fitted slope is appropriately close to 1, for example requiring that the confidence interval for the slope includes 1.00, or more ideally that the confidence interval for the fitted slope is fully contained within a pre-specified acceptable range around 1.00.
6. The correction factor for your assay (analogous to a laboratory-specific conversion factor) is defined as the reciprocal of the anti-log_{10} of the Intercept. This correction factor can be applied to any observed sample values falling within the range of the primary standards to derive values on the IS.

### ii. The calibration of BCR-ABL1 secondary standards.

If you also need to calibrate multiple secondary standards to the IS, for example if your secondary standards will extend beyond the range of the primary standards, follow these additional steps:

7. You should have at least 2 data points for each dilution of your secondary standards. Exclude all failed reactions, but if you have less than 2 data points for each level of BCR-ABL1 then start again from step 3.
8. Confirm a parallel relationship between the primary and secondary standards in your assay using the following procedure and the worked example overleaf (Suggested Method (B): The assignment of IS values to your BCR-ABL1 assay and secondary standards using a linear regression approach);
8.1. For your secondary standards, convert expected and testing lab (observed) % BCR-ABL1/control gene values to \( \log_{10} \) values.

8.2. Perform a linear regression analysis of the \( \log_{10} \) testing lab (observed) % BCR-ABL1/control gene values against the \( \log_{10} \) expected values. Your analysis should provide assurance that there is a linear relationship between observed and expected values over the range of the secondary standards, for example requiring that the R Square value is greater than 0.95. Similarly, your analysis should provide assurance that the fitted slope is appropriately close to 1, for example requiring that the confidence interval for the slope includes 1.00, or more ideally that the confidence interval for the fitted slope is fully contained within a pre-specified acceptable range around 1.00.

9. Apply the correction factor calculated in step 6 to the mean testing lab (observed) value for each of your secondary standards to derive values on the IS.

### Suggested Method (B): The assignment of IS values to your BCR-ABL assay and secondary standards using a linear regression approach

<table>
<thead>
<tr>
<th>Material</th>
<th>Ampoule</th>
<th>BCR-ABL/ABL %</th>
<th>Log(_{10}) transformed BCR-ABL/ABL %</th>
<th>Mean Testing lab (observed)</th>
<th>Corrected IS value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear (Primary Standards)</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linear (Secondary Standards)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

### SUMMARY OUTPUT

<table>
<thead>
<tr>
<th>Material</th>
<th>Ampoule</th>
<th>BCR-ABL/ABL %</th>
<th>Log(_{10}) transformed BCR-ABL/ABL %</th>
<th>Mean Testing lab (observed)</th>
<th>Corrected IS value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear (Primary Standards)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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