UK NEQAS (BTLP) update session

Bill Chaffe
Jenny White
Clare Milkins
November 2014

UK NEQAS

Staff

- Megan Rowley – Director
- Clare Milkins – Manager and Deputy Director
- Jenny White – Deputy Manager
- Bill Chaffe – project lead for TACT
- Claire Whitham – senior EQA scientist and TACT lead
- Arnold Mavurayi – EQA scientist
- Scientific staff shared with UK NEQAS (H) for FMH, and all admin and logistics staff shared

Updates

- TACT
- Genotyping pilot
- ABO titration pilot
- Learning points from last 12 months exercises

Claire Whitham MSc MIBMS, Snr EQA Scientist, UK NEQAS BTLP

Bill Chaffe FIBMS, Snr EQA Scientist, UK NEQAS BTLP

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The Bad News – TACT costs money!!!

- Subscription and Membership purchase launched 03 Nov 2014
- Subscription FREE
- Memberships:
  - Single membership @ £15
  - Up to 5 @ total cost of £50
  - Up to 10 @ total cost of £100
  - Up to 15 @ total cost of £138.75
  - Up to 20 @ total cost of £185
  - Up to 25 @ total cost of £212.50
  - Up to 30 @ total cost of £255
  - Up to 50 @ total cost of £300

Valid until 31st March 2015 – payment by one off purchase order

From 01 April 2015 can be financed through EQA Re-registration

Fees from April 2015

- Single membership £60
- 2-10 memberships £40 per member
- 11-20 memberships £37 per member
- 21-30 memberships £34 per member
- 31+ memberships £30 per member

24/7/365 access except for maintenance and upgrade periods

The Good News – TACT delivers what YOU want

- Initially a single scenario – Routine Request Handling
- Scenario's in the pipeline – Major haemorrhage / Trauma management, Neonatal & Paediatric management, Antenatal management, Transplant management, General laboratory housekeeping and quality **PLUS your suggestions**
- SAG meeting later this month
- Roadmap of ongoing developments to be published

And there's more

- Easy to understand manager and member dashboards coming soon with ability to review cases
- Variable assessment targets for each assessed area dependant on your laboratory priorities
- Six areas of automated assessment guided by BCSH guidelines
- Facilitates local policy and procedure

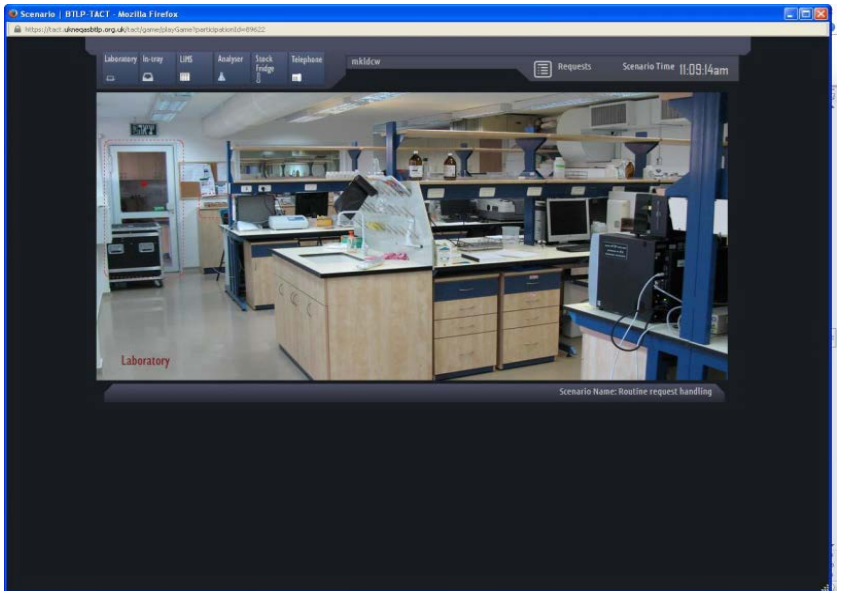
How does TACT help me?

- TACT is a knowledge based activity
- TACT gives a measure of competency
- TACT meets requirements of HCPC, CPA and BSQR's
- TACT provides a comprehensive CPD record
- TACT is transferable between organisations at no cost
- TACT can be undertaken at work, at home or on public transport

Not seen it yet?

What lies through the door?

Could you navigate round this lab?



Demonstration in here AFTER the meeting has finished – will only take 10mins

Join in the fun

- As of close of play 10/11/2014:-
  - 18 subscriptions representing 22 hospital sites
  - 17 in England
  - 3 in ROI
  - 2 in Scotland
  - 8 orders for a total number of 290 memberships

UK NEQAS / ISBT
Red cell Genotyping
pre-pilot exercise 2014

UK NEQAS

Background

- Increasing use of molecular techniques for blood grouping
  - Resolution of serological anomalies
  - Patient typing
  - Mass donor screening
- NHSBT ref labs starting to test – need EQA
- ISBT red cell genotyping workshops / sample exchanges (biennial)

Joint enterprise!

ISBT

- Workshop contacts
- Advice on current practice
- Wording of questions
- Verifying analysis
- Feedback
- Billing for participation

UK NEQAS

- Registration via SurveyMonkey
- Provision of material
- Distribution of exercise
- SurveyMonkey Q for results
- Analysis of results
- Feedback

ISBT International Society of Blood Transfusion

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Distribution 14G1

Australia	Japan
Austria	New Zealand
Brazil	Norway
Canada	Poland
Chile	Portugal
China	Slovenia
Denmark	South Africa
Finland	Spain
France	Kuwait
Germany	Sweden
Holland	Switzerland
India	Thailand
Ireland	The Netherlands
Israel	UK
Italy	USA

- ISBT working party contacts + others
- Distributed to 55 laboratories in 30 countries
- 52/55 (95%) returned results!

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General questions clinical practice

- Scope of testing
- Platforms used
- Volume of sample required
- Range of antigens tested
- Format of reporting (genotype and / or predicted phenotype)

Profile of participating labs

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

Format(s) reported	Number (%)
Genotype and predicted phenotype	34 (65%)
Genotype only	7 (13%)
Phenotype only	9 (17%)
None	1 (2%)
Not stated	1 (2%)

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Exercise 14G1

- 3 whole blood samples from donors – selected only for different Rh phenotypes

- Extract DNA
- Test using routine methods for:
  - D, Cc, Ee, MN, Ss, Kk, Fy^a Fy^b Fy, Jk^a Jk^b, Do^a Do^b
- Report genotypes and predicted phenotypes using ISBT terminology

Patient 1 – expected results

Antigens	Genotype	Predicted phenotype
D	*RHD*01* **or** *RHD*01/01N.01*	**D positive**
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+)**

Terminology example - D Patient 1

Just D (27)	D and deletion (15)
RHD*01 (13)	RHD*01/RHD*01N.01 (3)
RH*01 (2)	RHD*01/01N.01 (2)
RHD*01 positive (2)	RH*01; RH*01/01N.01 (RH*Dd) (1)
RH*1 (1)	RHD*01/01N.01, RHD*Pseudogene neg (1)
RHD*D (1)	RHD*01 (ex1 pos, int 4 pos, ex5 pos, D psi neg, ex7 pos, ex 10 pos; neg for RHD*weak D type 1, 2, 3, 4.0, 4.1, 4.2, 5, 11, 14, 15) / RHD*01N.01 (1)
RHD+ (2)	RH*01/01N (1)
D+ (1)	RHD*01, RHD*01N.01. (ie Dd) (1)
RHD pos (exon 5,4,3,7,6,9 tested) (1)	RHD*01 emizygote (D/d) (1)
RHD (2)	Dd (1)
Apparently non-negative (1)	D/d (1)
RH001 (1)	RHD*D/d (1)
	D/- (heterozygous) with both exons 4 & 7 (1)
D not reported = 10	

Errors

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

- 4 reported a single incorrect predicted phenotype
  - 2 based on an incorrect genotype (Labs A and B)
  - 2 ? error in reporting or interpretation as based on correct genotype (C and D)
- 1 (Lab E) reported an incorrect genotype, but no predicted phenotype
- 1 (Lab F) appears to have reported the results for Patient 2 as Patient 3, resulting in three incorrect genotypes and predicted phenotypes.

EQA issues

- Frequency of exercises
- Selection of samples – degree of difficulty?
- Exercise duration - stability of samples?
- Establishing ‘correct’ result – consensus?
- Range of antigens to report
- Free text or tickbox? - leading answers?
- Genotype & predicted phenotype – assess both?
- Interpretations in context of testing?
- Eventual scoring - exact terminology?
- Format of report to participants
- Funding

What next?

- Continued collaboration with ISBT – steering committee / advisory group
- Second pre-pilot (focussed)
- Pilot Scheme ? January 2015

ABO titration pilot update 2014

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UK NEQAS ABOT Pilot 2010 – to date

- Aim = to support ABOi transplant
- ABOi pilot EQA Scheme guided by ABOi SAG
- Development of standard technique
  - IAT and DRT DiaMed, prescribed volumes, end point etc.
  - facilitate EQA
  - transferrable results across centres
- Highlight variability in titres to clinicians
- Developing ABO ‘standards’ with NIBSC

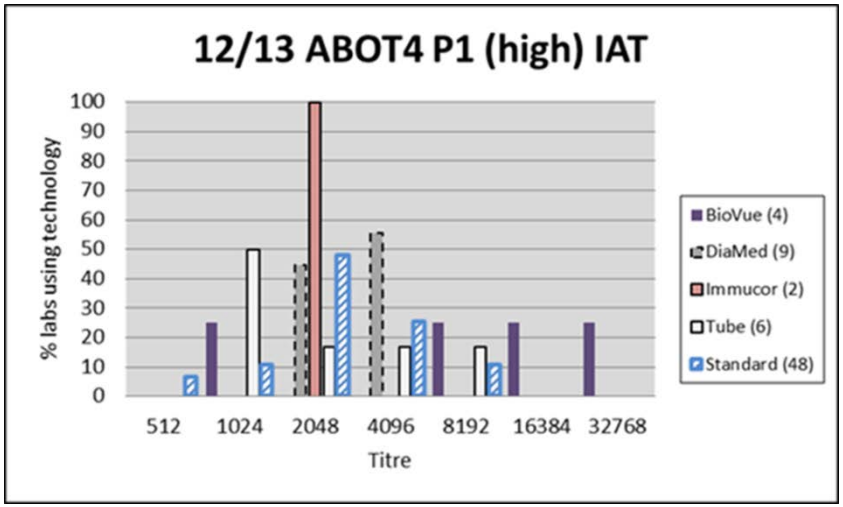
ABOi pilot 2012-13

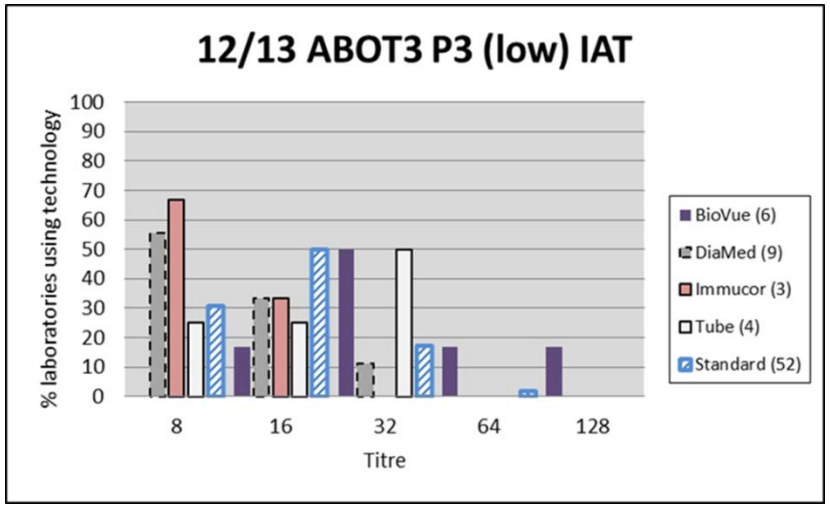
4 exercises per year

- 3 plasma samples for titration vs. A cells provided
- Replicate samples in 3 consecutive exercises
- Duplicate sample within an exercise
- Reporting individual result to each lab and method medians
- Comparing in-house and standard techniques
- Questions on clinical use of results

- 69 labs (37 UK), 38 supporting ABOi transplant and 31 others

Example individual results





Inter laboratory results spanned a wide range, e.g.:

512 – 32000 by IAT for a high titre sample ABOT4 P1 (standard median 2048)

8 – 128 by IAT for low titre sample ABOT3 P3 (standard median 16)

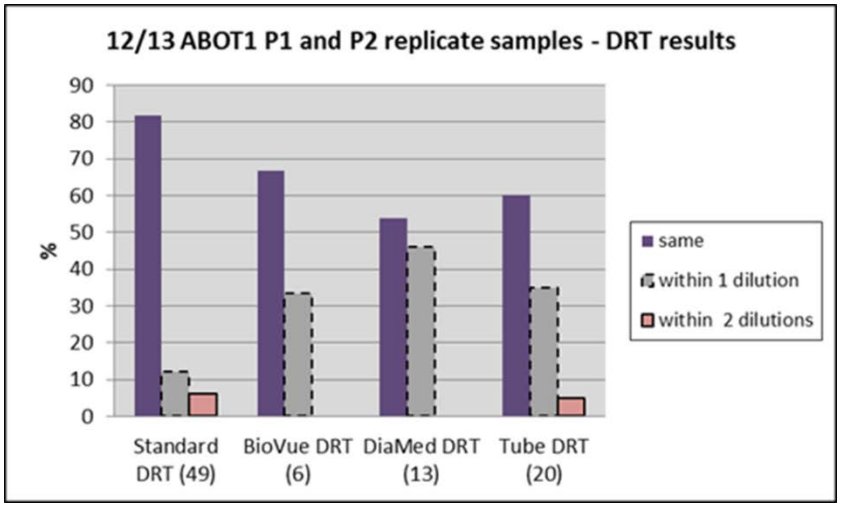
Replicate samples over 3 exercises

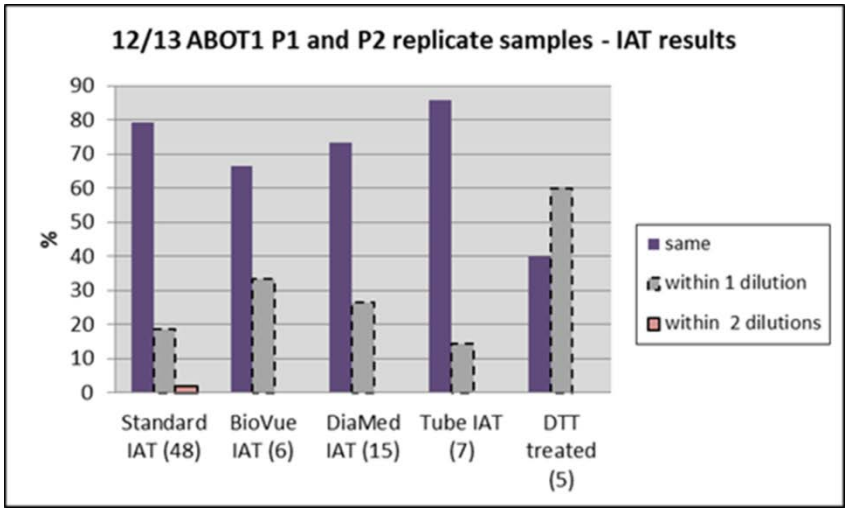
% results for replicate samples the same or within 1 or more dilution

Method (number)	Same each time	Within 1 dilution	Within 2 dilutions	>2 dilutions apart
Std DRT (33)	5 (15%)	12 (36%)	13 (39%)	3 (9%)
IH DRT (28)	4 (14%)	15 (54%)	7 (25%)	2 (7%)
IH DiaMed DRT (11)	2 (18%)	8 (73%)	1 (9%)	0 (0%)
IH Tube DRT (12)	0 (0%)	6 (50%)	4 (33%)	2 (17%)
Std IAT (38)	16 (42%)	19 (50%)	3 (8%)	0 (0%)
IH IAT (12)	1 (8%)	7 (58%)	2 (17%)	2 (17%)
IH IAT DTT (3)	0 (0%)	1 (33%)	1 (33%)	1 (33%)

- 92% sets of standard IAT results within 1 DD *cf.* 66% IH IAT.
- 51% sets of standard DRT results within 1 DD *cf.* 68% DRT IH
- Only 1/3 sets of IAT DTT treated plasma was within 1 DD

Duplicate samples in the same exercise

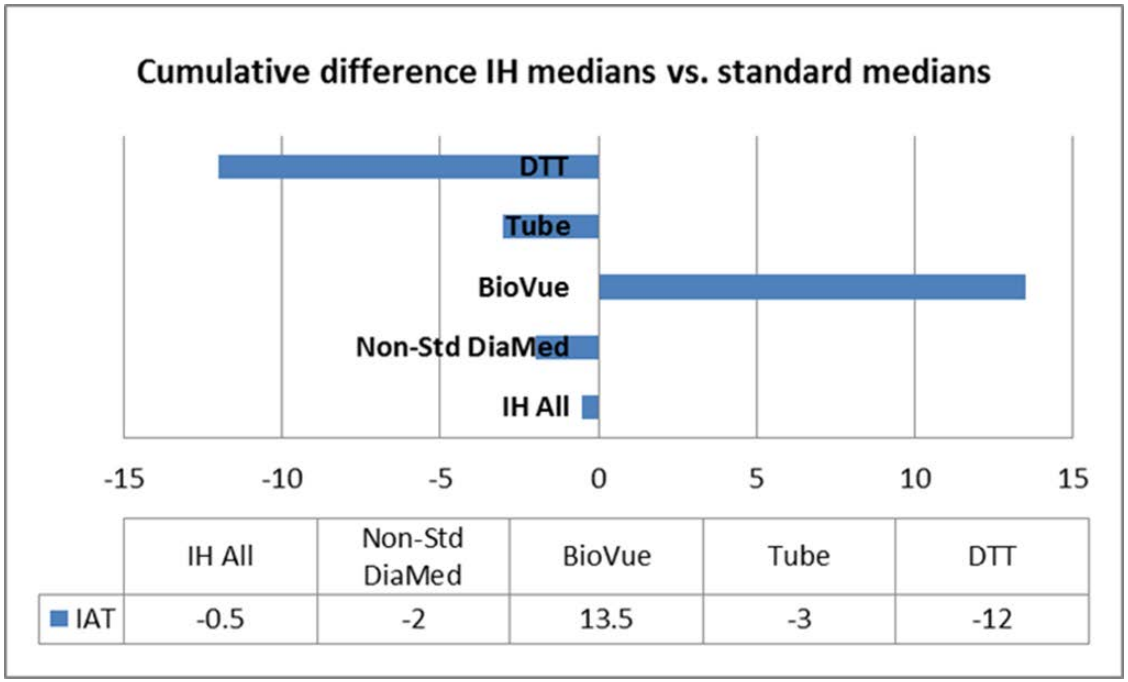




95.5% results by DRT and 98.8% by IAT were within one dilution

78% of IAT (non-DTT) results and 72% DRT results identical

In-house median vs. std median (IAT)



	IH All	Non-Std DiaMed	BioVue	Tube	DTT
IAT	-0.5	-2	13.5	-3	-12

The IAT BioVue median result was higher than that for the IAT 'standard technique' (DiaMed) in 11/12 (92%) samples

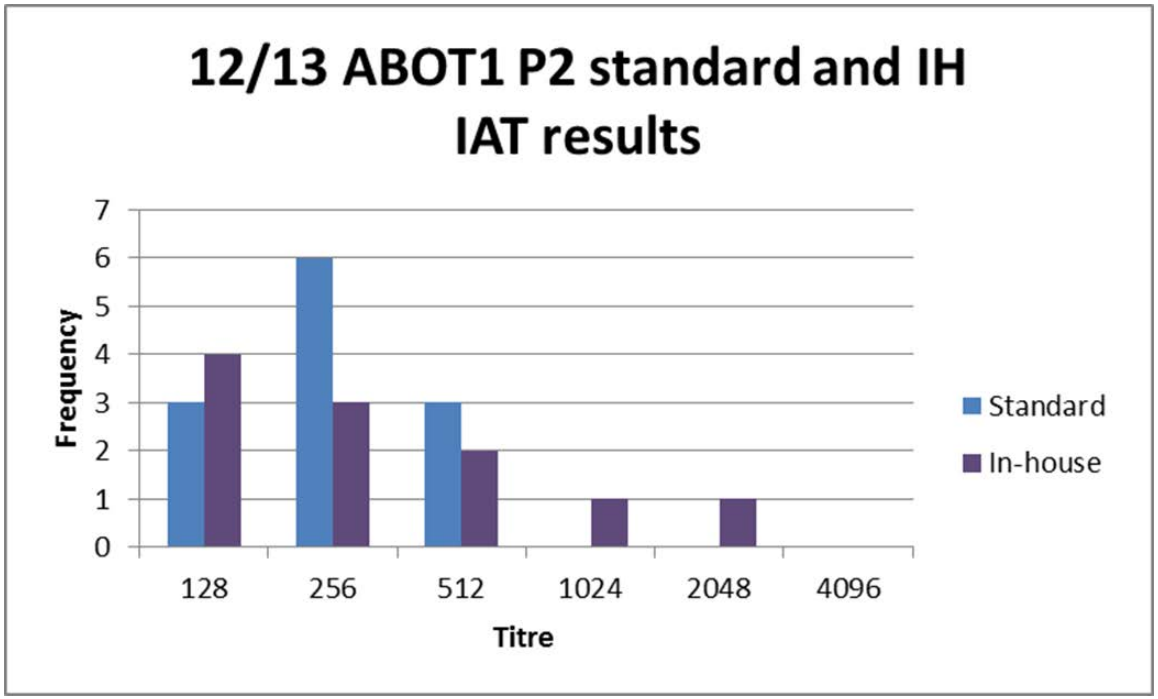
- Median for each sample by each IH technology assigned a score of 1 for each dilution above or -1 for each dilution below the standard median.
- Where median between two dilutions, results either side assigned 0.5.
- Scores totalled to give a cumulative score.

2012/13 ABOT Pilot – testing

- Still variation in in-house methodology
- Increasing use of ‘standard’ method (EQA & clinical practice)
  - 50/60 labs returned results of standard method (31 also IH method)
- IAT more reproducible than DRT
- Reproducibility generally good
- Standard IAT results more reproducible than IH IAT results
- Std. results tighter range than Tube
- BioVue IAT titre consistently higher than Std. IAT titre

Clinical use of results

- 14 UK transplant centres surveyed in 2013
- Maximum patient ABO antibody titres
  - 128-4096 for acceptance ABOi renal transplant programmes
  - 2-16 for a transplant to go ahead on the day



Example of IAT results (for a single EQA sample) submitted by laboratories providing ABO titration results to these centres

- **No correlation result with cut-off values**

NHSBT strategy group for incompatible renal transplant

- Preliminary meeting to discuss outcome of 2012 – 2013 ABOT data
- National workshop Oct 2014 where agreed that standardisation of titration results was required across all centres
  - Equitable access to shared donor programs
  - Safe ‘cut-off’ values pre-transplant
  - Potential reduction in pre-transplant treatment to bring down ‘high’ antibody levels

NIBSC reference preparation anti-A and anti-B

- Trial fill 2013
- NIBSC have agreement for WHO standard
- NHSBT collecting HT plasma (group O)
- Fill Jan 15 followed by international trial
- Potential use:
  - Control reproducibility of IH testing
  - Compare other methods vs. ‘standard’ method

EQA

- Continue as pilot scheme (2014/15)
- Work towards becoming substantive EQA
- Shadow ‘scoring’
  - Based on being within 1 doubling dilution
  - Reproducibility of testing replicates (all methods)
  - Standard technique vs. median value
- Better sample quality – filtration of plasma

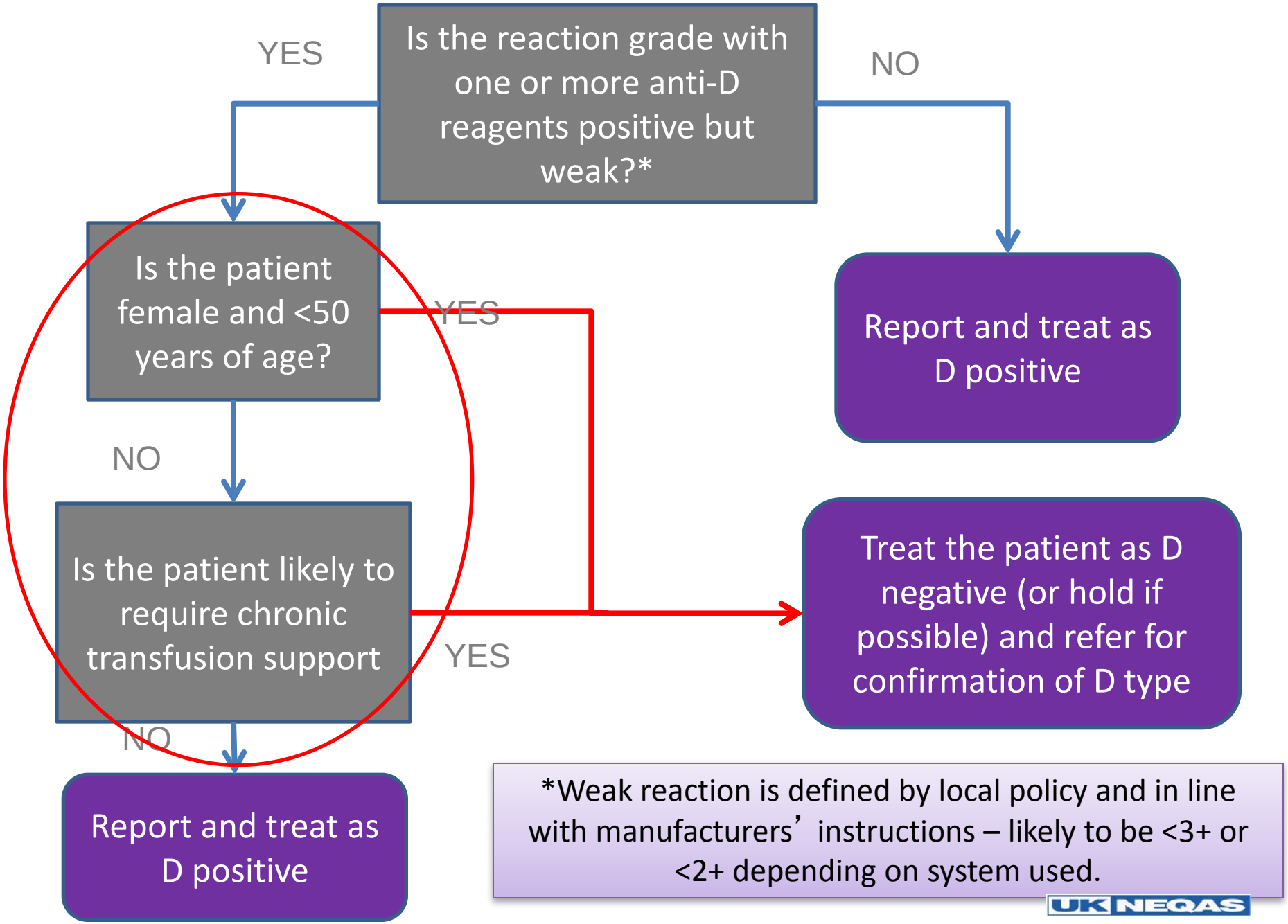
Learning points from exercises

UK NEQAS 14R1 January 2014

- D typing for a D weak patient and result interpretation in context of age and gender
- Selection of D pos/D neg red cells for transfusion
- Relationship between reaction grades and reagents

- Patient 1 - Group O D weak, inert (female, age 30, not transfusion dependent)
  - *Prepared from a pool of (uncategorised) weak D donations*

- Donor W - O D positive R_1R_1 (CDe/CDe), K-


Is the reaction grade with one or more anti-D reagents positive but weak?*
YES
NO
Report and treat as D positive
Is the patient female and <50 years of age?
YES
NO
Is the patient likely to require chronic transfusion support
YES
NO
Treat the patient as D negative (or hold if possible) and refer for confirmation of D type
Report and treat as D positive
*Weak reaction is defined by local policy and in line with manufacturers' instructions – likely to be <3+ or <2+ depending on system used.
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D typing: Reaction grades recorded

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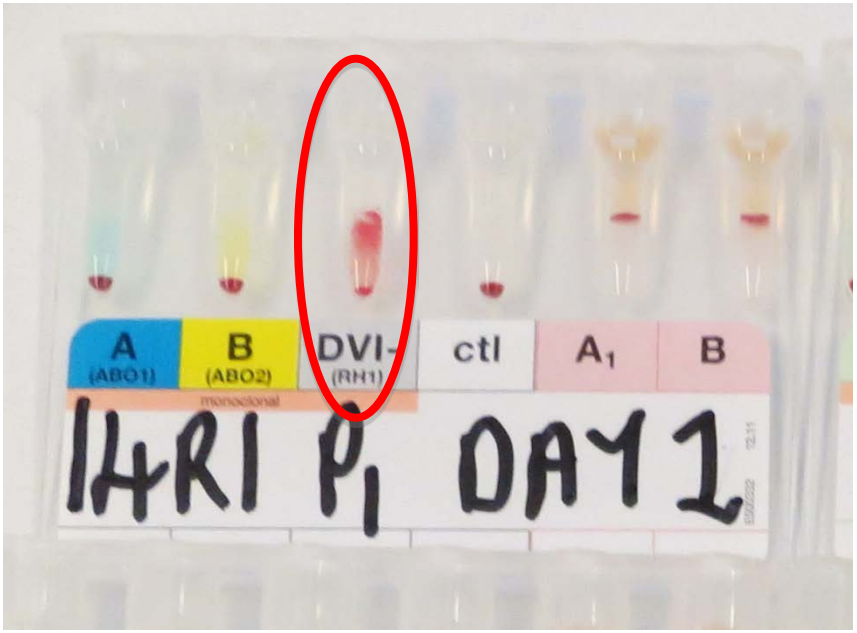
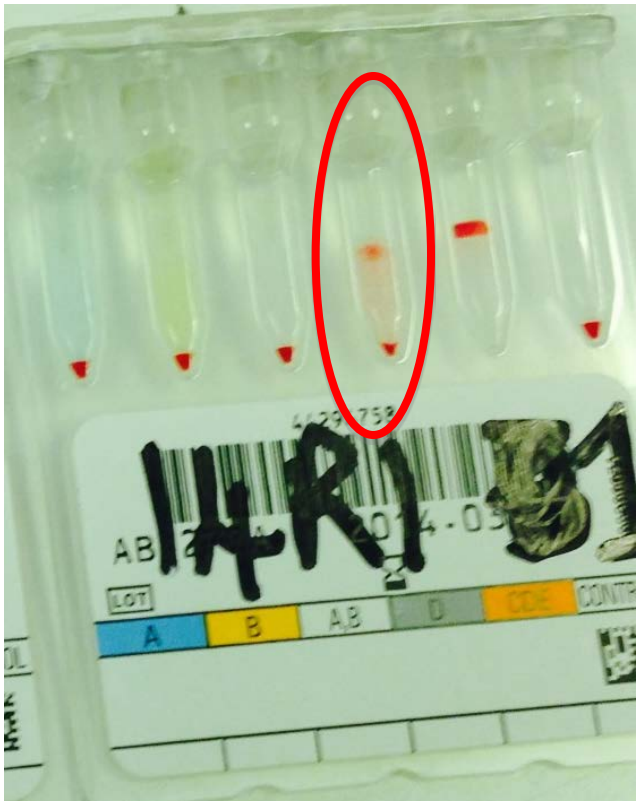
Interpretation (number)	Combination of reactions recorded with anti-D reagent(s)			
	Includes a weak pos[1]	Includes MF	Strong pos only[1]	Neg only[1]
D Variant (191)				
D Positive (121)				
D UI [2] (66)				
D Negative (16)				
Total (394)	293	63	24	14

[1] With one or two anti-D reagents
[2] Unable to interpret

356/394 (90%) recorded anomalous reactions with one or more than one anti-D reagent

In-house ABO/D typing results





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D typing: Reaction grades and interpretations recorded

Interpretation (number)	Combination of reactions recorded with anti-D reagent(s)			
	Includes a weak pos[1]	Includes MF	Strong pos only[1]	Neg only[1]
D Variant [3] (191)	177	13	0	1
D Positive (121)	94	3	24	0
D UI (66)	21	45	0	0
D Negative (16)	1	2	0	13
Total (394)	293	63	24	14

[1] With one or two anti-D reagents
[2] Unable to interpret
[3] Weak or partial

97/394 (25%) reported D positive based on anomalous D typing reactions = 27% of the 356 recording anomalous reactions

4/86 (5%) stated that they used an extended partial D typing kit

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Most common configuration of reagents

* No. Using this as a single test for P1

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	9	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	6	1	1

Selection of red cells

Interpretation P1 D type (number)	Result for Donor W (D positive) vs. Patient 1 (weak D)	
	Compatible – Would transfuse	Would not select/transfuse
D Variant (189)	71	118
D Positive (118)	108	10
D UI (65)	14	51
D Negative (16)	3	13
Total (388)	196	192

88/196 (45%) issuing the D positive unit reported D variant, D UI or D neg

7/88 (8%) said that they used an extended partial D typing kit

81/270 (30%) who made an interpretation other than D positive, would have transfused the D positive unit without knowing the variant subtype

Summary

- Variation in reaction grades even with same reagents and techniques
- 27% made an interpretation of D positive following anomalous D typing results (only 4 used an extended D typing kit)
- 30% of those who reported an anomalous D type, stated that they would have issued the D positive donation

Use of additional techniques

- 14E8 anti-e (±C)
  - 2 participants reported anti-C
  - Would have identified anti-e with an enzyme panel
- 14E2 anti-c+Fy^{a}
  - One lab reported anti-c+C^{w}
  - Could have excluded anti-C^{w} with enzyme panel

10% UK labs registered for antibody identification do not have an enzyme panel

Use of additional techniques

- 14E2 ant-c+Fy^a
  - 2 labs reported and-c+N
  - 13 UI submissions where labs could not distinguish between anti-Fy^a and anti-N by IAT

A room temperature panel would have excluded anti-N

Investigate fully

- 14E5 – anti-S+K (anti-S titre 1)
  - 2 labs reported anti-K only
    - One recorded negative reactions with K-S+ cells, but did not investigate an equivocal reaction with one K-SS cell
    - In retrospect they also noted a positive reaction with a K-S+ cells on the screening panel
- 14E2 - anti-c+Fy^{a}
  - One lab missed the anti-Fy^{a}, which was masked

Investigate weak positive reactions and don't forget the results of the screening panel!

Systematically exclude all antibodies of likely clinical significance

Treating EQA samples as clinical samples

- Number of transcription and transposition errors that occur because EQA patient samples are not ‘booked in’ to the LIMS

- Annual questionnaire suggests that 32% UK labs do not book the samples in.

Reasons for 93 labs (32%) not booking EQA samples in

Reason	Number
Format of samples (separate plasma)	34
Problems with cumulative data from EQA 'patients'	27
Interference with workload statistics	13
Problems with shared databases	14
Custom and practice	40
Other	13

23 (25%) cited custom and practice as the only reason

Low frequency/ low clinical significance antigens

- Kp^a
- C^w
- Lu^a
- Wr^a

- No need to detect in antibody in the screen
- No need to exclude in panel

9 participants received penalty points over the year because they stated that one of the corresponding antibodies was present (with another antibody). Probably unable to exclude it.

At least 8 made a UI submission because they were unable to exclude one of the corresponding antibodies

Where was the American Declaration of Independence signed?

At the bottom.