UK NEQAS
Haematology and Transfusion

UK NEQAS (BTLP)
PO Box 133
WATFORD
WD18 0WP
T: + 44 (0)1923 217933
F: + 44 (0)1923 217934
E: btlp@ukneqas.org.uk
W: www.ukneqasbtlp.org

Red cell genotyping pilot

Report of exercise 17/18G2
(distributed 4th September 2017)

Introduction

Two whole blood samples were provided, representing samples from haemoglobinopathy patients, referred for genotyping to facilitate transfusion support. Laboratories were requested to undertake red cell genotyping in the same way as for a similar clinical sample, and report the method used, and the genotype and predicted phenotype for D, Cc, Ee, MN, Ss, Kk, Fy^a, Fy^b, Fy, Jk^a, Jk^b, Do^a and Do^b. Additional questions were asked regarding the scope of testing and method of reporting in clinical practice.

Participation

The exercise was distributed to 43 laboratories in 23 different countries. 42/43 (97.7%) participants returned results, but not all centres reported a full set of genotypes and predicted phenotypes, presumably reflecting the testing that would be undertaken on a similar clinical sample.

Routine clinical practice in participating laboratories

Responses to the additional questions on clinical reporting of genotype and / or predicted phenotype are summarised in tables 1, 2 and 3.

Table 1 - How are genotyping results routinely translated to predicted phenotypes? n=42

Genotype translated to predicted phenotype	Number (%)
By the testing platform software	19 (45%)
Manually	17 (41%)
Using other IT	3 (7%)
Never report a predicted phenotype	3 (7%)

Table 2 - In clinical practice, how do results routinely get transferred for reporting? n=42

Results transferred for reporting	Number (%)
Transcribed manually to paper report	6 (14%)
Transcribed manually to an IT system	22 (53%)
Transmitted from testing platform via an electronic interface to an IT system[1]	14 (33%)

[1] In 10 of these the genotype is translated to predicted phenotype by the testing platform software or other IT

Table 3 - How do you routinely report results of patient testing in different clinical settings?

Format of results	Reference centre undertaking genotyping	Hospital transfusion lab	Clinician in haematology / transfusion	Another clinician managing the patient
Genotype and predicted phenotype	14	18	19	14
Genotype only	7	6	2	2
Predicted phenotype only	8	16	12	14
Not applicable / no response	13	2	9	12

Results

Six laboratories reported result(s) outwith consensus as detailed in Table 4; the anomalous elements are highlighted.

Table 4 – Laboratories with anomalous genotype and / or predicted phenotyping results

Laboratories with errors	Patient sample	Consensus genotype	Reported genotype	Consensus predicted phenotype	Reported predicted phenotype
A	2	*RHCE*C/C*	***RHCE*C/c***	C+ c-	**C+ c+**
A	2	*FY*01/01, GATA mutation not present*	***FY*01/02, GATA mutation not present***	Fy(a+b-)	**Fy(a+b+)**
A	2	*DO*01/01*	***DO*01/02***	Do(a+b-)	**Do(a+b+)**
B	2	*RHCE*C/C*	***RHCE*C/C***	C+ c-	**C- c+**
C	1	*FY*01/02, GATA mutation not present*	***FY*01/02*** ***Heterozygous for GATA mutation***	Fy(a+b+)	**Fy(a+b+)**
D	2	*The consensus of those testing for zygosity was RHD*01/01*	***RHD*01/01N.01***	D positive	**D positive**
E	1	*RHD*01N.01/01 N.01*	***RHD*01 (zygosity not determined)***	D negative	**D negative**
F	1	*KEL*02/02*	***KEL*01/01***	K- k+	**K+ k-**
F	1	*FY*01/02, GATA mutation not present*	***FY*01/01,*** ***Heterozygous for GATA mutation***	Fy(a+b+)	**Fy(a+b-)**
F	2	*KEL*02/02*	***KEL*01/01***	K- k+	**K+ k-**
F	2	*FY*01/01, GATA mutation not present*	***FY*01/02*** ***GATA mutation not present***	Fy(a+b-)	**Fy(a+b+)**

Report Comments

Two laboratories (coded A and F) made multiple errors, in which the incorrect genotype and corresponding predicted phenotype were reported, suggesting that the errors may have been in the testing. The remaining four laboratories each made a single error involving only the genotype or predicted phenotype.

Laboratory B reported a correct genotype for Patient 2 *(*RHCE*C/*C)*, but predicted the phenotype to be C-c+, possibly due to data entry error. Laboratory E may also have made a data entry error, as whilst an incorrect *RHD* genotype of *RHD*01* (zygosity not determined) was reported for Patient 1, the predicted phenotype matched the consensus (D negative).

Laboratory D reported Patient 2 as *RHD*01/01N.01*, predicted phenotype D positive, where the consensus *RHD* result for those reporting zygosity was *RHD*01/01*. Whilst there would not have been clinical consequences of this error in the scenario given in this exercise, errors in *RHD* zygosity testing are potentially significant in an antenatal setting.

Laboratory C, whilst correctly reporting the *FY* genotype as *FY*01/02*, incorrectly reported Patient 1 as heterozygous for the GATA mutation. A predicted phenotype of Fy(a+b+) was reported, and whilst this matches the consensus predicted phenotype, it is inconsistent with the reported genotyping results.

The effect of a 'heterozygous GATA mutation' on the expression of the Fy^b antigen on the red cell surface depends on the *FY*01* and *FY*02* status. The mutation that encodes for changes in a promoter region preventing the transcription factor GATA-1 from binding to facilitate the transcription of the *FY* gene, is almost always associated with *FY*02* rather than *FY*01.* Therefore, if an individual (as erroneously reported by Laboratory C for Patient 1) is heterozygous for the 'GATA mutation' and both the *FY*01* and *FY*02* genes are present, the mutation is highly likely to be associated with *FY*02* and consequently Fy^b will not be expressed on the red cells. The presence of a 'heterozygous GATA mutation' where there are two copies of the *FY*02* gene, would mean that only one would be affected and that Fy^b would still be expressed on the red cells. Laboratory F erroneously reported Patient 1 as *FY*01/01*, heterozygous for the GATA mutation. This is an unlikely genotype since an *FY*01* allele containing the GATA mutation is extremely rare.

Some of the errors seen might have occurred during data entry to SurveyMonkey, but there is potential for similar errors to occur in clinical practice where results are interpreted and transcribed manually. Only 10/42 (24%) laboratories recorded that they had automated both the translation of genotypes to predicted phenotypes and the transfer of results for reporting.

34/42 (81%) laboratories reported all results using ISBT terminology and did not indicate that any alternative terminology would be reported to clinicians. The eight laboratories that did report alternative terminology either as an EQA result or to clinicians all returned results that could have been expressed in ISBT terminology. In some cases the alternative terminology was misleading, e.g. 'd' or 'dd' to denote D negative.

Exercise 17/18G2 individual results

Laboratory Code: Gxxxx

Result(s) outwith consensus: No

Tables 5 and 6 show the consensus result for genotype and predicted phenotype for 17/18G2 Patients 1 and 2 respectively, and the results reported by your laboratory.

Table 5: Your results for Patient 1 compared to the consensus results

Antigens	Consensus results		Your results				
	Genotype	Predicted phenotype	**Genotype**	**Specify 'other' genotype[1]**	**Predicted phenotype**	**Specify 'other' phenotype**	**Other terminology used for reporting to clinicians[1]**
D	*RHD*01N.01/01N.01*	D negative	***RHD*01N.01/01N.01***		**D negative**		
Cc	*RHCE*c/c*	C- c+	***RHCE*c/c***		**C- c+**		
Ee	*RHCE*e/e*	E- e+	***RHCE*e/e***		**E- e+**		
MN	*GYPA*01/02*	M+ N+	***GYPA*01/02***		**M+ N+**		
Ss	*GYPB*03/04*	S+ s+	***GYPB*03/04***		**S+ s+**		
Kk	*KEL*02/02*	K- k+	***KEL*02/02***		**K- k+**		
Fy^a, Fy^b, Fy	*FY*01/02, GATA mutation not present*	Fy(a+b+)	***FY*01/02, GATA mutation not present***		**Fy(a+b+)**		
Jk^a Jk^b	*JK*01/01*	Jk(a+b-)	***JK*01/01***		**Jk(a+b-)**		
Do^a Do^b	*DO*01/02*	Do(a+b+)	***DO*01/02***		**Do(a+b+)**		

[1]These responses have been inserted directly from free text reported through SurveyMonkey, and it has not been possible to collect genotypes in italics.

Laboratory Code: Gxxxx

Table 6: Your results for Patient 2, compared to the consensus results

Antigens	Consensus results		Your results				
	Genotype	Predicted phenotype	**Genotype**	**Specify 'other' genotype[1]**	**Predicted phenotype**	**Specify 'other' phenotype**	**Other terminology reported to clinicians[1]**
D	*RHD*01 (zygosity not determined)[2]*	D positive	***RHD*01/01***		**D positive**		
Cc	*RHCE*C/C*	C+ c-	***RHCE*C/C***		**C+ c-**		
Ee	*RHCE*e/e*	E- e+	***RHCE*e/e***		**E- e+**		
MN	*GYPA*01/02*	M+ N+	***GYPA*01/02***		**M+ N+**		
Ss	*GYPB*03/04*	S+ s+	***GYPB*03/04***		**S+ s+**		
Kk	*KEL*02/02*	K- k+	***KEL*02/02***		**K- k+**		
Fy^a, Fy^b, Fy	*FY*01/01, GATA mutation not present*	Fy(a+b-)	***FY*01/01, GATA mutation not present***		**Fy(a+b-)**		
Jk^a Jk^b	*JK*01/01*	Jk(a+b-)	***JK*01/01***		**Jk(a+b-)**		
Do^a Do^b	*DO*01/01*	Do(a+b-)	***DO*01/01***		**Do(a+b-)**		

[1] These responses have been inserted directly from free text reported through SurveyMonkey, and it has not been possible to collect genotypes in italics.

[2] **The consensus of those testing for zygosity was *RHD*01/01***