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EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION
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International collaborative study to evaluate a proposed 1st WHO reference reagent for anti-human platelet antigen-15b (anti-HPA-15b)
Code: 18/220

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NOTE:
This document has been prepared for the purpose of inviting comments and suggestions on the proposals contained therein, which will then be considered by the Expert Committee on Biological Standardization (ECBS). Comments MUST be received by **5 October 2020** and should be addressed to the World Health Organization, 1211 Geneva 27, Switzerland, attention: Technologies, Standards and Norms (TSN). Comments may also be submitted electronically to the Responsible Officer: **Dr Ivana Knezevic** at email: knezevici@who.int.

Summary

Twenty-five laboratories from sixteen countries participated in an international collaborative study to evaluate a freeze-dried, recalcified plasma preparation (coded 18/220) as an anti-Human Platelet Antigen-15b IgG antibody reference reagent. The participants tested doubling dilutions of the material in glycoprotein-specific assays used for the detection of anti-HPA-15 antibodies and recorded the largest dilution at which the antibody could be detected. Results from the study indicate that a 1 in 8 dilution of the material should be assigned to 18/220 as the minimum potency; when tested at this dilution by clinical laboratories, a positive result would validate the sensitivity of the test method.

1. Introduction

To date a total of 41 platelet alloantigens have been defined serologically, of which 12 are grouped in 6 bi-allelic systems (Human Platelet Antigens; HPA -1, -2, -3, -4, -5, -15) [1,2]. The molecular basis of the 41 antigens has been resolved and in all but one (HPA-14b), the difference between self and non-self is defined by a single nucleotide polymorphism (SNP) in the gene encoding the relevant membrane glycoprotein [2]. Alloantibodies against human platelet antigens are involved in foetal/neonatal alloimmune thrombocytopenia (FNAIT), a disease in which foetal/neonatal platelets are destroyed by IgG alloantibodies from the incompatible, HPA sensitised mother. Alloantibodies are also involved in platelet refractoriness (PR), the repeated failure to achieve the desired level of blood platelets following transfusion and in post-transfusion purpura (PTP), the delayed reaction to a transfusion caused by recipient alloantibodies to incompatible transfused platelet antigens. Identification of the HPA antibody specificity is essential to the diagnosis and treatment of the patient. In severe cases of thrombocytopenia that require treatment with a platelet transfusion, it is important that the transfused platelets are negative for the target alloantibody specificity. HPA antibody detection has thus become commonplace in blood transfusion centres and in larger hospitals. Anti-HPA-1a is the most common antibody associated with FNAIT and PTP [3,4,5,6].

A variety of techniques are in use across the world to detect HPA antibodies. The Monoclonal Antibody-specific Immobilization of Platelet Antigen (MAIPA) assay has long been

considered the gold standard method for the determination of platelet (IgG) alloantibody specificity. The method involves using specific HPA-typed platelets to capture alloantibodies in human serum/plasma. Mouse monoclonal antibodies specific for glycoproteins on which the platelet antigens of interest are located are used to capture the platelet antigen – alloantibody complexes after solubilisation of the platelets. This design ensures the accurate identification of alloantibody specificity and avoids false positives resulting from, for example, HLA antibodies. The MAIPA does not routinely distinguish between IgG alloantibody subclasses in patient samples. To date there are three WHO minimum potency reference reagents established (anti-HPA-5b, anti-HPA-3a and anti-HPA-1a) [7,8,9] which are used for assay quality control. These reagents contain low titre antibodies and are used to validate the minimum sensitivity of tests for the respective HPA antibodies.

In addition to HPA-1a, -3a and -5b, HPA-15 is of clinical relevance. Some studies have shown this antigen to be as immunogenic as HPA-5 and alloantibodies against HPA-15b can be detected in patients receiving multiple transfusions and mothers with FNAIT [10,11,12]. The platelet-based methods used for the detection of anti-HPA-15 antibodies can be unreliable and vary in their sensitivity because CD109, the glycoprotein on which HPA-15b is located, is expressed in low numbers by platelets and is labile [13,14]. An anti-HPA-15b minimum potency reference reagent would allow clinical laboratories to validate their methods and would facilitate 'between test' comparability. The need for this important reference material was emphasized in the proficiency testing scheme organised by NIBSC in 2017 showing that anti-HPA-15b detection in the samples distributed was generally poor. Furthermore, it was concluded in the report of the 19th International Platelet Immunology Workshop organised by the International Society of Blood Transfusion (ISBT) in 2018 [15] that a more standardised approach to the CD109 MAIPA is required. Here, the authors describe the evaluation of a candidate anti-HPA-15b lyophilised reference reagent (18/220) in an international collaborative study involving 25 laboratories across the globe. The authors propose the minimum potency that should be assigned to the candidate preparation based on the endpoint titres reported by participants. Clinical laboratories testing the established reference reagent at the assigned minimum potency should expect a positive result if the sensitivity of their method is acceptable.

2. Aims of study

The aim of the international collaborative study was to evaluate a candidate WHO anti-HPA-15b reference reagent (minimum potency) for use as a quality control reagent in glycoprotein-specific assays used to detect/identify alloantibody specificity. Results from the study were used to assign a maximum dilution to the candidate material at which laboratories can be expected to detect the antibody.

3. Methods

Participants

The invitation (shown in Appendix 1) was distributed to participants of the ISBT Platelet Immunology Workshop and to participants of the HPA antibody detection quality assessment

scheme organised by the National External Quality Assessment Site UK (NEQAS). Twenty-seven clinical laboratories located across the globe accepted the invitation to participate in the study (see Appendix 2).

Candidate Material

The material used to generate the candidate preparation was provided to NIBSC by the National Blood Service, Oxford, UK. All donations of recalcified plasma came from just one consenting donor and each was screened using the MAIPA to identify the anti-HPA-15b titre. Those with the highest titres were pooled to make a bulk (coded 18/220) prior to filling 0.5 ml/ampoule into 1896 glass ampoules (size 2.5 ml). Filled material was freeze-dried under conditions established *in house* for the routine lyophilisation of WHO serum standards/reference reagents; a summary of the product information is shown in Table 1. Visual inspection revealed that robust cakes were formed. The individual donations from which the candidate reference material was prepared, and the pooled bulk were found to be negative for HBsAg, anti-HIV1+2 and anti-HCV. HCV PCR testing of the pooled bulk was also negative and no microbial contaminants were detected.

A trial-sized plasma pool containing the same proportions of plasma donations as for the definitive bulk (18/220) and selected single donations used to prepare 18/220 were evaluated by two independent clinical laboratories with considerable experience of performing MAIPA assays. Both laboratories were able to detect anti-HPA-15b antibodies but could not detect antibodies with any other HPA specificity. Both laboratories also confirmed the presence of anti-HLA Class 1 antibodies, the following specificities were detected: A2, A68, A69, C5, C8 and C15. Furthermore, constituent donations of the definitive pool have been evaluated in previous proficiency testing schemes organised by NIBSC; no other antibody specificities were reported other than anti-HPA-15b and anti-HLA.

Study Design

Four ampoules of the definitive freeze-dried material (coded 18/220) were sent to each laboratory with instructions for storage and reconstitution (see Instructions For Use in Appendix 5). Participants were asked to reconstitute the material immediately before testing and to titrate the material at doubling dilutions in one or more assay method(s) routinely used in clinical practice. Participants were also required to use a CD109 glycoprotein-specific method such as the MAIPA because the material contains anti-HLA antibodies which could otherwise cause false positive results (see Study Protocol in Appendix 3). Clinical laboratories would normally be required to use such a method since patient samples can also contain HLA antibodies. Two ampoules were to be tested, each on a different day, with HPA-15bb and HPA-15aa platelets or similar on both days. Ideally, different platelet donors with the same HPA-15 type were to be used over the two days. Laboratories were asked to report their interpretation of the results for each test by recording ‘positive’ or ‘negative’ for each dilution on the Results Report Form we provided (see Appendix 4). The endpoint dilution (or antibody titre) for 18/220 in each test was assigned as the largest (maximum) dilution which the laboratory reported to be ‘positive’. Laboratories reporting results as ‘weak positive’ or results that were defined as borderline in ‘grey zones’ of defined optical density ranges, were also deemed a negative result.

Stability Studies

To predict loss in stability over time, accelerated thermal degradation studies were performed at NIBSC using ampoules of lyophilised 18/220 stored at -70, -20, +4, +20, +37 and +45^{o}C for 13 months. Reconstituted material from two ampoules at each temperature was tested in duplicate at a range of two-fold serial dilutions in the MAIPA assay with HPA-15bb platelets. Absorbance readings were used to calculate the relative potencies of the accelerated thermal degradation samples by parallel-line analysis using the -70 sample as the reference. Relative potency calculations were performed using the European Directorate for the Quality of Medicines software CombiStats, version 6.0 using a sigmoid curve model and logit transformation of responses. The Arrhenius equation, relating degradation rate to absolute temperature assuming first-order decay (16), was used to predict the degradation rates when stored at a range of temperatures.

4. Results

Twenty-seven clinical laboratories accepted the invitation to participate in the study, however, only 25 laboratories returned results. Each laboratory was assigned a code number which does not reflect the order of listing shown in Appendix 2. The methods used by participants are summarised in Table 2. All 25 laboratories performed glycoprotein-specific assays, all of which were a version of the MAIPA using a monoclonal antibody specific for CD109. Some laboratories provided supplementary datasets from additional platelet donors or from a second method. A full summary of the results from each laboratory and details of their assay protocols are shown in Appendix 6.

Reported endpoint dilutions against HPA-15bb antigen

The majority of laboratories tested two ampoules of the material on two separate occasions, using different donors of HPA-15bb platelets, as described in the study protocol. Laboratory 20 reported results from 4 different HPA-15bb donors using 3 of the ampoules provided and laboratory 1 reported results using 3 different HPA-15bb donors from 3 ampoules provided. Out of 25 laboratories, 24 were indeed able to detect antibodies with HPA-15b specificity in preparation 18/220 with all HPA-15bb platelet donors/cells used and, in all methods performed. The remaining 1 laboratory reported a positive result for undiluted material using both HPA-15aa and HPA-15bb platelets but failed to titrate the material and so endpoint dilutions could not be recorded. Another laboratory which did detect antibodies with HPA-15b specificity, failed to titrate the material enough, consequently, an endpoint dilution could not be recorded for this laboratory either. Figure 1 shows the endpoint dilutions reported by 23 laboratories for each method and for each donor of HPA-15bb platelets/cells. The reported endpoint dilutions were normally distributed with a modal dilution of 1 in 16 and a range from 1 in 2 to 1 in 128 as shown in Figure 1. The intra-laboratory variation in reported endpoint dilutions was significant in this study. Several laboratories (13 in total) reported different endpoint dilutions for 18/220 in each independent assay using the same method but where different HPA-15bb platelet donors were used. For example, 3 of the 5 laboratories that reported poor endpoint dilutions (i.e. 1 in 2 or 1 in 4), only did so for 1 of 2 donors, with endpoint dilutions for a second donor of either 1 in 8 or 1 in

16. This difference could be attributed to the levels of CD109 expression on the platelets. The remaining 2 of the 5 laboratories reported 1 in 2 or 1 in 4 for both donors used.

Almost all laboratories carrying out MAIPA assays used CD109-specific, mouse monoclonal antibody TEA 2/16 from BD Pharmingen. However, two laboratories used an anti-CD109 monoclonal antibody from a different company, two laboratories used an in-house CD109-specific monoclonal antibody and one laboratory used a CD109-specific biotinylated antibody for a flow cytometric method. There was no clear trend in reported endpoint dilutions for 18/220 relating to the different CD109-specific monoclonal antibodies used a shown in Figure 1. As expected, the majority of laboratories used fresh HPA-15bb platelets, however some used frozen or lyophilised platelets. Laboratory 4 used frozen platelets characterised as having high CD109 expression levels prior to freezing and achieved an endpoint dilution in a MAIPA of 1 in 8 for 18/220 using the anti-CD109 clone TEA 2/16 and an endpoint dilution of 1 in 16 using the anti-CD109 clone HU17. Laboratory 17 compared lyophilised and fresh platelets in the MAIPA, with comparable endpoint dilutions being reported for both types (1 in 8 and 1 in 16 for two donors of lyophilised platelets and 1 in 16 and 1 in 32 for two donors of fresh platelets).

Participants were asked several questions regarding the procedure of their chosen method(s) as shown on the Results Report Form (see Appendix 4). Indeed, there was considerable variation amongst the MAIPA methods performed, the most notable differences (further to those described above) being the number of platelets and sample volume added to wells of the test plates. Platelet numbers ranged from 0.4×10^6 to 300×10^6 per well and sample volumes ranged from 20 to 120 µl per well.

Reported endpoint dilutions against HPA-15aa antigen

To confirm the specificity of the HPA-15 antibodies within candidate material, laboratories were also asked to test 18/220 with HPA-15aa platelets from 2 different donors where possible at doubling dilutions and to report the endpoint dilution; these results are shown in Figure 2. There were only two laboratories that reported results from just one HPA-15aa donor. In summary, as expected, nearly all laboratories reported that for all HPA-15aa platelet donors/cells used with each method performed, they were unable to detect HPA-15a antibodies or reported a positive result only for the undiluted (neat) material. However, 3 labs did report endpoint dilutions of 1 in 2, 1 in 4 and 1 in 8 with one or more donors. This could be explained by the presence of anti-HLA antibodies at high serum concentrations and incomplete solubilisation of the platelet membrane during the MAIPA procedure. Indeed, both laboratories which reported endpoint dilutions of 1 in 4 or 1 in 8, each did so for two different HPA-15aa platelet donors suggesting that it is not a rare donor-specific phenomenon but rather a procedural effect. One laboratory reported an endpoint dilution of 1 in 32 (result not shown in Figure 2); this appears to be anomalous in comparison with the results from all other laboratories. This may have arisen from the incorrect typing of the alleged HPA-15aa platelets or may be attributed to the fact that these platelets were 9 days old. Indeed, the same laboratory reported 'neat' as the endpoint for a second donor of HPA-15aa platelets which were only 6 days old. Whilst some laboratories have reported a positive result with HPA-15aa platelets at high concentrations of 18/220, there still remains a clear distinction between reported endpoint dilutions with HPA-15bb and HPA-15aa types.

Stability

Estimates of the potency of 18/220 stored at elevated temperatures for a period of 13 months, relative to 18/220 stored at -70°C for 13 months are summarised in Table 3. Tests for non-

parallelism and non-linearity in the parallel-line analysis to estimate relative potencies were not statistically significant (p=0.14 and 0.59 respectively). There was insufficient degradation at the elevated temperatures, even after 13 months of storage, to fit the Arrhenius model, with only a clear relative potency loss at +45°C. This indicates that 18/220 will be stable for long-term storage at -20°C and sufficiently stable to allow for shipment of ampoules at ambient temperature.

5. Conclusions

The aim of the study was to prepare and validate a stable, minimum potency reference reagent for anti-HPA-15b detection that clinical laboratories can use to assess and validate the sensitivity of their routine, glycoprotein-specific assays. The collaborative study has shown that candidate preparation 18/220 contains anti-HPA-15b antibody that could be detected at a dilution of 1 in 8 by 21 of 23 laboratories with at least one donor. The reporting of positivity with HPA-15aa donors has meant that setting the minimum potency at 1 in 8 removes any ambiguity at low dilutions (high concentrations). The results of the study in general show good consistency across most laboratories but also indicate that some laboratories should consider further improving the sensitivity of their assay method.

A report of the 19th ISBT Platelet Immunology Workshop gave a comprehensive summary of the variations in the MAIPA procedure used by workshop participants and concluded that the MAIPA is far from harmonised which may contribute to variations in results [15]. Whilst our study also showed a lack of inter-laboratory harmonisation of the CD109 MAIPA procedure, we were also able to demonstrate notable intra-assay variability in reported endpoint dilutions that are likely due to the differential levels in CD109 expression by platelets from different donors, as previously reported [14]. Therefore, the harmonisation of test methods using optimised assay conditions may well improve the sensitivity of testing across clinical laboratories to some degree but cannot overcome test variability as a result of donor-donor differences. The use of the anti-HPA-15b reference reagent will at least guarantee the sensitivity of the platelets used.

6. Recommendation

It is proposed that preparation 18/220 is established as the 1st WHO Reference Reagent for Human IgG Antibodies against HPA-15b. Since the material contains an anti-HLA component (as may any patient clinical sample), it should only be used in techniques that are glycoprotein-specific (i.e. for CD109) such as the ‘gold standard’ MAIPA which all laboratories used in this study or where it can be ensured that the anti-HLA antibodies will not cause a false positive reaction (i.e. through chloroquine treatment of platelets to remove HLA-Class 1 epitopes). The preparation should be used at a dilution of 1 in 8 for assay validation (i.e. the recommended minimum potency which should test positive) and may be used to qualify ‘in house’ controls. This recommendation was accepted by the study participants (see Appendix 7) and has been endorsed by the ISBT Platelet Immunobiology Working Party (see letter in Appendix 8).

7. Instructions for Use

The draft Instructions for Use to accompany 18/220 are provided in Appendix 5.

8. Acknowledgments

We thank the H&I Department, NHSBT, Filton and the Platelet & Leucocyte Department, Sanguin Diagnostic Services for their laboratory support and advice throughout the study. We also thank the ISBT Platelet Immunobiology Working Party and UK NEQAS H&I Wales for distributing the collaborative study invitations and finally, we thank the participants of the collaborative study.

9. References

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Tables and Figures

Table 1: Product Summary

Code number	18/220
Presentation	Heat sealed, 2.5 ml glass ampoules
Number of ampoules available	1830
Date filled	1st March 2019
Mean fill mass (n=96)	0.52 g
Fill mass CV (n=96)	0.65 %
Residual moisture by coulometric Karl Fischer titration (n=6)	0.20 %
Residual moisture CV (n=6)	24.8 %
Mean dry weight (n=6)	0.04 g
Dry weight CV (n=6)	0.44 %
Mean oxygen in head space by Lighthouse FMS670 (n=6)	0.20 %
Oxygen in head space CV (n=6)	44.69 %
Storage conditions	-20°C
Address of processing facility	NIBSC, Potters Bar, EN6 3QG, UK
Address of custodian	NIBSC, Potters Bar, EN6 3QG, UK

Table 2: Laboratory Method Summary

Method	**Laboratory code**	**Total number of entries**
Rapid MAIPA	3, 4*, 4a*$^{+}$, 5, 7, 7a†, 9, 11, 17, 17a**, 18, 21, 23	13
2-Day MAIPA	1, 10^{+}, 14, 16	4
In-House MAIPA	2, 6, 12, 13, 15, 19, 20, 22	8
Other	8$^{\#+}$, 18a$^{\#}$	2

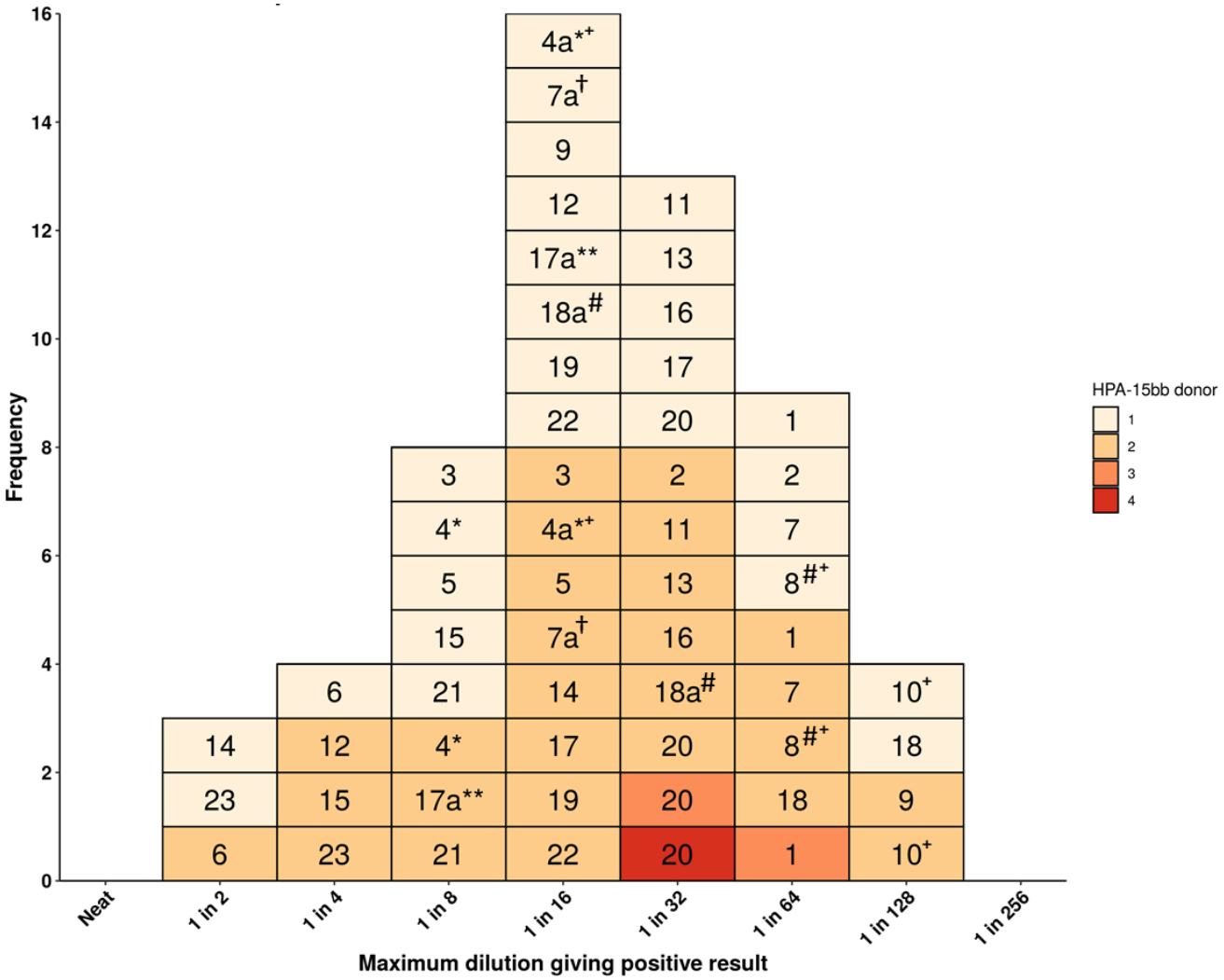
*Frozen platelets, **Lyophilised platelets, † K562 Recombinant cells used, $^{\#}$ Modified MAIPA method, $^{+}$ CD109 antibody other than mAb TEA 2/16. Labs providing supplementary datasets from a second method have an 'a' suffix.

Table 3: Accelerated Degradation Studies

Storage temperature (°C)	Relative potency (-70°C reference, n=4)	95% lower confidence limit	95% upper confidence limit
-20	1.00	0.92	1.09
+4	1.28	1.18	1.40
+20	1.13	1.04	1.23
+37	0.98	0.90	1.07
+45	0.72	0.66	0.79

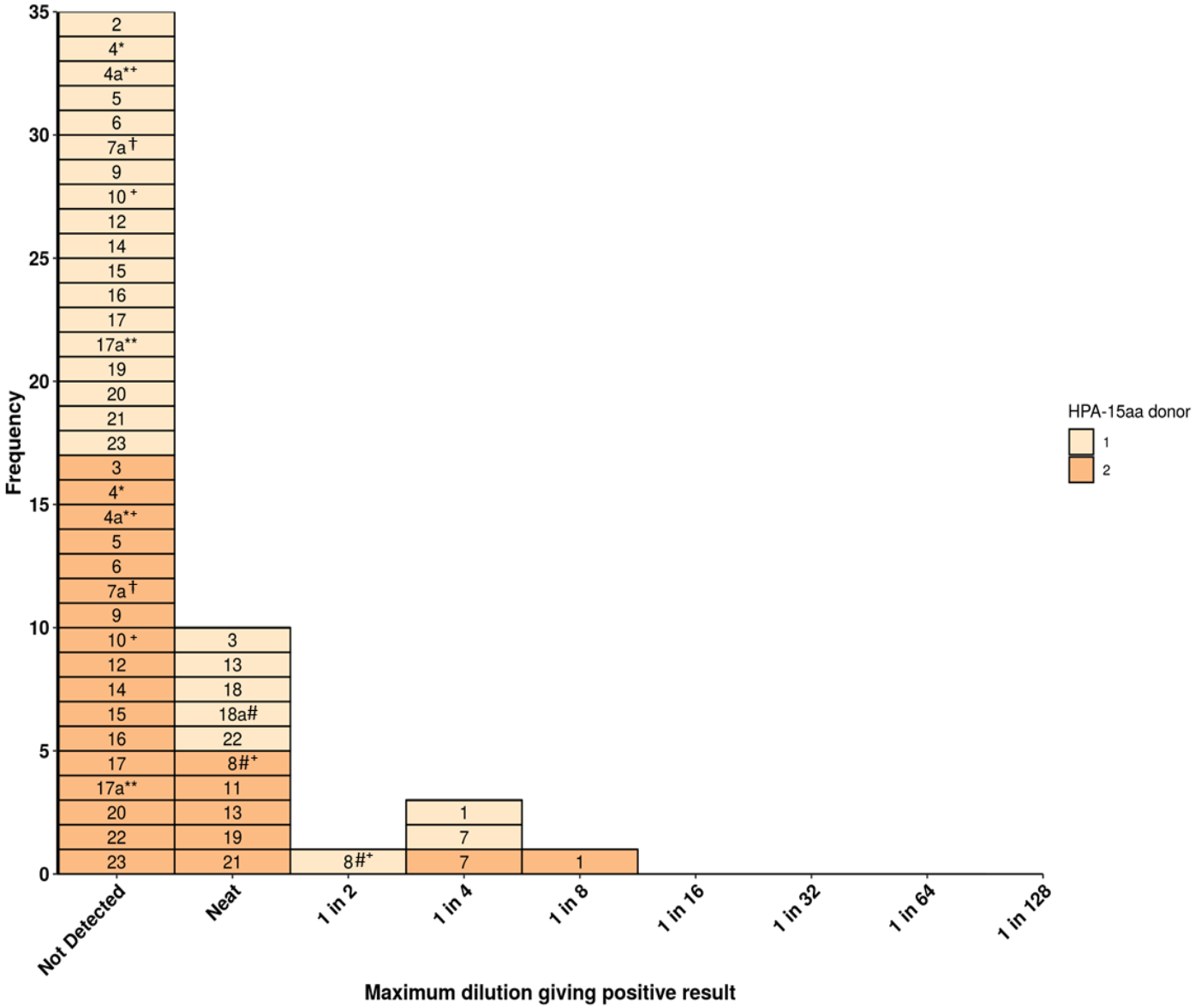
Note: ampoules stored for 13 months at each temperature.

Figure 1: Endpoint dilutions of 18/220 reported by laboratories for HPA-15bb platelets/cells; laboratory codes are shown in boxes.



a : Additional data sets submitted * : Frozen platelets ** : Lyophilised platelets † : Recombinant cells
: Modified MAIPA + : Anti-CD109 clone used other than TEA 2/16

Figure 2: Endpoint dilutions of 18/220 reported by laboratories for HPA-15aa platelet/cells; laboratory codes are shown in boxes.



a : Additional data sets submitted * : Frozen platelets ** : Lyophilised platelets † : Recombinant cells
: Modified MAIPA + : Anti-CD109 clone used other than TEA 2/16

Appendix 1: Invitation to participate in the collaborative study

International Collaborative Study to Evaluate a Candidate WHO Anti-Human Platelet Antigen-15b (anti-HPA-15b) Reference Reagent.

The National Institute for Biological Standards and Control (NIBSC) currently has available WHO anti-HPA antibody minimum potency reference reagents for HPA-1a, -3a and -5b intended for use to validate the sensitivity of HPA antibody detection methods. In October 2017, WHO Expert Committee on Biological Standardization (ECBS) endorsed a new NIBSC initiative to prepare a WHO anti-HPA-15b minimum potency reference reagent. We have recently prepared a candidate preparation and would like to invite your laboratory to participate in an international collaborative study to evaluate this material which will begin in the second quarter of 2019. There will be no charge for participation in the study.

Each participant will be required to titrate the candidate preparation at doubling dilutions in the Monoclonal Antibody-Specific Immobilization of Platelet Antigens (MAIPA) assay using HPA-15b15b and HPA-15a15a typed platelets. The results of the collaborative study will determine the maximum dilution (lowest concentration) of the candidate material that anti-HPA-15b antibody can be detected by study participants. This dilution will be assigned to the material as the minimum potency and the established preparation will be made available for use to validate assay sensitivity. Participants will be invited to comment on the final report prior to submission to WHO ECBS for endorsement. Results from participants will be anonymised in the report and in any subsequent publications.

Whilst participants are encouraged to use their own MAIPA assay protocol used for routine clinical testing, a protocol for the Rapid MAIPA can be found here: NIBSC - Blood cell and platelet immunology. Participants may perform an alternative method to the MAIPA in the study that is used in routine clinical testing, but it should be considered that the material contains anti-HLA antibodies. Please indicate below your choice of method(s).

Please complete the following information if you wish to participate:

Anti-HPA-15b antibody detection method(s) you intend to perform:

Contact name(s):

Laboratory:

Full Address:

Telephone (including international dialling code):

Email(s):

If test materials and protocol are to be sent somewhere different to the above, please indicate here:

Contact name(s):

Laboratory:

Full Address:

Telephone (including international dialling code)**:**

Email(s):

Please return this completed form by the end of March to:

Dr Lucy Studholme **and** Giles Sharp

Lucy.Studholme@nibsc.org, Giles.Sharp@nibsc.org

Appendix 2: List of participating laboratories

Institute	Country	Primary Contact
Australian Red Cross Blood Service, Victoria	Australia	Denise Herbert
Australian Red Cross Blood Service, Queensland	Australia	Gail Pahn / Mark Burton
Hospital Sirio Libanês	Brazil	Rita Fontão-Wendel
Canadian Blood Services	Canada	Lynette Beaudin
Institute of Blood Transfusion, Zhejiang Blood Centre	China	Xian-Guo Xu
Institute of Blood Transfusion, Guangzhou Blood Centre	China	Yuan Shao
French Blood Establishment (EFS), Lille	France	Anne Delsalle / Alluin Gaulthier
French Blood Establishment (EFS), Rennes	France	Gérald Bertrand
French Blood Establishment (EFS), Auvergne-Rhône-Alpes	France	Yves Mérieux
Centre Hospitalier Universitaire de Nantes	France	Marie Audrain/ Hans Kerchrom
Institut National de la Transfusion Sanguine	France	Rachel Petermann
Red Cross Blood Transfusion Services, NSTOB	Germany	Hartmut Kroll
Zentrum für Transfusionsmedizin und Zelltherapie, Berlin	Germany	Oliver Meyer / Mirelle Möckel
Institute of Clinical Immunology and Transfusion Medicine	Germany	Ulrich Sachs
National Blood Centre	Malaysia	Nurul Munira Yahya
Sanquin Diagnostic Services	Netherlands	Leendert Porcelijn

Institute	Country	Primary Contact
University Hospital of North Norway	Norway	Maria Therese Ahlen
Institute of Hematology and Transfusion Medicine	Poland	Ewa Brojer / Patrycja Łopacz
Banc de Sang i Teixits	Spain	Eduardo Muñiz-Diaz / Carme Canals
Karolinska University Hospital	Sweden	Agneta Wikman
University Hospital Geneva	Switzerland	Françoise Boehlen
The Thai Red Cross Society	Thailand	Sirilak Phiancharoen
National Health Service Blood and Transplant (NHSBT), Filton	UK	Anthony Poles
Welsh Blood Service	UK	Emma Burrows / Laura Williams
National Institute for Biological Standards and Control (NIBSC)	UK	Giles Sharp
Versiti	USA	Brian Curtis
Bloodworks	USA	Gayle Teramura

Appendix 3: Collaborative study protocol sent to participants

16th April 2019

International Collaborative Study to establish the 1st WHO anti-HPA-15b Reference Reagent (Collaborative study number: CS640)

Dear Colleague

Thank you for agreeing to take part in this study.

Aim of study

The aim of this international collaborative study is to test the suitability of the candidate anti-HPA-15b material for use as a WHO reference material. The results of the collaborative study will determine the maximum dilution (lowest concentration) of the candidate material that anti-HPA-15b antibody can be detected by study participants. The dilution will be assigned to the material as the minimum potency and the established preparation will be made available for use to validate assay sensitivity.

Candidate Material

The candidate material is recalcified plasma collected from a consenting blood donor with an anti-HPA-15b antibody and 0.5ml aliquots have been freeze-dried in heat-sealed glass ampoules for long term stability. You are provided with **4** ampoules of the candidate material Two ampoules are the minimum requirement to perform 2 independent MAIPAs using the standard protocol. Additional ampoules are supplied for laboratories carrying out additional detection methods or they can be treated as spare sample.

Storage and Reconstitution

Store all unopened ampoules below -20°C. Immediately before use, open the ampoules following the Instructions For Use (attached) and resuspend the contents in **0.5ml** distilled water with gentle mixing. If it is not possible to remove an aliquot with your usual pipette tips, then use the Pasteur pipettes provided.

Protocol

The pilot study has demonstrated that there are no other HPA specificities, but it **does contain anti-HLA**. Therefore, only glycoprotein specific assays such as the Monoclonal Antibody-Specific Immobilization of Platelet Antigens (MAIPA) will be suitable to use in this study. There is no need to test for other HPA or HLA antibodies.

Titrate the candidate preparation from neat in a replicate doubling dilution series in the MAIPA and/or using another appropriate method. The aim is to identify the largest dilution (lowest concentration) which gives a positive result. Use **HPA-15bb** and **HPA-15aa** antigens in each assay. For each method, test the sample twice, with each assay on separate days using a fresh ampoule. For the MAIPA method, ideally use different donors of fresh platelets to that of the previous day (i.e. 2 different HPA15bb donors and 2 different HPA15aa donors).

Results

For each dilution of the candidate material, please record your interpretation of the result (e.g. positive or negative) on the results form provided and the mean of the replicate raw data values, after subtracting the blank, if relevant. If you use more than one technique, please copy the form and enter your results on a different sheet. Please also complete all questions of the reverse of the 'results form' and then return by email to Giles.Sharp@nibsc.org or Lucy.Studholme@nibsc.org by the 31st July 2019.

Please complete and return the **Acknowledgement of receipt** form as soon as you receive the reference material for testing.

If anything is unclear in the protocol, please email either Giles Sharp or Lucy Studholme

Thank you in advance for your co-operation,

Best wishes

Giles Sharp
Senior Scientist

Enclosed

i) Protocol
ii) Results sheet
iii) Acknowledgement of Receipt form
iv) Instruction for Use, including Liability and Material Safety Sheet

Appendix 4: Results report form sent to participants

Medicines & Healthcare products Regulatory Agency

NIBSC

Titration of Candidate anti-HPA-15b standard, 18/220

Name of Laboratory: **Laboratory code number (NIBSC use only):**

Technique: Rapid MAIPA, 2 Day MAIPA, In-House MAIPA, Other: ..... (delete as required). Please copy this form and enter your results on a different sheet for each technique used.

DAY 1 Ampoule 1		Reciprocal dilution of HPA-15b standard										
		Neat	2	4	8	16	32	64	128	256	512	Negative Control
HPA-15bb platelets Donor 1	Mean Value*											
	Result: Pos/Neg											
HPA-15aa platelets Donor 2	Mean Value*											
	Result: Pos/Neg											

DAY 2 Ampoule 2		Reciprocal dilution of HPA-15b standard										
		Neat	2	4	8	16	32	64	128	256	512	Negative Control
HPA-15bb platelets Donor 3	Mean Value*											
	Result: Pos/Neg											
HPA-15aa platelets Donor 4	Mean Value*											
	Result: Pos/Neg											

*Optical Density or units of measurement after subtraction of value of 'blank' wells, if relevant. Units of measurement: _____

Please email to Dr Lucy Studholme: Lucy.Studholme@nibsc.org and Giles Sharp: Giles.Sharp@nibsc.org

Medicines & Healthcare products Regulatory Agency

NIBSC

Additional Information:

1) **For MAIPA assays please confirm that fresh platelets were used Yes/No ……**
2) **How many platelets were added to each well? ……..**
3) **For MAIPA assays what was the clone of the CD109 monoclonal antibody? ……; Manufacturer? ……**
4) **Volume of test material (diluted or undiluted) added to well of test plate?.....**
5) **Assay cut off criteria for a positive result?.....(e.g. OD Cut Off or Ratio Cut Off)**
6) **Please attach assay raw data to this data summary report sheet.**

Any other comments:

Please email to Dr Lucy Studholme: Lucy.Studholme@nibsc.org and Giles Sharp: Giles.Sharp@nibsc.org

Appendix 5: Instructions for Use for 18/220

Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

WHO Reference Reagent
Anti-HPA-15b (minimum potency)
NIBSC code: 18/220
Instructions for use
(Version 1.00, Dated)

1. INTENDED USE

This preparation, when reconstituted and diluted as described below, should be used as a reference reagent for minimum acceptable potency for the detection of antibodies against Human Platelet Antigen-15b (HPA-15b). It should not be used for HPA-15b typing or any other purpose.

2. CAUTION

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

The preparation contains material of human origin, and either the final product or the source materials, from which it is derived, have been tested and found negative for HBsAg, anti-HIV and HCV RNA. 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

No units are assigned to this material.

4. CONTENTS

Country of origin of biological material: United Kingdom.
Each ampoule contains the residue after freeze-drying of 0.5 ml recalcified plasma. The plasma was collected from a single consenting donor. The immunoglobulin class of the anti-HPA-15b antibodies is IgG. Antibodies against other HPA antigens have not been detected in this preparation but **antibodies against HLA Class 1 antigens are present, therefore this material should only be used in glycoprotein-specific assays (e.g. MAIPA).** The HLA Class 1 antibody specificities detected are A2, A68, A69, C5, C8 and C15.

5. STORAGE

Store 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.

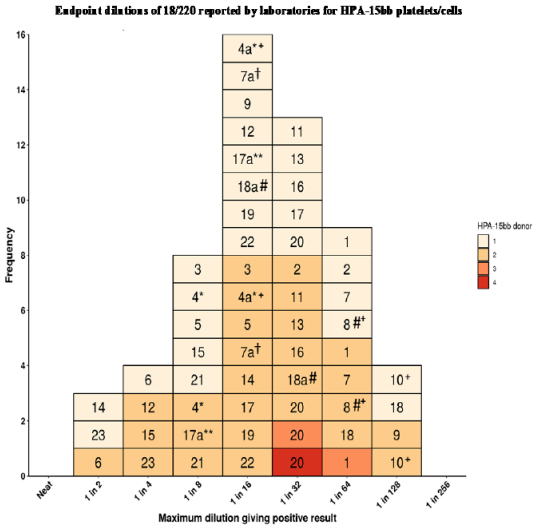
6. DIRECTIONS FOR OPENING

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

Reconstitute the contents of one ampoule with 0.5 ml distilled water immediately before use, mix gently until fully reconstituted. Dilute immediately before use by adding 1 volume of reconstituted material to 7 volumes of diluent (e.g. Tris-buffered saline containing 0.2% (w/v) bovine serum albumin). Diluted material should then be tested for the presence of IgG anti-HPA-15b antibodies using HPA-15bb platelets or antigens in glycoprotein specific assays (i.e. MAIPA assays). This dilution (1 in 8) is the minimum dilution expected to give a positive result. However, many laboratories can detect anti-HPA-15b at larger dilutions of 18/220, as shown in the following histogram.



Data from collaborative study: titration of anti-HPA-15b in individual laboratories. Numbers in boxes indicate lab code number only.

8. STABILITY

Reference materials are held at NIBSC within assured temperature controlled 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 minimum potency value to remain valid until the material is withdrawn or replaced. The studies have shown that the material is suitably stable for shipment at ambient temperature without any effect on the minimum potency value.

Users who have data supporting any deterioration in the characteristics of any reference preparation are encouraged to contact NIBSC.

9. REFERENCES

10. ACKNOWLEDGEMENTS

We thank the participants of 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:

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

Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

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: Pale yellow freeze-dried powder		Corrosive:	No
Stable:	Yes	Oxidising:	No
Hygroscopic:	No	Irritant:	
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: 0.04g
Toxicity Statement: Toxicity not assessed
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.

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

Appendix 6: Summary of results and assay details

Lab code	Method	Endpoint dilution for HPA-15bb	Endpoint dilution for HPA-15aa	Anti-CD109 mAb clone & supplier	Fresh, frozen or lyophilised platelets	Platelet/ cell conc. ($x10^6$ per well)	Volume of sample (µl/well)	Positive/ negative cut-off criteria (as reported)	Other comments
1	2-Day MAIPA	Donor 1 = 1 in 64	Donor 1 = 1 in 4	TEA 2/16 BD Pharmingen	Fresh	200 x 10^6	50 µl	OD >0.2	
		Donor 2 = 1 in 64	Donor 2 = 1 in 8						
		Donor 3 = 1 in 64	Donor 3 = N/A						
2	In-house MAIPA	Donor 1 = 1 in 64	Donor 1 = Not detected	TEA 2/16 BD Pharmingen	Fresh	25 x 10^6	50 µl	OD >0.3	Platelets used were either day 0 or day 1
		Donor 2 = 1 in 32	Donor 2 = N/A						
3	Rapid MAIPA	Donor 1 = 1 in 8	Donor 1 = Neat	TEA 2/16 BD Pharmingen	Fresh	10 x 10^6	25 µl	OD >0.15; ratio >3	
		Donor 2 = 1 in 16	Donor 2 = Not detected						
4	Rapid MAIPA	Donor 1 = 1 in 8	Donor 1 = Not detected	TEA 2/16 BD Pharmingen	Frozen	10 x 10^6	25 µl	OD >0.15; ratio >3	HPA-15aa donors the same for day 1 & 2 Panel of donors with high CD109 expression identified and frozen down for anti-HPA-15b assays
		Donor 2 = 1 in 8	Donor 2 = Not detected						
4a	Rapid MAIPA	Donor 1 = 1 in 16	Donor 1 = Not detected	HU17 Invitrogen	Frozen	10 x 10^6	25 µl	OD >0.15; ratio >3	HPA-15aa donors the same for day 1 & 2
		Donor 2 = 1 in 16	Donor 2 = Not detected						
5	Rapid MAIPA	Donor 1 = 1 in 8	Donor 1 = Not detected	TEA 2/16 BD Pharmingen	Fresh	300 x 10^6	50 µl	Ratio >2 (to neg control)	
		Donor 2 = 1 in 16	Donor 2 = Not detected						
6	In-house MAIPA	Donor 1 = 1 in 4	Donor 1 = Not detected	TEA 2/16 BD Pharmingen	Fresh	20 x 10^6	50 µl	OD >0.2	
		Donor 2 = 1 in 2	Donor 2 = Not detected						
7	Rapid MAIPA	Donor 1 = 1 in 64	Donor 1 = 1 in 4	TEA 2/16 BD Pharmingen	Fresh	25 x 10^6	50 µl	OD >0.16	HPA-15bb and HPA-15aa donors the same for day 1 & 2
		Donor 2 = 1 in 64	Donor 2 = 1 in 4						
7a	Rapid MAIPA	Donor 1 = 1 in 32	Donor 1 = Not detected	BIORAD MCA1227	Recombinant K562 cells	25 x 10^6	50 µl	OD >0.28	
		Donor 2 = 1 in 32	Donor 2 = Not detected						

Lab code	Method	Endpoint dilution for HPA-15bb	Endpoint dilution for HPA-15aa	Anti-CD109 mAb clone & supplier	Fresh, frozen or lyophilised platelets	Platelet/ cell conc. ($x10^6$ per well)	Volume of sample (µl/well)	Positive/ negative cut-off criteria (as reported)	Other comments
8	Modified MAIPA	Donor 1 = 1 in 64 Donor 2 = 1 in 64	Donor 1 = 1 in 2 Donor 2 = Neat	BAF4385 R&D Systems	Fresh	75 x 10^6	100 µl	MFI 3x neg control	Flow cytometric method with MFI readings
9	Rapid MAIPA	Donor 1 = 1 in 16 Donor 2 = 1 in 128	Donor 1 = Not detected Donor 2 = Not detected	TEA 2/16 BD Pharmingen	Fresh	40 x 10^6	50 µl	OD >0.2	
10	2-Day MAIPA	Donor 1 = 1 in 128 Donor 2 = 1 in 128	Donor 1 = Not detected Donor 2 = Not detected	15E10 / Sanquin	Fresh	60 x 10^6	120 µl	OD >0.3	
11	Rapid MAIPA	*Donor 1 = 1 in 32 **Donor 2 = 1 in 32	*Donor 1 = 1 in 32[#] ***Donor 2 = Neat	TEA 2/16 BD Pharmingen	Fresh	10 x 10^6	25 µl	OD >3x+/- SD neg control	#Anomalous result not included *Day 9 platelets used **Day 3 platelets used ***Day 6 platelets used
12	In-house MAIPA	Donor 1 = 1 in 32 Donor 2 = 1 in 4	Donor 1 = Not detected Donor 2 = Not detected	TEA 2/16	Fresh	100 x 10^6	20 µl	OD >0.2	
13	In-house MAIPA	Donor 1 = 1 in 32 Donor 2 = 1 in 32	Donor 1 = Neat Donor 2 = Neat	TEA 2/16 BD Pharmingen	Fresh	20 x 10^6	50 µl	OD ≥0.1 (or mean neg control + 2SD if this >0.1)	CD109 levels measured by flow cytometry to check for adequate expression and comparable levels
14	2-Day MAIPA	Donor 1 = 1 in 16 Donor 2 = 1 in 2	Donor 1 = Not detected Donor 2 = Not detected	TEA 2/16 BD Pharmingen	Fresh	20 x 10^6	50 µl	OD >neg control + 2 SD	
15	In-house MAIPA	Donor 1 = 1 in 8 Donor 2 = 1 in 4	Donor 1 = Not detected Donor 2 = Not detected	TEA 2/16 BD Pharmingen	Fresh	25 x 10^6	50 µl	OD >0.2	
16	2-Day MAIPA	Donor 1 = 1 in 32 Donor 2 = 1 in 32	Donor 1 = Not detected Donor 2 = Not detected	TEA 2/16 BD Pharmingen	Fresh	40 x 10^6	50 µl	Ratio >2x neg control and OD >0.15	

Lab code	Method	Endpoint dilution for HPA-15bb	Endpoint dilution for HPA-15aa	Anti-CD109 mAb clone & supplier	Fresh, frozen or lyophilised platelets	Platelet/ cell conc. ($x10^6$ per well)	Volume of sample (µl/well)	Positive/ negative cut-off criteria (as reported)	Other comments
17	Rapid MAIPA	Donor 1 = 1 in 32	Donor 1 = Not detected	TEA 2/16 BD Pharmingen	Fresh	25 x 10^6	50 µl	OD >0.2	
		Donor 2 = 1 in 16	Donor 2 = Not detected						
17a	Rapid MAIPA	Donor 1 = 1 in 16	Donor 1 = Not detected	TEA 2/16 BD Pharmingen	Lyophilised	25 x 10^6	50 µl	OD >0.2	
		Donor 2 = 1 in 8	Donor 2 = Not detected						
18	Rapid MAIPA	Donor 1 = 1 in 128	Donor 1 = neat	TEA 2/16 BD Pharmingen	Fresh	20 x 10^6	25 µl	0.02 OD above negative control	
		Donor 2 = 1 in 64	Donor 2 = N/A						
18a	Modified MAIPA	Donor 1 = 1 in 16	Donor 1 = Neat	TEA 2/16 BD Pharmingen	Fresh	20 x 10^6	25 µl	0.02 OD above negative control	
		Donor 2 = 1 in 32	Donor 2 = N/A						
19	In-house MAIPA	Donor 1 = 1 in 16	Donor 1 = Not detected	TEA 2/16 BD Pharmingen	Fresh	4 x 10^5	50 µl	Ratio >2	
		Donor 2 = 1 in 16	Donor 2 = Neat						
20	In-house MAIPA	Donor 1 = 1 in 32	Donor 1 = Not detected	TEA 2/16 BD Pharmingen	Fresh	100 x 10^6	50 µl	OD >0.2	
		Donor 2 = 1 in 32	Donor 2 = Not detected						
		Donor 3 = 1 in 32	Donor 3 = N/A						
		Donor 4 = 1 in 32	Donor 4 = N/A						
21	Rapid MAIPA	Donor 1 = 1 in 8	Donor 1 = Not detected	TEA 2/16 BD Pharmingen	Fresh	20 x 10^6	25 µl	OD >0.2	Day 1 platelets
		Donor 2 = 1 in 8	Donor 2 = Neat						
22	In-house MAIPA	Donor 1 = 1 in 16	Donor 1 = Not detected	TEA 2/16 BD Pharmingen	Fresh	20 x 10^6	25 µl	OD >0.2	
		Donor 2 = 1 in 16	Donor 2 = Neat						
23	Rapid MAIPA	Donor 1 = 1 in 4	Donor 1 = Not detected	TEA 2/16 BD Pharmingen	Fresh	20 x 10^6	25 µl	OD >0.150	
		Donor 2 = 1 in 2	Donor 2 = Not detected						

Appendix 7: Participant comments on collaborative study report

Email distributed to participants between 14th-15th May 2020.

Dear colleagues

Please see attached the participants report for the **PROPOSED 1st WHO INTERNATIONAL REFERENCE REAGENT (Minimum Potency) FOR ANTI-HPA-15b**

Your laboratory code is:

1. Please check the report and confirm:
   - Your details on the participants list (Appendix 2) are correct.
   - Your results have been interpreted and reported correctly **in Figures 1 and 2.**
   - You agree with the draft conclusions and proposals.
2. Any other comments or alternative proposals?

Please send your comments by email to Giles.Sharp@nibsc.org and Lucy.Studholme@nibsc.org by **10th June 2020**. **If I do not hear from you by this date, due to the deadline for submission to WHO, I will assume everything is correct and that you agree with the proposals.**

Summary of Responses

Laboratory Code	Participant Comments & Corrections Made
1	Report conclusions and proposals accepted
2	No comments submitted
3	No comments submitted
4	Report conclusion and proposals accepted. General question regarding the platelet numbers used by laboratories for the CD109 MAIPA compared to a conventional MAIPA for other glycoproteins. **Appendix 6 added to report to include assay specifics for each laboratory.**
5	No comments submitted
6	No comments submitted
7	Report conclusions and proposals accepted. Additional data set using recombinant cells was using the Rapid MAIPA method and NOT a Modified MAIPA method. **Report changed accordingly.** Participant commented on the number of HPA alloantigens quoted, **report corrected accordingly.**
8	No comments submitted
9	No comments submitted
10	Report conclusions and proposals accepted
11	No comments submitted

Laboratory Code	Participant Comments & Corrections Made
12	No comments submitted
13	Report conclusions and proposals accepted
14	No comments submitted
15	No comments submitted
16	No comments submitted
17	No comments submitted
18	Report conclusions and proposals accepted. Name correction and suggestion to change title from 'Participant' to 'Primary contact' in Appendix 2. **Report changed accordingly.** 18a data-set evaluation re-checked and verified as participant had concerns. Question asked about assay platelet numbers used by laboratories. **Appendix added to report to include assay specifics for each laboratory.**
19	No comments submitted
20	No comments submitted
21	No comments submitted
22	No comments submitted
23	Report conclusions and proposals accepted. Suggestion made to state the HLA antibody specificities in the report. **Specificities added to report and to IFU.**
Prof. Ulrich Sachs Chair, Working Party on Platelet Immunobiology of the ISBT	Endorsement from the ISBT Platelet Immunobiology working party. See Appendix 8.

Appendix 8: Endorsement from the ISBT Working Party on Platelet Immunobiology

Working Party on Platelet Immunobiology
Chairs: **Nelson H. Tsuno** (Tokyo, Japan) & **Ulrich J. Sachs** (Giessen, Germany)

Ulrich J. Sachs, MD, PhD
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June 8, 2020

Letter of Endorsement

The Biotherapeutics Department of the National Institute for Biological Standards and Control, Potters Bar, United Kingdom, has provided anti-human platelet antigen-15b (anti-HPA-15b) under the code 18/220 to be evaluated by 25 national reference laboratories for platelet immunobiology, organised in the Working Party on Platelet Immunobiology of the International Society of Blood Transfusion.

The aim of the study was to validate a minimum potency reference reagent for anti-HPA-15b detection that clinical laboratories can use to assess and validate the sensitivity of their routine assays. The collaborative study has shown that candidate preparation 18/220 contains anti-HPA-15b antibody that could be detected at a dilution of 1 in 8. The results of the study in general showed good consistency across laboratories.

The ISBT Working Party on Platelet Immunobiology supports the proposal that preparation 18/220 is established as a WHO Reference Reagent for Human IgG Antibodies against HPA-15b for the use in glycoprotein-specific (CD109) assays. The preparation should be used at a dilution of 1 in 8 as the recommended minimum potency which should test positive.

Giessen, Germany
June 8, 2020

Prof. Ulrich J. Sachs
Chair, Working Party on Platelet Immunobiology
of the International Society of Blood Transfusion

End of Report