The reliability of A_1 phenotyping results in an External Quality Assessment (EQA) programme supporting ABO incompatible renal transplantation

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Introduction

Most individuals who type as A_1 negative are of the A_2 phenotype which differs from the A_1 phenotype both qualitatively and quantitatively. It is recognised that some individuals cannot be defined as A_1 or A_2; some will be of rare subgroups such as A_3 and A_4, and some fall into what appears to be an intermediate category A_{int}[1].

A_1 phenotyping is currently an unscored part of the UK NEQAS BTLP ABO titration EQA programme, which supports ABO-incompatible organ transplantation. Obtaining the correct A_1 typing result is important in selection of an ABO-mismatched renal transplant donor to minimise the risk of rejection associated with ABO-incompatibility. Material for the UK NEQAS exercises is prepared from leucodepleted red cell donations previously typed as either A_1 positive or A_1 negative by the supplier. Sometimes, material is used to prepare more than one EQA sample, but this is not known by the participants and therefore should not influence the results reported.

Method

Analysis of A_1 typing results was undertaken for 14 EQA exercises, each containing three samples. The correct result for each sample is based on the consensus.

Results

In total, 42 samples made from 30 pools of material were distributed, and 3437 A_1 typing results analysed.

Twenty-five samples (from 19 pools) had a consensus result of A_1 positive (matching the material supplier's designation), and in all cases >90% of laboratories achieved the consensus; these results are shown in Table 1.

Table 1 – Consensus result A_1 positive

Exercise	Sample	Nº reported A_1 positive (%)	Nº reported A_1 negative (%)	Nº reported A_1 not determined (%)
1920ABOT3	Y	79 (97.5)	2 (2.5)	0
	Z	80 (98.8)	1 (1.2)	0
1920ABOT4	Z	72 (94.7)	3 (3.9)	1 (1.3)
20ABOT2	W	76 (97.4)	2 (2.6)	0
20ABOT3	W	79 (100)	0	0
20ABOT4	W	79 (100)	0	0
	Y*	79 (100)	0	0
	Z*	71 (100)	0	0
21ABOT1	Y*	80 (98.8)	1 (1.2)	0
	Z*	80 (98.8)	1 (1.2)	0
21ABOT2	W	84 (100)	0	0
21ABOT3	W*	83 (100)	0	0
	Y*	83 (100)	0	0
21ABOT4	W	85 (98.8)	0	1 (1.2)
	Y*	85 (98.8)	0	1 (1.2)
	Z*	85 (98.8)	0	1 (1.2)
22ABOT1	W	83 (100)	0	0
22ABOT2	W	79 (95.2)	0	4 (4.8)
22ABOT2	Y	80 (96.4)	2 (2.4)	1 (1.2)
22ABOT3	Y*	82 (98.8)	1 (1.2)	0
	Z*	82 (98.8)	1 (1.2)	0
22ABOT4	W	83 (97.6)	1 (1.2)	1 (1.2)
23ABOT1	W	87 (98.9)	1 (1.1)	0
	Y*	87 (98.9)	1 (1.1)	0
	Z*	86 (97.7)	2 (2.3)	0
	Overall	2029 (98.6)	19 (0.9)	10 (0.5)

*Material in the same exercise is from the same pool

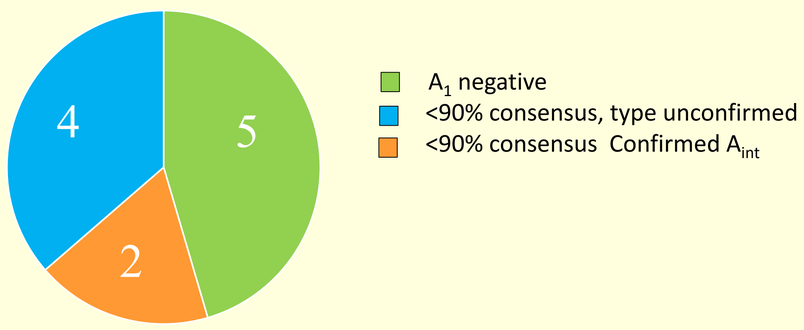
Seventeen samples (from 11 pools) had a consensus result of A_1 negative, matching the material supplier's designation, (see Table 2). In 9/17 (52.9%) samples >90% of laboratories achieved the consensus. In 8/17 (47.1%) samples <90% of laboratories achieved the consensus (range 51.8% to 87.7% - highlighted yellow in the table). Two pools of material (making up three of the eight samples) were confirmed by the International Blood Group Reference Laboratory as the A_{int} phenotype (text red in the table): 22ABOT1 Samples Y and Z (negative consensus 51.8% and 53.0% respectively), and 22ABOT2 Sample Z (negative consensus 73.2%). The other samples were not sent for further testing and were reported by UK NEQAS as '*possibly of the A_{int} phenotype*'.

Table 2 – Consensus result A_1 negative

Exercise	Sample	Nº reported A_1 positive (%)	Nº reported A_1 negative (%)	Nº reported A_1 not determined (%)
1920ABOT3	W	6 (7.4)	70 (86.4)	5 (6.2)
1920ABOT4	W*	2 (2.7)	73 (97.3)	0
	Y*	3 (3.9)	73 (96.1)	0
20ABOT2	Y*	0	78 (100)	0
	Z*	2 (2.6)	76 (97.4)	0
20ABOT3	Y*	0	79 (100)	0
	Z*	1 (1.3)	78 (98.7)	0
21ABOT1	W	7 (8.6)	71 (87.7)	3 (3.7)
21ABOT2	Y*	15 (17.9)	59 (70.2)	10 (11.9)
	Z*	18 (21.4)	57 (67.9)	9 (10.7)
21ABOT3	Z	3 (3.6)	80 (96.4)	0
22ABOT1	Y*	29 (34.9)	43 (51.8)	11 (13.3)
	Z*	28 (33.7)	44 (53.0)	11 (13.3)
22ABOT2	Z	17 (20.7)	60 (73.2)	5 (6.1)
22ABOT3	W	8 (9.6)	71 (85.5)	4 (4.8)
22ABOT4	Y*	3 (3.5)	79 (92.9)	3 (3.5)
	Z*	3 (3.5)	79 (92.9)	3 (3.5)
	Overall	145 (10.5)	1170 (84.8)	64 (4.6)
	>90 consensus	17 (2.4)	695 (96.8)	6 (0.8)
	<90% consensus	128 (19.4)	475 (71.8)	58 (8.8)

*Material in the same exercise is from the same pool

Figure 1. Results of pools supplied as A_1 negative



2/11 (18.2%) pools of material identified by the material supplier as A_1 were confirmed to be A_{int}. Four other pools had a <90% consensus of A_1 negative but were not tested further (see Figure 1).

Conclusion / Discussion

When testing an organ donor there are risks associated with incorrect A_1 results. False negative results could increase the risk of rejection due to ABO-incompatibility. False positive results present a lesser but still significant risk of a suitable organ not being utilised for transplant causing delay to the intended recipient.

The A_{int} phenotype does not produce reliable results with different A_1 reagents, and positive reactions did not appear to be associated with a particular reagent. It is not known what affect this phenotype has on ABO-incompatible organ transplantation.

Commercial anti-A_1 reagents are usually prepared from Dolichos biflorus lectin. In its raw form this agglutinates cells of A_1 and A_2 phenotypes, but at a suitable dilution it will not react with A_2 cells. The use of lectin reagents instead of a true anti-A_1 antibody, may be the reason for a higher rate of false positive than false negative results seen in the UK NEQAS exercises. It is worth noting that samples created from the same pool of material did not always show identical distribution of results, demonstrating that some laboratories did not get the same result for two identical samples when using the same reagent.

[1] Daniels G. Human blood groups. 2nd ed. Oxford (GB): Blackwell Scientific Ltd; 2002.