Observations for UK NEQAS (BTLP) data

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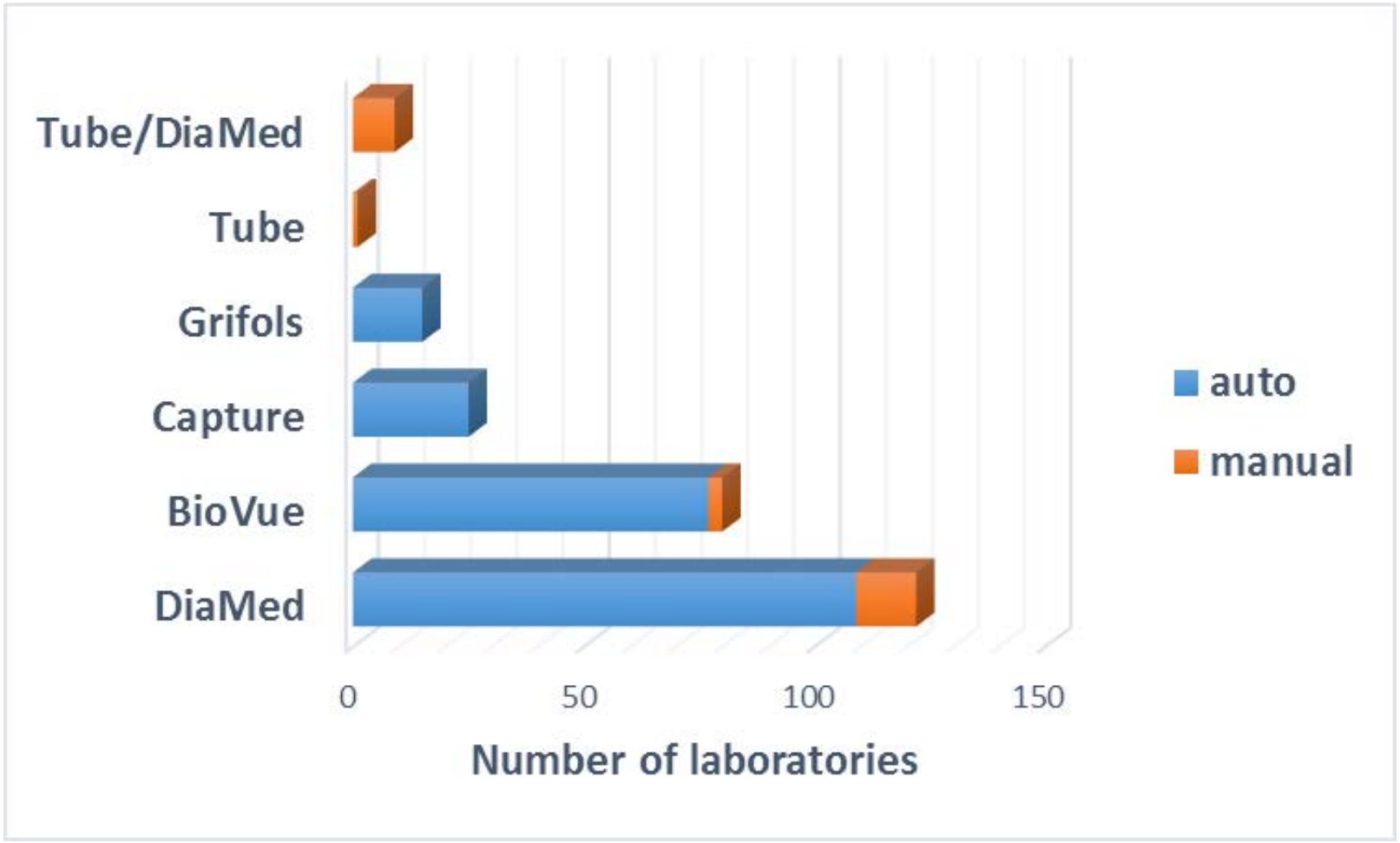
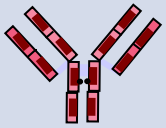
UK NEQAS Q data 2016
Technology used for routine G+S



Inherent differences in IAT technology

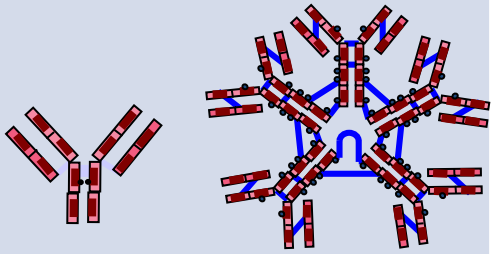
Solid phase

- Easy to automate
- Adherence of cell membranes to wells, so no agglutination phase, so detects IgG only
- May detect ‘enzyme only antibodies’



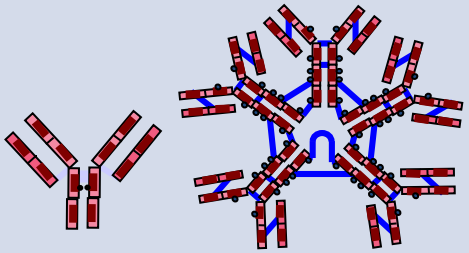
CAT

- Easy to automate
- Agglutination phase, so some IgM detected even if anti-IgG AHG used

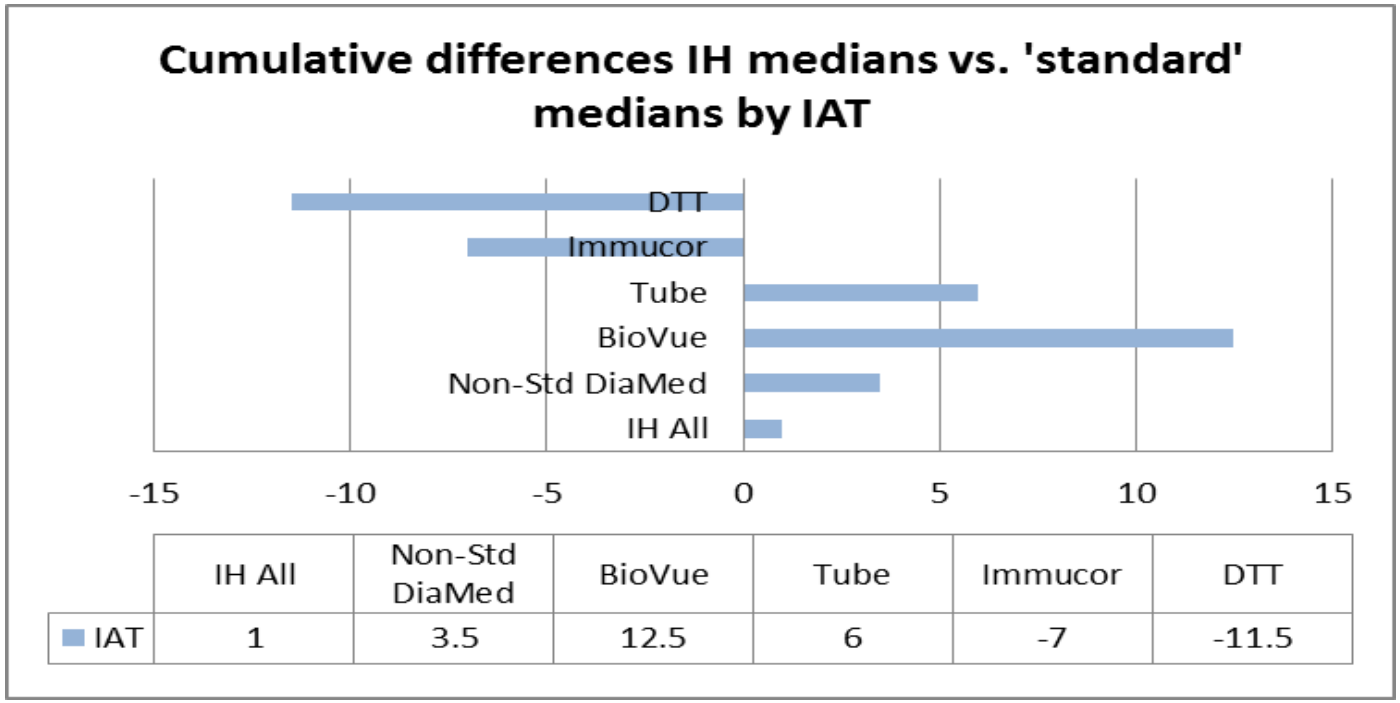


Tube

- Difficult to automate
- Agglutination phase, so some IgM detected even if anti-IgG AHG used



Antibody titration – anti-A
UK NEQAS ABOT pilot 2014/15



	IH All	Non-Std DiaMed	BioVue	Tube	Immucor	DTT
IAT	1	3.5	12.5	6	-7	-11.5

- Measurement of IgG only can be useful – no need to DTT treat
- More difficult to compare to other technologies
- BioVue at opposite extreme

Anti-E+C^{w} Exercise 15E5 P2

- Anti-E only 5.32% n=(19)
  - 15/19 using Immucor Capture R

In-house testing 15E5P2

Results - Ready ID

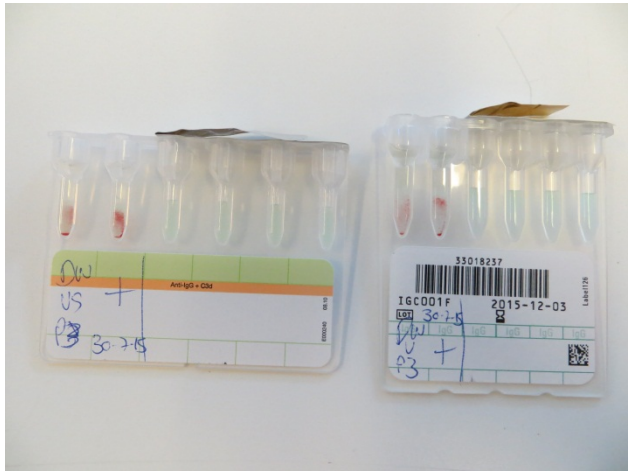
Sample ID	Interp.	Flags	R-ID 1	R-ID 2	R-ID 3	R-ID 4	R-ID 5	R-ID 6	R-ID 7	R-ID 8
15E5P2	Complete	*	3+	0	3+	0	0	3+	0	0
										
Sample ID	**Interp.**	**Flags**	**R-ID 9**	**R-ID 10**	**R-ID 11**	**R-ID 12**	**R-ID 13**	**R-ID 14**	**Pos Ctrl**	**Neg Ctrl**
15E5P2	Complete	*	0	0	0	0	0	0	4+	0
										

- Not scored - no requirement for Kp(a+) or Cw(+) on screening panel
- IgM only antibodies not usually clinically significant

ABO antibodies in XM 15R7

Expected results

	Donor W (A)	Donor Y (A)	Donor Z (A)
Patient 3 (B)	I	I	I



- 5 labs missed incompatibility P3 vs. DW – serological XM
  - 3 positive on repeat (CAT)
  - 2 using Capture – still negative on repeat
- IT systems would have detected ABOi, but if IT down need RT XM

2/2 Capture Laboratories performing serological XM

	Donor W (A_{2*})	Donor Y (A_1)	Donor Z (A_1)
Patient 3 (B)	0	4	4

* Negative vs. anti-A_1

Enzyme non-specific (ENS) antibody

Exercise	Antibody	Titre of specific antibody (Tube IAT)	Acceptable responses	% reporting ENS
14E8	Anti-S+ENS	8 vs. Ss cells	Anti-S, Anti-S+ENS	42%

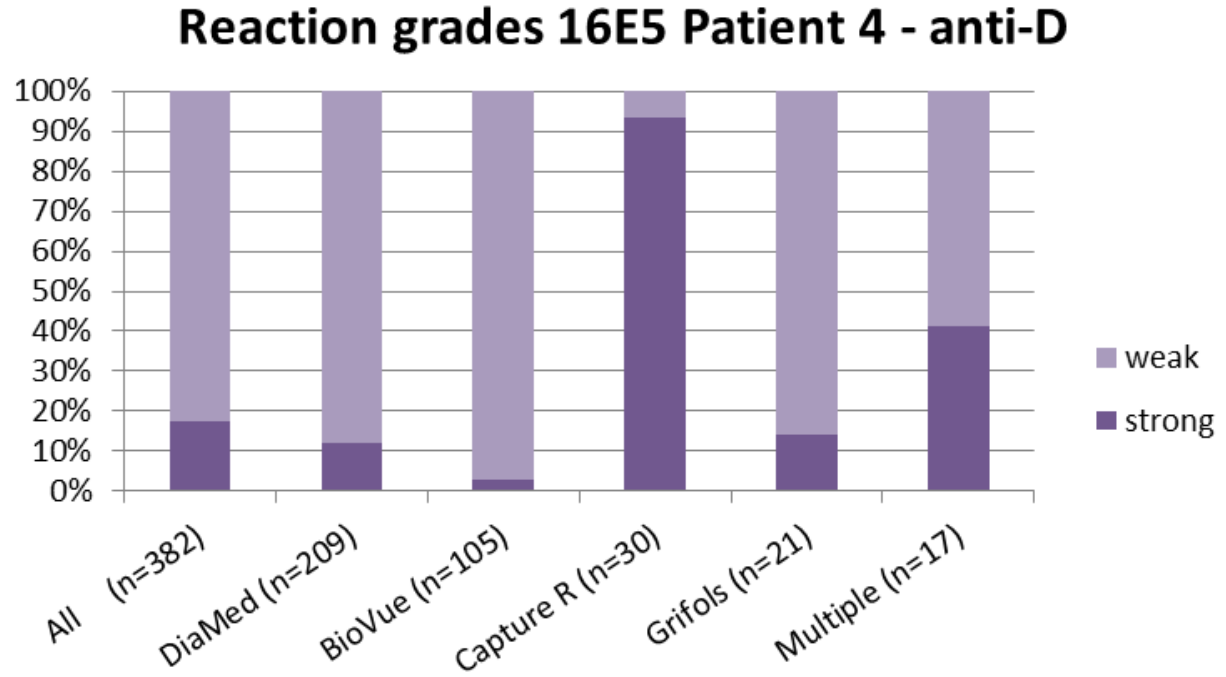
- UK NEQAS in house testing
  - Anti-S 4+ reactions
  - ENS (+/- reactions) throughout by Capture
- Reports of stronger reactions in some labs making it difficult to identify anti-S with Capture alone.

Non-specific antibodies reacting by IAT can make it difficult to identify or exclude other clinically significant antibodies

Detection of weak examples of clinically significant antibodies, e.g. anti-Jk^{a} useful

Sensitivity - UK NEQAS ‘standard’ anti-D

All UK laboratories detected anti-D:



Diluents and sensitivity

- 10R4 anti-s and anti-S (weak antibodies)
- No correlation XM errors with technology overall, but some DiaMed users reported positive reactions with Diluent 2 when missed originally in CellStab
- DiaMed recommend Diluent 2

- 11R4 Anti-E missed in XM by 19 BioVue users
- 8/9 contacted used addition 3-5% cells + BLISS
- Ortho validated methods for addition (BLISS) and suspension (0.8% diluent)
- Ortho recommend 0.8% diluent

? Similar problem in 15R1

Crossmatch weak anti-Jk^a vs. Jk(a+b+)

- 22% of BioVue users reported a false negative IAT *cf.* 1% of DiaMed users
- No correlation automation vs. manual (BioVue)
- 1+ reaction obtained during in-house at closing using manual BioVue with a 0.8% diluent.

Choice of IAT technology BCSH guidelines 2012

5.2. Choice of IAT technology

5.2.1. Automated and manual techniques for antibody screening vary in sensitivity and specificity, and should be evaluated in consideration of local requirements.

5.2.2. A low ionic strength solution (LISS) IAT is considered to be the most suitable for the detection of clinically significant antibodies because of its speed, sensitivity and specificity. **Different technologies (e.g. column agglutination, solid-phase) have *different strengths and weaknesses* and should be *subject to local validation* before their introduction into routine use.**

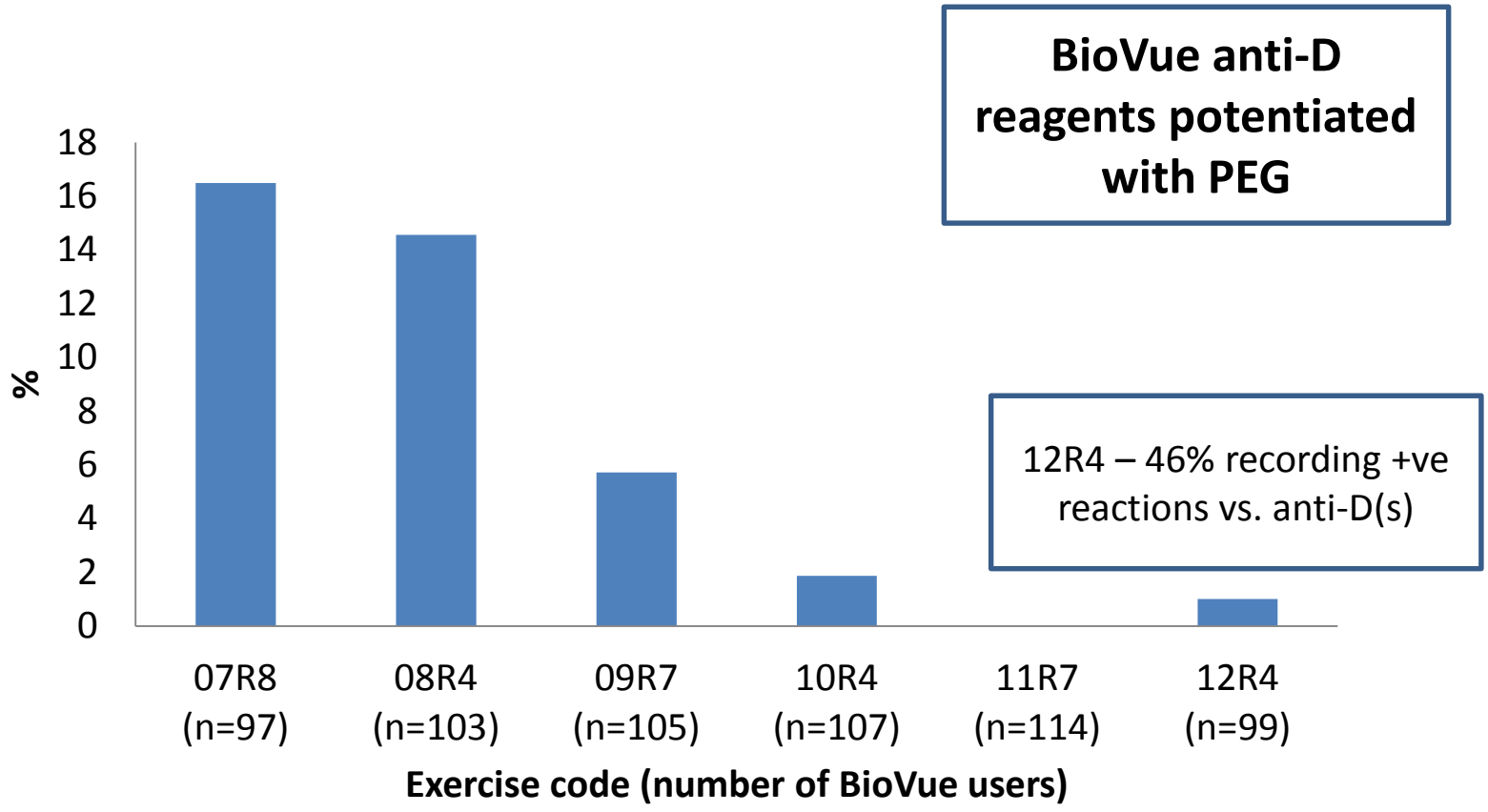
UK NEQAS antibody detection stakes!

ABO/D typing

- D typing rr DAT positive red cells
- Weak D
- Detecting dual populations

- Limitation of 6 wells (not Grifols or Immucor)

% UK BioVue users reporting a false positive D type for a rr DAT+ sample 2007-2012



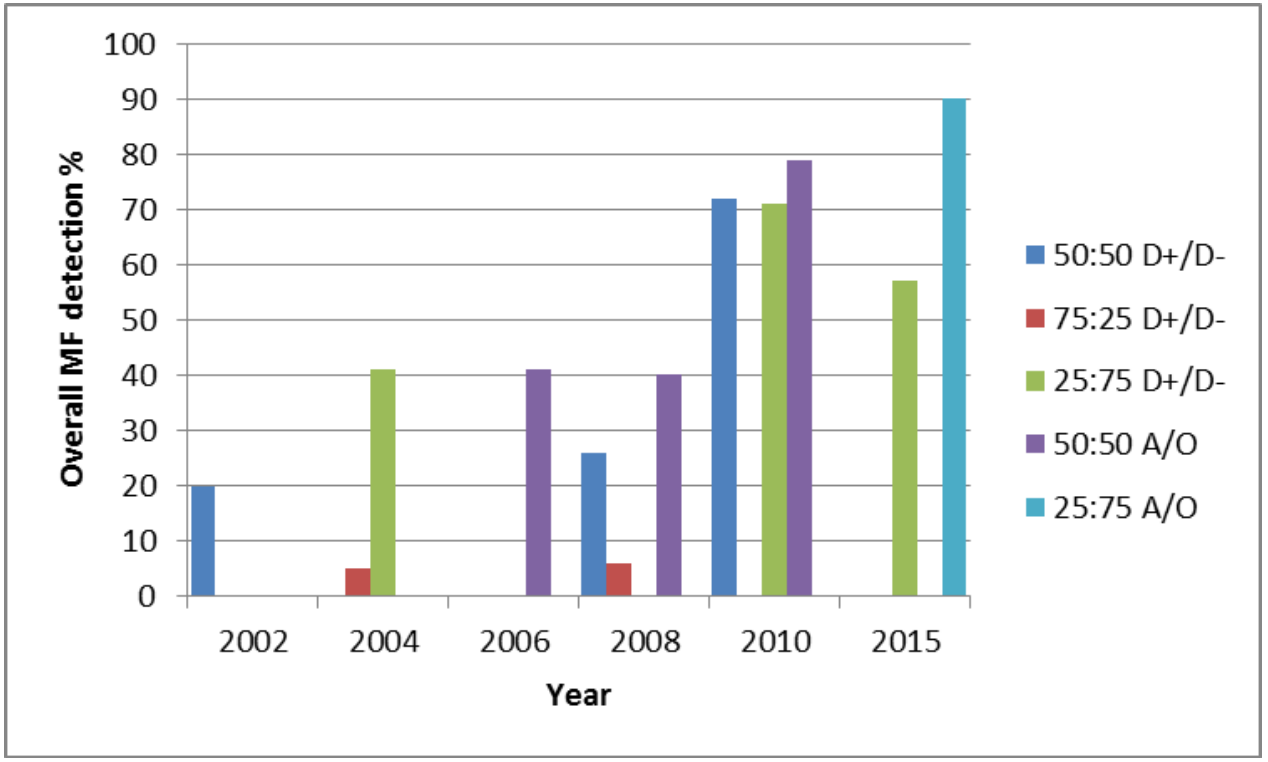
No false positive reactions recorded vs. anti-D or controls in 14R7 or 15R9 …..but weaker DAT+

14R1 – Patient 1 D weak (not scored)

Manufacturer and configuration	Clones	No.	No*	Str	Wk	MF	Neg
BioVue							
ABORh Combo(A B D Ctrl rev rev)	D7B8	82	46	2	19	25	0
ABODD (A B AB D D Ctrl)	D7B8 + RUM-1	15	0	1	5	3	0
DiaMed							
ABO/D Rev (A B D Ctrl rev rev)	LDM3 + 175-2	126	95	14	79	1	1
ABO/D Rev (A B D D rev rev)	5 clones	44	32	4	23	5	0
LPM - Immucor							
Immuclone & Novoclone	RUM-1 + D175+D415	33	12	0	7	0	5
Grifols							
A B D D Ctrl N N (+ K or N)	P3x61 + MS-201	9	8	0	8	0	0
Tube							
Various	RUM-1 + BS-201	14	9	1	7	1	1

* Number using this as a single test for P1

Detection rates for dual populations in EQA exercises 2002 - 2015



Exercise 15R4
Detection of dual populations

Technology	Number detecting MF / number* using technology (%)	
	Vs. anti-A for Patient 1 A /O (25:75)	Vs. anti-D for Patient 2 D +/D - (25:75)
BioVue	77/79 (97%)	34/87 (39%)
DiaMed	158/173 (91%)	128/177 (72%)
Grifols	15/15 (100%)	10/15 (67%)
LPMP[1]	4/8 (50%)	1/30 (3%)
Tube	10/14 (71%)	7/13 (54%)
Total	**264/289 (91%)**	**180/322 (56%)**

* = number using as a single technology or same technology twice

DiaMed Auto/man	MF anti- D	MF anti-A
Auto (n=120)	71%	95%
Manual (n=34)	71%	76%

15R4 P2 MF (D pos/neg 25:75)

Reactions other than MF recorded for Patient 2 vs. anti-D, by technology

Technology	Total	Negative	Weak positive	Strong positive
BioVue	53	33 (62%)	18 (34%)	2 (4%)
DiaMed	49	34 (69%)	3 (6%)	12 (24%)
Grifols	5	5 (100%)	0 (0%)	0 (0%)
LPMP[1]	29	28 (97%)	0 (0%)	1 (3%)[2]
Tube	6	1 (17%)	3 (50%)	2 (33%)

[1] LPMP = liquid phase microplate, and includes those stating Capture or solid phase
[2] manual testing

Questions raised

- Why is there a difference in detection rates of MF reactions between ABO and D typing?
- Why is this not consistent within and between technologies?

Excessive shaking in liquid phase?

Shear forces?

Centrifugation speeds and time?

Antibody affinity?

Potentiators?

…a combination of these things?

DAT – false positive reactions

- 15R7 DAT pilot sample DAT2 (4+ IgG coating)
  - 21 false positive vs. anti-C3d
    - I DiaMed
    - 20 BioVue
      - 4/20 BioVue positive internal negative control
- 11/42 BioVue users commented on a positive reaction in an internal negative control

- Few reports of unexpected positive DATs on EQA ‘donors’ during crossmatching using Grifols

Effectiveness of technology depends on

- Design of the technology itself
- Reagents - avidity, affinity, potentiators
- Automation, centrifuging, incubation, temperature, timing
- Adherence to manufacturer's instructions

New CAT technologies spotted at ISBT….

– 1 minute groups (including reverse)