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Haematology and Transfusion

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A UK NATIONAL EXTERNAL QUALITY ASSESSMENT SERVICE (UK NEQAS) PILOT SCHEME FOR THE DIRECT ANTIGLOBULIN TEST (DAT) – AN ASSESSMENT OF SENSITIVITY BY TECHNOLOGY

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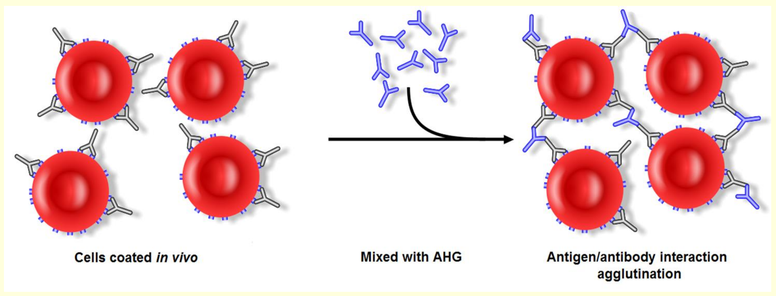
Background and Aims

Between July 2015 and January 2017, UK NEQAS (BTLP) sent twelve red cell samples to participants in the DAT pilot, with the intention of establishing a full EQA scheme. These samples comprised of four negatives (uncoated), one C3d coated and seven IgG coated sets of cells, with varying reaction grades between weak to 4+. Four of the samples coated with IgG had reaction grades of 2+, and data relating to these four samples has been collated for this study.

The aim of the study was to assess the sensitivity of the DAT by technology for the four IgG coated samples with an intended reaction grade of 2+ vs. anti-IgG.

Figure 1. Schematic of red cells coated with antibody, showing the principal of the DAT.



Methods

Participants were asked to process the DAT samples, using the same technique as they would for a similar clinical sample, within seven days of issue. Participants were asked to provide their observed reaction grades and specify the technology used for each sample, via an online SurveyMonkey questionnaire. The data from 200 UK and Republic of Ireland laboratories was collated and analysed for reported reaction grades vs. polyspecific anti-human globulin (AHG) and anti-IgG by each technology, where available. Mixed field reactions, transcription or transposition errors, and where details of technologies were not provided were excluded from the analysis. Technologies with less than 12 users were also not included in the comparison. Overall, 792 sets of results with reaction grades were included in the analysis.

Results

The overall median reaction grade vs. polyspecific AHG and anti-IgG was 2+ for all 4 samples.

Table 1 and figure 2 show reported reaction grades tested vs. polyspecific AHG by technology. Table 2 shows the number of reported reaction grades stronger, weaker and the same as the median (2+) vs. polyspecific AHG by technology.

Table 1. Reaction grades reported vs. polyspecific AHG by technique (n=306).

	Reaction grades vs polyspecific AHG (%)					
Technique	**4+**	**3+**	**2+**	**1+**	**Weak**	**Negative**
BioRad (n=206)	4 (1.9)	47 (22.8)	136 (66.0)	18 (8.7)	1 (0.5)	0 (0)
Ortho (n=58)	1 (1.7)	4 (6.9)	22 (37.9)	18 (31.0)	10 (17.2)	3 (5.2)
Grifols (n=26)	0 (0)	13 (50.0)	12 (46.2)	1 (3.8)	0 (0)	0 (0)
Tube (n=18)	1 (5.6)	2 (11.1)	9 (50.0)	4 (22.2)	1 (5.6)	1 (5.6)

Figure 2. Percentage of reaction grades reported vs. polyspecific AHG by technique (n=306).

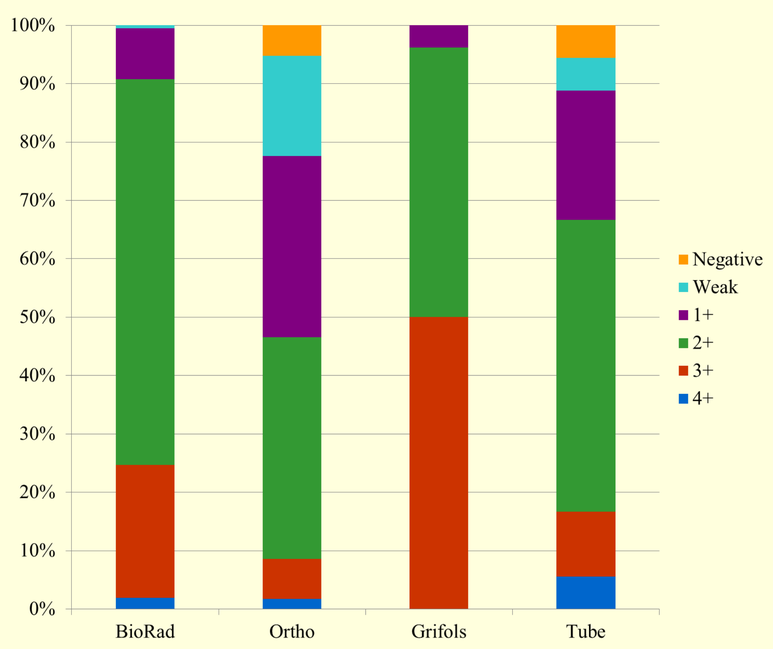


Table 2. Number of reaction grades stronger, weaker and the same as the median (2+) versus polyspecific AHG by technology.

	Reaction grades vs. polyspecific AHG (%)		
Technique	**Stronger**	**2+ (median)**	**Weaker**
BioRad (n=206)	51(24.8)	136(66.0)	19(9.2)
Ortho (n=58)	5(8.6)	22(37.9)	31(53.4)
Grifols (n=26)	13(50.0)	12(46.2)	1(3.8)
Tube (n=18)	3(16.7)	9(50.0)	6(33.3)

When tested vs. polyspecific AHG, 24.8% and 50.0% of reported reaction grades in BioRad (n=206) and Grifols (n=26) respectively, were stronger than the median, whilst 9.2% and 3.8% respectively, were weaker. Conversely, 52.6% and 29.4% of reported reaction grades in Ortho (n=58) and tube (n=18) respectively were weaker than the median, whilst 8.8% and 17.6% were stronger vs. polyspecific AHG.

Table 3 and figure 3 show reported reaction grades tested vs. anti-IgG by technology. Table 4 shows the number of reported reaction grades stronger, weaker and the same as the median (2+) versus anti-IgG by technology.

Table 3. Reaction grades reported vs. anti-IgG by technique (n=471).

	Reaction grades vs anti-IgG AHG (%)					
Technique	**4+**	**3+**	**2+**	**1+**	**Weak**	**Negative**
BioRad (n=303)	4 (1.3)	78 (25.7)	192 (63.4)	24 (7.9)	4 (1.3)	1 (0.3)
Ortho (n=124)	3 (2.4)	10 (8.1)	48 (38.7)	40 (32.3)	18 (14.5)	5 (4.0)
Grifols (n=26)	0 (0)	11 (42.3)	13 (50.0)	2 (7.7)	0 (0)	0 (0)
Tube (n=19)	0 (0)	2 (10.5)	9 (47.4)	7 (36.8)	1 (5.3)	0 (0)

Figure 3. Percentage of reaction grades reported vs. anti-IgG by technique (n=306).

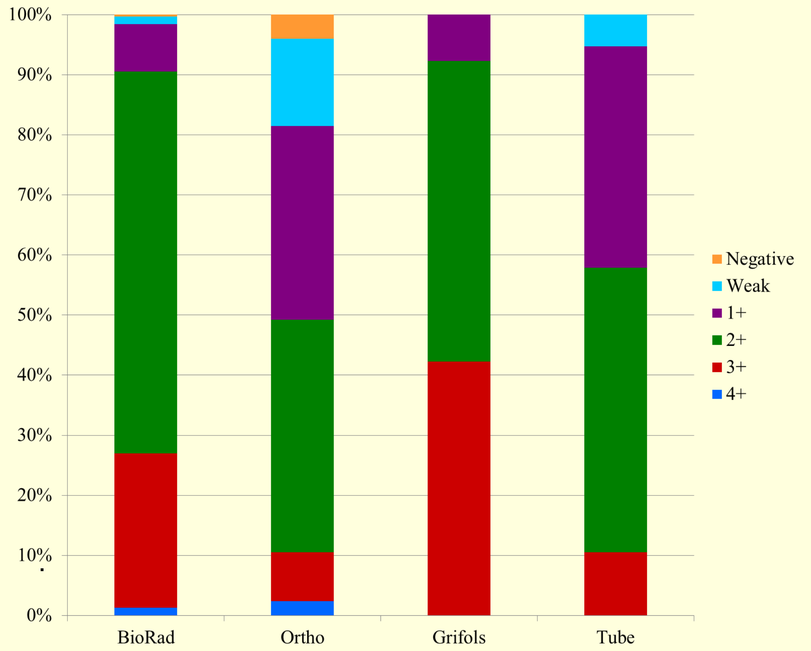


Table 4. Number of reaction grades stronger, weaker and the same as the median (2+) versus anti-IgG by technology.

	Reaction grades vs. anti-IgG AHG (%)		
Technique	**Stronger**	**2+ (median)**	**Weaker**
BioRad (n=303)	82(27.1)	192(63.4)	29(9.6)
Ortho (n=124)	13(10.5)	48(38.7)	63(50.8)
Grifols (n=26)	11(42.3)	13(50)	2(7.7)
Tube (n=19)	2(10.5)	9(47.4)	8(42.1)

A similar picture was noted with anti-IgG, with 27.2% and 42.3% of reported reaction grades in BioRad (n=303) and Grifols (n=26) respectively were stronger than the median, and 9.3% and 7.7% respectively were weaker. Conversely, 50.0% and 42.1% of reported reaction grades in Ortho (n=124) and tube (n=19) respectively, were weaker than the median, whilst 10.7% and 10.5% respectively were stronger. This data includes 8 false negative reactions in Ortho (by 3 participants), 1 in BioRad, 1 in tube and none in Grifols.

Conclusions

When compared to the median reaction grade of 2+, the data showed that a majority of BioRad and Grifols users reported the grade as same or stronger vs. both polyspecific AHG and anti-IgG, and that a majority of Ortho and tube users reported the reaction grade as the same or weaker than the median. The majority of the false negative reactions occurred in Ortho, although six of these were reported by one participant. From this data, we conclude that BioRad and Grifols technologies demonstrate the greatest level of sensitivity for detecting a 2+ DAT (coated with IgG), and Ortho and tube a lower level of sensitivity. A false negative DAT can have an impact upon the diagnosis of clinical conditions, such as HDFN and acute HTR. Conversely, false positive reactions can cause a delay in patient care while extra unnecessary investigations are performed. Future work will also examine false positive reactions obtained through the pilot study.

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