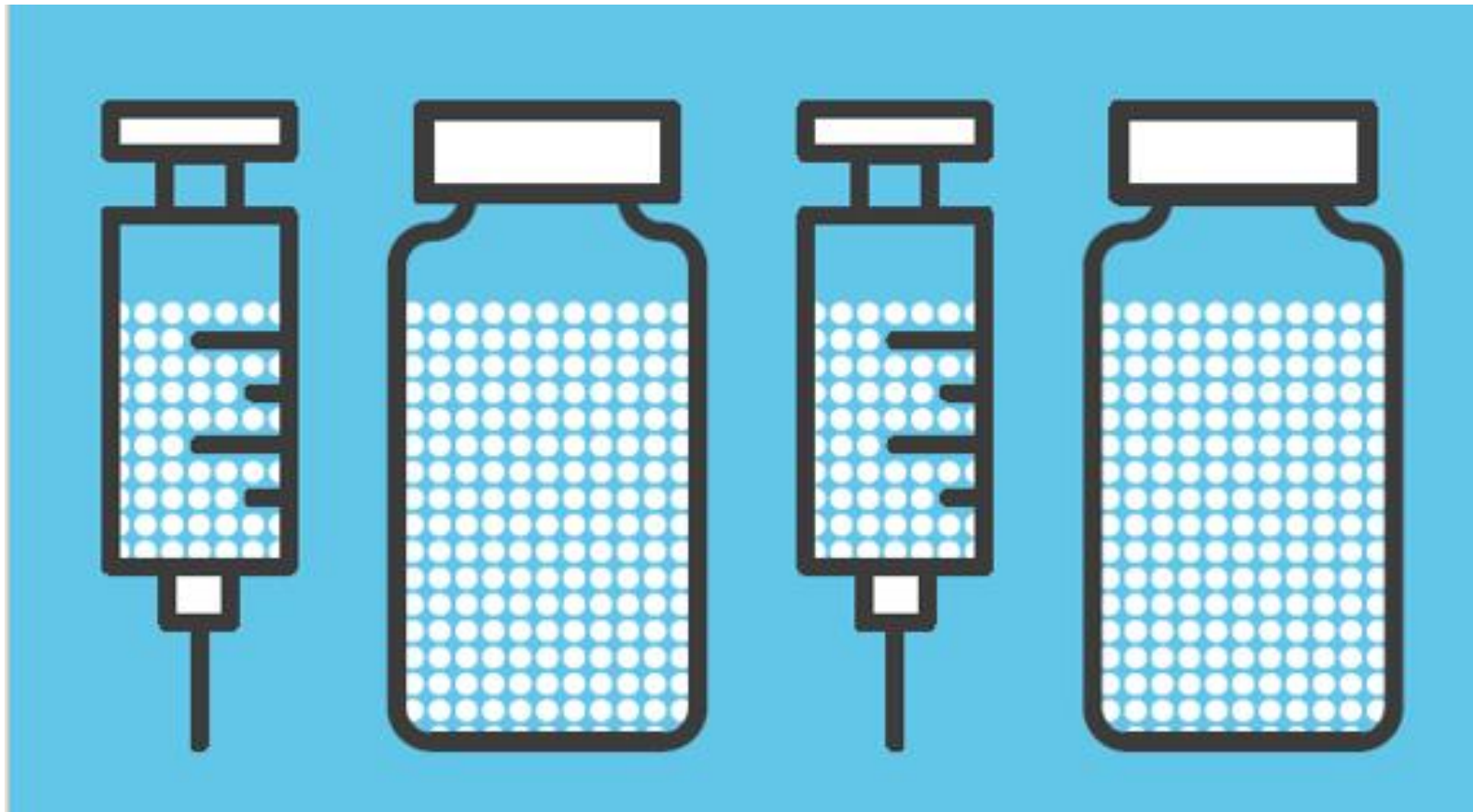


UK NEQAS

Blood Transfusion Laboratory Practice



Antibody Identification UI:

**What, When,
How, Why?**

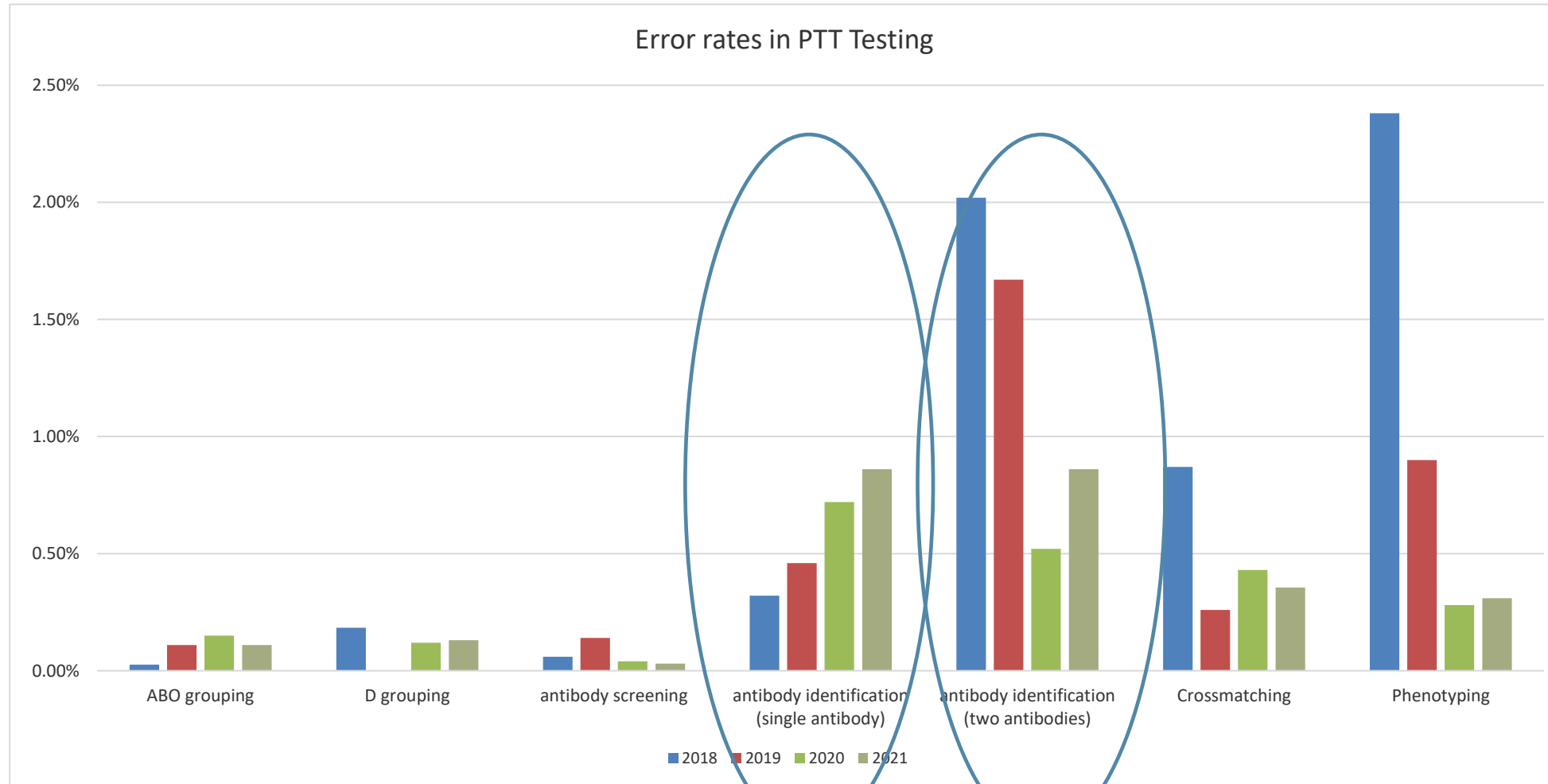
Richard Haggas & Katy Veale

UK NEQAS BTL

Summary

- How good are we at antibody identification
- What are the key BSH rules for antibody identification
- Tips on identification
- What happens in a UK NEQAS exercise when you cannot complete ID
 - The UI process
 - How to submit a UI
 - Assessment of a UI
 - Scoring of UIs

How good are we at antibody ID



BSH Guidelines on ABID

- When a positive antibody screen is obtained an investigation must be undertaken to determine the specificity, and the likely clinical significance
- If the patient is known to have already formed a red cell antibody, each new sample should be fully tested to exclude the presence of further alloantibodies
- The patient's plasma must be tested against an identification panel of reagent red cells which must include an IAT Panel

Inclusion of a specificity

- Antibody specificity should only be assigned when the plasma is reactive with at least two examples of reagent red cells expressing the antigen and non-reactive with at least two examples of reagent red cells lacking the antigen
- When one antibody specificity has been identified, the presence of additional specificities must be excluded
- Failure to recognise all of the antibody specificities within a sample may lead to a haemolytic transfusion reaction (SHOT, 1996-2010).

Excluding a specificity

- In particular, the presence of anti-c, anti-Jk^a, anti-Jk^b, anti-S, anti-s, anti-Fy^a, and anti-Fy^b should be excluded using red cells having homozygous expression of the relevant antigen.
- A single example only of each phenotype is sufficient for exclusion.
- It is acceptable to exclude Rh antibodies using validated techniques incorporating enzyme treated cells, clinically significant antibodies in other blood group systems must be excluded using IAT

Techniques and Technology

- Different technologies are better at detecting some specificities
 - E.g Capture-R does not detect IgM antibodies
- Enzyme techniques can help with ID of weak antibodies and antibody mixtures
- Know which specificities are denatured by enzymes
 - Anti-Fy^a / Fy^b, anti-S (usually), anti-s, anti-M, anti-N

Enzymes

- Not all antibodies work in enzyme techniques
- Don't fully believe those charts we have in our labs
- Standard 2-stage enzyme panel (saline enzyme technique) may not detect all examples of anti-K or anti-Jk^a
- Enzyme IAT will detect those antibodies, but not usually a validated technique and not all technologies work using enzyme IAT

Tips on identification

- Have a means to record what you know you have included / excluded
 - E.g. Checklist

		Hom							Hom	Hom	Hom	Hom	Hom	Hom				not compulsory	not compulsory	not compulsory
D	C	c	E	e	K	k	M	N	S	s	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	P ₁	C ^w	Kp ^a	Lu ^a

- Perform a phenotype – this can also help with exclusion

Tips on identification

- Make sure all positive reactions are accounted for
- Remember to check the reactions in the antibody screen
- Remember some antibodies will have similar patterns
 - Anti-S and anti-M (linkage disequilibrium in MNS system)
- Antibody ID gets easier the more you do it
 - Practice – write out panels for your colleagues
 - Encourage your lab manager to take out a TACT subscription



UI

Un Identifiable

UI – Why?

- Can't complete an antibody ID
 - Would probably refer
- We assess your ABID process
- Is it safe
- According to BSH Guidance

UI – When?

- If can't **positively ID** the antibodies to satisfaction
- Not sure what **clinically significant** antibody(s) are there
- Can't exclude **clinically significant** antibodies

- A lot if a mixture of antibodies

- Maximum 2 antibodies

UI – When not to?

- Don't need a UI if:
- Positively identified 2 antibodies
- Can't exclude non-clin sig
 - e.g. C^w, Lu^a, Kp^a
 - Not available to tick

UI – When not to?

Antibody specificities positively identified (currently a maximum of 2 in any sample)	Positively identified	Specificities that cannot be excluded	Cannot exclude	
<input type="checkbox"/> D <input type="checkbox"/> C <input checked="" type="checkbox"/> c+/-E <input type="checkbox"/> E <input type="checkbox"/> e+/-C <input type="checkbox"/> C ^w <input type="checkbox"/> M <input type="checkbox"/> N <input type="checkbox"/> S <input type="checkbox"/> s <input type="checkbox"/> P ₁ <input type="checkbox"/> Lu ^a	<input type="checkbox"/> K <input type="checkbox"/> k <input type="checkbox"/> Kp ^a <input type="checkbox"/> Le ^a <input type="checkbox"/> Le ^b <input type="checkbox"/> Fy ^a <input type="checkbox"/> Fy ^b <input type="checkbox"/> Jk ^a <input type="checkbox"/> Jk ^b <input type="checkbox"/> Wr ^a <input type="checkbox"/> Enz non-specific <input checked="" type="checkbox"/> UI¹	<input type="checkbox"/> D <input type="checkbox"/> C <input type="checkbox"/> c+/-E <input type="checkbox"/> E <input type="checkbox"/> e+/-C <input checked="" type="checkbox"/> M <input checked="" type="checkbox"/> S	<input type="checkbox"/> s <input type="checkbox"/> K <input type="checkbox"/> Fy ^a <input checked="" type="checkbox"/> Fy^b <input type="checkbox"/> Jk ^a <input type="checkbox"/> Jk ^b	M S Fyb

¹ UI = Unable to interpret

Please note that if you have positively identified 2 specificities there is no need to make a UI submission as UK NEQAS samples currently do not contain more than two. Anti-c+/-E is counted as a single specificity for the purposes of EQA, as is anti-e+/-C.

You may indicate commonly encountered antibodies of potential clinical significance that cannot be positively identified but might be present (cannot be excluded) based on your testing and the phenotype provided.

UI – How 1

Antibody specificities positively identified (currently a maximum of 2 in any sample)	Positively identified		
<input type="checkbox"/> D <input type="checkbox"/> C <input checked="" type="checkbox"/> c+/-E <input type="checkbox"/> E <input type="checkbox"/> e+/-C <input type="checkbox"/> C ^w <input type="checkbox"/> M <input type="checkbox"/> N <input type="checkbox"/> S <input type="checkbox"/> s <input type="checkbox"/> P ₁ <input type="checkbox"/> Lu ^a <input type="checkbox"/> K <input type="checkbox"/> k <input type="checkbox"/> Kp ^a <input type="checkbox"/> Le ^a <input type="checkbox"/> Le ^b <input type="checkbox"/> Fy ^a <input type="checkbox"/> Fy ^b <input type="checkbox"/> Jk ^a <input type="checkbox"/> Jk ^b <input type="checkbox"/> Wr ^a <input type="checkbox"/> Enz non-specific <input type="checkbox"/> UI ¹	c+/-E		
<p>¹ UI = Unable to interpret</p> <p>Please note that if you have positively identified 2 specificities there is no need to make a UI submission as UK NEQAS samples currently do not contain more than two. Anti-c+/-E is counted as a single specificity for the purposes of EQA, as is anti-e+/-C.</p>			

UI – How 2

www.ukneqash.org says

Please remember to make a UI submission in the following section.
See Antibody Identification Notes for more information

OK

UI – How 3

The screenshot shows the user interface for the UK NEQAS Blood Transfusion Laboratory Practice. At the top left is the UK NEQAS logo with the text 'Haematology and Transfusion'. The main header is 'UK NEQAS Blood Transfusion Laboratory Practice' with 'version: 3.15' on the right. Below the header is a navigation bar with the following elements: 'Sample Entry Details' (a link), 'Back to List' (a button), 'Summary' (a button), 'Reports' (a button), 'Data entry form and instructions' (a button circled in red), 'Email scheme' (a button), and 'Exercise Instructions' (a button). Below the navigation bar is a section titled 'Pre-Transfusion Testing'.

UI – How 4

Blank Data Sheets

	Upload date & time	Description
PTT 'E' Exercise blank data sheet	15-07-2021 10:42	PTT 'E' Exercise blank data sheet
PTT 'R' Exercise blank data sheet	15-07-2021 10:43	PTT 'R' Exercise blank data sheet

Instructions

	Upload date & time	Description
UI Portal Instructions	23-04-2018 13:53	UI Portal Instructions
Web data entry instructions	09-03-2020 12:12	Web data entry instructions
UI rules and fax cover sheet	15-07-2021 10:45	UI rules and fax cover sheet

UI – How 5

Antibody specificities positively identified (currently a maximum of 2 in any sample)	Positively identified	Specificities that cannot be excluded	Cannot exclude
<input type="checkbox"/> D <input type="checkbox"/> C <input checked="" type="checkbox"/> c+/-E <input type="checkbox"/> E <input type="checkbox"/> e+/-C <input type="checkbox"/> C ^w <input type="checkbox"/> M <input type="checkbox"/> N <input type="checkbox"/> S <input type="checkbox"/> s <input type="checkbox"/> P ₁ <input type="checkbox"/> Lu ^a	<input type="checkbox"/> K <input type="checkbox"/> k <input type="checkbox"/> Kp ^a <input type="checkbox"/> Le ^a <input type="checkbox"/> Le ^b <input type="checkbox"/> Fy ^a <input type="checkbox"/> Fy ^b <input type="checkbox"/> Jk ^a <input type="checkbox"/> Jk ^b <input type="checkbox"/> Wr ^a <input type="checkbox"/> Enz non-specific <input checked="" type="checkbox"/> UI¹	<input type="checkbox"/> D <input type="checkbox"/> C <input type="checkbox"/> c+/-E <input type="checkbox"/> E <input type="checkbox"/> e+/-C <input type="checkbox"/> M <input type="checkbox"/> S <input type="checkbox"/> s <input type="checkbox"/> K <input type="checkbox"/> Fy ^a <input type="checkbox"/> Fy ^b <input type="checkbox"/> Jk ^a <input type="checkbox"/> Jk ^b	
<p>¹ UI = Unable to interpret</p> <p>Please note that if you have positively identified 2 specificities there is no need to make a UI submission as UK NEQAS samples currently do not contain more than two. Anti-c+/-E is counted as a single specificity for the purposes of EQA, as is anti-e+/-C.</p>	<p>c+/-E UI</p>	<p>You may indicate commonly encountered antibodies of potential clinical significance that cannot be positively identified but might be present (cannot be excluded) based on your testing and the phenotype.</p>	
UI Submission		UI Instructions	
Upload Document		Explain briefly why identification could not be confirmed in-house, including reasons why specificities could not be positively identified or excluded.	
<input type="checkbox"/> All screen profiles including results have been uploaded		<div style="border: 1px solid #ccc; height: 60px; width: 100%;"></div>	
<input type="checkbox"/> All panel profiles including results have been uploaded			
<input type="checkbox"/> The investigation process has been fully described			

UI – How 6

The screenshot shows a web form titled "UI Submission" with a "UI Instructions" tab. A red box highlights the "Upload Document" button. Another red box highlights a text input field with the instruction: "Explain briefly why identification could not be confirmed in-house, including reasons why specificities could not be positively identified or excluded." A third red box highlights a list of three checkboxes:

- All screen profiles including results have been uploaded
- All panel profiles including results have been uploaded
- The investigation process has been fully described

Positively ID'd anti-c

Other positive reaction, could be anti-K, anti-Fy^b, anti-M, anti-S – *please tick the ones that can't be excluded*

Suspect anti-K, but can't positively identify anti-K as only one c- K+ cell available

Can't exclude anti-M as no c- M+N- cells

Can't exclude anti-Fy^b as no c- Fy(a-b+) cells

Can't exclude anti-S as no c- S+s- cells

UI – What we do with them

- Look at uploads
- Log them in a spreadsheet
- Check if have actually ticked UI at submission
- Two people check
- Agree / disagree with you
- Agree / disagree with ourselves
- Amend to UI acceptable = 0 points
- Write a letter = 40 or 50 points

UI – Example 1

Actual Response	Excluded Response	Designated Response
c+/-E,UI	M,K	c+/-E,K

Patient 1

Explain briefly why identification could not be confirmed in-house, including reasons why specificities could not be positively identified or excluded.

Unable to distinguish between Anti-K and anti-M with screen and panel in the presence of anti-c. Would refer sample to reference laboratory and provide K- c- M- units whilst awaiting report. Unable to exclude either antibody using phenotype. Also attached incomplete antigam for NHSBT panel but not performed as would not aid in differentiating between anti-K and anti-M in the presence of anti-c.

UI – Example 1

CONCLUSION: Anti-e Unable to exclude
Excluded by phenotype on 11-14, on 11-14.

0:8% Resolve® Panel C Ficin Treated
 0:8% Resolve® Panel C Untreated
 ANTIGRAM® Antigen Profile

63520

Cell#	Rh-hr	Donor Number	Rh-hr							KELL				DUFFY		KIDD		Sex Linked	LEWIS			MNS				P	LUTHERAN		Special Antigen Typing	Test Results														
			D	C	E	c	e	f	Cw	V	K	k	Kpa	Kpb	Jsa	Jsb	Fya		Fyb	Jka	Jkb	Xga	Lea	Leb	S	s	M	N		P ₁	Lu ^a	Lu ^b	Cell#	I	E									
1	R1wR1	500749	+	+	0	0	+	0	+	+	+	0	+	0	+	0	+	+	+	0	+	0	+	0	+	+	0	+	+	0	+	+	0	+	+	0	+	+	0	+				
2	R1R1	503106	+	+	0	0	+	0	0	+	0	+	0	+	+	0	0	+	+	0	+	+	0	0	+	+	0	+	+	0	+	+	0	+	+	0	+	+	0	+				
3	R2R2	503107	+	0	+	+	0	0	0	+	0	+	0	+	+	+	+	0	0	0	0	+	0	+	+	+	+	0	+	+	+	+	+	0	+	+	0	+	+	0	+			
4	Ror	503108	+	0	0	+	+	+	0	+	0	+	0	+	+	0	+	0	+	0	0	+	0	+	0	+	0	+	+	0	+	+	0	+	+	0	+	+	0	+	+	0	+	
5	r'r	503109	0	+	0	+	+	+	0	+	0	+	+	+	+	+	+	+	0	+	+	+	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	+	@	+	0	+	
6	r'r	502593	0	0	+	+	+	+	0	+	0	+	+	+	+	0	+	0	+	0	+	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	+	@	+	0	+	
7	rr	503110	0	0	0	+	+	+	0	+	+	0	+	+	+	0	+	+	0	+	0	+	0	+	+	+	+	+	+	+	+	+	+	+	+	+	0	+	@	+	0	+		
8	rr	502504	0	0	0	+	+	+	0	+	+	+	+	+	0	+	0	+	0	+	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	+		+	0	+		
9	rr	503111	0	0	0	+	+	+	0	+	0	+	+	+	0	+	+	+	+	0	+	0	+	0	+	0	+	+	+	+	+	+	+	+	+	+	0	+		+	0	+		
10	rr	503112	0	0	0	+	+	+	0	+	0	+	+	+	0	0	+	+	0	+	0	+	0	+	0	+	0	+	+	+	+	+	+	+	+	0	+	HLA+	+	0	+			
11	R1R1	503113	+	+	0	0	+	0	0	+	+	0	+	+	0	+	+	+	+	0	+	0	+	+	0	+	+	0	+	+	+	0	+	+	0	+	+	0	+	+	0	+		
	Patient Cells																																											
Mode of			Rh-hr							KELL				DUFFY		KIDD		Sex Linked	LEWIS			MNS				P	LUTHERAN		Special Antigen Typing	Test Results														
Cell#	Rh-hr	Donor Number	D	C	E	c	e	f	Cw	V	K	k	Kpa	Kpb	Jsa	Jsb	Fya		Fyb	Jka	Jkb	Xga	Lea	Leb	S	s	M	N		P ₁	Lu ^a	Lu ^b	Cell#	I	E									
1	R1wR1	500097	+	+	0	0	+	0	+	+	+	0	+	+	0	+	+	+	0	+	+	0	0	+	+	+	+	+	0	+	+	+	+	+	+	0	+			1	3			
2	R2R2	500817	+	0	+	+	0	0	0	+	0	+	0	+	+	0	+	0	+	0	+	+	+	0	0	+	0	+	0	+	+	+	+	+	+	+	0	+			2	3		
3	rr	503119	0	0	0	+	+	+	0	+	0	+	+	+	0	+	+	0	+	0	+	0	+	0	+	0	+	+	0	+	+	+	+	+	+	+	0	+			3	3		

UI – Example 2

Actual Response	Excluded Response	Designated Responses
c+/-E,UI	S,K,Fyb	c+/-E,K

Patient 1

Explain briefly why identification could not be confirmed in-house, including reasons why specificities could not be positively identified or excluded.

Unable to exclude the presence of Anti-Fyb, Anti-S and Anti-K. Only one antigen positive cell non reactive in IAT, others masked by presence of Anti-c. Local policy requires two non-reacting cells to formally exclude.
Other available panel antigrams included in the PDF.

- *“From the results obtained and additional cells available, it is possible to identify anti-c+/-E and anti-K and exclude all other common specificities and therefore your results remain as anti-c+/-E and UI.”*

UIs

- We want to agree with you
- We don't always agree with ourselves
- Give us an explanation
- Give us your antigrams
- Are you following BSH guidance
- You can always appeal

Any Questions?

Feedback Feedback Feedback

BTLP@ukneqas.org.uk