Workshop on Molecular Blood Group Genotyping 2014
Report of pre-pilot exercise 14G1 (April 2014)

Introduction

Three whole blood samples were provided, representing samples from haemoglobinopathy patients, referred for genotyping to facilitate transfusion support. The samples were sourced from UK blood donors, and were not selected on any criteria other than a range of Rh phenotypes. Laboratories were requested to undertake red cell genotyping in the same way as for similar clinical samples, and report the genotype and predicted phenotype for D, Cc, Ee, MN, Ss, Kk, Fy^a, Fy^b, Fy, Jk^a, Jk^b, Do^a, Do^b using ISBT nomenclature. There was an accompanying questionnaire requesting information regarding the scope of routine practice and methods used. Participants were asked for feedback on the format of the exercise, and suggestions for development of a potential EQA Scheme. Initial findings were presented at ISBT Seoul in June 2014.

Participation

55 laboratories from 30 countries registered and 52/55 (95%) returned results, although not all reported a full set of genotypes and predicted phenotypes.

Questionnaire regarding clinical practice

Sections 1 – 7 are a summary of the data collected regarding routine clinical practice.

1. Scope of practice

Table 1 – categories of samples tested in clinical practice

Category of clinical samples tested	Number (%)
Patient samples (transfusion related)	49 (94%)
Patient samples (maternal / paternal)	29 (56%)
Cell free fetal DNA in maternal plasma (cffDNA)	21 (40%)
Donor samples (mass screening)	23 (44%)
Donor samples (blood grouping anomalies)	36 (69%)

- 3 do not test transfusion related patient samples
  - 2 pregnancy related samples only (i.e. maternal /paternal and cffDNA,)
  - 1 mass donor screening only.
- 19/29 (66%) of those testing maternal /paternal samples also undertake cffDNA testing
- 7 undertake testing on all categories of patient and donor samples
- 14 undertake all categories of patient testing, including cffDNA.
- 24 undertake all categories of patient testing, except for cffDNA

2. Testing platform

Table 2 –Platform(s) used for routine testing

Testing platform	Number of laboratories
Progenika BLOODChip	4
Progenika IDCORE+	3
Progenika IDCORE XT	6
HEA BeadChip	11
Inno-Train FluoGene	4
Inno-Train Ready-Gene	19
Sequenom MASSarray	4
BAG gene	7
Lifecode	1

- 30/52 (58%) have an in-house system
    - 21 in addition to at least one commercial platform
    - 9 for all testing (including 2 in process of installing commercial platforms)
    - 7 include sequencing, and 1 next generation sequencing for fetal samples

3. Extraction of DNA

- 37/52 extract DNA robotically (including 1 for cffDNA only), including:
    - 19 using Qiagen systems
    - 5 using Maxwell
    - 5 using Roche MagnaPure

4. Minimum sample volume required for DNA extraction

Table 3 – volume of whole blood required for DNA

Volume (microliters)	Number of laboratories
100	2
200	22
250	1
300	4
350	4
400	8
500	4
600	1
1000	4
1500	1
4000	1
Total	**52**

- No apparent correlation with platform(s) used, e.g.
    - Inno-Train Ready Gene (n=19): range 200 -1000 microlitres
    - HEA Beadchip (n=11): range 200 – 4000 microlitres

- No apparent correlation with robotic extraction of DNA:
    - Manual extraction (n=15): range 100 – 4000 microlitres
    - Robotic extraction (n=37): range 100 – 1000 microlitres

Volume of whole blood required for extraction of cffDNA

Table 4 – volume of whole blood required for cffDNA

Volume (microliters)	Number of laboratories
800	3
1000	6
1200	1
1300	1
2000	1
2500	1
3000	1
5000	1
6000	3
7500	1
20000	1
Not stated	1
Total	**21**

5. Reporting of clinical results

- 34 report both genotype and predicted phenotype to clinicians
- All of those undertaking mass screening of donors report predicted phenotype +/- genotype
- 7 report only the genotype:
    - 6 testing patient transfusion related samples (+/- antenatal and donor testing)
    - 1 testing only antenatal samples
- 9 report only a predicted phenotype:
    - All patient transfusion related testing (+/- antenatal and donor testing)
- 1 does not report clinical results (for research only), and 1 did not state a reporting method

6. Range of antigens reported in clinical practice

- Many different combinations are reported for both patient and donor testing, to some extent dependent on kit, but also on clinical demand
- Extra testing for patients include: C^w C^x VS; $Kp^a/^b$ $Js^a/^b$; U GPMur; $Di^a/^b$; Hy Jo^a; $Co^a/^b$; $Yt^a/^b$; and D variants
- Of those undertaking mass donor screening, four screen only for high frequency antigens and 2 only for D variants

Results of testing samples from 'Patients' 1, 2 and 3

The final three pages of this report show the genotype and predicted phenotype consensus results for each of the three samples (in ISBT terminology), and also the results reported by your laboratory.

Not all laboratories were able to report results for all of the antigens requested, and many supplied additional information. The majority of results were not reported using ISBT terminology making comparison with an expected response difficult.

However, our interpretation of the responses show that six laboratories reported eight results that did not fit with the consensus and these are summarised in Table 5:

Table 5 – errors in testing and / or reporting

Laboratory	Patient	Consensus Genotype	Consensus Predicted phenotype	Reported Genotype	Reported predicted phenotype
A	2	*RHCE*e/e*	E- e+	*RHCE*E/e*	E+ e+
B	3	*FY*01/02*	Fy(a+b+)	*FY*A*	Fy(a+b-)
C	3	*RHCE*c/c*	C- c+	*RHCE*01*	C+ c+
D	3	*RHCE*e/e*	E- e+	*RHe/RHe*	E- e-
E	3	*RHCE*e/e*	E- e+	*RHCE*cE/cE*	Not reported
F	3	*RHCE*c/c*	C- c+	*RHCE*ce, RHCE*Ce*	RH:2,4
F	3	*GYPA*M/N*	M+ N+	*GYPA*M*	MNS:1,-2
F	3	*GYPB*s/s*	S- s+	*GYPB*S, GYPB*s*	MNS:3,4

Four laboratories reported a single incorrect predicted phenotype, with two based on an incorrect genotype (coded A and B), and the other two were most likely due to error in reporting or interpretation as the correct genotype has been recorded (C and D). Laboratory E reported an incorrect genotype, but no predicted phenotype. One laboratory (F) appears to have reported the results for Patient 2 as Patient 3, resulting in three incorrect genotypes and predicted phenotypes.

Discussion of results

Terminology

The most diverse terminology was used to report CcEe, (see example in Table 6). This was caused to some extent by the format of the questionnaire, where results for Cc and Ee were requested separately, rather than as a combined result for CcEe. When reporting CcEe together, ISBT nomenclature allows the format *RHCE*ce/Ce* or a numerical format e.g. *RHCE*01/02* (where 01=ce, 02=Ce, 03=cE and 04=CE). However, the latter is easily confused with the phenotype reporting method RH:1,2,-3,4,5 (where 1=D 2=C 3=E 4=c 5=e).

Another common problem was incomplete reporting of homozygous genotypes, e.g. DO*B instead of DO*B/B, i.e. using the absence of *DO*A* to infer homozygosity for *DO*B*. When the alleles are heterozygous, e.g. JK*A/B, most laboratories reported this correctly.

There were many instances of genotypes being reported in ISBT (and other) terminology intended for reporting predicted phenotypes.

Table 6 - Cc genotyping results (n=46) reported for Patient 1 – result (number of labs)

ISBT terminology ***RHCE*c/c* or *RHCE*01/01 (or RHCE*ce/ce)***	RHCE*c/c or in combination with RHEe: RHCE*01/01 **(1)**
RHCE*c **(6)**	RHCE*01/01 **(1)**
RHCE*c/c **(2)**	RHCE*ce/ce **(1)**
RHCE*c/RHCE*c **(2)**	RHCE*01/RHCE*01 or RHCE*ce/RHCE*ce **(2)**
RHCE*01 **(2)**	RHCE*c/c **(1)**
RHCE*c/*c **(2)**	C-, c+ **(1)**
cc **(2)**	RHC- negative, RHc-positiv, HEX3: negative **(1)**
RHCE*01/*01 or RHCE*c/*c **(1)**	RH*-2,4 **(1)**
RHCE*01/RHCE*01.01 **(1)**	RH*04/04 **(1)**
RH004 **(1)**	RHCE*01 / 01.01 **(1)**
RHCE*c RHCE*ce/RHCE*ce **(1)**	RHCE*c/RHCE*c (RHCE*01/RHCE*01) **(1)**
c/c **(1)**	RH*04/RH*04 **(1)**
RH*04 **(1)**	RHCE*01/01 (RHCE*ce/ce) **(1)**
RHCE*04/04 **(1)**	Rh cc **(1)**
RH*c/c **(1)**	RHCE*4 **(1)**
c+ (unable to perform RhC as sample is D+) **(1)**	RHCE*c RHCE*c **(1)**
RHCE*cc **(1)**	RHc/RHc **(1)**
RHCE*01 (ie RHc) **(1)**	RHCE*01/RHCE*01 **(1)**

Reporting on the GATA mutation

There was considerable variation in reporting of the GATA mutation (see results for Patient 1 in Table 7)

Table 7 –Fy[a], Fy[b], Fy genotyping results (n=46) reported for Patient 1 – result (number of labs)

Including GATA mutation (n=9)	**GATA not reported (n=37)**
FY*01, FY*02, No Fy (GATA), No Fyx **(1)**	FY*01/02 **(9)**
FY*01/02 (no FY null, no FYX) **(1)**	FY*01/*02 **(4)**
FY*01/02, FY*01N.01 neg, FY*02N.01 neg, FY*02M.01 neg **(1)**	FY*01 (ie FY*A), FY*02 (ie FY*B) **(1)**
FY*01/*02 or FY*A/*B; FY*01N.01 neg; FY*02W.01 neg **(1)**	FY*01/FY*02 **(6)**
FY*01/FY*02 FyGATA neg **(1)**	FY*01, FY*02 **(1)**
FY*A/FY*B [FY*01N.01 not detected] **(1)**	FY*1,2 **(1)**
FY*A/FY*B, FY*-67T, FY*265C **(1)**	FY*1/*2 **(2)**
Fya+, Fyb+, Fyx- **(1)**	FY*1/2 **(1)**
FYA-positive, FYB-positive, FY-GATA-negative **(1)**	FY*A FY*B **(1)**
	FY*A, FY*B **(1)**
	FY*A,FY*B **(1)**
	FY*A/B **(4)**
	FY*A/FY*B **(1)**
	FY001 FY002 **(1)**
	FYA/FYB **(2)**
	Fya/Fyb **(1)**

Patient 2 RHD

43 laboratories reported a genotype and predicted phenotype for Patient 2. Of these, 32 reported the predicted phenotype as D positive, and 11 reported anomalous results (see comments in Table 8).

Table 8 – Free text comments on Patient 2 RhD

RH*01.DUC-3/01N D partial (DUC-3)
RHD*neg(1)-D(2-10)
RHD*D (variant D? unclear - Sequencing would be helpful)
RH001 (Rh D type is not reported). Patient 2 was positive for Exon-10 and Exon-5 and negative for Exon-1 however the phenotype was D positive.)
RH*var D (exon 1 - missing or point mutation, maybe RH*CE(1)-D(2-10); RH*01/01N.01 (RH*Dd)
"RHD*01"/RHD*01N.01 with "RHD*01" actually being an unknown hybrid RHCE(p-132)-D(i1[+18]-10)
Discrepant (Exons 5 + 10 detected, Exon 1 not detected)
RHD*01/RHD*01N.01 (unclear RHD allele) Genotyping revealed RHD allele with negative PCR-SSP for Promoter/Exon1 region (Inno-Train RHCDE: reaction 1). Sequencing of this region would be necessary for exact determination of RHD allele. Preliminary result: Donor RhD positive, recipient RhD negative
RHD exon 1: in RBC-Ready Gene, the RHD exon 1 reaction was negative; in RBC-FluoGene the RHD exon 1 reaction was weak positive. We assume a mutation in the primer binding site of the 3' primer of the respective reaction that affects the primer binding of RHD-exon 1 primers of Ready Gene more than FluoGene. Nevertheless, it seems that the whole RHD gene is present. This sample could be interesting for sequencing. For safety reasons we would recommend to declare such a sample to be RHD as a donor and RHd as a recipient.
Inconclusive required sequencing

On investigation at IBGRL Bristol, PCR amplification of RHD exon 1 in this sample was very poor. However, sequencing results suggest that there is a normal RHD exon 1 present, which fits with the normal serology observed. We would speculate that a mutation in one or other of the RHD primer sites is responsible for the poor amplification. This primer site mutation is difficult to prove due to the limited number of differences between the RHD and RHCE genes. It is also possible that this is a RHCE(1)-RHD(2-10) hybrid, and work is continuing at IBGRL Bristol to seek a resolution.

One laboratory reported the variant DUC-3 (which is RHD*48G>C). This is the only nucleotide difference between RHCE*C and RHD*D in exon 1, and it is possible RHCE*C has been amplified by the laboratory reporting this change.

Conclusions

- There is wide variation in technology used, the scope of testing and in the format for reporting red cell genotyping.
- The overall error was rate low. Three errors appear to have occurred during testing but it is not possible to say whether this is a failure of technology or user error. The remaining errors were due to interpretation (genotype to predicted phenotype) and procedure, i.e. possible transposition of sample results.
- A wide variety of terminology used, making transfer of results between centres difficult and potentially leading to misinterpretation in a clinical setting.
- An EQA Scheme will be useful with expanding clinical use of genotyping, but would need to be reformatted to collect results via ‘tickbox’ or dropdown standard responses, and allow registration for different levels of participation depending on scope of testing.

Exercise 14G1 individual results – Patient 1

Laboratory Code: For info. only

Antigens	Consensus Genotype (ISBT nomenclature)	Genotype Your result	Consensus Predicted phenotype (ISBT notation)	Predicted phenotype Your result
D	***RHD*01/01N.01***		**D +**	
Cc	***RHCE*c/c* or *RHCE*01/01 (or RHCE*ce/ce)***		**C- c+**	
Ee	***RHCE*e/e* or *RHCE*01/01 (or RHCE*ce/ce)***		**E- e+**	
MN	***GYPA*01/02 or GYPA*M/N***		**M+ N+**	
Ss	***GYPB*03/04 or GYPB*S/s***		**S+ s+**	
Kk	***KEL*02/02***		**K- k+**	
Fy^a, Fy^b, Fy	***FY*01/02, FyGATA neg***		**Fy(a+b+), Fy:-3**	
Jk^a Jk^b	***JK*01/02***		**Jk(a+b+)**	
Do^a Do^b	***Do*02/02***		**Do(a-b+)**	

Exercise 14G1 individual results – Patient 2

Laboratory Code: For info. only

Antigens	Consensus Genotype (ISBT nomenclature)	Genotype Your result	Consensus Predicted phenotype (ISBT notation)	Predicted phenotype Your result
D	***RHD*01 (possibly variant)***		**D positive***	
Cc	***RHCE*C/c* or *RHCE*01/02 (or RHCE*ce/Ce)***		**C+ c+***	
Ee	***RHCE*e/e* or *RHCE*01/02 (or RHCE*ce/Ce)***		**E- e+***	
MN	***GYPA*01/01 or GYPA*M/M***		**M+ N-**	
Ss	***GYPB*03/04 or GYPB*S/s***		**S+ s+**	
Kk	***KEL*02/02***		**K- k+**	
Fy^a, Fy^b, Fy	***FY*02/02, FyGATA neg***		**Fy(a-b+), Fy:-3**	
Jk^a Jk^b	***JK*01/01***		**Jk(a+b-)**	
Do^a Do^b	***Do*02/02***		**Do(a-b+)**	

***Also confirmed by serology**

Exercise 14G1 individual results – Patient 3

Laboratory Code: For info. only

Antigens	Consensus Genotype (ISBT nomenclature)	Genotype Your result	Consensus Predicted phenotype (ISBT notation)	Predicted phenotype Your result
D	***RHD*01N.01***		**D negative**	
Cc	***RHCE*c/c* or *RHCE*01/01 (or RHCE*ce/ce)***		**C- c+**	
Ee	***RHCE*e/e* or *RHCE*01/01 (or RHCE*ce/ce)***		**E- e+**	
MN	***GYPA*01/02 or GYPA*M/N***		**M+ N+**	
Ss	***GYPB*04/04 or GYPB*s/s***		**S- s+**	
Kk	***KEL*02/02***		**K- k+**	
Fy^a, Fy^b, Fy	***FY*01/02, FyGATA neg***		**Fy(a+b+), Fy:-3**	
Jk^a Jk^b	***JK*01/02***		**Jk(a+b+)**	
Do^a Do^b	***Do*02/02***		**Do(a-b+)**	