Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

WHO International Standard
1st WHO International Genetic Reference Panel for the quantitation of BCR-ABL1 translocation
NIBSC code: 09/138
Instructions for use
(Version 9.0, Dated 22/10/2020)

1. INTENDED USE

The 1st WHO International Genetic Reference Panel for the quantitation of BCR-ABL1 mRNA was established in 2009 by the Expert Committee on Biological Standardization (ECBS) of the World Health Organization (WHO; NIBSC product code 09/138) [1]. The panel comprises four individually coded standards/ampoules, each containing freeze-dried cells. Each standard has a different defined value for BCR- ABL1 on the International Scale (BCR-ABL^{IS}) depending on which control gene is used. Together they are intended for use as primary standards in the calibration of kits, assays, and secondary standards (see Section 7). These materials should not be put to any other use. Please note the materials have been validated only for BCR-ABL1 mRNA detection in the range 0.01% to 10% on the International Scale (IS). They have not been validated for other direct applications e.g. IS measurements >10%, or for directly determining assay sensitivity <0.01%, although these may be determined through the derivation of secondary standards (see Section 7). These materials contain the BCR-ABL1 e14a2 transcript (also known as b3a2); their suitability for the quantification of other transcripts including BCR-ABL1 e13a2 (b2a2) in the end-user's assay should be verified before use, for example by ensuring equally efficient PCR amplification of the transcripts.

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^{IS} values were obtained for each of the 4 primary standards using the three most commonly used control genes.

Ampoule Code	BCR-ABL1^{IS} (*ABL1*)	BCR-ABL1^{IS} (*BCR*)	BCR-ABL1^{IS} (*GUSB*)
08/192	0.0118	0.0195	0.0071
08/194	0.1112	0.1753	0.0749
08/196	1.1672	1.6627	0.8295
08/198	10.7469	16.3129	10.1235

4. CONTENTS

Country of origin of biological material: Germany & United Kingdom.
The ampoules contain freeze-dried K562 cells (expressing the BCR-ABL1 translocation e14a2, also known as 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.

The below table shows estimated RNA yields for each material. Note these values are for information only, not for formal use

Ampoule Code	Mean RNA yield post-extraction (µg)
08/192	14.86
08/194	14.34
08/196	14.16
08/198	17.42

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. Various types of ampoule breaker are available commercially. To open the ampoule, tap the ampoule gently to collect material at the bottom (labelled) end and follow manufactures instructions provided with the ampoule breaker.

7. USE OF MATERIAL

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

Before proceeding, please read the suggested assay and secondary standard calibration methods on pages 3-5.
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.

a. Open the ampoules as described in section 6. above.
b. Reconstitute each freeze-dried material at room temperature with 1ml Trizol, 600 µl RLT buffer or equivalent for other RNA extraction systems.
c. Ensure all cells are lysed by repeated aspiration with a pipette tip or needle. Incubate for 5 min.
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. (If following the suggested method, prepare 2 batches of cDNA from each RNA material; see page 3).
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.

If you require further information on how to use these materials, contact grmteam@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 -20ºC 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.https://www.nibsc.org/documents/ifu/SupplementaryInformation/09-138/WHO_BS_09.2106_eng.pdf

National Institute for Biological Standards and Control,
Potters Bar, Hertfordshire, EN6 3QG. T +44 (0)1707 641000, nibsc.org
WHO International Laboratory for Biological Standards,
UK Official Medicines Control Laboratory

World Health Organization

2. White H.E. *et al.*, Establishment of the 1st World Health Organization International Genetic Reference Panel for quantitation of BCR-ABL mRNA. Blood. 2010 Nov 25;116(22):e111-7. doi: 10.1182/blood-2010-06-291641.

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

Physical and Chemical properties			
Physical appearance: Freeze-dried solid		Corrosive:	No
Stable:	Yes	Oxidising:	No
Hygroscopic:	Yes	Irritant:	No
Flammable:	No	Handling:	See caution, Section 2
Other (specify):	Contains material of human origin		

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 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_biolefstandardsrev2004.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 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-ABL1* translocation.

This suggested method will allow you to calibrate your assay and any secondary standards to the *BCR-ABL1* International Scale (IS).

If you have previously purchased the panel, you may have used a difference plot approach based on the publication Branford *et al.* [Blood. 2008 Oct 15;112(8):3330-8. doi: 10.1182/blood-2008-04-150680], using 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. This method is available on request from grmteam@nibsc.org.

We now suggest the below linear regression approach to assign values on the *BCR-ABL1* IS as it is mathematically simpler and likely provides a more familiar visualisation of the data. This method generates results highly similar to the Branford *et al.* approach. The suggested criteria are derived from extensive quality assessment datasets for *BCR-ABL1* measurement in external laboratories.

This method uses regression analysis of observed vs. expected values for the primary standards 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 any secondary standards, using the 1st WHO International Genetic Reference Panel for the quantitation of *BCR-ABL1* 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:

Ampoule code	BCR-ABL1^{IS} (*ABL1*)	BCR-ABL1^{IS} (*BCR*)	BCR-ABL1^{IS} (*GUSB*)
08/192	**0.0118**	**0.0195**	**0.0071**
08/194	**0.1112**	**0.1753**	**0.0749**
08/196	**1.1672**	**1.6627**	**0.8295**
08/198	**10.7469**	**16.3129**	**10.1235**

1. Order up to 5 of the 1st WHO International Genetic Reference Panel for the quantitation of *BCR-ABL1* mRNA, which will contain 1 each of the above ampoules.
   Notes:
   - the use of 1-3 panels is recommended for ongoing calibrations;
   - the use of up to 5 panels is recommended for new calibrations carried out after September 2020.
2. Reconstitute each of the 4 primary standards (ampoules) 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.
3. For each of the primary standards, make 2 independent batches of cDNA. Test each of these cDNA batches independently at least in triplicate, by your usual method to generate a minimum of 6 datapoints for each primary standard. If you are evaluating secondary standards, also prepare cDNA from this RNA in parallel, and also test these in at least duplicate in the same assay(s).

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

4. You should now have at least 6 data points for each of the primary standards. Exclude all failed reactions, but if you

have fewer than 6 data points for each *BCR-ABL1* level 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 on page 5;
   5.1. Convert assigned IS (expected) and testing lab (observed) % *BCR-ABL1*/control gene values to log10
   5.2. Perform a linear regression analysis of the log10 testing lab (observed) % *BCR-ABL1*/ control gene values against the log10 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 e.g. the R Square value is greater than 0.97. Similarly, your analysis should provide assurance that the fitted slope is appropriately close to 1 e.g. the 95% confidence interval on the fitted slope must be fully contained within [0.83, 1.20].
6. The correction factor for your assay (analogous to a laboratory-specific conversion factor) is defined as the reciprocal of the anti-log10 of the Intercept. This correction factor can now 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 are calibrating secondary standards which fully fall within the range of the primary standards i.e. 0.01 to 10% *BCR-ABL1*/ control gene, you can simply apply your assay's correction factor (as derived in step 6) to the secondary standards' values.

If your secondary standards will extend beyond the range of the primary standards e.g. to less than 0.01% *BCR-ABL1*/ control gene, you must determine parallelism of the secondary standards' to the primary standards' values, to ensure your assay's correction factor is applicable across the whole range of the secondary standards. Follow these additional steps;

7. Secondary standards extending beyond the range of the primary standards must be prepared by serial dilution in order to have an expected value with which to perform the linear regression analysis.
8. 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.
9. Confirm a parallel relationship between the primary and secondary standards in your assay using the following procedure and the worked example on page 5;
   9.1. For your secondary standards, convert expected and testing lab (observed) % *BCR-ABL1*/ control gene values to $\log_{10}$.
   9.2. Perform a linear regression analysis of the log10 testing lab (observed) % *BCR-ABL1*/ control gene values against the log10 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 e.g. the R Square value is greater than 0.97. Similarly, your analysis should provide assurance that the fitted slope is appropriately close to 1 i.e. the 95% confidence interval on the fitted slope must be fully contained within [0.83, 1.20].
10. 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.

An 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, contact grmteam@nibsc.org .

WORKED EXAMPLE

Please note that the worked example presented here is for one panel only.

Example data for the assignment of International Scale values to your BCR-ABL1 assay and secondary standards using a linear regression approach

Table 1. Primary Standards

Material	cDNA batch	Assay replicate	BCR-ABL1/ABL1 %		Log_{10} transformed BCR-ABL1/ABL1 %	
			IS value (expected)	Testing lab (observed)	IS value (expected)	Testing lab (observed)
08/192	1	1	0.0118	0.0261	-1.9281	-1.5826
		2	0.0118	0.0177	-1.9281	-1.7514
		3	0.0118	0.0261	-1.9281	-1.5836
	2	4	0.0118	0.0290	-1.9281	-1.5381
		5	0.0118	0.0119	-1.9281	-1.9242
		6	0.0118	0.0188	-1.9281	-1.7265
08/194	1	1	0.1112	0.2311	-0.9539	-0.6362
		2	0.1112	0.1973	-0.9539	-0.7049
		3	0.1112	0.1892	-0.9539	-0.7231
	2	4	0.1112	0.1839	-0.9539	-0.7354
		5	0.1112	0.1842	-0.9539	-0.7348
		6	0.1112	0.1894	-0.9539	-0.7225
08/196	1	1	1.1672	2.0203	0.0671	0.3054
		2	1.1672	2.2358	0.0671	0.3494
		3	1.1672	2.2934	0.0671	0.3605
	2	4	1.1672	2.2099	0.0671	0.3444
		5	1.1672	1.8396	0.0671	0.2647
		6	1.1672	1.6579	0.0671	0.2196
08/198	1	1	10.7469	16.3350	1.0313	1.2131
		2	10.7469	14.1758	1.0313	1.1515
		3	10.7469	17.1538	1.0313	1.2344
	2	4	10.7469	15.9942	1.0313	1.2040
		5	10.7469	15.6497	1.0313	1.1945
		6	10.7469	17.5578	1.0313	1.2445

Figure 1. Regression Analysis for Primary Standards

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.9975
R Square	0.9950
Adjusted R Square	0.9948
Standard Error	0.0801
Observations	24

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	28.1831	28.1831	4394.9263	0.0000
Residual	22	0.1411	0.0064		
Total	23	28.3242			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	0.2167	0.0176	12.2952	0.0000	0.1801	0.2532
IS value (expected)	0.9791	0.0148	66.2942	0.0000	0.9484	1.0097

Correction factor: 0.6072

Table 2. Secondary Standards

Material / Dilution	BCR-ABL1/ABL1 %		Log_{10} transformed BCR-ABL1/ABL1 %		BCR-ABL1/ABL1 %	
	Expected	Testing lab (observed)	Expected	Testing lab (observed)	Mean Testing lab (observed)	Corrected IS value
1	0.0010	0.0012	-3.0000	-2.9208		
	0.0010	0.0010	-3.0000	-3.0044		
	0.0010	0.0008	-3.0000	-3.0969	0.0010	0.0006
2	0.0100	0.0101	-2.0000	-1.9957		
	0.0100	0.0104	-2.0000	-1.9830		
	0.0100	0.0098	-2.0000	-2.0088	0.0101	0.0061
3	0.1000	0.0910	-1.0000	-1.0410		
	0.1000	0.1030	-1.0000	-0.9872		
	0.1000	0.1100	-1.0000	-0.9586	0.1013	0.0615
4	1.0000	0.9600	0.0000	-0.0177		
	1.0000	0.9800	0.0000	-0.0088		
	1.0000	1.0500	0.0000	0.0212	0.9967	0.6052
					Correction factor:	0.6072

Figure 2. Regression Analysis for Secondary Standards

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.9993
R Square	0.9986
Adjusted R Square	0.9985
Standard Error	0.0452
Observations	12

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	15.0511	15.0511	7371.2959	0.0000
Residual	10	0.0204	0.0020		
Total	11	15.0715			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	0.0024	0.0218	0.1110	0.9138	-0.0462	0.0511
Expected	1.0017	0.0117	85.8563	0.0000	0.9757	1.0277

Figure 3. Linear Regression Analysis: Primary and Secondary Standards