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**WHO Reference Reagents to standardise blood group genotyping  
Report of the international collaborative study to evaluate four candidate  
WHO Reference Reagents**

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## Summary

Four lyophilised genomic DNA preparations from genotyped and phenotyped donors were evaluated for their suitability to serve as WHO Reference Reagents to standardise blood group genotyping procedures for common Caucasian and Black African alleles, in an international collaborative study.

## Introduction

The molecular bases of almost all the clinically significant blood group polymorphisms are known and blood group genotyping is becoming increasingly used as it has a number of advantages over conventional blood group serology: when reliable and consistent serological reagents are unavailable to type minor antigens and variants; for blood typing red cells with a positive direct antiglobulin test; typing multiply transfused patients. Genotyping can also be used to resolve reagent typing discrepancies and predicting fetal Rh D phenotype and some other clinically important blood groups from cell-free fetal DNA in maternal blood.

International Society of Blood Transfusion (ISBT) workshops on molecular blood group genotyping have been held at 2 yearly intervals since 2004 [1-3] with the multiple aims of functioning as an external quality assurance scheme, improving communication between genotyping laboratories, identifying methodology, and assessing the accuracy and reliability of methods and laboratories. The report from the Third ISBT workshop, held in 2008, noted an overall high level of diagnostic accuracy with a small improvement from previous workshops, but still room for further improvement [3]. Similar conclusions were drawn from a fourth workshop in 2010 (unpublished). For example, in the third workshop, only 24 of 33 laboratories obtained completely correct results for samples obtained from a patient with abnormal anti-D serology and two multiply transfused patients. Thus a requirement for standardisation in molecular blood group genotyping through the availability reliable and well validated DNA reference materials of consistent quality and DNA concentration was identified.

The aim of the present study was to evaluate the first four of an envisaged larger panel of lyophilised DNA preparations produced from consenting blood donors for their suitability to serve as World Health Organization (WHO) Reference Reagents for common alleles found in Caucasian and black African populations, in an international collaborative study organised under the auspices of the ISBT.

## Materials and methods

### *Candidate WHO Reference Reagents for blood group genotyping*

Blood samples were collected from consenting donors of previously determined genotype and phenotype according to local ethical practice. Each donor was tested and found negative for HIV, HTLV, Hepatitis B, Hepatitis C, and Syphilis. Red cells were stored frozen in aliquots at -80°C and in liquid nitrogen for further serological phenotyping if necessary, and lymphoblastoid cell lines were established following EBV transformation. Master and working cell banks were produced to ensure a continual future cell supply. Four cell lines, termed RBC1, RBC4, RBC5 and RBC12 were selected for bulk production of DNA for the first 4 candidate reference reagents. Large-scale cell culture was carried out and genomic DNA extracted from cell pellets using Genra Puregene chemistry (Qiagen, UK). Genomic DNA extracted using the same purification procedure from EBV-transformed cell lines did not show EBV infectivity. Each of the genomic DNA preparations was diluted to 10 µg/ml in 2 mM Tris, 0.2 mM EDTA containing 3 mg/ml trehalose, dispensed into glass ampoules (0.25 ml/ampoule) and lyophilised. The preparations were coded as shown in Table 1. The ampoules were stored in the dark at -20°C, except for a small number that were stored at temperatures ranging from -150°C to +56°C for accelerated degradation studies. Upon reconstitution with 50 µl distilled/deionised water

(nuclease-free), the DNA concentration was approximately 50 µg/ml in 10 mM Tris, 1 mM EDTA containing 15 mg/ml trehalose. The quality of the extracted and freeze-dried DNA preparations was confirmed by agarose gel electrophoresis, spectrophotometry and real-time QPCR.

Full details of the product characterisation and fills are located in the relevant Reference Material Design Dossier at NIBSC.

Fill details are summarised in Table 1.

#### ***Acquisition of bulk material for replacement reagents***

Master cell banks of each of the 4 lymphoblastoid cell lines used as the source of DNA have been established at NIBSC. These allow for consistent genotype replacement upon exhaustion of the present ampoule stocks, and provide a source of DNA for the addition of further alleles to the genotype profile.

#### ***Collaborative study participants***

A total of 29 laboratories in 19 countries from Europe, North and South America, the Middle East, China and Australia participated in the study (Appendix 1). Each was assigned a code number, which does not reflect the order of listing.

#### ***Study design***

Each participant was provided with 3 ampoules of each of the candidate Reference Reagents 10/232, 10/234, 10/236 and 10/238 which were coded as shown in Table 1. Participants were requested to perform blood group genotyping on the reconstituted contents using their usual method(s) for the genes listed in Table 3, analysing ampoules A-D on day 1, ampoules E-H on day 2 and ampoules I-L on day 3. Participants were requested to indicate which genes they detected in the reconstituted contents of each ampoule on results sheets provided. They were not asked to report predicted phenotypes to avoid possible transcriptional errors or use of inconsistent terminology. The study protocol is given in Appendix 2.

## **Results**

Results were returned by 29 laboratories. A range of methods was used by the laboratories, the most common being classical PCR using allele or sequence specific primers (Table 2). Ten laboratories used different methods for detecting different genes; laboratory 14 returned 2 complete sets of results using PCR-SSP and BeadChip array, so these data were considered separately (laboratory 14a and 14b, respectively) to give a total of 30 sets of results. Table 3 shows the number of laboratories that tested for each gene, the serological phenotypes of the samples, and the phenotypes predicted by the study organisers from the genotypes returned by the laboratories.

#### ***RBC1 (10/232; D, E, K), RBC4 (10/236; A, H, J) and RBC5 (10/238; C, F, L)***

These samples had been selected to represent a range of common alleles in homozygous and heterozygous combinations. There were relatively few genotyping errors in the results returned by the study participants as judged both from the serology and the consensus predicted phenotypes, although only 11 laboratories tested for all genes requested. A further laboratory (lab 10) tested for all genes with the caveat that their methodology would not detect all *GYP A\* N* alleles. Errors were of two types: intra-laboratory inconsistency where the result from one of the replicated samples was inconclusive or did not agree with those of the others replicates (shown by labs 3, 14b, 19, 22, 26 for one or more samples/genes) and consistent but incorrect genotyping (Kidd genotype of RBC1 by lab 2, Duffy genotype of RBC5 by lab 16, and Dombrock genotype of RBC1, RBC4 and RBC5 by lab 16). Twenty-four sets of data were free

from any errors, but many laboratories did not test for a number of genes. Laboratory 14 returned an error-free set of data using conventional PCR (lab 14a), but data with several anomalies using BeadChip array (lab 14b).

**RBC12 (10/234; B, G, I)**

This sample was selected to contain alleles that are common in individuals of African origin, but extremely rare in other populations. This sample is D-, but has the inactive *RHD* $\Psi$  gene. It is also V+ VS+ and so has a nucleotide change in *RHCE*, and is Fy(a-b-) and so is homozygous for a nucleotide change in the promoter region of *DARC*. One laboratory did not test for *RHD* $\Psi$  and so incorrectly predicted a D+ phenotype. The other laboratories recognised the presence of *RHD* $\Psi$  apart from one that could not distinguish *RHD* $\Psi$  from *RHD*\**DVa*. No laboratories reported testing for the VS polymorphism. All laboratories identified the *FY*\**Bnull* allele and so correctly predicted an Fy(a-b-) phenotype.

**Stability**

In NIBSC's experience, lyophilised DNA extracted from cell lines shows good stability. Preliminary accelerated degradation studies on ampoules of RBC1, 4, 5 and 12 stored at -150°C, -20°C, +45°C and +56°C for 6 months were carried out. DNA quantification (Nanodrop spectrophotometer readings) showed that all ampoules had concentrations of  $\geq 50$  ng/ $\mu$ l after reconstitution (mean 56.8ng/ $\mu$ l; range 52.8 – 60.7; SD 2.8; Figure 1). All samples looked in good condition using agarose gel electrophoresis (Figure 2), and appeared to have better integrity than the commercial control sample (lanes 2 & 19). There was an indication of slight degradation in some of the 45°C and 56°C samples as judged from a small decrease in DNA size compared with the ampoules stored at -20°C and -150°C. Some degradation in three of the 56°C samples, and possibly 2 of the 45°C samples was also suggested by increased cycle threshold values from real-time QPCR of a 486 bp sequence on chromosome 11 (Table 4). The data were analysed using the DEGTEST program where the percentage amount of DNA in each of the ampoules stored at -20°C, +45°C and +56°C was expressed relative to the relevant sample stored at -150°C. The analysis did not run successfully for 10/236 and 10/238 as insufficient loss was observed. The predicted losses of activity for 10/232 and 10/234 are shown below:

<b>Code</b>	<b>Predicted percentage loss per year at -20°C</b>
10/232	0.197%
10/234	0.120%

Thus, based on the preliminary data, the preparations should be sufficiently stable at -20°C for many years, particularly as they are intended only as qualitative controls in PCR.

It is proposed to test ampoules stored at elevated temperatures at 1-2 yearly intervals to monitor degradation and confirm that the preparations are still fit for purpose i.e., give the appropriate reactions in the relevant genotyping tests.

**Instructions for Use**

The draft Instructions for Use to accompany this reference material are provided in Appendix 3.

**Participant feedback**

All of the participants accepted the recommendation to establish RBC1, RBC4, RBC5 and RBC12 as WHO Reference Reagents for blood group genotyping.

## Discussion

This study demonstrates an overall high level of accuracy in blood group genotyping for common alleles, but the identified errors and inconsistencies, and the limited genotyping capabilities of many laboratories, indicate a need for validated reference materials to control test procedures and ensure they are sufficiently sensitive. The finding that the majority of laboratories reported genotypes in accordance with those determined by the study organisers and the serological phenotypes validates the use of RBC1, RBC4, RBC5 and RBC12 as reference reagents for blood group genotyping, subject to the ongoing demonstration of adequate stability. The reference reagents are intended for qualitative use only i.e., to give positive or negative results for a particular allele from which red cell phenotype can be inferred; quantification of DNA is not required. It is therefore recommended that RBC1, RBC4, RBC5 and RBC12 are established as the first four WHO Reference Reagents for blood group genotyping to control test procedures for common alleles in Caucasian and black African populations as listed in the IFU. The success of the present project allows for the possibility of expanding the current panel both in terms of extending the allelic profiles of the same 4 DNA preparations, and the preparation of DNA from additional consenting donors to cover, for example, clinically important alleles found in other populations. As the cost of molecular genotyping decreases, it is anticipated that such methodology will become increasingly used world-wide.

## Proposal

It is proposed that RBC1 (10/232), RBC4 (10/236), RBC5 (10/238) and RBC12 (10/234) are established as the first four WHO Reference Reagents for blood group genotyping to control test procedures for common alleles in Caucasian and black African populations.

## Implementation plan

Blood group genotyping laboratories and manufacturers of genotyping kits will be informed about the availability of the Reference Reagents directly and through the ISBT e.g., through the external quality assurance workshops.

The report of the collaborative study will be published in an international scientific journal.

## Acknowledgements

The study organisers thank Dr Paul Matejtschuk and Chris Halls, NIBSC, for lyophilisation developmental work and staff of the Centre for Biological Reference Materials, NIBSC, for lyophilising the genomic DNA bulks and sample despatch. We thank Peter Rigsby, NIBSC, for stability predictions.

We are grateful to Dr Sylvia Armstrong-Fisher and Pete Martin for evaluating trial fill samples, and the study participants for contributing data.

## References

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2. Daniels G, van der Schoot CE, Olsson ML. Report of the second international workshop on molecular blood group genotyping. *Vox Sang* 2007; 93: 83-8.
3. Daniels G, van der Schoot CE, Gassner C, Olsson ML. Report of the third international workshop on molecular blood group genotyping. *Vox Sang* 2009; 96: 337-43.

Table 1 Summary of fill details

	<i>Cell line (phenotype)</i>			
	<i>RBC1 (AR<sub>1</sub>R<sub>1</sub>)</i>	<i>RBC4 (OR<sub>1</sub>R<sub>2</sub>)</i>	<i>RBC5 (Brr)</i>	<i>RBC12 (OψD)</i>
<i>NIBSC code</i>	10/232	10/236	10/238	10/234
<i>Study code</i>	D, E, K	A, H, J	C, F, L	B, G, I
<i>Mean weight of the dispensed solution (number of fill weights measured)</i>	0.26 g (102)	0.27 g (102)	0.27 g (77)	0.27 g (72)
<i>Imprecision of the filling (coefficient of variation)</i>	0.33%	0.65%	0.72%	0.49%
<i>Residual moisture</i>	1.1%	1.1%	1.6%	0.89%
<i>Number of ampoules for distribution as WHO reference reagent</i>	2785	1776	2682	1951

**Table 2 Methods used by participating laboratories**

<b>Lab</b>	<b>PCR-ASP or PCR-SSP</b>	<b>PCR-RFLP</b>	<b>Multiplex SSP or RFLP PCR</b>	<b>Real time PCR</b>	<b>BeadChip array</b>	<b>BloodChip array</b>	<b>Luminex array</b>	<b>5' nuclease assay</b>
<b>1</b>	x							
<b>2</b>	x							
<b>3</b>					x			
<b>4</b>	x			x				
<b>5</b>	x	x						
<b>6</b>	x							
<b>7</b>	x			x				
<b>8</b>								x
<b>9</b>							x	
<b>10</b>	x							
<b>11</b>	x	x						
<b>12</b>	x							
<b>13</b>	x							
<b>14a</b>	x							
<b>14b</b>					x			
<b>15</b>	x			x				
<b>16</b>	x	x						
<b>17</b>	x							
<b>18</b>	x			x				
<b>19</b>				x				
<b>20</b>	x							
<b>21</b>	x		x		x			
<b>22</b>	x							
<b>23</b>			x		x			
<b>24</b>	x							
<b>25</b>	x					x		
<b>26</b>	x							
<b>27</b>	x				x			
<b>28</b>			x					
<b>29</b>					x			

**Table 3 Comparison of serological phenotype with predicted phenotype from blood group genotyping for samples A-L**

Polymorphism	Serology	Number of labs testing for gene	Predicted phenotype from reported results	
			Correct for all replicates tested <sup>a</sup>	Incorrect or inconsistent results
<b>RBC1 (D, E, K)</b>				
MN	M+N-	19 ( <i>GYPA*M</i> , <i>GYPA*N</i> )	M+N- (17) <sup>b</sup> ; M+N- with caveat that not all N alleles are detected (lab 10) <sup>c</sup>	
Ss	S+s+	23 ( <i>GYPB*S</i> , <i>GYPB*s</i> )	S+s+ (23)	
D	D+	25 ( <i>RHD</i> ) 23 ( <i>RHDψ</i> )	D+ (25); <i>RHDψ</i> not detected by 23 labs)	
Cc	C+c+	27 ( <i>RHCE*C</i> ) 29 ( <i>RHCE*c</i> )	C+c+ (27); c+ (2; <i>RHCE*C</i> not tested <sup>d</sup> )	
Ee	E+e+	30 ( <i>RHCE*E</i> ) 28 ( <i>RHCE*e</i> )	E+e+ (28); E+ (2; <i>RHCE*e</i> not tested <sup>d</sup> )	
Kk	K+k+	29 ( <i>KEL*1</i> , <i>KEL*2</i> )	K+k+ (29) <sup>c</sup>	
Duffy	Fy(a-b+)	28 ( <i>FY*A</i> , <i>FY*B</i> ) 27 ( <i>FY*BNull</i> )	Fy(a-b+) (28) <sup>c</sup>	
Kidd	Jk(a-b+)	28 ( <i>JK*A</i> , <i>JK*B</i> )	Jk(a-b+) (27) <sup>c</sup>	Jk(a+b+) (lab 2)
Dombrock	not tested	20 ( <i>DO*A</i> , <i>DO*B</i> )	Do(a-b+) (19) <sup>c</sup>	Do(a+b+) (lab 16)
<b>RBC4 (A, H, J)</b>				
MN	M+N+	19 ( <i>GYPA*M</i> , <i>GYPA*N</i> )	M+N+ (18) <sup>b</sup>	
Ss	S-s+	23 ( <i>GYPB*S</i> , <i>GYPB*s</i> )	S-s+ (23)	
D	D+	25 ( <i>RHD</i> ); 23 ( <i>RHDψ</i> )	D+ (25); <i>RHDψ</i> not detected by 23 labs)	
Cc	C+c-	27 ( <i>RHCE*C</i> ) 28 ( <i>RHCE*c</i> )	C+c- (26); c+ (1; <i>RHCE*C</i> not tested <sup>d</sup> )	C+c- for samples A and J, C+ but c inconclusive for sample H (lab 14b)
Ee	E-e+	29 ( <i>RHCE*E</i> ) 28 ( <i>RHCE*e</i> )	E-e+ (26); E- (2; <i>RHCE*e</i> not tested <sup>d</sup> ); e+ (1, <i>RHCE*E</i> not tested)	Inconclusive results for both E and e for samples A and H, E-e+ for sample J (lab 14b)
Kk	K-k+	29 ( <i>KEL*1</i> , <i>KEL*2</i> )	K-k+ (29)	
Duffy	Fy(a-b+)	28 ( <i>FY*A</i> ,	Fy(a-b+) (27); Fy(a-b+)	Fy(a-b+) for sample A, Fy(a-b-

		<i>FY*B</i> ) 27 ( <i>FY*BNull</i> )	in absence of <i>FY*BNull</i> result (1) <sup>f</sup>	) for samples H and J (lab 16)
Kidd	Jk(a+b+)	28 ( <i>JK*A</i> , <i>JK*B</i> )	Jk(a+b+) (28)	
Dombrock	not tested	20 ( <i>DO*A</i> , <i>DO*B</i> )	Do(a-b+) (18)	Do(a+b+) (lab 16); Do(a-b-) for sample A (lab 19)
<b>RBC5 (C, F, L)</b>				
MN	M-N+	19 ( <i>GYPA*M</i> , <i>GYPA*N</i> )	M-N+ (16) <sup>b</sup>	M-N+ for samples C and F, M- N- for sample L (lab 3); M-N+ for samples F and L, no results for sample C (lab 22)
Ss	S-s+		S-s+ (22)	S-s+ for samples F and L, no results for sample C (lab 22)
D	D-	24 ( <i>RHD</i> ); 23 ( <i>RHDψ</i> )	D- (23)	D- for samples F and L, no results for sample C (lab 22)
Cc	C-c+	28 ( <i>RHCE*C</i> ) 29 ( <i>RHCE*c</i> )	C-c+ (26); c+ (1; <i>RHCE*C</i> not tested)	C-c+ for samples C and F (lab 3), C-c- for sample L (lab 3); C-c+ for samples F and L, no results for sample C (lab 22)
Ee	E-e+	29 ( <i>RHCE*E</i> ) 29 ( <i>RHCE*e</i> )	E-e+ (27); E- (1; <i>RHCE*e</i> not tested); e+ (1, <i>RHCE*E</i> not tested)	E-e+ for samples F and L, no results for sample C (lab 22)
Kk	K+k-	29 ( <i>KEL*1</i> , <i>KEL*2</i> )	K+k- (27)	K+k- for samples C and F, K- k- for sample L (lab 3); K+k- for samples F and L, no results for sample C (lab 22)
Duffy	Fy(a+b+)	28 ( <i>FY*A</i> , <i>FY*B</i> ) 27 ( <i>FY*BNull</i> )	Fy(a+b+) (23); Fy(a+b+) in absence of <i>FY*BNull</i> result (1)	Fy(a+b+) for samples C and F, Fy(a-b-) for sample L (lab 3); Fy(a-b+) (lab 16); Fy(a+b+) for samples F and L, no results for sample C (lab 22); Fy(b+), <i>FY*A</i> inconclusive (sample C only tested; lab 26)
Kidd	Jk(a+b-)	28 ( <i>JK*A</i> <i>JK*B</i> )	Jk(a+b-) (26)	Jk(a+b-) for samples C and F, Jk(a-b-) for sample L (lab 3); Jk(a+b-) for samples F and L, no results for sample C (lab 22)
Dombrock	not tested	20 ( <i>DO*A</i> , <i>DO*B</i> )	Do(a-b+) (19)	Do(a+b+) (lab 16)
<b>RBC12 (B, G, I)</b>				
MN	M+N+	19 ( <i>GYPA*M</i> , <i>GYPA*N</i> )	M+N+ (17) <sup>b</sup>	M+N- with caveat that not all N alleles are detected (lab 10) <sup>c</sup>
Ss	S-, s not tested	23 ( <i>GYPB*S</i> , <i>GYPB*s</i> )	S-s+ (23)	
D	D-	25 ( <i>RHD</i> ) 23 ( <i>RHDψ</i> )	D- ( <i>RHD</i> and <i>RHDψ</i> detected (6); exon 5 negative and <i>RHDψ</i> detected (1); exon 5 negative (1); <i>RHDψ</i>	D+ ( <i>RHDψ</i> not tested; lab19)

			detected (15); <i>RHD</i> $\psi$ or <i>RHD</i> * <i>Dva</i> detected (1)	
Cc	C-c+	27 ( <i>RHCE</i> * <i>C</i> ) 29 ( <i>RHCE</i> * <i>c</i> )	C-c+ (27); c+ (2; <i>RHCE</i> * <i>C</i> not tested <sup>d</sup> )	
Ee	E-e+	29 ( <i>RHCE</i> * <i>E</i> ) 28 ( <i>RHCE</i> * <i>e</i> )	E-e+ (27); E- (2; <i>RHCE</i> * <i>e</i> not tested <sup>d</sup> ); e+ (1, <i>RHCE</i> * <i>E</i> not tested)	
Kk	K-, k not tested	29 ( <i>KEL</i> * <i>I</i> , <i>KEL</i> * <i>2</i> )	K-k+ (29)	
Duffy	Fy(a-b-)	28 ( <i>FY</i> * <i>A</i> , <i>FY</i> * <i>B</i> ) 27 ( <i>FY</i> * <i>BNull</i> )	Fy(a-b-) (28) <sup>g</sup>	
Kidd	Jk(a+b+)	28 ( <i>JK</i> * <i>A</i> <i>JK</i> * <i>B</i> )	Jk(a+b+) (28)	
Dombrock	not tested	20 ( <i>DO</i> * <i>A</i> , <i>DO</i> * <i>B</i> )	Do(a-b+) (19)	Do(a+b+) (lab 16)

<sup>a</sup>Numbers of laboratories obtaining each result are shown in parentheses; laboratory 26 tested samples A-D only

<sup>b</sup>Laboratory 18 tested samples E-H only for *GYP A*\**M* and *GYP A*\**N*

<sup>c</sup>Laboratory 10 offers *GYP A*\**M* typing only as not all *GYP A*\**N* alleles are detected

<sup>d</sup>Laboratory 15 only tested samples C, F, L for *RHCE*\**C* and *RHCE*\**e* as the assays could only be performed on *RHD*-negative samples

<sup>e</sup>Lab 22 did not test sample D

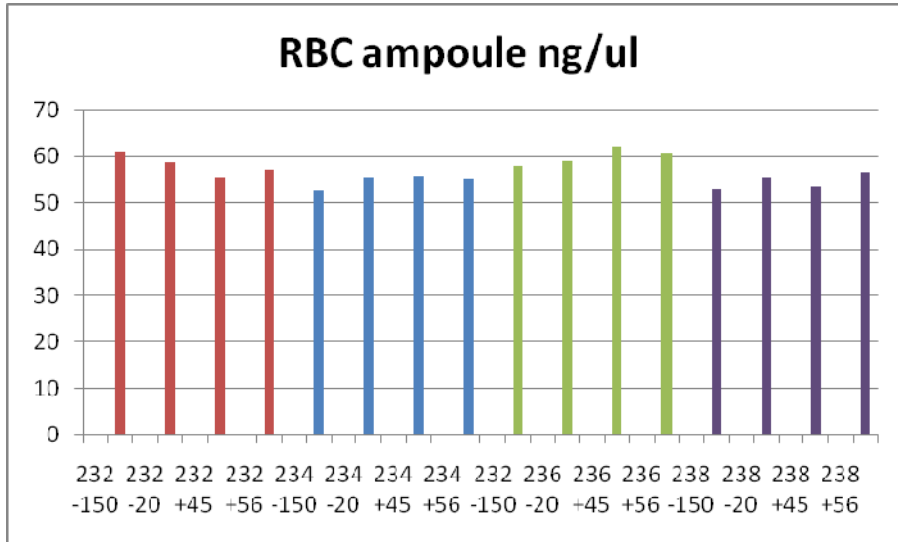
<sup>f</sup>Lab 14a did not test sample H for *FY*\**A*, *FY*\**B*

<sup>g</sup>Laboratory 21 reported detection of homozygous GATA mutation, hence phenotype is Fy(b-)

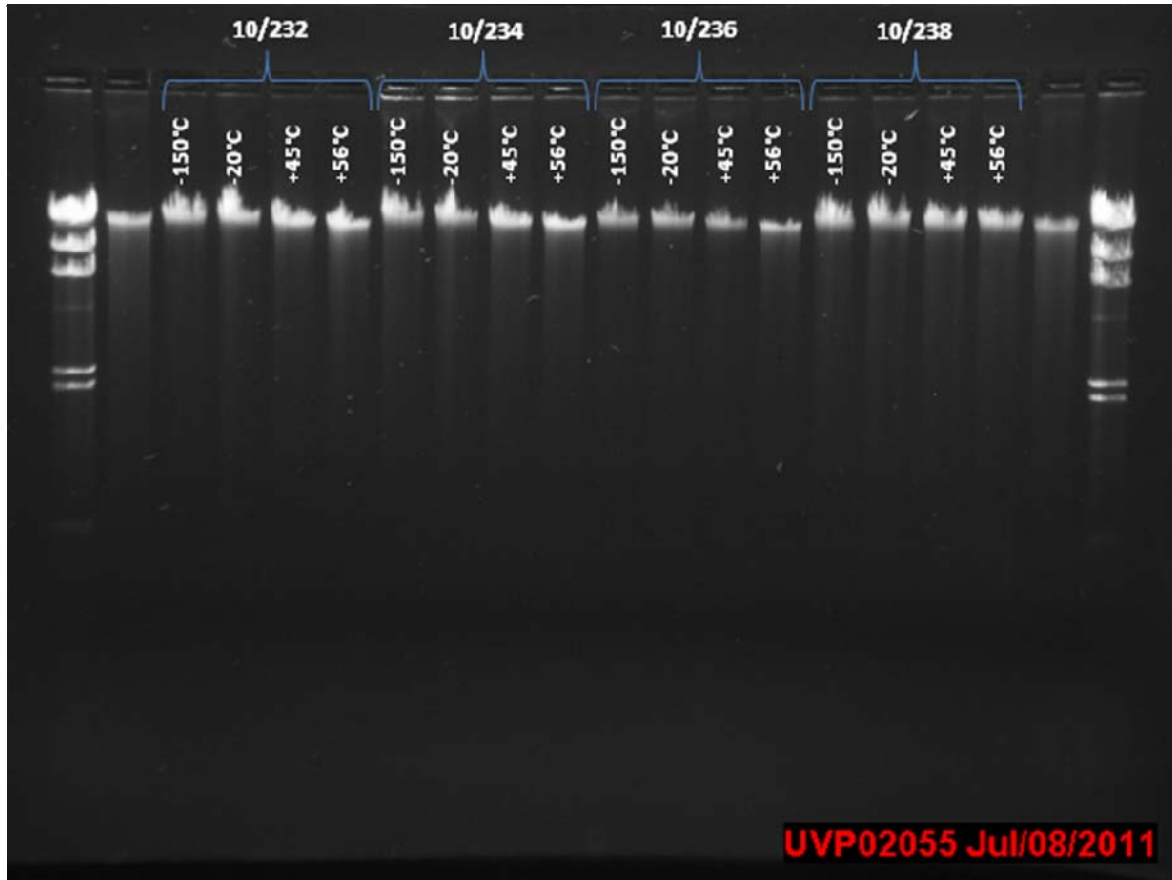
**Table 4 Cycle threshold (Ct) values from real-time QPCR**

<b>Preparation</b>	<b>Storage temperature °C</b>			
	<b>-150</b>	<b>-20</b>	<b>+45</b>	<b>+56</b>
10/232 (RBC 1)	20.00	19.92	20.34	20.69
10/234 (RBC 12)	20.13	20.02	20.37	20.61
10/236 (RBC 4)	20.68	20.73	20.68	21.18
10/238 (RBC 5)	20.27	20.23	20.22	20.33

**Figure 1 Nanodrop spectrophotometer readings of reconstituted contents of ampoules of RBC1 (232), RBC4 (236), RBC5 (238) and RBC12 (234) stored at -150°C, -20°C, +45°C and +56°C for 6 months**



**Figure 2** Agarose gel electrophoresis of RBC1 (10/232), RBC4 (10/236), RBC5 (10/238) and RBC12 (10/234) stored at -150°C, -20°C, +45°C and +56°C for 6 months



**Appendix 1 Participants (in alphabetical order of country)**

Natalie Cowley, Australian Red Cross Blood Service, Kelvin Grove, Queensland, Australia

Christof Jungbauer, Austrian Red Cross Blood Service for Vienna, Lower Austria and Burgenland, Vienna, Austria

Rita Fontão-Wendel, Blood Bank, Hospital Sírio Libanês, São Paulo, Brazil

Mariza Mota, Hospital Israelita Albert Einstein, São Paulo, Brazil

Lilian Castilho, Hemocentro-Unicamp, Campinas, Brazil

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## Appendix 2 Collaborative study protocol

### COLLABORATIVE STUDY TO EVALUATE LYOPHILISED DNA PREPARATIONS FOR THEIR SUITABILITY TO SERVE AS INTERNATIONAL (WHO) REFERENCE REAGENTS FOR BLOOD GROUP GENOTYPING (CS459)

#### COLLABORATIVE STUDY PROTOCOL

#### AIM

To evaluate lyophilised DNA preparations for their suitability to serve as International (WHO) Reference Reagents for blood group genotyping.

#### MATERIALS PROVIDED

12 ampoules of human DNA lyophilised in trehalose labelled as A-L. Store unopened ampoules at -20°C or below.

#### RECONSTITUTION

Do not open ampoules or reconstitute until the day of assay.

If necessary, tap the ampoules to ensure the lyophilised plug is visible at the bottom, and open according to the Instructions for Use (IFU) accompanying the materials. Reconstitute the contents of each ampoule with 50 µl deionized or distilled water according to the IFU. Check that the contents have dissolved completely before use. Following reconstitution, the concentration of DNA is ~50 ng/µl in 10 mM Tris 1 mM EDTA containing 15 µg/µl trehalose.

**Please take into account that these preparations contain material of human origin and apply all relevant safety procedures for use and subsequent disposal.**

#### METHODS

Use your usual method(s) for blood group genotyping, e.g., microarray and/or PCR kits.

#### ASSAY DESIGN

DAY 1: Reconstitute and analyse ampoules A-D for the genes listed on the results sheet.

DAY 2: Reconstitute and analyse ampoules E-H for the genes listed on the results sheet.

DAY 3: Reconstitute and analyse ampoules I-L for the genes listed on the results sheet.

Please enter the results on the assay results sheets provided.

Return the results to: Dr SJ Thorpe by 15<sup>th</sup> April 2011.

Tel: +44 (0)1707 641251; Fax: +44 (0)1707 641057; email: [susan.thorpe@nibsc.hpa.org.uk](mailto:susan.thorpe@nibsc.hpa.org.uk)

**Collaborative Study to assess lyophilised DNA preparations as candidate International Reference Reagents**

**ASSAY RESULTS SHEET DAY 1**

Name:

Laboratory:

Method:

Date:

**Please indicate the genes detected with X**

	Ampoule			
	A	B	C	D
<b>Genes</b>				
<i>GYPA</i> *M				
<i>GYPA</i> *N				
<i>GYPB</i> *S				
<i>GYPB</i> *s				
<i>RHD</i>				
<i>RHD</i> Ψ				
<i>RHCE</i> *C				
<i>RHCE</i> *c				
<i>RHCE</i> *E				
<i>RHCE</i> *e				
<i>KEL</i> *1				
<i>KEL</i> *2				
<i>FY</i> *A				
<i>FY</i> *B				
<i>FY</i> *BNull				
<i>JK</i> *A				
<i>JK</i> *B				
<i>DO</i> *A				
<i>DO</i> *B				

**Collaborative Study to assess lyophilised DNA preparations as candidate International Reference Reagents**

**ASSAY RESULTS SHEET DAY 2**

Name:

Laboratory:

Method:

Date:

**Please indicate the genes detected with X**

	Ampoule			
	E	F	G	H
<b>Genes</b>				
<i>GYPA</i> *M				
<i>GYPA</i> *N				
<i>GYPB</i> *S				
<i>GYPB</i> *s				
<i>RHD</i>				
<i>RHD</i> Ψ				
<i>RHCE</i> *C				
<i>RHCE</i> *c				
<i>RHCE</i> *E				
<i>RHCE</i> *e				
<i>KEL</i> *1				
<i>KEL</i> *2				
<i>FY</i> *A				
<i>FY</i> *B				
<i>FY</i> *BNull				
<i>JK</i> *A				
<i>JK</i> *B				
<i>DO</i> *A				
<i>DO</i> *B				

Collaborative Study to assess lyophilised DNA preparations as candidate International Reference Reagents

ASSAY RESULTS SHEET DAY 3

Name:

Laboratory:

Method:

Date:

Please indicate the genes detected with X

	Ampoule			
	I	J	K	L
<b>Genes</b>				
<i>GYPA</i> *M				
<i>GYPA</i> *N				
<i>GYPB</i> *S				
<i>GYPB</i> *s				
<i>RHD</i>				
<i>RHD</i> Ψ				
<i>RHCE</i> *C				
<i>RHCE</i> *c				
<i>RHCE</i> *E				
<i>RHCE</i> *e				
<i>KEL</i> *1				
<i>KEL</i> *2				
<i>FY</i> *A				
<i>FY</i> *B				
<i>FY</i> *BNull				
<i>JK</i> *A				
<i>JK</i> *B				
<i>DO</i> *A				
<i>DO</i> *B				

## Appendix 3 Draft Instructions for Use



International Ref. Reagent  
International Reference Reagents for blood group genotyping  
RBC1, RBC4, RBC5, RBC12  
NIBSC code: 10/232, 10/236, 10/238, 10/234  
Instructions for use  
(Version 1.00, Dated )

This material is not for in vitro diagnostic use.

### 1. INTENDED USE

Blood group genotyping.

### 2. CAUTION

This preparation is not for administration to humans.

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

N/A

### 4. CONTENTS

Country of origin of biological material: United Kingdom.  
Each ampoule contains 2.5 µg DNA and 0.75 mg trehalose. Upon reconstitution with 50 µl distilled or de-ionised water, the final concentration of DNA will be 50 ng/µl (2500 ng/ml) in 10 mM Tris 1 mM EDTA containing 15 µg/µl trehalose.  
The DNA was prepared from lymphoblastoid cell lines established from phenotyped and genotyped consenting blood donors.

### 5. STORAGE

Store unopened ampoules at -20°C or below.

### 6. DIRECTIONS FOR OPENING

DIN ampoules have an 'easy-open' coloured stress point, where the narrow ampoule stem joins the wider ampoule body.

Tap the ampoule gently to collect the material at the bottom (labeled) end. Ensure that the disposable ampoule safety breaker provided is pushed down on the stem of the ampoule and against the shoulder of the ampoule body. Hold the body of the ampoule in one hand and the disposable ampoule breaker covering the ampoule stem between the thumb and first finger of the other hand. Apply a bending force to open the ampoule at the coloured stress point, primarily using the hand holding the plastic collar.

Care should be taken to avoid cuts and projectile glass fragments that might enter the eyes, for example, by the use of suitable gloves and an eye shield. Take care that no material is lost from the ampoule and no glass falls into the ampoule. Within the ampoule is dry nitrogen gas at slightly less than atmospheric pressure. A new disposable ampoule breaker is provided with each DIN ampoule.

### 7. USE OF MATERIAL

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

Reconstitute with 50 µl distilled or deionised water.

Reagents RBC1, RBC4, RBC5 and RBC12 have been validated for blood group genotyping in an international collaborative study. The genotypes are shown in Table 1.

### 8. STABILITY

Reference materials are held at NIBSC within assured, temperature-controlled storage facilities. Reference Materials should be stored on receipt as indicated on the label.

NIBSC follows the policy of WHO with respect to its reference materials.

### 9. REFERENCES

N/A

### 10. ACKNOWLEDGEMENTS

These reagents were produced under the auspices of the International Society for Blood Transfusion in collaboration with Dr Geoff Daniels and Professor Stan Urbaniak.

### 11. FURTHER INFORMATION

Further information can be obtained as follows:

This material:

enquiries@nibsc.hpa.org.uk

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.who.int/biologicals/reference\\_preparations/en/](http://www.who.int/biologicals/reference_preparations/en/)

Ordering standards from NIBSC:

[http://www.nibsc.ac.uk/products/ordering\\_information/frequently\\_asked\\_questions.aspx](http://www.nibsc.ac.uk/products/ordering_information/frequently_asked_questions.aspx)

NIBSC Terms & Conditions:

[http://www.nibsc.ac.uk/terms\\_and\\_conditions.aspx](http://www.nibsc.ac.uk/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.hpa.org.uk

### 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

Physical and Chemical properties	
Physical appearance: lyophilisate	Corrosive: No
Stable: Yes	Oxidising: No
Hygroscopic: No	Irritant: Unknown
Flammable: No	Handling: See caution, Section 2
Other (specify): contains human DNA	
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.

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A World Health Organization Laboratory for Biological Standards





#### 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

Information provided by the Institute is given after the exercise of all reasonable care and skill in its compilation, preparation and issue, but it is provided without liability to the Recipient in its application and use.

It is the responsibility of the Recipient to determine the appropriateness of the standards or reference materials supplied by the Institute to the Recipient ("the Goods") for the proposed application and ensure that it has the necessary technical skills to determine that they are appropriate. Results obtained from the Goods are likely to be dependant on conditions of use by the Recipient and the variability of materials beyond the control of the Institute.

All warranties are excluded to the fullest extent permitted by law, including without limitation that the Goods are free from infectious agents or that the supply of Goods will not infringe any rights of any third party.

The Institute shall not be liable to the Recipient for any economic loss whether direct or indirect, which arise in connection with this agreement.

The total liability of the Institute in connection with this agreement, whether for negligence or breach of contract or otherwise, shall in no event exceed 120% of any price paid or payable by the Recipient for the supply of the Goods.

If any of the Goods supplied by the Institute should prove not to meet their specification when stored and used correctly (and provided that the Recipient has returned the Goods to the Institute together with written notification of such alleged defect within seven days of the time when the Recipient discovers or ought to have discovered the defect), the Institute shall either replace the Goods or, at its sole option, refund the handling charge provided that performance of either one of the above options shall constitute an entire discharge of the Institute's liability under this Condition.

#### 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: 0.0016 g  
Toxicity Statement: Toxicity not assessed  
Veterinary certificate or other statement if applicable.  
Attached: No

Table 1

+ indicates genes present; - indicates genes absent

	Reference Reagent			
	RBC1 (AR <sub>1</sub> R <sub>1</sub> )	RBC4 (OR <sub>1</sub> R <sub>1</sub> )	RBC5 (Brr)	RBC12 (O <sub>ψ</sub> D)
	10/232	10/236	10/238	10/234
<i>GYP</i> A*M	+	+	-	+
<i>GYP</i> A*N	-	+	+	+
<i>GYP</i> B*S	+	-	-	-
<i>GYP</i> B*s	+	+	+	+
<i>RHD</i>	+	+	-	-
<i>RHD</i> ψ	-	-	-	+
<i>RHCE</i> *C	+	+	-	-
<i>RHCE</i> *c	+	-	+	+
<i>RHCE</i> *E	+	-	-	-
<i>RHCE</i> *e	+	+	+	+
<i>KEL</i> *1	+	-	+	-
<i>KEL</i> *2	+	+	-	+
<i>FY</i> *A	-	-	+	-
<i>FY</i> *B	+	+	+	(+)
<i>FY</i> *BNull	-	-	-	+
<i>JK</i> *A	-	+	+	+
<i>JK</i> *B	+	+	-	+
<i>DO</i> *A	-	-	-	-
<i>DO</i> *B	+	+	+	+