Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

WHO Reference Panel
1st International Reference Panel for Genomic JAK2 V617F
NIBSC code: 16/120
Instructions for use
(Version 4.0, Dated 25/04/2020)

1. INTENDED USE

The panel comprises seven individually coded ampoules, each containing freeze-dried purified genomic DNA extracted from human cell lines. Each primary standard/ampoule has a different defined value for *JAK2* V617F as a percentage of total *JAK2* (NM_004972.3). The panel is intended for use as primary standards for calibrating secondary standards, assays and kits for *JAK2* V617F detection. The materials are not intended as run controls. The materials were tested by external laboratories to show suitability as standards by giving satisfactory results in allele-specific (AS)-QPCR, digital PCR, allelic discrimination-based endpoint PCR (including AS-PCR, Amplification Refractory Mutation System-PCR (ARMS-PCR) and Matrix Assisted Laser Desorption/Ionization-Time of Flight (MALDI-TOF) mass spectrometric analysis) and next-generation sequencing.
The panel was established in 2016 by the Expert Committee on Biological Standardization of the World Health Organization (WHO) as the WHO 1st International Reference Panel for Genomic JAK2 V617F, NIBSC code 16/120[1].
These materials should not be put to any other use. Data analysis must be focussed on the *JAK2* locus. No attempt must be made to identify source material donors.

2. CAUTION

This preparation is not for administration to humans or animals in the human food chain.

The cell lines used in the preparation of this panel were tested and found to be negative for HIV1, HTLV1, HBV and HCV by PCR. An Epstein Barr virus (EBV)-transformed lymphoblastoid cell line was used in the preparation of the panel. EBV is a category 2 pathogen as classified by the UK Advisory Committee on Dangerous Pathogens. EBV sequences may be present in these materials, but the DNA has been prepared using a protocol in which proteins are denatured and removed, thus likely inactivating the virus. However, the potential for viable virus to survive cannot be eliminated. 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 29 laboratories and 38 testing methods. The following median values for *JAK2* V167F as a percentage of total *JAK2* were obtained;

Ampoule Code	% *JAK2* V617F / total *JAK2*
15/172	0
15/170	0.03[2]
15/168	1.00
15/166	10.8
15/244	29.6
15/246	89.5
15/164	100

[2]The consensus value for material 15/170 was lower than expected (compared with in-house droplet digital PCR data). It is noted that of the 27 (statistically appropriate) quantitative methods for material 15/170, 5 methods reported a mean value of 0%, which may be due to either the limit of detection of the method or the reduced sampling frequency of a low level target. Results from digital PCR methods only (n= 6), all of which reported a value of greater than 0%, showed a mean value of 0.04% *JAK2* V617F (±0.01%; 95% confidence interval). Material 15/170 is clearly positive for *JAK2* V617F when compared with material 15/172 (0% *JAK2* V617F).

4. CONTENTS

Country of origin of biological material: United Kingdom and Germany.
The panel comprises seven individually coded ampoules, each containing approximately 5µg freeze-dried purified genomic DNA extracted from human cell lines. The genomic DNAs were extracted using a 'salting out' method, combined in varying proportions to produce a range of values for *JAK2* V617F as a percentage of total *JAK2*, and diluted in Tris-EDTA buffer with 5mg/ml Trehalose before freeze-drying.

5. STORAGE

Store all unopened ampoules of the freeze-dried materials 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

a. Open the ampoule as described in section 6. above.
b. Reconstitute each freeze-dried material at room temperature with 100µl nuclease-free water to give purified genomic DNA in 1x Tris-EDTA buffer (10mM Tris, 1mM EDTA).
c. Transfer the entire contents of each ampoule to a nuclease-free tube using a pipette.
d. Allow the material to reconstitute for 1 hour at room temperature and pipette well to mix before use.
e. Measure the DNA concentration before use; add the required amount to your assay.
f. If using the standards for assay calibration purposes, determine the correction factor for your *JAK2* V617F assay by analysing observed vs. expected values for these standards. For further details see the suggested calibration method for the 1st WHO International Genetic Reference Panel for the quantitation of BCR-ABL1 translocation (NIBSC product code 09/138; https://www.nibsc.org/documents/ifu/09-138.pdf)
g. If you are calibrating secondary standards, test this genomic DNA at the same time as the primary standards and apply the associated correction factor. For further details see the suggested calibration method for the 1st WHO International Genetic Reference Panel for the quantitation of BCR-ABL1 translocation (NIBSC product code 09/138; https://www.nibsc.org/documents/ifu/09-138.pdf)
h. It is highly recommended that the material is used on the day it is reconstituted and is not stored. However, in-house analysis determined reconstituted freeze-dried genomic DNA to be stable for at least 4 days at +4°C (or 2 months at -20°C). Care should be taken to avoid cross-contamination with other samples.

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

8. STABILITY

NIBSC follows the policy of WHO with respect to its reference materials. It is the policy of the WHO not to assign an expiry date to their international reference materials. They remain valid with the assigned values and status until withdrawn or amended.
Reference materials are held at NIBSC within assured, temperature-controlled storage facilities. Reference materials should be stored on receipt as indicated on the label.

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

Accelerated degradation studies have indicated that these materials are 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 materials are suitably stable for shipment at ambient temperature without any effect on the assigned values.
Users who have any data supporting any deterioration in the characteristics of materials are encouraged to contact NIBSC.

9. REFERENCES

1. WHO document WHO/BS/2016.2293

10. ACKNOWLEDGEMENTS

We gratefully acknowledge the significant contributions of all collaborative study participants. Particular thanks go to MPN&MPNr-Euronet for their knowledgeable contribution to the project, especially Dr Niels Pallisgaard, Roskilde, Denmark and Dr Sylvie Hermouet, Nantes, France. We value the endorsement of this panel by both MPN&MPNr-Euronet and European LeukemiaNet. We also extend our gratitude to Professor Walter Fiedler, Hamburg, Germany for donating the UKE-1 cell line used for the preparation of the materials. We would also like to thank Dr Véronique Laloux of Qiagen, Courtaboeuf, France for connecting us with MPN&MPNr-Euronet and laboratories in South America.

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
NIBSC Genomic Reference Materials:
http://www.nibsc.org/science_and_research/advanced_therapies/genomic_reference_materials.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: white crystalline 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 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

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: 3.5g 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.

Appendix I. QIAGEN ipsogen JAK2 RGQ PCR kit CE

Following the international collaborative study, and at the request of the *JAK2* V617F diagnostic community, a small performance evaluation of the 1st International Reference Panel for Genomic JAK2 V617F with the QIAGEN ipsogen JAK2 RGQ PCR kit CE was performed, to determine both the panel's usability with the kit, and the alignment of the results to that of other commonly-used *JAK2* V617F diagnostic techniques. There was no significant difference between the kit and collaborative study data, demonstrating both the appropriateness of the panel's use with this kit, and the suitability of the kit in providing *JAK2* V617F data which are in agreement with other commonly-used diagnostic techniques; for data see https://www.nibsc.org/science_and_research/advanced_therapies/genomic_reference_materials/jak2_v617f_(who).aspx

The data from this performance evaluation are not intended to contribute to the panel members' consensus values, but indicate the similar performance of the panel with this kit, and thus enable better standardisation across *JAK2* V617F diagnostic approaches with a range of commonly-used techniques.

Appendix II. *JAK2* copies/µl in the 1st International Reference Panel for Genomic JAK2 V617F

Also at the request of the *JAK2* V617F diagnostic community, *JAK2* copies/µl were calculated for each material of the 1st International Reference Panel for Genomic JAK2 V617F. Data are copies/µl of the reconstituted freeze-dried genomic DNA (in 100µl, as per Section 7 above) and corrected for the Qubit BR-verified DNA concentration at the time of reconstitution.

Data are derived from 3 independent in-house ddPCR assays (n=15 replicates total) using the BioRad *JAK2* V617F kit, with 20ng input genomic DNA per 20µl ddPCR reaction.

Ampoule Code	% *JAK2* V617F / total *JAK2*	*JAK2* V617F copies/µl (n=15)		*JAK2* wild-type copies/µl (n=15)		*JAK2* total copies/µl (n=15)	
		Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation
15/172	0	0.00	0.00	16064.73	2220.45	16064.73	2220.45
15/170	0.03	3.91	3.19	12889.72	1129.76	12893.63	1130.89
15/168	1.00	176.11	32.39	15428.73	1463.85	15604.84	1482.21
15/166	10.8	1417.22	124.87	11424.67	580.45	12841.90	682.45
15/244	29.6	4967.94	696.78	11343.65	1257.07	16311.59	1875.11
15/246	89.5	15249.63	2133.31	1761.67	263.07	17011.30	2381.81
15/164	100	10475.61	1345.94	1.28	2.64	10476.89	1345.68

Please note this information is supplementary information only, and not to be used for diagnostic purposes.