UK NEQAS Histocompatibility & Immunogenetics

UK NEQAS H&I

Annual Participant's Meeting 2020-21

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UK NEQAS

International Quality Expertise

Welcome and Introduction

Dr Judith Worthington

Chair of UK NEQAS for H&I Steering Committee

GIG CYMRU NHS WALES
Ymddiriedolaeth GIG Prifysgol Felindre
Velindre University NHS Trust

Welsh Blood Service

Meet The Team!

Director: Dr Tracey Rees

Deputy Director: Deborah Pritchard

Operations Manager: Amy De'Ath

Deputy Manager: Melanie Bartley

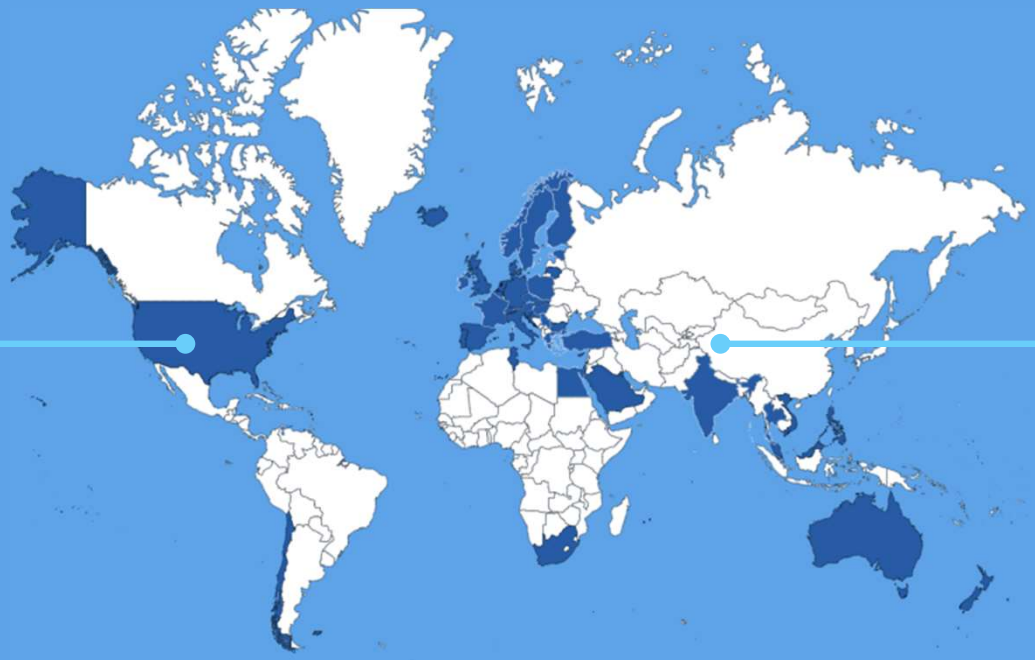
Healthcare Scientist Practitioner: Geraint Clarke

QA Technical Officer: Jack Jefferies

MLA: Owain Seldon

UK NEQAS for H&I: An Overview

Over 350 participants...



In >50 countries.

UK NEQAS International Quality Expertise Histocompatibility & Immunogenetics

UK NEQAS for H&I Steering Committee 2021

Judith Worthington (Chair)
Arthi Anand
Katy Derbyshire
James Kelleher
Sylvia McConnell
Anthony Poles
Rommel Ravanan (Clinical Representative)
Elizabeth Wroe (BSHI Representative to UK NQAAP)

Kathryn Robson (Expert Advisor Scheme 5B)
Marian Hill (Expert Advisor Scheme 5B)
Tim Clench (Expert Advisor Scheme 5B)

Key Data from the Schemes

Amy De'Ath
UK NEQAS for H&I Operations Manager

Things To Note...

Presentation Focus...

Performance, key trends, discussion points and 2021 changes

Further Details...

The presentation will be available to view on our website.

Lab Locations...

1-100 = UK & Ireland.
101+ = Rest of the world.

Please ask questions using the Q&A function!

Scheme Assessments

- Most Schemes assessed on a consensus basis using a 75% consensus level i.e. 75% of reports must agree on a result for it to be assessed.
- Reference typing results are used for typing/disease schemes if consensus not reached plus educational schemes where required:
  - *e.g. Scheme 8: HLA Genotyping for Coeliac and Other HLA Associated Diseases*
    Scheme 4A1: HLA Typing at 1st Field Resolution - DPB1 assessment using a reference result

- Equivocal result only accepted for Scheme 2B.
- All Not Tested (NT) results excluded from assessment.
- Labs that fail to return results or do not a provide valid reason for NT are assessed as unacceptable.

Unsatisfactory Performance (UP)

- Each scheme has minimum annual performance criteria:
  - *HLA Typing schemes 90%*
  - *Crossmatching 85%*
  - *Disease Association Schemes 100%*
  - *Antibody Specificity 75%*
  - *Antibody Detection 80%*

- Participants that do not meet the minimum criteria are classed as unsatisfactory performers.
- Must complete a root cause analysis and CAPA form.

Changes for 2021-22

Steering Committee

New member rec

rticipant's Portal

s

We will also be launching our new website which is currently under development!

New

MLA

Scheme Changes

3: Optional DQA and DPA assessment
8: Allopurinol Hypersensitivity

Scheme 2A

Cytotoxic Crossmatching

Scheme 2A – Cytotoxic Crossmatch

Purpose

Assess participants ability to determine cell/serum cytotoxicity crossmatch status

Consensus

At least 75% agreement on pos/neg result

Satisfactory Performance

85% of reports agree with consensus in distribution year for each cell/DTT type

10 blood samples, 40 serum samples over 5 distributions

Scheme 2A: Performance

All cells with and without DTT	2016 +DTT	2017	2018	2019	2020
Number of Participants (UK&I)	64 (18)	75 (19)	71 (18)	71 (22)	66 (16)
Number with Unsatisfactory Performance (< 85%) (UK&I)	13 (6)	16 (6)	16 (7)	5 (1)	7 (0)
% Unsatisfactory Performance (UK&I)	20.3% (33.3%)	21.3% (31.6%)	22.5% (38.8%)	7.0% (4.5%)	10.6% (0)

2020: 7 Unsatisfactory Performers (0 UK & Ireland)

Scheme 2A: UK&I Performance

	PBL	PBL +DTT	T Cell	T Cell +DTT	B Cell	B Cell +DTT
Crossmatches assessed (n=40)	31	31	40	39	34	34
% NT	13.3%	16.7%	15.0%	16.3%	21.8%	27.5%
NT	41	49	74	94	97	134
% incorrect assignments	2.2%	3.4%	2.4%	3.8%	5.4%	6.6%
False Positive	5	4	9	12	6	15
False Negative	2	6	3	10	18	17

Scheme 2A: Unacceptable Performers 2020

	PBL -DTT	T -DTT	B -DTT	PBL + DTT	T + DTT	B + DTT	Lab Identified Error
116		81%			83%		Cell viability
145		80%	82%				Sample mix up error
159			77%			82%	Cell viability
235				82%			
351		0%	0%		0%	0%	Sample delays & no results returned
411			76%			83%	
1349	74%	78%	56%	68%	74%	65%	Procedural/testing issues

Scheme 2A: Do Cell Seperation Methods Affect B Cell Viability?

Method of Cell Separation Used by All Participants in 2019-20 (n=84)	Average % Cell Viability Reported	Number Submitting Viability Info	Number Reported Using Method
Invitrogen Dynabeads	87%	15	19 (22%)
Stem Cell EasySep	81%	17	22 (26%)
One Lambda Fluorobeads	74%	4	9 (11%)
Miltenyi Biotec MACSprep	88%	5	6 (7%)
Other Methods e.g. Ingen-Eurobio/ Lagitre/ Nylon Fiber Columns	89%	3	3 (4%)
Not Known	85%	17	25 (30%)

Method of Cell Separation Used by All Participants in 2020-21 (n=77)	Average % Cell Viability Reported	Number Submitting Viability Info	Number Reported Using Method
Invitrogen Dynabeads	81%	12	17 (22%)
Stem Cell EasySep	88%	17	22 (29%)
One Lambda Fluorobeads	88%	7	7 (9%)
Miltenyi Biotec MACSprep	79%	2	7 (9%)
Other Methods e.g. Ingen-Eurobio/ Lagitre/ Nylon Fiber Columns	71%	1	4 (5%)
Not Known	86%	6	20 (26%)

- Most widely used methods are Dynabeads and Stem Cell EasySep
- Viability varies between kits
  - Miltenyi users average 88%
  - Fluorobead users average 74%

- Most widely used methods are Dynabeads and Stem Cell EasySep
- Viability varies between kits
  - EasySep + Fluorobead users average 88%
  - Other method users average 71%

Scheme 2A: Do Cell Seperation Methods Affect B Cell Viability and Performance?

2019	2A-01	2A-02	2A-03	2A-04	2A-05	2A-06	2A-07	2A-08	2A-09	2A-10	Average	B cell Performance Without DTT
Invitrogen Dynabeads	78	72	76	86	71	68	67	68	73	77	**87**	**94**
StemCell EasySep	69	69	58	58	69	53	64	62	85	85	**81**	**97**
One Lambda Fluorobeads	60	63	60	64	86	60	92	94	79	80	**74**	**99**
Miltenyi MACSprep	93	83	89	89	89	69	73	76	75	75	**88**	**93**
Average	**75**	**72**	**71**	**74**	**79**	**62**	**74**	**75**	**78**	**79**	**74**	**96**

2020	2A-01	2A-02	2A-03	2A-04	2A-05	2A-06	2A-07	2A-08	2A-09	2A-10	Average	B cell Performance Without DTT
Invitrogen Dynabeads	77.6	79.2	77.8	74.4	82.8	72.2	83.3	86.7	89.3	87.8	**81**	**92.5**
StemCell EasySep	84	90	90	91	93	69	92	92	93	88	**88**	**97**
One Lambda Fluorobeads	80.6	84.5	84.1	82.7	87.9	70.8	80.0	80.0	91.0	70.0	**81**	**81.4**
Miltenyi MACSprep	90.0	93.3	94.3	94.7	92.8	45.0	94.2	92.5	92.7	92.7	**88**	**96.9**
Average	**83**	**87**	**87**	**86**	**89**	**64**	**87**	**88**	**91**	**85**	**85**	**92**

- Highest reported cell viability not always associated with best performance (2019 v 2020)

Scheme 2A: Do Cell Seperation Methods Affect B Cell Viability and Performance?

Looking at B cell performance without DTT in comparison to cell viability:

2019 B cell Without DTT	Average Cell Viability	Overall Performance
Dynabeads (n=15)	87	94
StemCell EasySep (n=17)	81	97
One Lambda Fluorobeads (n=4)	74	99
Miltenyi MACSprep (n=5)	88	93

- Fluorobead users who reported the lowest cell viability had the best overall performance in the scheme
- Dynabead and Miltenyi users who reported the highest cell viability had the worst overall performance in the scheme

Note: data will be affected by number of users (Dynabead n=15, Flourobeads n=4 and Miltenyi n=5)

2020 B cell Without DTT	Average Cell Viability	Overall Performance
Dynabeads (n=10)	81	93
StemCell EasySep (n=16)	88	97
One Lambda Fluorobeads (n=2)	81	81
Miltenyi MACSprep (n=6)	88	97

- StemCell and Miltenyi users who reported the highest cell viability had the best overall performance in the scheme
- Dynabead and Flourobead users reported the same average cell viability but Dynabead users had better overall performance

Note: data will be affected by number of users (Dynabead n=10, Flourobeads n=2)

Scheme 2A: Discussion

- Not all Scheme 2A results will reach consensus (that's ok!)
- B-cells are difficult (transport, non-specific binding)
- Only partially emulates clinical practice
- 2A is a technical assessment of cytotoxic crossmatching and should not be 'interpreted'
- Lab's need to ensure that all test parameters and acceptance criteria are met prior to reporting NEQAS samples
    - CDC assays are not quantitative so reliant on subjective assessment

Scheme 2B

Crossmatching by Flow Cytometry

Scheme 2B: Crossmatching by Flow Cytometry

Purpose

Assess participants ability to determine cell/serum flow crossmatch status

Consensus

At least 75% agreement on pos/neg or equivocal result

Satisfactory Performance

85% reports agree with consensus in distribution year for each cell type

10 blood samples, 40 serum samples over 5 distributions

Scheme 2B: Performance

All cells with and without DTT	2016	2017	2018	2019	2020
Number of Participants (UK&I)	76 (23)	85 (22)	83 (22)	84 (23)	80 (21)
Number with Unsatisfactory Performance (< 85%) (UK&I)	13 (1)	8 (1)	15 (2)	12 (1)	11 (0)
% Unsatisfactory Performance (UK&I)	17.1% (4.3%)	8.7% (4.5%)	18.1% (9.1%)	14.2% (4.3%)	13.8% (0)

2020: 11 Unsatisfactory Performers (0 UK & Ireland)

Scheme 2B: Summary

	T Cells			B Cells		
	UK&I	RoW PC	RoW WB	UK&I	RoW PC	RoW WB
Number of participants	21	30	28	20	27	28
Number of XM assessed (>75% consensus)	38/40	38/40	39/40	39/40	36/40	38/40
Number of Positive XM	27	22	28	32	31	36
Number of Negative XM	11	16	11	7	5	2
Number of incorrect assignments	20 (2.5%)	46 (4.0%)	54 (4.9%)	18 (2.3%)	30 (3.1%)	45 (4.2%)
Number of False Pos	9	28	9	11	7	2
Number of False Neg	11	18	45	7	23	43
Number of equivocal assignments	0 (0%)	0 (0%)	6 (0.5%)	2 (0.3%)	2 (0.2%)	5 (0.5%)
Number of NT assignments	26 (3.3%)	117 (10.3%)	129 (11.8%)	23 (2.9%)	83 (8.5%)	133 (12.5%)

UK&I and RoW receive different blood samples

Scheme 2B: Unacceptable Performers 2020

Lab	T Cell	No. of results submitted	B Cell	No. of results submitted	Error
119	82.9%	36/40	91%	36/40	Poor cell viability/sample delays
142	74.4%	40/40	95%	40/40	Interpretation issues
143	78.9%	20/40	N/A	N/A	Technical issues/low cell viability
147	84.2%	40/40	94%	40/40	
186	92%	40/40	84.2%	40/40	
191	82.1%	40/40	55.3%	40/40	Reporting/results issues
235	78.4%	39/40	89%	40/40	
245	50%	8/40	71.4%	8/40	Testing suspended/reagents under validation
311	0%	0/32	0%	0/32	No results returned
351	21.7%	8/40	14.3%	8/40	Cell count low/no results returned
374	66.7%	40/40	81.6%	40/40	

11 labs with UP (<85%)

Scheme 2B: Equivocal Results

- In 2020 Equivocal results were assessed

  - i.e. if 75% or more of participants report positive/negative, any laboratories reporting 'equivocal' were assessed as 'unacceptable'
  - If a 75% consensus result is not reached when including the equivocal reports, the sample was not assessed.

    - Technical issues and invalid results (e.g. control failures, replicate issues, sample quality issues) should be reported as 'Not Tested' with the reason stated.

Scheme 2B: Reporting of Equivocal Results

- 2020 Summary
  - 7 T cell equivocal results (from 3083 = 0.2%)
  - 11 B cell equivocal results (from 2929 = 0.4%)
  - 6 T cell equivocal results assessed as unacceptable (0.2%)
  - 9 B cell equivocal results assessed as unacceptable (0.3%)

2020	No of Labs Reporting Equivocal	No. of Labs Reporting >1 Equivocal Result
UK (n=21)	1 (4.8%)	0 (0%)
OS (n= 58)	9 (15.5%)	4 (6.9%)
Total (n=79)	10 (12.6%)	4 (5.1%)

2020	T cell Equivocal Results	Total Results	B cell Equivocal Results	Total Results	Equivocal Assessed as Unacceptable Result	
					T cell	B cell
1+2	2	602	3	575	2	2
3+4	2	627	2	593	2	2
5+6	0	611	0	582	0	0
7+8	2	629	3	596	2	3
9+10	1	614	3	583	0	2
Totals	7	3083	11	2929	6	9

Scheme 2B: Do Cell Seperation Methods Affect Performance?

- Analysis of cell preparation methods reported in 2020-21

		Average Performance	
Technique	Number of Labs (n=78)	B cell	T cell
Ficoll	26 (33%)	94.7	94.2
Lymphoprep	8 (10%)	98	97.3
Lympholyte	5 (6%)	97.9	96.3
Unspecified Density Gradient	7 (9%)	96.4	96.9
Miltenyi MACSprep	2 (3%)	96.2	98.7
StemCell EasySep	2 (3%)	98.6	100
Other	9 (11%)	95.1	91.8
Unknown	13 (17%)	89.3	84.8
Pre-prepped cells	6 (8%)	96.4	97.6

- 58% participants use some form of density gradient separation media
- The percentage of acceptable T cell crossmatches was highest in those labs that use Miltenyi and StemCell (6% participants)
- The percentage of acceptable B cell crossmatches was highest in those labs that use Lymphoprep, Lympholyte and StemCell (19% participants)

Scheme 6

HLA Antibody Detection

Scheme 6: HLA Antibody Detection

Purpose

Assess participants ability to determine **presence or absence** of HLA antibodies

Consensus

At least 75% agreement on presence/absence of HLA antibodies

Satisfactory Performance

80% reports agree with consensus in distribution year

12 serum samples over 2 distributions

Scheme 6: Performance

2 Unsatisfactory Performers (0 UK&I)

	2016	2017	2018	2019	2020
Number of Participants (UK&I)	98 (24)	101 (24)	88 (25)	82 (25)	74 (25)
Number with Unsatisfactory Performance (< 80%) (UK&I)	18 (4)	21 (0)	5 (0)	8 (0)	2 (0)
% Unsatisfactory Performance	18.4% (16.7%)	20.8% (0%)	5.7% (0%)	9.7% (0%)	2.7% (0%)

The 2 labs with unacceptable performance:

- x1 used One Lambda kits; x1 no information

Scheme 6: Not Assessed Samples

28/1680 (1.7%) results out of consensus (6 UK&I)

2020 Sample	2019 Sample	2018 Sample	Class I All Labs (n=90)	Class I UK&I (n=25)		Class II All Labs (n=88)	Class II UK&I (n=24)
601	601	601	92.9%	96%		97.5%	100%
602*	602	602	90.5%	100%		98.8%	100%
603*	603	603	90.4%	96%		91.3%	96%
604	604*	604*	56.6%	52%		100%	100%
605	605*	605	100%	100%		100%	100%
606	606	606	95.2%	100%		61.3%	60%
607*	607	607	98.8%	100%		100%	100%
608	608*	608	75.3%	100%		100%	100%
609	609	609	100%	100%		100%	100%
610*	610	610	100%	100%		100%	100%
611	611	611*	70.2%	52%		100%	100%
612	612*	612*	74.1%	56%		51.2%	62.5%

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* Denotes samples were sourced from non-transfused male donors

Scheme 6: Not Assessed Samples

Not Assessed Samples from Non-Transfused Males		Class I	Class II
2020-21	602		
	603		
	607		
	610	<2,500 A26 <2,000 A25, ?A66, B37	
2019-20	604		*Not Tested*
	605		<5,000 DQ9 DQ8 ?DQ7
	608		
	612		
2018-19	604	<1,500 A23	
	611	<7,000 B45 <3,000 Cw4 <1,500 ?A34 A43 A66 Cw14	
	612	<1,500 A80	<1,500 ?DP11 ?DP13 ?DP1

Scheme 3

HLA Antibody Specificty Analysis

Scheme 3: HLA Antibody Specificity Analysis

Purpose

Assess participants ability to determine **specificity** of HLA antibodies

Consensus

At least 75% agreement on presence of HLA antibodies, 95% agreement on absense.

Satisfactory Performance

75% reports agree with consensus in distribution year

10 serum samples over 2 distributions

Scheme 3: Performance

Class I		2016	2017	2018	2019	2020
Number of Participants (UK&I)		85 (24)	72 (24)	73 (25)	70 (25)	64 (24)
Number with Unsatisfactory Performance (UK&I)	Presence	8 (0)	10 (0)	15 (1)	3 (0)	1 (0)
	Absence	3 (0)	3 (0)	5 (0)	2 (0)	1 (0)
% Unsatisfactory Performance	Presence	9.4%	13.8%	20.5%	4.2%	1.6%
	Absence	3.5%	4.2%	6.8%	2.6%	1.6%

- CI 1 Unsatisfactory Performer (0 UK&I)

- CII 3 UP (0 UK&I)

Class II		2016	2017	2018	2019	2020
Number of Participants (UK&I)		85 (24)	72 (24)	75 (25)	69 (25)	63 (24)
Number with Unsatisfactory Performance (UK&I)	Presence	5 (0)	5 (0)	12 (0)	5 (0)	2 (0)
	Absence	4 (0)	2 (0)	3 (0)	2 (0)	1 (0)
% Unsatisfactory Performance	Presence	5.9%	6.9%	16.0%	7.2%	3.2%
	Absence	4.7%	2.8%	4.0 %	2.8%	1.6%

Scheme 3: Unacceptable Performers 2020

3 labs (0 UK&I) with UP (<75%)

Lab	Class I		Class II		Kit
	Presence	Absence	Presence	Absence	
169	98%	96%	89%	71%	LABScreen
302	73%	63%	56%	94%	No info
1349	89%	100%	72%	100%	Lifecodes

Scheme 3: Class I Assessment

	Number of HLA Class I Specificities (n=64)										
	301	302	303	304	305	306	307	308	309	310	Total
Present (≥75%)	20	48	21	17	15	25	36	5	0	7	194
Absent (<5%)	19	13	27	8	24	18	31	30	23	29	252
Absent 0%	0	13	14	61	37	37	3	10	61	42	308
Not Assessed (5-74%)	20	15	23	2	12	8	19	14	5	10	128

574 (absent 0% not included in analysis) specificities reported over 10 samples

33.8% reached consensus presence

43.9% reached consensus absence

22.3% specificities were not assessed

Scheme 3: Class II Assessment

DPB included in assessment in 2020

	Number of HLA Class II Specificities (DR, DQ, DP) (n=63)										
	301	302	303	304	305	306	307	308	309	310	Total
Present (≥75%)	14	0	0	0	10	0	13	19	8	0	64
Absent (<5%)	21	27	9	2	9	18	15	6	11	15	133
Absent 0%	0	6	27	42	23	29	13	0	11	27	178
Not Assessed (5-74%)	11	13	10	2	3	0	6	15	16	3	99

296 specificities (absent 0% not included in analysis) reported over 10 samples

21.6% reached consensus presence

44.9% reached consensus absence

33.4% specificities were not assessed

Scheme 3: DPB Only

	Number of HLA DPB Specificities (n=63)										
	301	302	303	304	305	306	307	308	309	310	Total
Present (≥75%)	11	0	0	0	0	0	11	6	0	0	28
Absent (<5%)	8	8	4	2	6	8	2	1	5	2	46
Absent 0%	0	6	10	16	11	11	1	6	7	17	74
Not Assessed (5-74%)	0	5	5	1	2	0	5	4	7	0	29

3 samples had DPB1 specificities that reached consensus

103 specificities reported over 10 samples

- 27.2% reached consensus presence
- 44.7% reached consensus absence
- 28.2% specificities were not assessed

Scheme 3: DQA and DPA Assessment

A survey was sent to Scheme 3 participants in August 2020 to ascertain if they would like the inclusion of DQA and DPA antibodies to form part of the assessment (46 responses).

24% participants would like to be assessed for DQA antibodies only
- UK&I - 21%
- RoW - 27%

48% would like to be assessed for DQA and DPA antibodies
- UK&I - 54%
- RoW - 41%

25% UK&I labs and 32% RoW would not like to be assessed for DQA/DPA antibodies

80% labs have a cut off for defining DQA/DPA antibodies
- 500 - 19%
- 000 - 27%
- 1500 - 5%
- 2000 - 32%

In UK&I most common response 2000 (52%)
In RoW most common response 500 (44%)

63% of labs consider DQA antibodies when assessing potential donor suitability
- UK&I - 96%
- RoW - 27%

44% of labs consider DPA antibodies when assessing potential donor suitability
- UK&I - 67%
- RoW - 18%

Scheme 3: Kit Use

Manufacturer	2019-20			2020-21		
	UK&I	RoW	Overall Use	UK&I	RoW	Overall Use
One Lambda LABScreen	11 **(42%)**	25 **(50%)**	**36 (47%)**	13 **(54%)**	22 **(55%)**	**35 (55%)**
Immucor Lifecodes	3 **(12%)**	13 **(26%)**	**16 (21%)**	1 **(4%)**	12 **(30%)**	**13 (20%)**
LABScreen and Lifecodes	10 **(38%)**	1 **(2%)**	**11 (15%)**	10 **(42%)**	4 **(10%)**	**14 (22%)**
Unknown	2 **(8%)**	11 **(22%)**	**13 (17%)**	0 **(0%)**	2 **(5%)**	**2 (3%)**
Total	26 **(34%)**	50 **(66%)**	**76**	24 **(38%)**	40 **(62%)**	**64**

Overall LABScreen kits are the most widely used

UK&I labs are more likely to use a combination of kits (38/42% compared to 2/10% RoW)

Immucor kit use more prevalent in RoW labs (26/30% compared to 12/4% UK&I)

Scheme 3: Testing Strategy 2020-21

A further breakdown by type of kits used for Scheme 3 and the testing strategy shows:

2020-21	Use in Testing Protocol	LABScreen Mixed (LSM12)	SA Class I (LS1A04)	SA Class II (LS2A01)	PRA Class I (LS1PRA)	PRA Class II (LS2PRA)	PRA Class I&II (LS12PRA)	Multi (LSMUTR)	Total LABScreen Use
UK&I	Selected Use	0	6	6	2	2	0	1	17
	Used for All Testing	7	17	17	0	0	0	1	42
RoW	Selected Use	0	4	4	0	0	0	0	8
	Used for All Testing	10	21	20	2	2	0	0	55
All	Selected Use	0	10	10	2	2	0	1	25 (20%)
	Used for All Testing	17	38	37	2	2	0	1	97 (80%)
	Total	17	48	47	4	4	0	2	122
	Percent	14%	39%	39%	3%	3%	0%	2%	68%

The most common manufacturer used is OL (66.5% 2019, 68% 2020) in comparison to Immucor (33.5% 2019, 32% 2020)

A testing kit is usually applied to all samples (~80% test all, ~20% test selected samples)

2020-21	Use in Testing Protocol	Lifecodes Lifescreen Mixed (LMX)	SA Class I (LSAI)	SA Class II (LSAII)	Class I ID (LM1)	Class II ID (LM2)	SA CI&CII (LSAI&II)	SA MIC (LSAMIC)	Total Lifecodes Use
UK&I	Selected Use	0	4	4	0	0	0	0	8
	Used for All Testing	1	7	6	0	0	0	0	14
RoW	Selected Use	1	1	0	0	0	0	0	2
	Used for All Testing	2	13	14	2	2	1	0	34
All	Selected Use	1	5	4	0	0	0	0	10 (17%)
	Used for All Testing	3	20	20	2	2	1	0	48 (83%)
	Total	4	25	24	2	2	1	0	58
	Percent	7%	43%	41%	3.5%	3.5%	2%	0%	32%

Scheme 11

HPA Antibody Detection/Specification

Scheme 11: HPA Antibody Detection/Specification

Purpose

Assess participants ability to correctly determine pesence and specificty of HPA antibodies.

Consensus

Specificity determined by at least 75% agreement and absence determined by at least 95% agreement.

Satisfactory Performance

At least 75% of specificities in agreement with the consensus result in a distribution year.

8 serum/plasma samples over 2 distributions

Scheme 11: Performance

- 3 Unsatisfactory Performer (0 UK&I)

	2017 Pilot	2018	2019	2020
Number of Participants (UK&I)	13 (3)	35 (4)	39 (5)	42 (4)
Number with Unsatisfactory Performance (< 75%) (UK&I)	N/A	1 (0)	1 (0)	3 (0)
% Unsatisfactory Performance	N/A	2.9%	2.6%	7.1%

Scheme 11: HPA Antibody Detection/Specification

- All samples could be assessed for HPA detection

2020 Sample	HPA Detection	HLA Detection	HPA Antibody ID	
			Presence	Absence
1	97.5% Neg	100% Pos	HPA-1a, 3a, 5a, CD109 2.5%; 15b 5%	
2	100% Neg	100% Pos	N/A	
3	97.6% Neg	89.5% Pos	HPA-5b 2.4%; HPA-5a 4.9%	
4	97.6% Neg	92.1% Pos	HPA-1b, 5a, 5b 2.4%	
5	97.6% Pos	95.2% Neg	HPA-5b 97.6%	HPA-5a 4.8%; GP1b 2.4%
6	92.9% Pos	100% Pos	HPA-1a 92.9%	HPA-3a, 4b 2.4%; 3b 4.8%
7	92.9% Pos	100% Pos	HPA-1b 81%, 5b 92.9%	HPA-2a, 15b, GP1a/11a 2.4%; CD109 4.8%
8	100% Neg	100% Pos	N/A	

Scheme 11: Methods Used

Method(s) used	Manufacturer	UK&I (n=5)	RoW (n=39)	Total (n=44)	Detection Limitation
Luminex	Immucor PAK-Lx	2	11	**13 (30%)**	Unable to detect HPA-6 and HPA-15 antibodies
MAIPA	2 use kit (ApDia)	1	8	**9 (20.5%)**	Depends on monoclonals used
Luminex-MAIPA	2 use kit (ApDia)	0	9	**9 (20.5%)**	
ELISA	Immucor PAKPlus	1	6	**7 (16%)**	
ELISA-Luminex	PAK-Lx, PAK-Plus	0	3	**3 (7%)**	
PITC-FC-MAIPA		1	0	**1 (2%)**	
PIFT-FC-Luminex	Pak-Lx	0	1	**1 (2%)**	
ELISA-MAIPA	PAK-Plus, ApDia	0	1	**1 (2%)**	

Methods used to detect HPA antibodies varies considerably

e.g. PAK-Lx HPA-1, -2, -3, -4, -5, GPIV and HLA CI difficulties with HPA-3a and HPA-5.

Cannot detect HPA-6 and -15.

- Even within MAIPA users there is variation in the use of monoclonals

Lab	GPIIb/IIIa (CD41, CD61)	GPIa/IIa (CD49b)	GPIb/IX (CD42b, CD42a)	GPV (CD42d)	CD109	GPIV (CD36)	HLA
20	Y2/51	31H4	PAB-5		TEA 2/16		
26	PAB1	P16	PAB-5	SW16	TEA2/16	FA6.152	w6/32+P43
379	P2	Gi9	SZ2		TEA2/16	FA6.152	W6/32
383	Anti-CD41a, Anti-CD61	Anti-CD49b	Anti-CD42a		Anti-CD109		B2-m
386	AP2	Gi9	AP1				
387	Y251	Gi9	SZ2		TEA2/16		W6/32
389	P2	Gi9	AK2		TEA2/16	FA6.152	W6/32
394	P2	Gi9	SZ2		TEA2/16		
395	P2, SZ21	Gi9	Sz1				
397	P2	AK7	SZ1	CLB-SW16			
400	ApDia Kit (+ PL1-64 & PL2-4)	ApDia Kit	ApDia Kit				ApDia Kit
1344	C17	10G11	MB45		1.50E+11		
1345	Anti-CD61	Anti CD49	Anti CD42	Anti CD42			

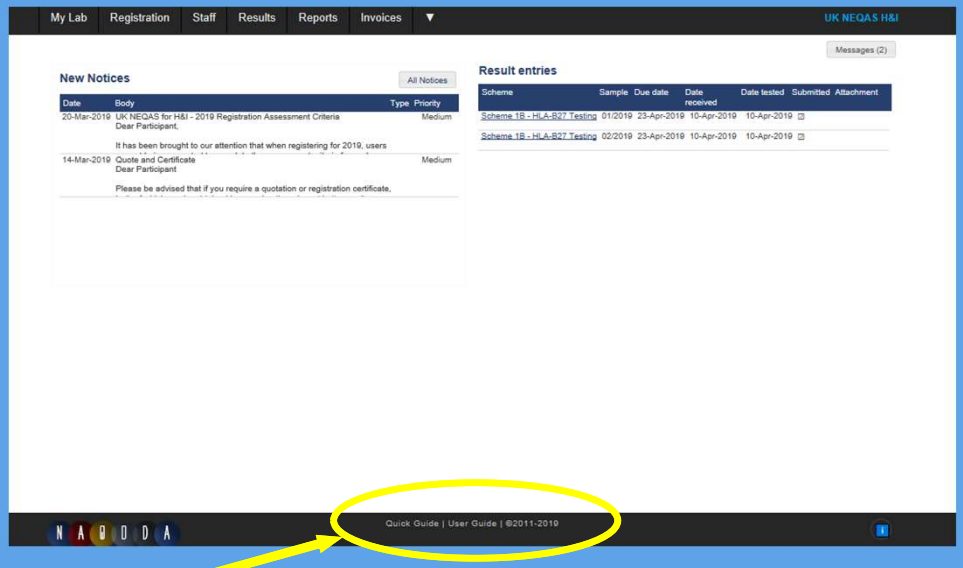
UK NEQAS
Histocompatibility
& Immunogenetics

Participant's Portal

Participant's Portal

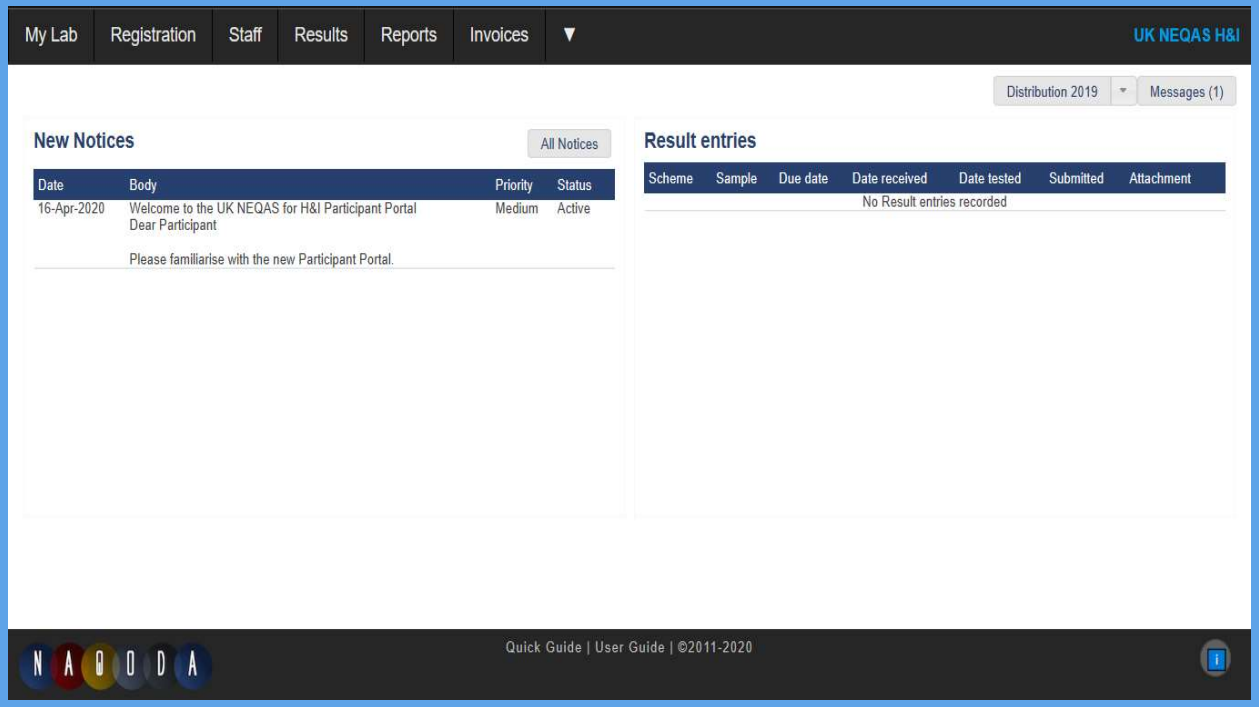
Participant's Portal

- Ease of use of the system
- Accessing reports
- Accessing result summary tables
- Data entry of results
- System generated notices



The System User Guide and the 'Quick Guide' are available in the footer section

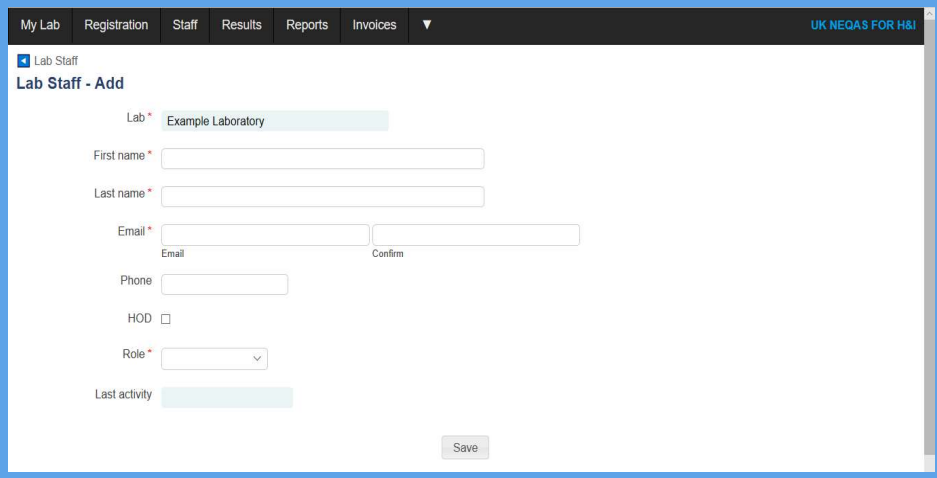
Participant's Portal: Notices



- New notices/messages from UK NEQAS for H&I are displayed on the homepage when a user logs in to the system
- Notices may contain important information so please read them regularly and mark as 'read' when finished
- Click on a notice to mark it as 'read' and remove it from the homepage.
- To view previously read notices click on All Notices

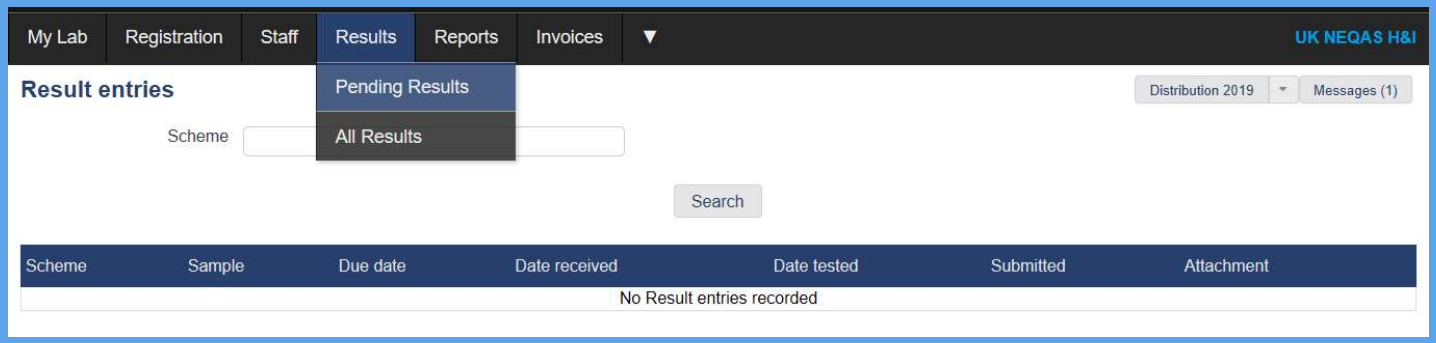
Participant's Portal: Users

- Click on the Add button in the top right corner of the 'Lab Staff' page
- Complete the required name and contact information and select the relevant user role
- Click save and the staff member will be sent an e-mail detailing how to access the system



	Participant System Function				
User Role	Administer Registration/Scheme assessment criteria	Manage Users	Enter results	View reports	View Invoices
Primary User	✓	✓	✓ All Schemes	✓ All Schemes	✓
Scheme User	×	×	✓ Assigned Schemes only	✓ Assigned Schemes only	×
Report Recipient	×	×	×	Assigned Schemes only	×

Participant's Portal: Results



- Only Primary Users or Scheme Users linked to relevant scheme can enter results
- To enter results, select Results > Pending Results, samples that have results due/open for entry will be listed here
- If relevant, the system will show you what assessment criteria you have chosen - this can be edited if incorrect in Registration > Scheme Entries
- Completion of selected assessment criteria is mandatory, denoted by *
- Only selected criteria will be assessed, however, other data can be entered for information only

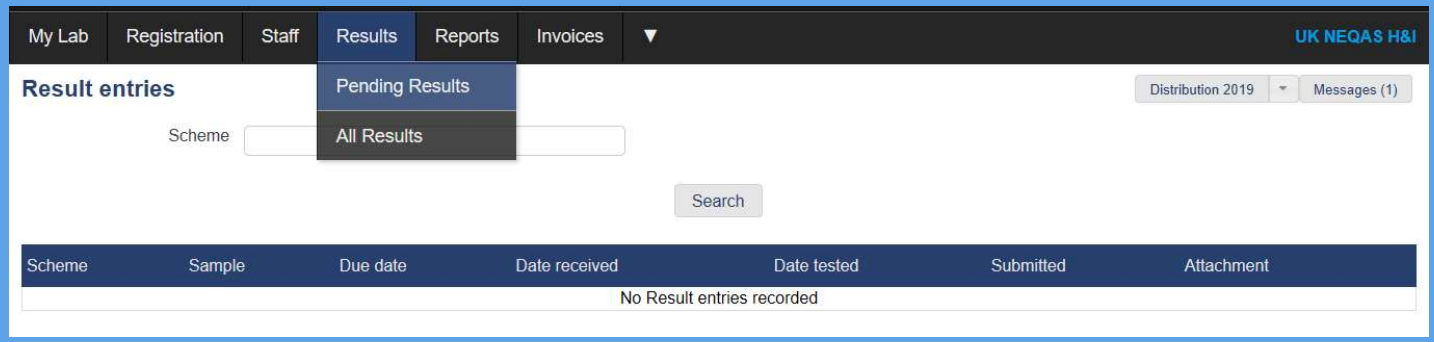
Participant's Portal: Results

Method Pages

- Complete your laboratory testing methods by completing the methodology questions. This only needs to be completed once, you can then skip to results entry on subsequent samples.

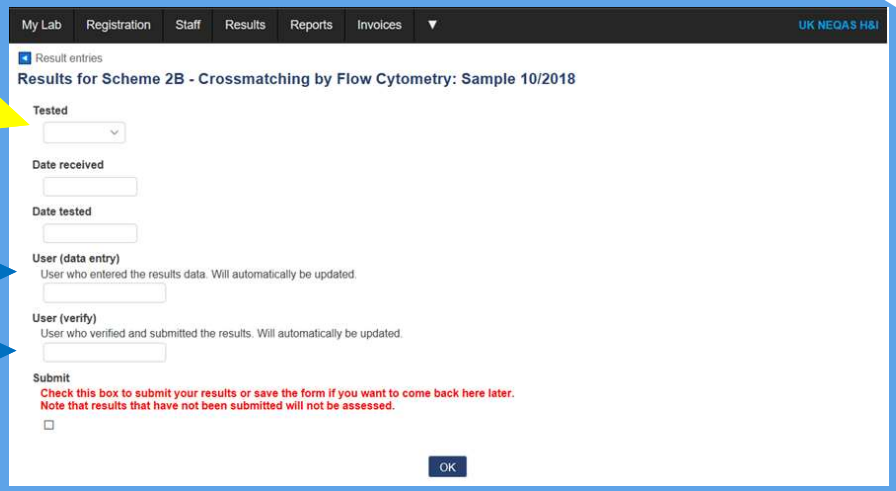
View/Save/Print Entered Results

- Select Results from the main menu and Pending Results or All Results.
- Click on the drop down arrow on the right of the 'result entries' table and select "Summary"



Participant's Portal: Results

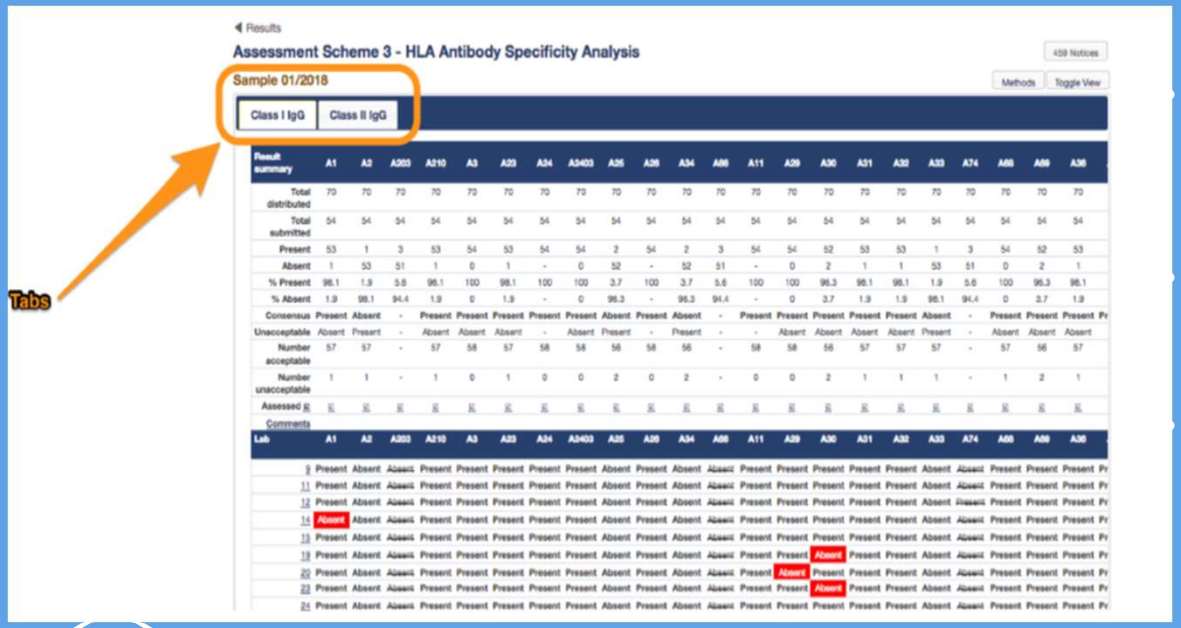
- Enter here if results were not tested and include a reason



- The User that completes the initial data entry will be named here:
- The User that ticks the "Submit" box will be named here:
- If the initial User ticks the "Submit" box, they will be named in both fields
- If verification is required by a second staff member, leave the "Submit" button unticked and press "OK"
- When satisfied with the results, the second staff member can tick the "Submit" box to show verification has been completed, then press "OK"
- Results can be amended up until the deadline
  - A reminder will be issued 2 days before the deadline
  - PLEASE NOTE: results must be formally submitted in order to be assessed. Failure to tick the "Submit" box before the deadline will result in Unsatisfactory Performance.

Participant's Portal: Performace Tables

- To view result summaries tables, select Reports > Performance Tables
- *All samples are separate entries in the system, even if in the same distribution.*



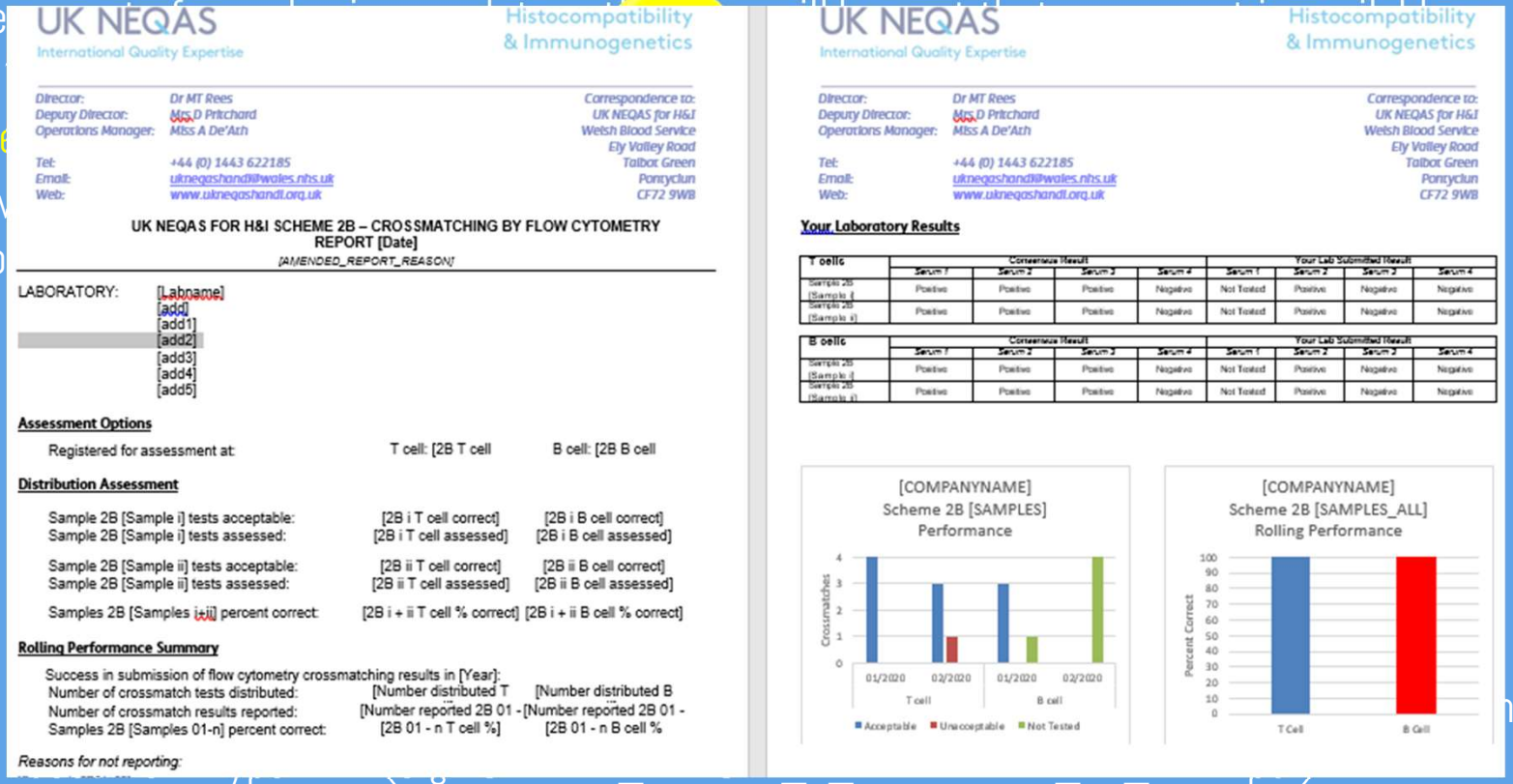
The summary tables will *highlight your lab

Performance tables can be downloaded as .xlsx files.

PLEASE NOTE: lab numbers in the Performance tables/downloaded spreadsheets are random for anonymity and therefore do not correlate to your UK NEQAS ID number

Participant's Portal: Result Reports

- Once asse
to view in
- Click on Re
- The table
notificatio
- ne



UK NEQAS
International Quality Expertise
Histocompatibility & Immunogenetics

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Ely Valley Road
Talbot Green
Pontyclun
CF72 9WB

UK NEQAS FOR H&I SCHEME 2B – CROSSMATCHING BY FLOW CYTOMETRY REPORT [Date]

[AMENDED_REPORT_REASON]

LABORATORY: [Labname]
[add]
[add1]
[add2]
[add3]
[add4]
[add5]

Assessment Options

Registered for assessment at: T cell: [2B T cell | B cell: [2B B cell

Distribution Assessment

Sample 2B [Sample i] tests acceptable:	[2B i T cell correct]	[2B i B cell correct]
Sample 2B [Sample i] tests assessed:	[2B i T cell assessed]	[2B i B cell assessed]
Sample 2B [Sample ii] tests acceptable:	[2B ii T cell correct]	[2B ii B cell correct]
Sample 2B [Sample ii] tests assessed:	[2B ii T cell assessed]	[2B ii B cell assessed]
Samples 2B [Samples i+ii] percent correct:	[2B i + ii T cell % correct]	[2B i + ii B cell % correct]

Rolling Performance Summary

Success in submission of flow cytometry crossmatching results in [Year]:

Number of crossmatch tests distributed:	[Number distributed T	[Number distributed B
Number of crossmatch results reported:	[Number reported 2B 01 -	[Number reported 2B 01 -
Samples 2B [Samples 01-n] percent correct:	[2B 01 - n T cell %]	[2B 01 - n B cell %

Reasons for not reporting:

UK NEQAS
International Quality Expertise
Histocompatibility & Immunogenetics

Director: Dr MT Rees
Deputy Director: Mrs D Pritchard
Operations Manager: Miss A De'Ath

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Pontyclun
CF72 9WB

Your Laboratory Results

T cells	Consensus Result				Your Lab Submitted Result			
	Serum 1	Serum 2	Serum 3	Serum 4	Serum 1	Serum 2	Serum 3	Serum 4
Sample 2B [Sample i]	Positive	Positive	Positive	Negative	Not Tested	Positive	Negative	Negative
Sample 2B [Sample ii]	Positive	Positive	Positive	Negative	Not Tested	Positive	Negative	Negative

B cells	Consensus Result				Your Lab Submitted Result			
	Serum 1	Serum 2	Serum 3	Serum 4	Serum 1	Serum 2	Serum 3	Serum 4
Sample 2B [Sample i]	Positive	Positive	Positive	Negative	Not Tested	Positive	Negative	Negative
Sample 2B [Sample ii]	Positive	Positive	Positive	Negative	Not Tested	Positive	Negative	Negative

The Virus, The Variants And The Vaccines – The COVID-19 Pandemic So Far

Guest Speaker

Ines Ushiro-Lumb

Clinical Microbiology Lead in Organ Donation and Transplantation, NHSBT

Key Data from the Schemes

Deborah Pritchard
UK NEQAS for H&I Deputy Director

Scheme 1A

HLA Phenotyping

Scheme 1A: HLA Phenotyping

Purpose

Assess participants ability to use serological and supplementary methods to correctly identify HLA phenotype

Satisfactory Performance

9 or more complete HLA phenotypes in agreement with consensus per distribution year.

Consensus

At least 75% agreement on each specificity.

10 blood samples over 2 distributions

Scheme 1A: Performance

- 3 labs with unsatisfactory performance (1 UK&I).

	2016	2017	2018	2019	2020
Number of Participants (UK&I)	41 (7)	38 (6)	38 (6)	38 (5)	34 (4)
Number with Unsatisfactory Performance (< 90%) (UK&I)	3 (0)	1 (0)	6 (1)	8 (1)	3 (1)
% Unsatisfactory Performance	7.3%	2.6%	15.8%	21.1%	8.8%

Scheme 1A: 2020 Incorrect Assignments

14/340 (4.1%) incorrect HLA types in 2020 reported by 6 labs:

5 reports that contained broad not split specificity (e.g. DQ3 v DQ7)
5 reports that contained an incorrect specificity (e.g. DR4 v DR13)
2 reports with molecular based nomenclature (e.g. A01 v A1)
2 reports that involved a sample mix up (complete HLA type incorrect)

CAPA responses

- Procedural error - low B cell count
- Errors not noticed during check steps
- EQA reporting procedures different to clinical samples

3/3 labs with unsatisfactory performance completed CAPA

Scheme 1A: 2020 Incorrect Assignments Resulting in UPs

Sample	Lab Number	Consensus	Report
1A 01	62	A1, A26; B37, B57	A01, A26; B37, B57
1A 02	62	A1, A29; B8, B44	A01, A29; B08, B44
1A 02	209	A1, A29; B8, B44; DR7, DR7; DQ2, DQ9	A1, A29; B8, B44; DR2, DR7; DQ2, DQ7
1A 03	209	A1, A2; B27, B60	A1, A2; B27, B40
1A 05	209	A2, A66; B41, B44; DR1, DR13; DQ5, DQ7	A2, A66; B41, B44; DR1, DR4; DQ5, DQ7
1A 05&06	193		SAMPLE MIX UP
1A 07&08	209		SAMPLE MIX UP
1A 09	209	A1, A24; B8, B35; DR1, DR17; DQ2, DQ5	A1, A24; B8, B35; DR1, DR17; DQ2, DQ1
1A10	209	A23, A24; B7, B44; DR4, DR7; DQ2, DQ8	A23, A24; B7, B44; DR4, DR7; DQ2, DQ3

Scheme 4A1

DNA Typing at 1st Field Resolution

Scheme 4A1: DNA Typing at 1st Field Resolution

Purpose

Assess participants ability to correctly determine HLA genotypes at the 1st field resolution.

Satisfactory Performance

9 or more full HLA types in agreement with consensus/reference result in a distribution year.

Consensus

At least 75% agreement on each allele. When consensus is not met, a reference result is used. Reference result is always used for DPB1 assessment

10 blood samples over 2 distributions

Scheme 4A1: Performance

- 8 labs with unsatisfactory performance (0 UK&I)

	2016	2017	2018	2019	2020
Number of Participants (UK&I)	102 (28)	106 (28)	105 (28)	100 (28)	88 (26)
Number with Unsatisfactory Performance (< 90%) (UK&I)	21 (4)	11 (1)	15 (1)	4 (1)	8 (0)
% Unsatisfactory Performance	20.6%	10.4%	14.3%	4%	9.1%

Scheme 4A1: 2020 Incorrect Assignments

- 27/835 (3%) incorrect HLA types reported by 18 different labs (5 UK&I)
  - 10 incorrect assignments (e.g. A*02 instead of A*03) (2 UK&I)
  - 8 incorrect uses of nomenclature (e.g. DQB1*2 instead of DQB1*02) (1 UK&I)
  - 6 missed null alleles (e.g. DRB4*01 instead of DRB4*01N) (2 UK&I)
  - 2 ambiguous assignments (e.g. reporting B*07 or 42 instead of B*07)
  - 1 missed assignment (e.g. reported homozygous when heterozygous)

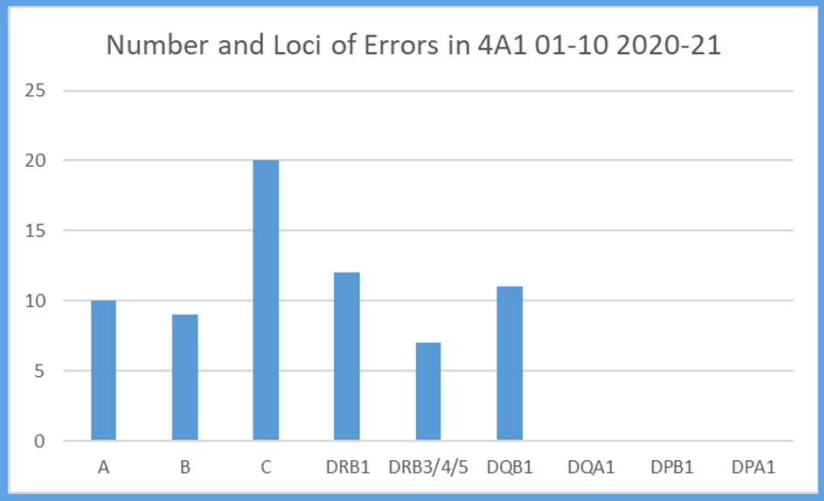
3/8 labs with unsatisfactory performance completed CAPA

CAPA responses

No analytical errors

- EQA reporting procedures different to clinical samples
- Transcription errors
- Known limitation of kit B*07/*42

8 HLA types with multiple errors = 69 allele errors



Scheme 4A1: 2020 Incorrect Assignments

20-21	A*	A*	B*	B*	C*	C*	DRB1*	DRB1*	DRB3*	DRB4*	DRB5*	DQA1*	DQA1*	DQB1*	DQB1*	DPA1*	DPA1*	DPB1*	DPB1*	Error Type	Not Reported	Incorrect Type
sample 1																						
209	1	2	7	8	7	7	3	7						2	2					*Nomenclature*	4	2
331	01	02	07 or 42	8			3	7												Ambiguity		
sample 2																						
209	2	31	27	40	2	3	4	13						3	6					*Nomenclature*	4	1
sample 3																						
209	2	68	40	44	3	5	7	15						2	6					*Nomenclature*	4	1
sample 4																						
172	02	03	07	35	03	1	04	15				01	03	03	06					Wrong type	4	2
209	2	3	7	35	3	7	4	15						3	6					*Nomenclature*		
sample 5																						
45	01	02	07	08	07	07	01	07	n/a	01	n/a	02	05	03	03			04	04	Missed Null	4	9
62	01	02	07	08	07	07	01	07	N/A	01	N/A	02	05	03	03	01	01	04:02:01	04:02:01	Missed Null		
126	01	02	07	08	07	07	01	07	N/A	01	N/A	02	05	03	03	01	01	04:02	04:02	Missed Null		
127	01	02	07	08	07	07	01	07	N/A	01	N/A	02	05	03	03					Missed Null		
195	01	02	07	08	07	07	01	07	N/A	01	N/A	02	05	03	03			04:02	04:02	Missed Null		
209	1	2	7	8	7	7	1	7						3	3					*Nomenclature*		
268	01	02	07	08	07	07	103	07				02	05	03	03					*Nomenclature*		
292	01	02	07	08	07	07	01	07	N/A	01	N/A	02	05	03	03					Missed Null		
331	01	02	07 or 42	08	NT	NT	vv	07												Ambiguity		
sample 6																						
42	01	26	08	14	07	7	07	14	02	01	N/A	01	02	02	05	01	02	02:01/05:	05:01/10	Wrong type	4	2
322	01	26	08	14	07	08	07	14				01	02	5	05					Wrong type		
sample 7																						
309	02	24	18	44	05	05	04	11	02	4	N/A	03	05	03	03	*Not*	*Not*	*Not*	*Not*	Wrong Type	4	2
322	02	24	5	5	4	11	3	3				03	05	03	03					Wrong Type		
sample 8																						
23	03	30	18	44	5	5	04	15	N/A	01	01	01	03	03	06	01	01	02	04	*Nomenclature*	4	3
230	03	30	18	44	05	05	4	15						03	06					*Nomenclature*		
374	2	30	18	44	05	05	04	15						03	06					Wrong Type		
sample 9																						
51	23	31	40	44	03	04	7	07	N/A	01	N/A	02	03	02	03	02	02	11	13	Wrong Type	4	3
209	23	24	40	44	03	04	04	07						02	03					Wrong Type		
322	23	31	40	44	7	04	04	07				02	03	02	03					Wrong Type		
sample 10																						
213	11:01	33:03:00	14:02	15:01	03:03	05:02	01:02	04:01				01:01	03:01	03:02	05:01					Wrong Type	4	2
322	11	33	14	15	01	04	01	04				01	03	03	05					Wrong Type		
Total	10		9		20		12		7			0		11		0		0			**40**	**27**

Scheme 4A1i

Interperative HLA Genotype

Scheme 4A1: Interpretive HLA Genotype

Purpose

Assess participants ability to correctly interpret their 4A1 genotype result to the 'split' specificity level.

Satisfactory Performance

9 or more full HLA types in agreement with consensus/reference result in a distribution year.

Consensus

At least 75% agreement on each specificity. When consensus is not met, a reference result is used.

10 HLA genotypes from Scheme 4A1

Scheme 4A1i: Performance

- 6 labs with unsatisfactory performance (2 UK&I)

	2017	2018	2019	2020
Number of Participants (UK&I)	36 (20)	40 (21)	44 (22)	44 (22)
Number with Unsatisfactory Performance (< 90%) (UK&I)	6 (1)	6 (0)	8 (1)	6 (2)
% Unsatisfactory Performance	16.7%	15.0%	18.1%	13.6%

Scheme 4A1i: Interpreted DNA Results

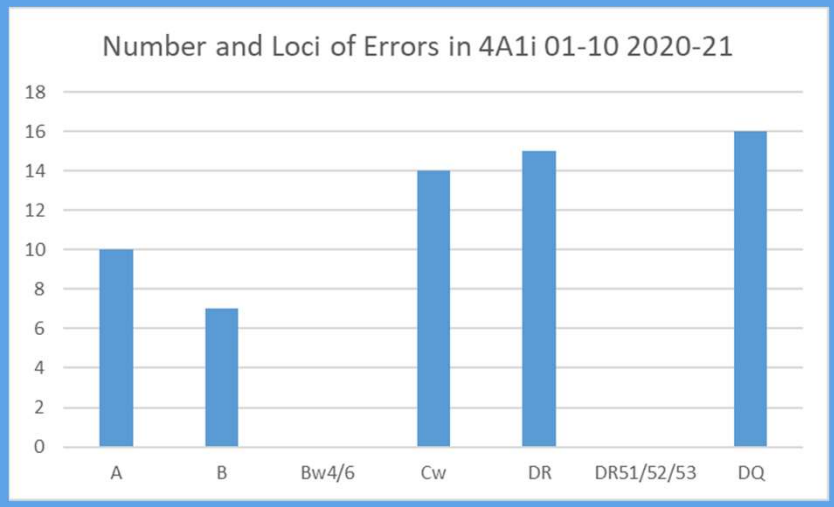
23/420 (5.5%) incorrect HLA types reported by 15 labs (7 UK&I)

- 12 reports using the wrong nomenclature (e.g. DQ02 rather than DQ2) (5 UK&I)
- 8 reports of the wrong type (e.g. DR1 instead of DR103, A24 instead of A31, B40 instead of B44) (4 UK&I)
- 2 reports of incorrect broad/split use (e.g. B40 instead of B60; DQ3 instead of DQ8)
- 1 ambiguous assignment (e.g. reported DQ7 (3) or DQ8 (3) instead of DQ7)

4/6 labs with unsatisfactory performance completed CAPA

CAPA responses

- EQA reporting procedures different to clinical samples
- Transcription errors



9 HLA types with multiple errors = 62 specificity errors

Scheme 4A1i: Interpreted DNA Results

20-21	A	A	B	B	Bw4	Bw6	Cw	Cw	DR	DR	DR51	DR52	DR53	DQ	DQ	Error Type	Not Reported	Incorrect Type
sample 1																		
58	1	2	7	8	Absent	Present	7	7	3	17	Absent	Present	Present	2	2	Wrong type	2	2
112	01	02	07	08			07	07	07	17	Absent	Present	Present	02	02	Nomenclature		
sample 2																		
14	2	31	27	60	Present	Present	02	10	4	13	Absent	Present	Present	6	8	Nomenclature	2	3
58	2	31	27	60	Present	Present	02	10	4	13	Absent	Present	Present	6	8	Nomenclature		
112	02	31	27	60			02	10	04	13	Absent	Present	Present	06	08	Nomenclature		
sample 3																		
14	2	68	44	60	Present	Present	5	10	7	15	Present	Absent	Present	02	06	Nomenclature	2	3
112	02	68	44	60			05	10	07	15	Present	Absent	Present	02	06	Nomenclature		
190	2	68	44	40	Present	Present	5	10	07	15	Present	Absent	Present	02	06	Broad/Split Nomenclature		
sample 4																		
112	02	03	07	35			07	09	04	15	Present	Absent	Present	06	07	Nomenclature	2	1
sample 5																		
9	1	2	7	8	Absent	Present	7	7	1	7	Absent	Absent	~~Absent~~	7	9	Wrong type	2	5
54	1	2	7	8	Absent	Present	7	7	1	7	Absent	Absent	~~Absent~~	7	9	Wrong type		
112	01	2	07	08			07	07	01	07	Absent	Absent	~~Present~~	07	09	Nomenclature		
190	1	2	7	8	Absent	Present	7	7	95	7	Absent	Absent	~~Absent~~	7	9	Wrong type		
220	1	2	7	8	Absent	Present	7	7	1	7	Absent	Absent	~~Absent~~	7	9	Wrong type		
sample 6																	1	1
42	1	26	8	64	Absent	Present	7	7	7	14	Absent	Present	Present	2	5	Wrong type		
sample 7																	1	0
sample 8																		
15	03	30	18	44	Present	Present	5	5	4	15	Present	Absent	Present	6	7	Nomenclature	1	3
101	3	30	18	44	Present	Present	05	05	04	15	Present	Absent	Present	6	7	Nomenclature		
260	3	30(	18(	44(	Present	Present	5	5	4	15(	Present	Absent	Present	6(1)	7(3),8(3)	Ambiguity		
sample 9																		
45	23	31	44	60	Present	Present	4	10	04	07	Absent	Absent	Present	2	8	Nomenclature	1	5
128	23	31	44	60			04	10	4	7				2	8	Nomenclature		
190	23	31	44	60	Present	Present	4	10	4	7	Absent	Absent	Present	2	3	Broad/Split		
209	23	24	44	60			4	10	4	7				2	8	Wrong type		
309	23	31	40	60	Present	Present	4	10	4	7	Absent	Absent	Present	2	8	Wrong type		
sample 10																	1	0
Total	10		7		0		14		15		0			16			15	23

Scheme 4A2

DNA Typing to 2nd or 3rd Field Resolution

Scheme 4A2: DNA Typing to 2nd or 3rd Field Resolution

Purpose

Assess participants ability to correctly determine HLA type to 2nd or 3rd field.

Consensus

At least 75% agreement on each allele. If consensus is not met, a reference result is used.

Satisfactory Performance

9 or more full HLA types in agreement with consensus/reference genotype in a distribution year.

10 blood samples over 2 distributions

Scheme 4A2: Performance

- 45/64 participants registered for 2nd field
- 19/64 participants registered for 3rd field

- 7 labs with unsatisfactory performance (0 UK&I)

5/7 labs with unsatisfactory performance completed CAPA

	2016	2017	2018	2019	2020
Number of Participants (UK&I)	63 (21)	66 (21)	63 (20)	62 (20)	64 (20)
Number with Unsatisfactory Performance (< 90%) (UK&I)	8 (2)	4 (0)	9 (2)	9 (1)	7 (0)
% Unsatisfactory Performance	12.7%	6.1%	14.3%	14.5%	11.0%

Scheme 4A2: Notice for 2021-22

- Assessment at 2nd field resolution
  - Resolve all ambiguities resulting from polymorphisms located within exon 2 and 3 for class I loci, and exon 2 for class II loci
- Assessment of 3rd field resolution
  - Participants must sequence all exons to resolve all ambiguities
    - E.g. DRB1*07:01:01/07:79 or DQB1*03:02:01/03:02:26 would be unacceptable as ambiguities in exon 4 have not been resolved
  - If you cannot unambiguously assign at the 3rd field please register for 2nd field
    - Labs are able to perform their own manual assessment at the 3rd field

Results at the 4th field can be reported, but will not be assessed

Scheme 4A2: Incorrect Assignments: 2nd Field

14/435 (3.2%) incorrect HLA types reported by 10 labs (3 UK&I)

- 5 reports of alleles in a string that should have been resolved

(e.g. A*02:01/07/09/20/24/30/53N/02:06)

- 4 reports of incorrect allele

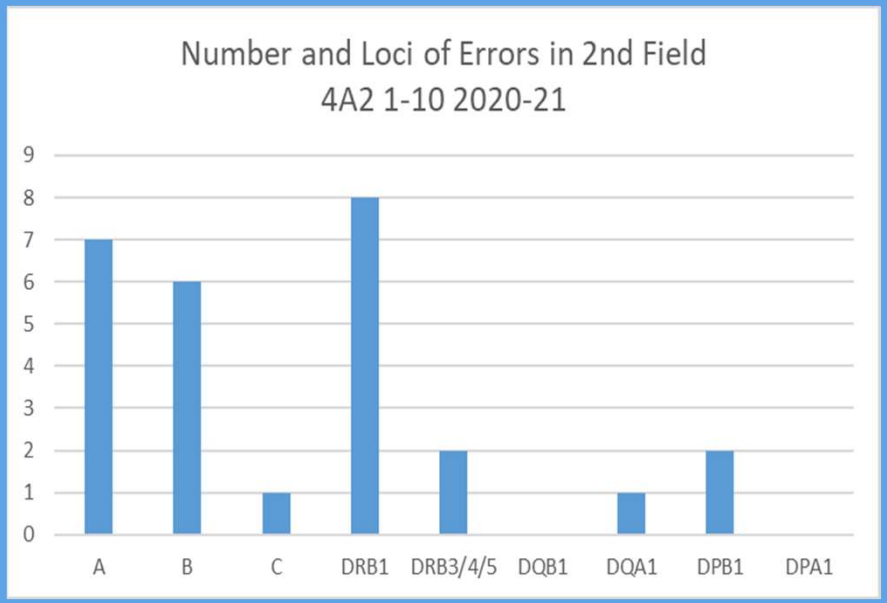
(e.g. B*37:68 rather than B*37:01)

- 3 reports incorrect at 1st field (1 UK&I)

(e.g. DPB1*23:01 rather than DPB1*04:01)

- 2 reports of homozygous type when heterozygous (2 UK&I)

(e.g. DRB1*01:01, - rather than DRB1*01:01, DRB1*01:03



CAPA responses

- Training issue of staff reporting results
- Interpretation error

6 HLA types with multiple errors = 27 allele errors

Scheme 4A2: Incorrect Assignments: 2nd Field

2nd Field	A*	A*	B*	B*	C*	C*	DRB1*	DRB1*	DRB3*	DRB3*	DRB4*	DRB4*	DRB5*	DRB5*	DQA1*	DQA1*	DQB1*	DQB1*	DPA1*	DPA1*	DPB1*	DPB1*	Error Type	Not Reported	Incorrect Type
sample 1																								0	3
112	02:01/07/09/20/24/	02:01/07/09/20/24/	15:01	44:02/05/27	04:01/09N	05:01	04:01/04/08	04:01/04/08							######	#######	03:01/19	03:02					Ambiguities should have been resolved		
142	02:01	02:01	15:01	44:02:00	04:01	05:01	04:01	04:04							Not	Not	03:01	03:02			03:01	23:01	Wrong Type		
367	02:01	02:01	15:01	44:02:00	04:01	05:01	04:08	04:13	N/A	N/A	01:03	01:03	N/A	N/A			03:01	03:02					Wrong Allele		
sample 2																								0	3
23	03:01	03:01	07:02	07:02	07:02	07:02	04:07	07:01	NA	NA	01:03	01:03	NA	NA	02:01	03:03	02:02	03:01	01:03	02:01	03:01	11:01	Homoygous when Heterozygous		
39	03:01	03:01	07:02	07:02	07:02	07:02	04:07	07:01	N/A	N/A	01:01	01:01	N/A	N/A	02:01	03:03	02:02/156	03:01/2	01:03	02:01	03:01	11:01	Homoygous when Heterozygous		
112	03:01/05	03:01/05	07:02/10	07:02/10	07:02	07:02	04:07	07:01							02:01	03:02/03	02:02	03:01/0					Ambiguities should have been resolved		
sample 3																								0	2
112	02:01/07/	25:01:00	18:01	46509.83	05:01	12:03	04:01	15:01/04							01:02	03:02/03	03:01/19	06:02					Ambiguities should have been resolved		
268	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	na	na	01:03	na	01:01	na	######	03:01:01	[illegible]	[illegible]	######	######	[illegible]	[illegible]	Wrong Allele		
sample 4																								0	1
112	02:01/07/	31:01:00	07:02	40:01:00	03:02/0	07:02	04:04	15:01/04							01:02	03:01	03:02	06:02					Ambiguities should have been resolved		
sample 5																								0	2
112	02:01/07/	30:02:00	18:01/03	40:01:00	03:04	05:01	03:01	13:01							01:03	05:01	02:01	06:03					Ambiguities should have been resolved		
226	02:01/78	30:02:00	18:01/14	40:01/379	03:04	05:01	03:02	13:01									02:01/109	06:03					Wrong Allele/Type		
sample 6																								0	0
sample 7																								0	0
sample 8																								0	0
sample 9																								0	2
267	01:01/24	31:01/11	37:/68	52:01/95	06:02/2	12:02/2	04:01/242/24	15:02/140/14									03:02/289	06:01/2			03:01/104:	05:01/1	Wrong allele		
328	01:01	31:01:00	37:01:00	52:01:00	06:02	12:02	04:01	05:02									03:02	06:01			03:01/104:	05:01	Wrong Type		
sample 10																								0	1
48	01:01/24	01:01/24	08:01/94/	14:01/28	06:02	07:01	07:01/79/93	07:01/79/93									02:02/97/	02:02/9			463:01:00	13:01	Wrong type		
Total	7		6		1		8		2						1		0		0		2			0	14

Scheme 4A2: Incorrect Assignments: 3rd Field

8/175 (4.6%) incorrect HLA types reported by 4 labs (0 UK&I)

- 5 reports of unresolved ambiguities

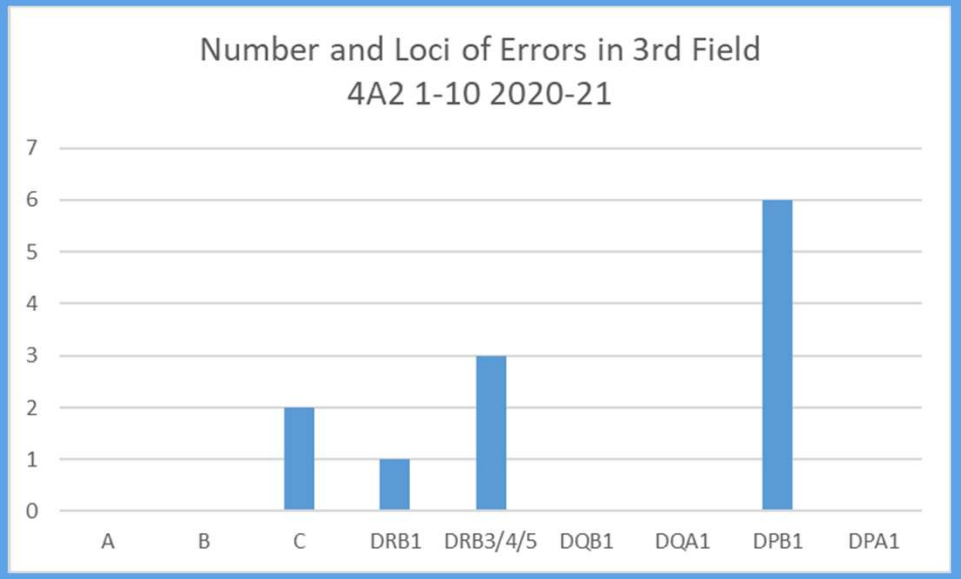
(e.g. DPB1*04:01:01/939:01)

- 2 reports at 2nd field only

(e.g. DRB1*03:01/147)

- 1 reports of incorrect allele

(e.g. DRB4*01:03:01 rather than 01:01:01)



CAPA responses

- Kit DRB4 issue
- Interpretation error
- Registration error (register for 2nd field)

3 HLA types with multiple errors = 12 allele errors

Scheme 4A2: Incorrect Assignments: 3rd Field

3rd Field	A*	A*	B*	B*	C*	C*	DRB1*	DRB1*	DRB3*	DRB3*	DRB4*	DRB4*	DRB5*	DRB5*	DQA1*	DQA1*	DQB1*	DQB1*	DPA1*	DPA1*	DPB1*	DPB1*	Error Type	Not Reported	Incorrect Type
Sample 1																								1	1
185	02:01:01	02:01:01	15:01:01	44:02:01	04:01:01	05:01:01	04:01:01	04:04:01	N/A	N/A	01:03:01	01:03:01	N/A	N/A	03:01:01	03:03:01	03:01:01	03:02:01	01:03:01	01:03:01	03:01:01	04:01:01/9	Ambiguities		
Sample 2																								1	1
176	03:01:01:0	03:01:01:0	07:02:01	07:02:01	07:02:01	07:02:01	04:07:01:01	07:01:01	N/A	N/A	01:03:01	01:03:01	N/A	N/A	02:01:01:0	03:03:01:0	02:02:01:0	03:01:01	01:03:01:0	02:01:01:0	03:01:01	11:01:01:0	01:03:01 instead of 01:01:01		
Sample 3																								1	2
185	02:01:01	25:01:01	18:01:01	44:02:01	05:01:01	12:03:01	04:01:01	15:01:01	N/A	N/A	01:03:01	N/A	01:01:01	N/A	01:02:01	03:03:01	03:01:01	06:02:01	01:03:01	01:03:01	04:01:01/9	04:01:01/9	Ambiguities		
411	02:01:01:0	25:01:01:0	18:01:01:0	44:02:01:0	08:02:01:01	12:04:02:0	04:01:01:01	15:01:01:0									03:01:01:0	06:02:01:0					Ambiguities		
Sample 4																								1	1
185	02:01:01	31:01:02	07:02:01	40:01:02	03:04:01	07:02:01	04:04:01	15:01:01	N/A	N/A	01:03:01	N/A	01:01:01	N/A	01:02:01	03:01:01	03:02:01	06:02:01	01:03:01	01:03:01	04:01:01/9	04:01:01/9	Ambiguities		
Sample 5																								1	2
176	02:01:01	30:02:01:0	18:01:01	40:01:02	03:04:01	05:01:01	03:01/147	13:01:01	02:02:01	02:02:01	N/A	N/A	N/A	N/A	01:03:01:0	05:01:01:0	02:01:01	06:03:01:0	01:03:01	01:03:01	02:02:01:0	04:01:01	2nd Field		
185	02:01:01	30:02:01	18:01:01	40:01:02	03:04:01	05:01:01	03:01:01	13:01:01	02:02:01	02:02:01	N/A	N/A	N/A	N/A	01:03:01	05:01:01	02:01:01	06:03:01	01:03:01	01:03:01	02:02:01	04:01:01/9	Ambiguities		
Sample 6																								3	0
Sample 7																								3	0
Sample 8																								3	0
Sample 9																								3	0
Sample 10																								3	1
380	01:01:01	01:01:01	08:01:01	14:01:01	06:02:01	07:01:01	07:01:01	07:01:01	N/A	N/A	01:01/03:0	01:01/03:0	N/A	N/A	02:01:01	02:01:01	02:02:01	02:02:01	01:03:01	01:03:01			2nd Field		
Total	0		0		2		1		3						0		0		0		6			20	8

Scheme 9

KIR Genotyping

Scheme 9: KIR Genotyping

Purpose

Assess participants ability to correctly determine the presence or absence of specific KIR genes.

Satisfactory Performance

9 or more full KIR genotypes in agreement with consensus/reference genotype in a distribution year.

Consensus

At least 75% agreement on the presence/abesence of each gene. Reference type used where consensus is not met

10 blood samples over 2 distributions

Scheme 9: KIR Genotyping

- Participants able to report any of the following: *KIR2DL1, KIR2DL2, KIR2DL3, KIR2DL4, KIR2DL5, KIR3DL1, KIR3DL2, KIR3DL3, KIR3DS1, KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS4, KIR2DS5, KIR2DP1, KIR3DP1.*
- Also able to report any other KIR polymorphisms they detected for information
- Participants can also report an 'A' or 'B' haplotype for each sample based on the gene content of the sample

Scheme 9: Performance

- 0 errors
- 0 labs with unsatisfactory performance

	2016 (Pilot)	2017	2018	2019	2020
Number of Participants (UK&I)	11 (2)	8 (3)	9 (1)	12 (1)	12 (1)
Number with Unsatisfactory Performance (UK&I)	*N/A*	0 (0)	1 (0)	3 (0)	0 (0)
% Unsatisfactory Performance	*N/A*	0%	11.1%	25%	0%

Scheme 10

HPA Genotyping

Scheme 10: HPA Genotyping

Purpose

Assess participants ability to correctly determine HPA polymorphisms.

Satisfactory Performance

9 or more full HPA types in agreement with consensus/reference genotype in a distribution year.

Consensus

At least 75% agreement on the presence/abesence of each allele. Reference type used where consensus is not met

10 blood samples over 2 distributions

Scheme 10: HPA Genotyping

- Participants able to report any of the following: *HPA-1, HPA-2, HPA-3, HPA-4, HPA-5, HPA-6, HPA-15*
  - 32/39 reported HPA-1, 2 , 3, 4, 5 and 15
  - 32/39 labs reported HPA-4
  - 27/39 labs reported HPA-6
- Also able to report any other HPA polymorphisms detected, *for information*

Scheme 10: HPA Genotyping

- 4 errors
- 0 labs with unsatisfactory performance

	2016 Pilot	2017	2018	2019	2020
Number of Participants (UK&I)	12 (4)	15 (5)	37 (6)	38 (6)	40 (0)
Number with Unsatisfactory Performance (< 100%) (UK&I)	N/A	1 (0)	1 (0)	3 (0)	0 (0)
% Unsatisfactory Performance	N/A	6.7%	2.7%	7.9%	0%

Scheme 10: HPA Genotyping

4 Errors:

Result Summary	HPA-1 a	HPA-1 b	HPA-2 a	HPA-2 b	HPA-3 a	HPA-3 b	HPA-4 a	HPA-4 b	HPA-5 a	HPA-5 b	HPA-6 a	HPA-6 b	HPA-15 a	HPA-15 b
Sample 1														
35	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Positive	Not Tested	Not Tested	Positive	Positive
Sample 4														
387	Positive	Positive	Positive	Positive	Negative	Positive	Positive	Negative	Positive	Positive	Positive	Negative	Positive	Positive
Sample 6														
180	Positive	Negative	Positive	Negative	Positive	Negative	Not Tested	Not Tested	Positive	Negative	Not Tested	Not Tested	Positive	Positive
Sample 9														
390	Negative	Positive	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative

Methods used for detection:

Lab with Error	Location of Error	Method	Source of Primers and Probes	Manufacturer	Detection System	Reagents/Kits Used	System
35	**HPA-15a**	PCR-SSP, RT-PCR	Commercial kits, Own design	**Innotrain-**	Fluorescence, Gel	Roche MagnaPure	Roche MagnaPure
387	**HPA-2b**	PCR-SSP	Commercial kits	**Bioarray Immucor**	Fluorescence	GeneAll	
180	**HPA-3b**	PCR - Melt curve analysis	Other	**Other**	Other	MagNA Pure Compact Nucleic	MagNA Pure Compact
390	**HPA-1a**	PCR-SSP	Commercial kits	**Protrans DNA box 500**	Fluorescence	FluoGene (BeDia Genomics)	Fluovista

Scheme 1B

HLA-B27 Testing

Scheme 1B: HLA-B27 Testing

Purpose

Assess participants ability to correctly determine HLA-B27/2708/B*27 status.

Consensus

At least 75% agreement on B27 status. Reference type used where consensus is not met

Satisfactory Performance

Making 10/10 reports that are in agreement with consensus in a distribution year.

10 donor samples sent over 5 distributions

Scheme 1B: Performance

- 12 labs with unsatisfactory performance (2 UK&I)

	2016	2017	2018	2019	2020
Number of Participants (UK&I)	123 (54)	127 (52)	133 (54)	133 (53)	141 (52)
Number with Unsatisfactory Performance (< 100%) (UK&I)	15 (6)	7 (2)	10 (3)	4 (1)	12 (2)
% Unsatisfactory Performance (UK&I)	12.2%	5.5%	7.5%	3.0%	8.5%

Scheme 1B: 2020 Incorrect Assignments

Sample	Result	Lab Number	Technique	HLA Type	Lab Identified Cause
1B 03	False neg	404	Molecular	B8 B27	Technical issue
1B 03&04	No results	7	Serological	B8 B27 & B8 B50	Late result entry
1B 03&04	False pos & neg	209	Molecular	B8 B27 & B8 B50	Sample mix-up
1B 05	False neg	295	Serological	B7 B27	Transcription error
1B 06	False neg	57, 154	Serological, Molecular	B27 B40	Procedural error, sample mix-up
1B 05&06	False neg	305	Molecular	B7 B27, B27 B40	No reply
1B 07&08	False pos & neg	317	Molecular	B7 B55, B27 B40	No reply
1B08	False neg	324	Serological	B27 B40	Unknown cause
1B 09	False pos	153	Serological	B7 B37	Interpretation issue
1B 10	False neg	198, 357	Serological	B27 B40	Unknown cause, no reply

- 5/10 samples distributed were HLA-B27 positive
- 14 errors: 9 false neg, 3 false pos, 1 lab did not return results
- 7/14 errors involved molecular technique
- 2 sample mix-up; 1 transcription error; 4 other

9/12 labs with unsatisfactory performance completed CAPA

Scheme

5A

HFE Typing

Scheme 5A: HFE Testing

Purpose

Assess participants ability to correctly determine HFE mutations.

3 mutations assessed:

- Codon 63: Histidine63Aspartic acid (H63D)
- Codon 282: cysteine282tyrosine (C282Y)
- Codon 65: Serine63Cysteine (S65C)

Satisfactory Performance

10 reports in agreement with consensus/reference result in a distribution year.

Consensus

At least 75% agreement on each HFE mutation. Reference type used where consensus is not met

10 donor samples sent over 3 distributions

Scheme 5A: Performance

- 1 labs with unsatisfactory performance (1 UK&I)

	2016	2017	2018	2019	2020
Number of Participants (UK&I)	58 (49)	56 (42)	58 (44)	51 (38)	49 (36)
Number with Unsatisfactory Performance (< 100%) (UK&I)	3 (2)	3 (2)	0 (0)	2 (1)	1 (1)
% Unsatisfactory Performance	5.2%	5.3%	0%	3.9%	2.0%

CAPA responses

- Human error - cross contamination of sample during testing procedures

Scheme 5B

Interpretive HFE genotype and Hereditary Haemochromatosis

Scheme 5B: Interpretive HFE genotype and Hereditary Haemochromatosis

Purpose

Assess participants ability to produce an accurate, clear and concise clinical report. HFE genotype and various clinical information provided

Satisfactory Performance

Must have <50% of available penalty points available to be considered acceptable.

Assessment

Reports must be identical in format to those typically produced by lab. Penalty points awarded for failure to cover interpretive criteria identified and agreed by the expert assessors.

Twice a year, 2 clinical scenarios

Scheme 5B: Performance

- 1 lab with unsatisfactory performance (0 UK&I)

	2016	2017	2018	2019	2020
Number of Participants	19	20	21 (18)	21 (17)	19 (15)
Number with Unsatisfactory Performance	0	0	1 (1)	3 (1)	1 (0)
% Unsatisfactory Performance	0%	0%	4.8%	14%	5.3%

Scheme 5B: Performance

- 2020 – All 4 scenarios:

  maximum 6 penalty points per scenario, 24 in total.

7	labs got	0 penalty points
2	labs got	0.5 penalty points
2	labs got	1 penalty point
2	labs got	1.5 penalty points
2	labs got	2 penalty points
1	lab got	2.5 penalty points
1	lab got	3.5 penalty points
1	lab got	4 penalty points
1	lab got	5 penalty points
1	lab got	16.5 penalty points

Scheme 7

HLA-B*57:01 Typing for Drug Hypersensitivity

Scheme 7: HLA-B*57:01 Typing for Drug Hypersensitivity.

Purpose

Assess participants ability to correctly determine HLA-B*57:01 status

Satisfactory Performance

Making 10 sample reports in agreement with the consensus/reference result in a distribution year.

Consensus

At least 75% agreement on the status of HLA-B*57:01. Reference result used when consensus not met.

10 donor sample over 3 distributions

Scheme 7: Performance

- 6/10 samples distributed were HLA-B*57:01 positive
- 2 labs with unacceptable performance

	2016	2017	2018	2019	2020
Number of Participants (UK&I)	62 (25)	64 (26)	67 (27)	67 (27)	67 (27)
Number with Unacceptable Performance (< 100%) (UK&I)	1 (1)	4 (1)	2 (0)	0 (0)	2 (0)
% Unsatisfactory Performance	1.6%	6.3%	3.0%	0.0%	3.1%

CAPA responses

- Human error - sample mix up

1/2 labs with unsatisfactory performance completed CAPA

Scheme 8

HLA Genotyping for Coeliac and other HLA Associated Disease

Scheme 8: HLA Genotyping for Coeliac and other HLA Associated Disease.

Purpose

Assess participants ability to correctly determine HLA type associated with carious diseases e.g. coeliac disease, narcolepsy.

Satisfactory Performance

Making 10 sample reports in agreement with the reference genotype in a distribution year.

Assessment

Lab results reported in format identical to clinical report. Reference HLA result used for assesment.

10 donor sample over 3 distributions

Scheme 8: Performance

- 17 Unsatisfactory Performers (5 UK&I)

	2016	2017	2018	2019	2020
Number of Participants (UK&I)	39 (8)	45 (9)	52 (10)	50 (11)	55 (12)
Number with Unsatisfactory Performance (< 100%) (UK&I)	8 (3)	15 (2)	14 (4)	13 (2)	17 (5)
% Unsatisfactory Performance	21% (38%)	33% (22%)	27% (40%)	26% (18%)	31% (42%)

CAPA responses

- Labelling error/ sample mix up
- Human error – not following checking procedures
- Transcription errors
- Kit interpretation error
- Reporting error

13/17 labs with unsatisfactory performance completed CAPA

Scheme 8: Unacceptable Performance by Disease

Disease	HLA Association	Number of Participants	No. of Participants with Unacceptable Performance
Coeliac	DQ2.5, DQ8, DQ2.2	53	21
Narcolepsy	DQB1*06:02	22	3*
Actinic Prurigo	DRB1*04:07	3	0
Birdshot Retinopathy	A*29	9	0
Behçet's	B*51	13	0
Rheumatoid Arthritis	DRB1*04	4	1
Diabetes	DR3, DR4	7	1
Psoriasis	C*06	1	0

**UP noted in CD and Narcolepsy*

Scheme 8: Example Incorrect Assignments

Reference HLA Type	Serotype	Lab Reported Result	Explanation of Error
DQB1*02:02, DQB1*03:01 DQA1*02:01, DQA1*03:03	DQ2.2, DQ7	Negative for **DQ2** and DQ8	**False DQ2 negative.** The alleles DQB1*02 and DQA1*02 which encode the DQ2.2 heterodimer are present. Although less frequent than DQ2.5 and DQ8, DQ2.2 is associated with CD, therefore CD could be incorrectly excluded on the basis of this result.
DQB1*03:01, - DQA1*03:03, DQA1*05:01	DQ7 homozygous	**DQB1*02:01**, DQB1*03:01 **DQA1*03:02**, DQA1*05:01	**False DQ2 positive.** DQB1*02 (DQ2) is not present in this individual. The DQA1*05 allele is present, which is part of the DQ2.5 heterodimer, but in this case the DQA1*05 allele is in association with DQB1*03:01 (DQ7). The DQA1*03 allele is also incorrectly reported as DQA1*03:02 instead of DQA1*03:03, although this would not alter clinical interpretation of the results.
DQB1*03:01, - DQA1*05:05, -	DQ7 homozygous	**Half DQ2 positive**	**Confusing/uninformative report.** The report does not state whether it is the alpha or beta part of the heterodimer that is positive, and is likely to be confusing for clinicians to interpret.
DQB1*02:01, 03:01 DQA1*03:03, 05:01	DQ2.5, DQ7	Positive for DQB1*02, DQB1*03/06, **DQA1***03*, DQA1*05, **DQA1***03:02/03, alpha-subunit HLA-DQ2.5, **alpha-subunit HLA-DQ8,** beta-subunit HLA-DQ2.5	**Overly complex and confusing report.** DQA1*03 reported twice (as DQA1*03 then DQA1*03:02/03). 'alpha-subunit HLA-DQ8' report potentially misleading as the presence of DQA1*03 without DQB1*03:02 (DQ8) has not been linked to CD.

Coeliac Guidelines

Laboratory Testing and Clinical Interpretation of HLA Genotyping Results in the Diagnosis of Coeliac Disease

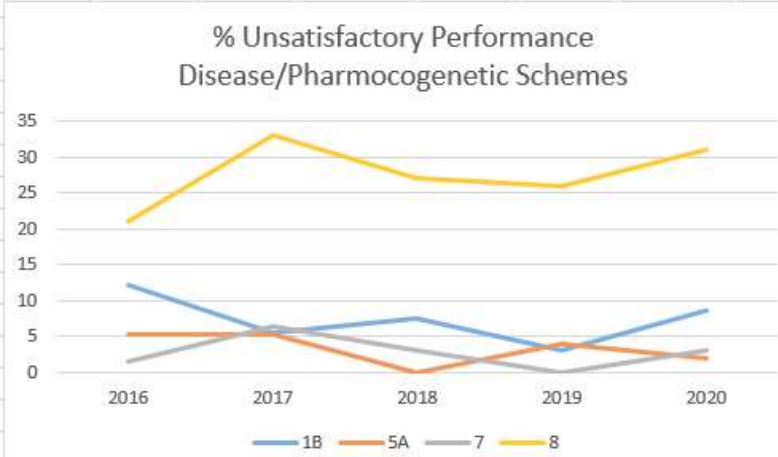
- In preparation to submit for publication
- Best practice guidelines for HLA testing and reporting for coeliac disease
- Includes suggested interpretive comments for clinical reports

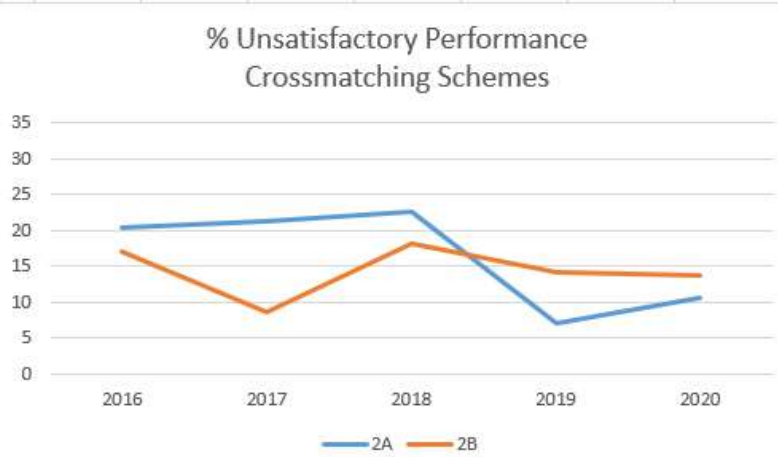
- Assessment of HLA results and interpretive comments as part of EQA Scheme

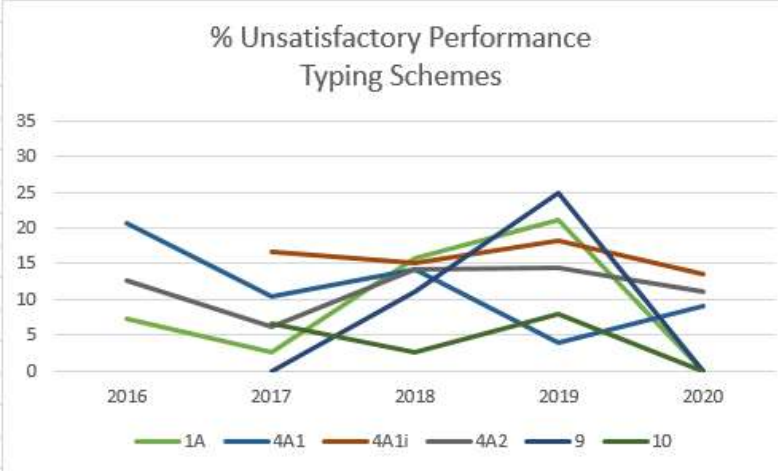
Scheme Summary

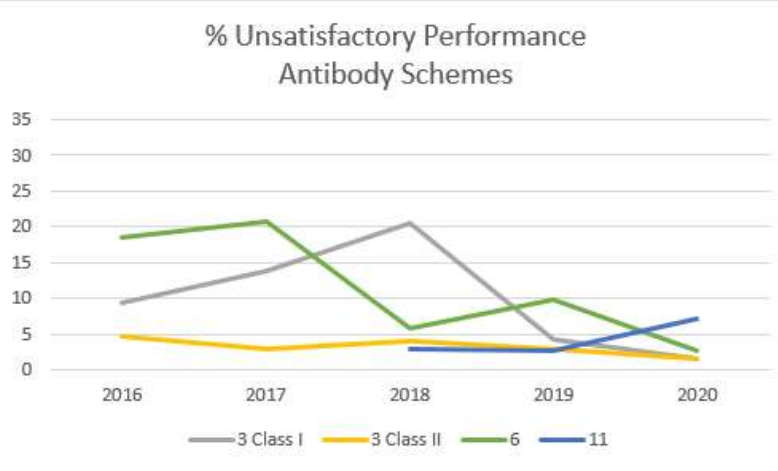
Performance Summary for all Schemes

5 Year Trends in Unsatisfactory Performance









UK Pathology EQA Governance

EQA Provider

- Identify and report persistent poor performing labs to relevant NQAAP
- Work with labs to investigate performance issues
- Monitor performance of test kits/IVDs

NQAAP

- **National Quality Assurance Advisory Panel**
- Support EQA providers to deliver high quality EQA schemes
- Harmonize standards between EQA providers
- Monitoring performance and escalating concerns

QAPC

- RCPath **Quality Assurance in Pathology Comm**
  (Formally Joint Working Group in Quality Assurar
- Oversight of performance in EQA in UK
- Contact head of department and CEOs
- Report to UKAS, CQC (or relevant devolved bodies)

https://www.rcpath.org/profession/patient-safety-and-quality-improvement/technical-eqa.html

RCPath: EQA Oversight Board & EQA Stakeholder Forum

- 2 year programme started in 2020 to develop framework for governance and oversight of EQA
- Work streams set up to deliver the new framework

Developing & implementing a governance and assurance framework

Agreeing & implementing a consistent approach to identifying & responding to poor performance

Amy De'Ath
Deb Pritchard

UK NEQAS Histocompatibility & Immunogenetics

UK NEQAS H&I

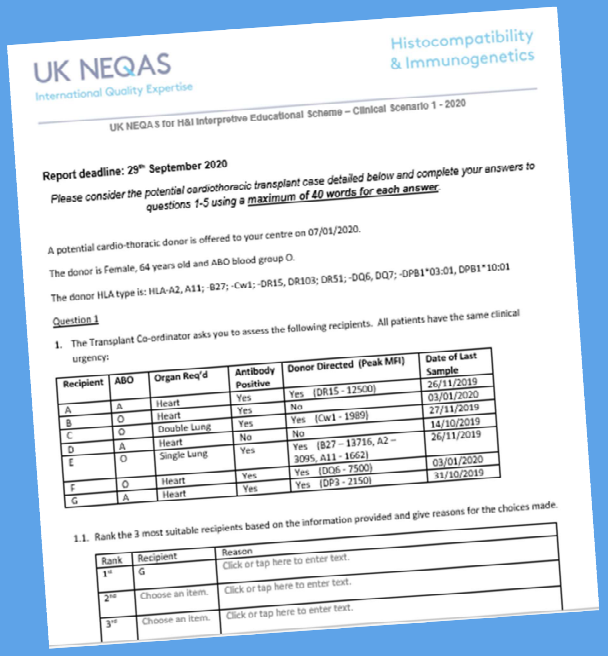
Interpretative Educational Scheme Results

@UKneqasHI

@UK_NEQAS

Table of Contents

Our Educational Schemes



UK NEQAS
International Quality Expertise

Histocompatibility & Immunogenetics

UK NEQAS for H&I Interpretive Educational Scheme – Clinical Scenario 1 - 2020

Report deadline: 29th September 2020

Please consider the potential cardiothoracic transplant case detailed below and complete your answers to questions 1-5 using a maximum of 40 words for each answer.

A potential cardio-thoracic donor is offered to your centre on 07/01/2020.

The donor is Female, 64 years old and ABO blood group O.

The donor HLA type is: HLA-A2, A11; -B27; -Cw1; -DR15, DR103; DR51; -DQ6, DQ7; -DPB1*03:01, DPB1*10:01

Question 1

1. The Transplant Co-ordinator asks you to assess the following recipients. All patients have the same clinical urgency:

Recipient	ABO	Organ Req'd	Antibody Positive	Donor Directed (Peak MFI)	Date of Last Sample
A	A	Heart	Yes	Yes (DR15 - 12500)	26/11/2019
B	O	Heart	Yes	No	03/01/2020
C	O	Double Lung	Yes	Yes (Cw1 - 1989)	27/11/2019
D	A	Heart	No	No	14/10/2019
E	O	Single Lung	Yes	Yes (B27 – 13716, A2 – 3095, A11 - 1662)	26/11/2019
F	O	Heart	Yes	Yes (DQ6 - 7500)	03/01/2020
G	A	Heart	Yes	Yes (DP3 - 2150)	31/10/2019

1.1. Rank the 3 most suitable recipients based on the information provided and give reasons for the choices made.

Rank	Recipient	Reason
1st	G	Click or tap here to enter text.
2nd	Choose an item.	Click or tap here to enter text.
3rd	Choose an item.	Click or tap here to enter text.

- Interpretative Educational Scenarios
  - 3 clinical scenarios a year
    - Solid organ, HSCT, platelet/transfusion
  - Based on patient cases
    - Provide relevant clinical details and test results
    - Questions on interpretation of results and clinical advice
- Educational Crossmatch Scheme
  - Combined crossmatch, HLA typing and antibody testing – testing and clinical interpretion

Not assessed

Provided free of charge

01

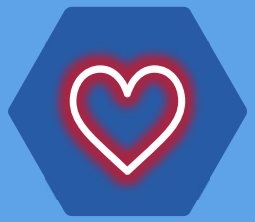
Educational Scheme (iED) Scenario 1: Solid Organ – Cardiothoracic Scenario

Solid Organ Scenarios

Year	Transfusion/Platelet	Returns
2013	Live kidney transplant	46
2014	Deceased kidney transplant	50
2015	Cardiothoracic transplant	50
2016	Deceased donor virtual XM	50
2017	Cardiothoracic transplant	45
2018	Live kidney transplant	53
2019	Kidney after heart transplant	53

- Dispatched on 1st September 2020
- 45 Responses
  - 20 from UK and Ireland (UK&I)
  - 25 from the Rest of the World (RoW)

Heart Transplant



- Heart tx performed on patients with end-stage heart failure, congenital heart disease or severe coronary artery disease.
- Average waiting time for a heart is 6 months.
- First human to human heart tx performed in 1967.

Lung Transplant

- Lung tx performed on patients with obstructive pulmonary diseases, pulmonary fibrosis, cystic fibrosis and pulmonary hypertension.
- Lungs can be totally or partially replaced.
- First lung tx performed in 1963.

UK NHSBT Cardiothoracic Advisory Group (CTAG) Guidelines

Risk Level	Immunological Risk	Description	MFI Level
I	Standard Risk	No detectable antibody	N/A
II	Additional Risk	Minimum risk of hyperacute rejection but greater than standard risk of rejection	<2,000
III	Medium/ Intermediate Risk	Low risk of hyperacute rejection but significant risk of early rejection and antibody mediated graft damage. Immediate pre-transplant antibody reduction advised.	2,000-5,000
IV	High Risk	Transplant veto apart from exceptional cases	>5,000

- Each positive HLA specificity should be assigned a risk based on its MFI level.
- Where a donor is homozygous for a mismatch the corresponding MFI should be doubled.

https://www.odt.nhs.uk/transplantation/cardiothoracic/

UK NHSBT Cardiothoracic Advisory Group Guidelines

Crossmatching Considerations

- Confirm no sensitising events since last antibody screen
- Patients currently HLA antibody negative can be transplanted without a prospective crossmatch.
- A retrospective crossmatch should be performed
- Patients with well defined HLA antibodies can be transplanted using a virtual XM and retrospective crossmatch
- Patients without fully defined HLA antibodies or a recent sensitising event must have a prospective crossmatch

UK NHSBT Cardiothoracic Advisory Group Guidelines

Post-Transplant Monitoring

- Standard risk tx should be tested every 3 months
- If risk >standard then test at day 7, 28 then 3 monthly
- If high risk then more frequent testing would be advised
- Re-test if suspected or diagnosed rejection episodes

UK NEQAS Scenario #1

A potential cardiothoracic donor is offered to your centre on 07/01/2020:

- Female
- 64 years old
- Blood group O
- HLA type: HLA-A2, A11; B27, -; Cw1, -; DR15, DR103; DR51; DQ6, DQ7; DPB1*03:01, DPB1*10:01

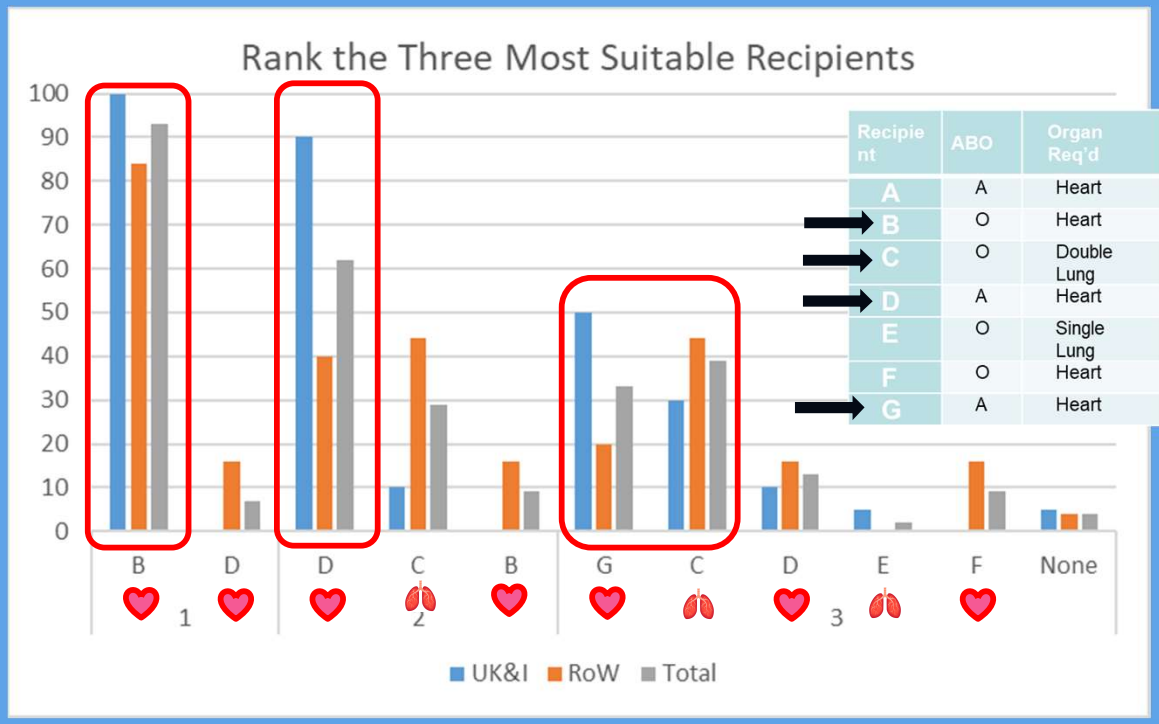
Q1: Selection of Recipient

- The Transplant Co-ordinator asks you to assess the following recipients (all with similar clinical urgency):

Recipient	ABO	Organ Required	Antibody Positive	Donor Directed (Peak MFI)	Date of Last Sample*
A	A	Heart	Yes	Yes (DR15 - 12500)	26/11/2019
B	O	Heart	Yes	No	03/01/2020
C	O	Double Lung	Yes	Yes (Cw1 - 1989)	27/11/2019
D	A	Heart	No	No	14/10/2019
E	O	Single Lung	Yes	Yes (B27 – 13716, A2 – 3095, A11 - 1662)	26/11/2019
F	O	Heart	Yes	Yes (DQ6 - 7500)	03/01/2020
G	A	Heart	Yes	Yes (DP3 - 2150)	31/10/2019

*Offer made on 7th January 2020

Q1: Selection of Recipient



Recipient	ABO	Organ Req'd	Antibody Positive	Donor Directed (Peak MFI)	Date of Last Sample
A	A	Heart	Yes	Yes (DR15 - 12500)	26/11/2019
B	O	Heart	Yes	No	03/01/2020
C	O	Double Lung	Yes	Yes (Cw1 - 1989)	27/11/2019
D	A	Heart	No	No	14/10/2019
E	O	Single Lung	Yes	Yes (B27 – 13716, A2 – 3095, A11 - 1662)	26/11/2019
F	O	Heart	Yes	Yes (DQ6 - 7500)	03/01/2020
G	A	Heart	Yes	Yes (DP3 - 2150)	31/10/2019

Q1: Selection of Recipient – Reasons For

NEQAS recommends

1 – Recipient B

Recipient B has no donor directed antibodies and last sample was 4 days ago (CTAG standard risk)

2 – Recipient D

Recipient D has no donor directed antibodies but ranked below B as the last serum sample was 14/10/2019 (CTAG standard risk)

3 – Recipient G

The remaining recipients all have donor directed antibodies. Recipient G has been ranked 3rd as it has the lowest MFI

3rd Choice could also be Recipient C

There is minimal difference in the MFI between C and G. Recipient C's last sample was more recent.

Recipient	Main Reasons for Selection
	Recent sample. Check if any sensitising events since last sample.
	tibody screen.
	S/BSHI
	l doubled. e.
	Recent antibody screening.
None	Could not select a 3rd recipient without discussion with the clinical team.

Q1: Selection of Recipient – Reasons Against

Recipient	Comments
A	• Antibody directed to DR15 (MFI 12,500) • **CTAG Level 4: veto to transplant except for exceptional cases.** • Increased chance of Hyperacute AMR. • CII Ab can be treated also evidence of accommodation. • Different blood group. • Likely positive B cell CDCXM.
B	• No comments received.
	• Different blood group.
E	• Multiple donor-specific antibodies (cumulative MFI 32,189; B27 homozygous so MFI doubled 'High' level B27 donor homozygous, 'Medium' level A2 and 'Low' level A11. • CTAG Level 4: veto to transplant except for exceptional cases. • Increased chance of Hyperacute AMR. • Likely cause a positive B- and T-cell CDC-crossmatch.
F	• Antibody directed to DQ6 (MFI 7,500). • CTAG Level 4: veto to transplant except for exceptional case. • Increased chance of Hyperacute AMR. • CII Ab can be treated also evidence of accommodation.
G	• Preformed Class II DSA gives an increased risk of cardiac allograft vasculopathy. • Donor specific anti HLA-DP3 antibody, MFI 2150, detected within 3 months. • CTAG Risk Level III. • ABO compatible (blood group A with access to group O and A donors). • Significant risk of early rejection and antibody mediated graft damage. • Could be considered if immediate antibody reduction feasible. • Cw and DPB1 are low expression antigens, BUT would require discussion with the clinical team.

NEQAS comments

- **Recipient A, E and F were not selected as they all have donor directed antibodies which, based on the MFI data, would represent too great an immunological risk (CTAG risk level IV)**

Q2: Donor Specific Antibodies

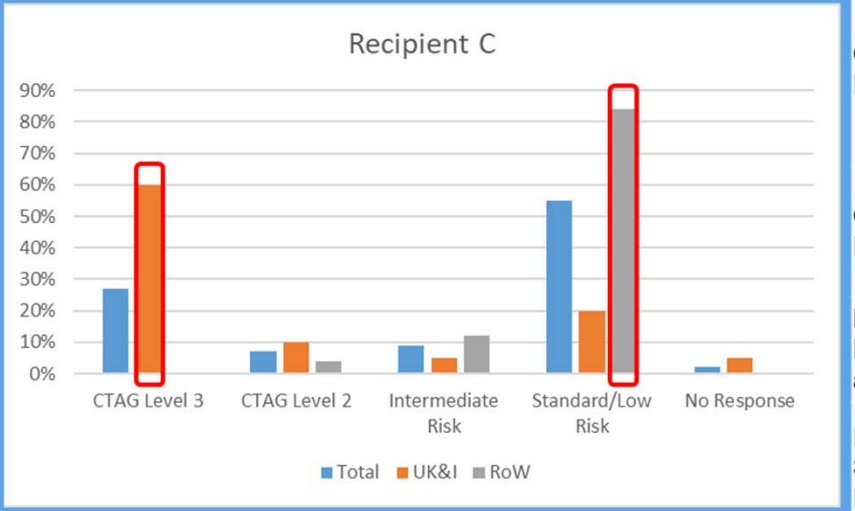
The heart was accepted for a super urgent patient at another centre. The antibody results from November 2019 for the two remaining lung patients were provided:

Donor Specific Antibody	Recipient C MFI	Recipient E MFI
A2	136-182	1650-3095
A11	229-254	992-1662
B27	183	244-13716
Cw1	1989	425
Class II Negative		

Donor HLA Type: HLA-A2, A11; B27, -; Cw1, -;

Q2: Immunological Risks (Patient C)

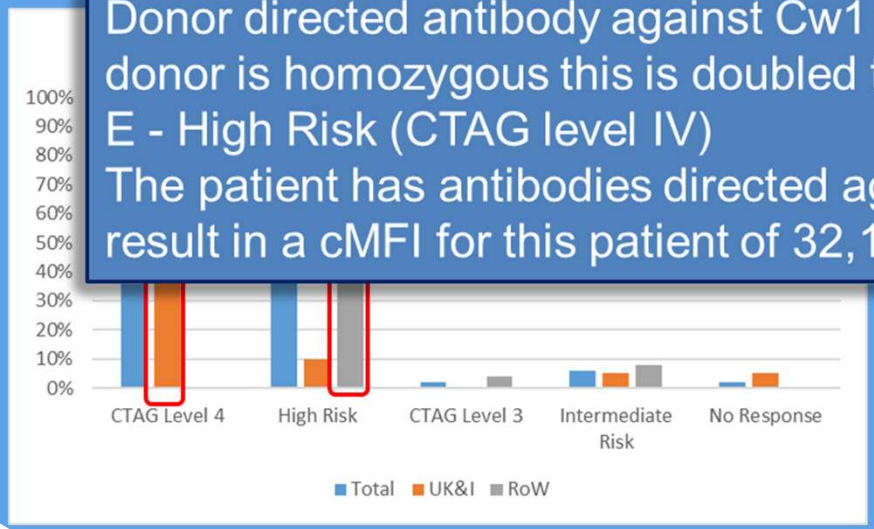
Select the immunological risk for each recipient and explain the reason



Risk	Total	UK &I	RoW	Reason
CTAG Level 3	**27%**	60%	0%	Cumulative MFI 3,978. Cw1 homozygous so MFI doubled. Lower expression HLA-Cw potentially more permissible. Low risk of hyperacute rejection but significant risk of early rejection/AMR.
CTAG Level 2	**7%**	10%	4%	Cw1 antibody at 1989 MFI. Minimum risk of HAR due to low level DSA. If following BSHI/BTS guidelines in doubling MFI due to homozygosity then would be risk level III.
Medium / Intermediate Risk	**9%**	5%	12%	Weak DSA Cw1 MFI 1989. FCXM likely to be negative. Check any sensitisation since the last sample.
Low / Standard Risk	**55%**	20%	84%	Cw1 = 1989. Cumulative MFI (doubling Cw1) just over 4000. Minimum risk of hyperacute rejection due to low level DSA but greater than standard risk of rejection.
No response	**2%**	5%	0%	N/A

Q2: Immunological Risks (Patient E)

Risk	Total	UK&I	RoW	Reason
CTAG Level 4	**36%**	80%	0%	The patient has a B27 DSA of 13,000 MFI, combined with an A2-3000 MFI and A11-1662 MFI. Cumulative donor-directed MFI is 32,189. Risk of hyperacute rejection, contraindication to transplantation.



NEQAS recommends

C - Intermediate Risk (CTAG Level III)

Donor directed antibody against Cw1 with a peak MFI of 1989. As the donor is homozygous this is doubled to give cMFI of 3978.

E - High Risk (CTAG level IV)

The patient has antibodies directed against donor mismatches that result in a cMFI for this patient of 32,186.

Q2: Crossmatch Test

Patient C was selected for transplant. Would you perform a prospective crossmatch?

YES

- DSA, <5,000 MFI
- Virtu
- Wou
  time
- Dete
  fixin
- Resu
  desensitisation/
  immunosuppression protocols

NEQAS recommends

- This patient would not require a prospective crossmatch - the last sample tested was within the last 6 weeks.
- Prospective crossmatching would delay the transplant.

NO

- Need to limit CIT
- Tx is low risk
- FI
- ect
- roceed
- Perform XM retrospectively

Q3: Crossmatching Results

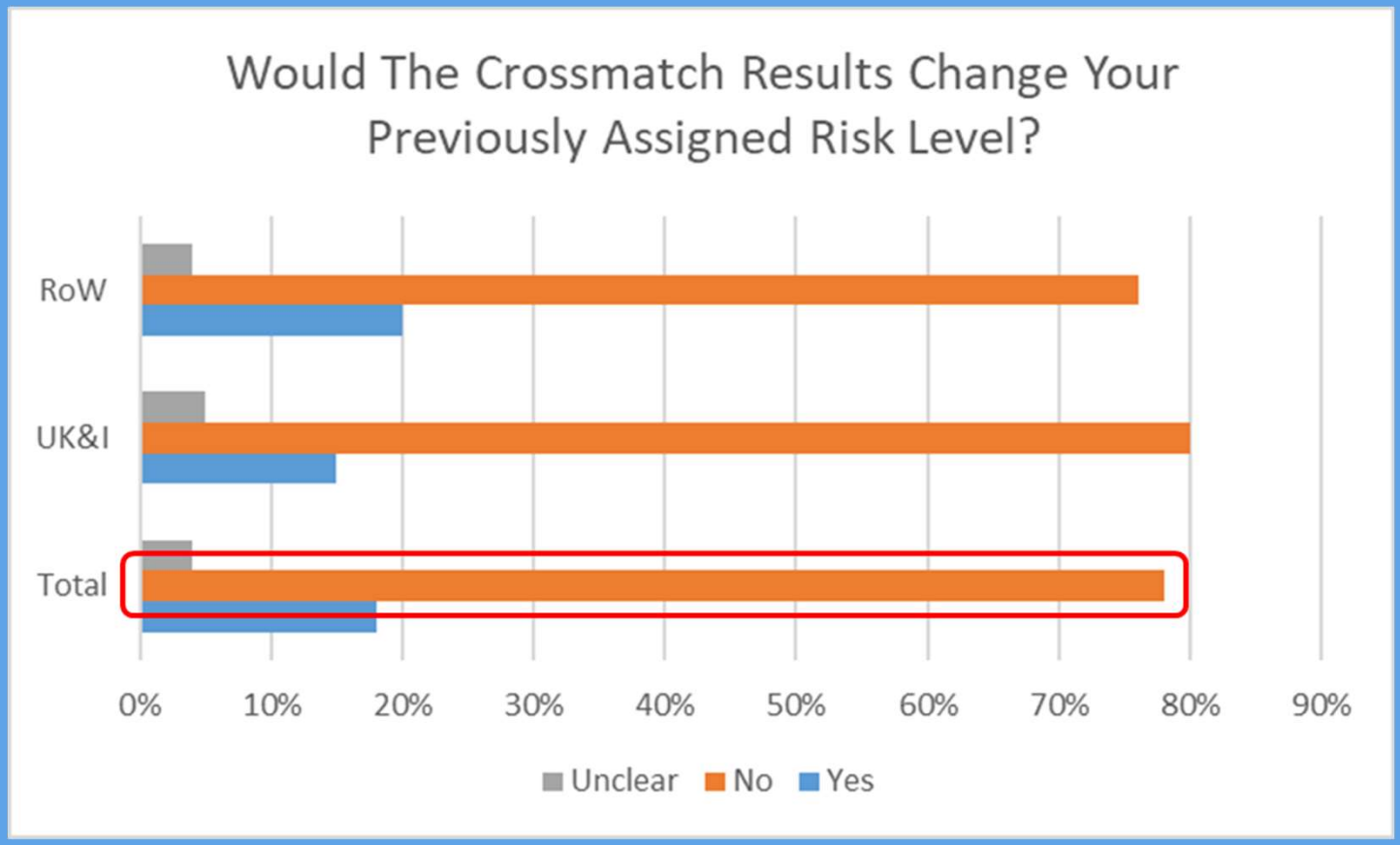
The crossmatching results for Patient C are provided:

	Cytotoxic Crossmatch		Flow Crossmatch		Single Antigen Bead
Serum	Spleen	Spleen + DTT	T cells Linear Channel Shift (LCS)	B cells Linear Channel Shift (LCS)	MFI of peak donor directed bead
22/01/2018	6	1	7.6	92.0	2965
27/11/2018	4	1	Not tested		1900
23/04/2019	2	1	7.7	29.7	1845
06/09/2019	2	1	Not tested		1765
27/11/2019	2	1	7.6	46.9	1989
07/01/2020	6	1	7.6	57.2	1800

LCS Thresholds

	Neg	Equivocal	Positive
T cells	<46		>=46
B cells	<35	>=35<63	>=63

Q3: Crossmatching Results



Q3: Crossmatching Results

Would this change the risk level previously assigned?

NEQAS recommends

- The crossmatch results for sample 22/01/2018 are the main area for concern – the cytotoxic crossmatch reduced with DTT but was not completely abrogated and the flow crossmatch is B cell positive. However, the MFI data is not supportive of a CDC crossmatch positive.
- The day of transplant sample is negative for IgG donor directed antibodies in the CDC assay and in the flow crossmatch raised but did not reached test cut off.
- Overall this would not change the risk level.

Need autologous XM results to interpret risk

Need medical history of medications or infections to interpret result

Q3: Crossmatching Results

What would you suggest, if anything, to the clinical team to increase the chances of successful transplant?

NEQAS recommends

- As the retrospective crossmatch is historically positive and currently negative, antibody removal is not indicated.
- Regular post-transplant monitoring (at 7 days post-transplant, 28 days post-transplant and quarterly thereafter for the first year or more frequently if clinically indicated) could be recommended with a low threshold for intervention if AMR suspected.

Test Graft Function More Frequently	1 (1%)	0	1 (3%)
No Comments	3 (4%)	0	3 (10%)

Q4: Post-Transplant Monitoring

Single antigen bead testing was performed on a post-transplant sample. The results are provided:

Pre-tx DSA Level	Recipient C MFI
Cw1	1989

Serum Date	PC bead MFI	NC bead MFI	Cw1 bead MFI
13/01/2020	8904	21	801
17/01/2020	10448	52	705
31/01/2020	14112	27	1271
12/02/2020	9510	23	1093
28/02/2020	13379	26	1240
16/03/2020	17440	37	4240
26/03/2020	14014	19	1220

Q4: Post-Transplant Monitoring

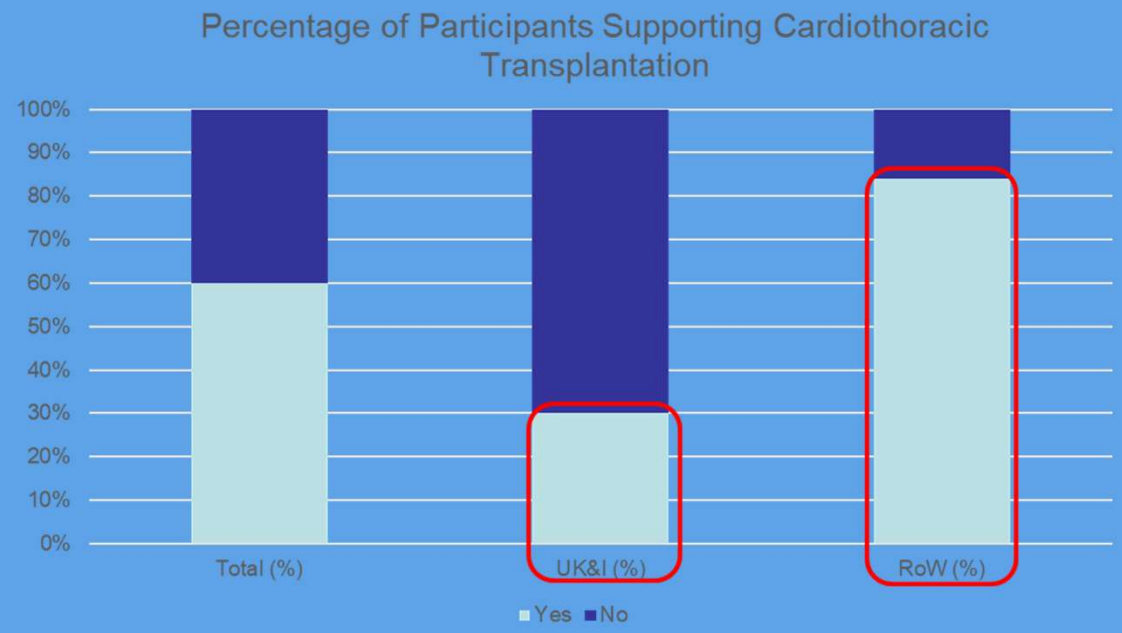
Based on these results, what would be your recommendations for further immunological monitoring?

NEQAS recommends

- With the exception of the sample received 16/03/2020, the MFI levels for the donor directed beads is lower than the pre-transplant samples.
- Also, the test for 16/03/2020 could be repeated. Is this a true increase as PC bead is also higher in this test than in the other samples?
- It may also be beneficial to request a further sample from the patient to see current status and continue to monitor DSA post-transplant every 3 months or when clinically indicated.

Your Laboratory

Does Your Laboratory Support Cardiothoracic Transplantation?



Response	Total	UK&I	RoW
YES	27 (60%)	6 (30%)	21 (84%)
NO	18 (40%)	14 (70%)	4 (16%)

Further Comments

- Would be useful to know recent sensitisation events as all patients would need HLA antibody testing prior to tx offer
- In most cases clinical urgency is taken in to account
- It would be useful to know the gender of recipients
- Our centre uses different MFI levels to stratify HLA antibodies as locally agreed with the transplant team:
  'Neg' = <1000 'Low' = 1000 - 1999
  'Medium' = 2000 - 3999
  'High' = > 4000
- A virtual crossmatch will be issued for sensitised cardiothoracic patients where up to 2 x 'Low' OR 1 x 'Medium' MFI level specificities are detected in the last sample. We would only perform a prospective crossmatch where patient HLA specificities couldn't be clearly defined

Follow Up and Discussion

- The patient this scenario was based on a real case.
- The patient has had no post-transplant complications. They have been shielding due to COVID-19 but as doing well.
  - Last antibody screening was performed on 18/08/2020 where the Cw1 DSA recorded an MFI of 1128.

- Consistency in responses but depended on whether labs would follow CTAG guidelines regarding doubling of MFI levels where the donor is homozygous

02

Educational Scheme (iED) Scenario 2: Haematopoietic Stem Cell Transplant

HSCT Scenarios

Year	HSCT	Returns
2013	Matched unrelated donor	27
2014	Mismatched unrelated donor	42
2015	Paediatric cord donor selection	43
2016	Donor search for patient with unusual HLA type	45
2017	Haploidentical donor selection	49
2018	Unrelated donor selection – permissive/non-permissive options	37
2019	Haploidentical donor selection with antibody	50

- Dispatched on 20th October 2020
- 49 Responses
  - 19 from UK and Ireland (UK&I)
  - 30 from the Rest of the World (RoW)

UK NEQAS Scenario #2

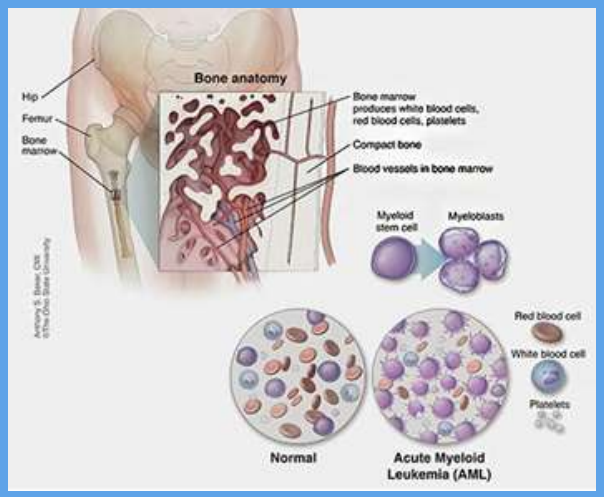
A patient with AML is referred to your laboratory:

- Female
- 49 years old
- Blood group O RhD pos, CMV neg

- Patient has 5 potential **related** donors:
  - one full sibling
  - four children
- All are sent for HLA genotyping. An unrelated search is initiated.

Acute Myeloid Leukaemia (AML)

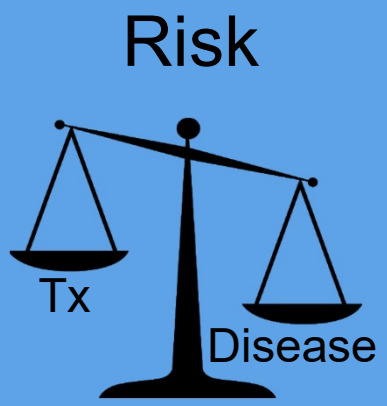
- Accumulation of immature myeloblasts
- Multiple subtypes identifies by cytology and genetic testing
- Most common acute leukaemia in adults but can occur in children
- Median age of diagnosis is 70
- Symptoms include fatigue, loss of appetite, enlarge lymph nodes and spleen

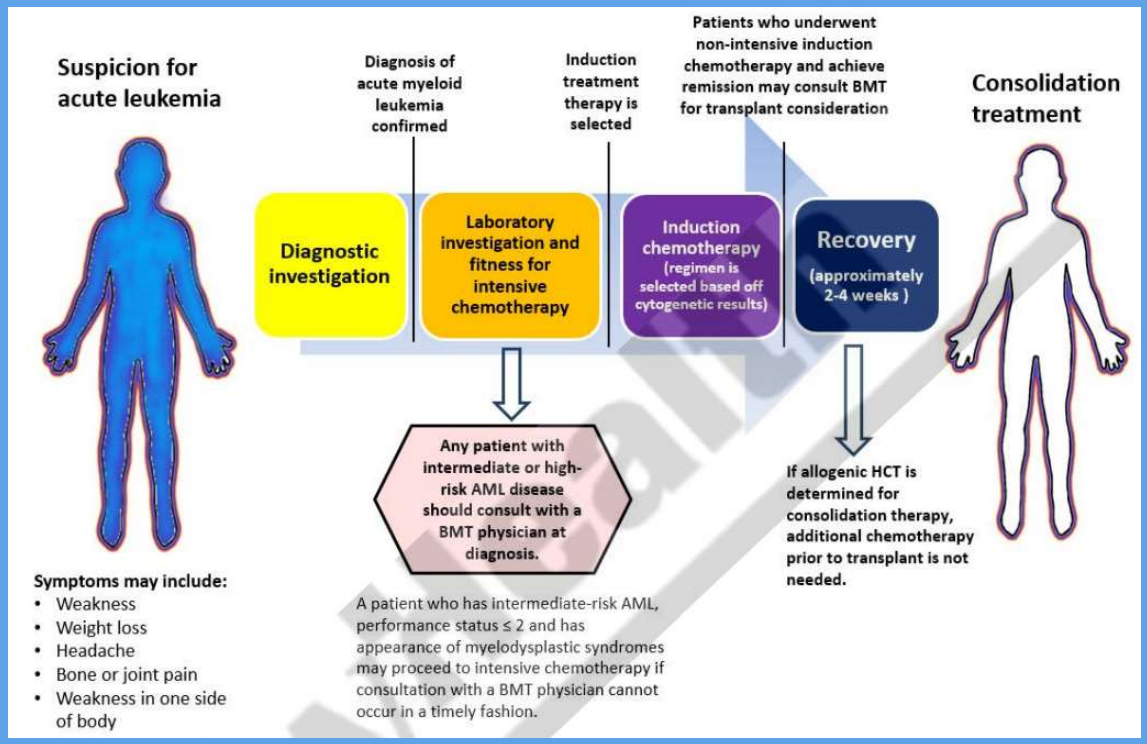


- Up to 40% relapse post-transplant Y1
- Relapse difficult to control
- Rapid growth
- Drug resistance

AML Treatment

- Depending on risk classification of disease subtype use allo-HSCT (high risk only), auto-HSCT or continual chemotherapy





Q1: Challenges of Unrelated Donor Search

- What aspects of the patient's HLA type make this a challenging unrelated donor search?

	HLA-A*		HLA-B*		HLA-C*		HLA-DRB1*		HLA-DQB1*		HLA-DPB1*	
Patient	02:01	25:01	56:01	57:01	01:02	06:02	04:01	07:01	03:01	03:03	04:01	13:01

- One common European haplotype (A2 B57 Cw6 DR7 DQ9)
- One rare Russian/Eastern European haplotype (A25 B56 Cw1 DR4 DQ7)
- Low frequency A*25 often in haplotype with B*18 and C*12
- B*56:01 has lots of HLA-C associations (issue if C not defined by registry)
- Patient has less common DR7 DR53N DQ9 type rather than more common DR7 DR53 DQ2 combination
- Potential for DQB mm as DR4 associated with DQ7 and DQ8

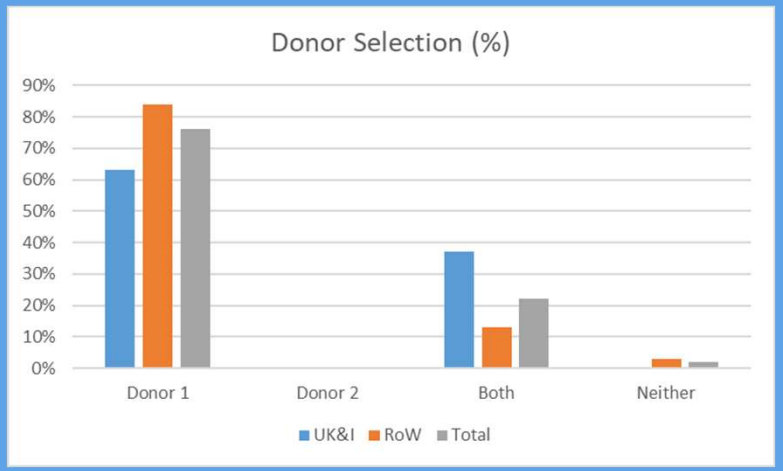
Q2: Unrelated Donor Selection

- An unrelated donor search revealed only two potential fully matched donors:

Donor	Registry	M/F	Age	ABO	CMV	HLA-A*	HLA-B*	HLA-C*	HLA-DRB1*	HLA-DQB1*	HLA-DPB1*
1	DE-ZKRD	M	21y	AB+	N	02:01; 25:01	56:01; 57:01	01:02; 06:02	04:01; 07:01/07:79	03:01; 03:03	04:01; 13:01
2	BR-REDOME	M	59y			02; 25	56; 57		04; 07		

Potential 12/12

- Would you pursue either donor listed?



Q2: Unrelated Donor Selection

Reasons for Making Selection	
Donor 1	Young (<30) male donor.
	Full 10/10 match.
	Possibility of 12/12 match grade to 2nd field (pending confirming ambiguity on DR).
	CMV matched.
	Major ABO mismatch.
	Need to confirm the ABO antibody titre status of the patient before proceeding.
	Donor is from a reliable and rapid Registry, especially important in patients with progressive diseases like AML.
	Brazilian Registry is not very responsive. Risk of Zika virus.
	CMV status unknown.
Both Donors	Donor 1 preferable.
	Donor 2 backup.
	We would request both as there are only two options available.
	Selecting both will provide a choice for the clinician between old donor or ABO incompatible.
	Depends also on urgency.
	As the patient has a rare HLA type we would test both donors.
Neither	Wait for the results of HLA typing of full sibling before pursuing an unrelated donor.

76%

22%

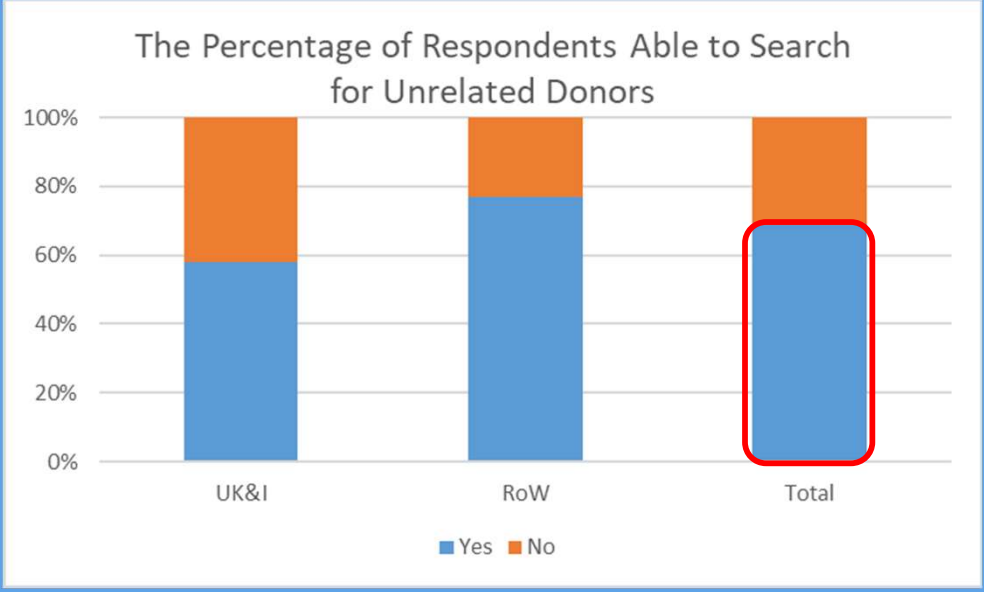
2%

NEQAS recommends
Donor 1 – male, <30 years old, CMV match, possible 12/12

Q3: Running Patient on Search Programme

If you are able, run the patient on a search programme.

	UK&I	Percentage (n=19)	RoW	Percentage (n=30)	Total	Percentage (n=49)
Yes	11	58%	23	77%	34	69%
No	8	42%	7	23%	15	31%



Q3: Donor Selection

Are there any potential donor options that may be recommended to the transplant consultant? *Give your two preferred options and reasons why.*

Most Common Results of Search	
First Preference	6939DKM0012331311817 - 11/12, DPB1 matched, CMV and ABO matched 22 year old male with an allelic DQB1*03 mismatch with no DSA.
	1 A-MM (GvH), DPB1 permissive, male, 26yrs
	9/10 in GvH direction (donors homozygote A*02:01) with permissive DPB1*.
	No potential fully matched donors but we would consider a 9/10 match, preferably at HLA-A or HLA-DQ.
	6/6 with no mismatch in A, B and C low resolution loci, but the rest of the genotyping is unknown and the donor is a women of 51y with a CMV+ status.
	9/10 matches one 27y old male with a DQB1mm (03:02 vs 03:01).
	12/12 match, male, age 21y, CMV negative.
Second Preference	9/10 (10/12 HLA-DPB1 permissive) HLA-A mismatch; CMV Negative (last tested 2019); ABO blood group mismatch; Male 28.
	Female, 25, CMV Neg, ZKRD, HLA 9/10 DRB1 mismatch (DRB1*11:01), DP permissive
	1 A mismatched (bidirectional), 1 DPB1 mismatched (GvH) German donor. Male, 28yrs. CMV negative (matched). However, ABO major mismatch.
	9/10 A mm, permissive DP mm, young male with recent CMV Neg status
	Mismatch at HLA-A, CMV negative, young male donor
	9/10, permissive DPB1 mismatched, ABO and CMV matched 25 year old female with a DR mismatch with no DSA.
	Male donor 5/6 with no mismatch in A and B loci and one mismatch in C, but the rest of the genotyping is unknown as well as the CMV status and the blood group
	26y old male donor with a HLA_A mm(direction of the Mm gvH, homozygous for -A02:01)
	Female 36 years with 1 non permissive DPB1 mismatch (HvG) and with potential pregnancy. Blood group and CMV unknown.
	1 A-MM, DPB1 permissive, female, 24yrs.
Other Comments	WMDR search did not return any 10/10 matched donors. The majority of the donors were 9/10 or 8/10 match with a mismatch on the HLA-A locus was the most common.
	No suitable donors.
	found in BMDW but at least four 8/8 with DQ8 instead of DQ7
	recommend either haplo-identical donor or 9/10 donor with HLA-A mismatch, preferred one unidirectional mismatch

Q4: Cord Search

Both unrelated donors were deleted from the registry so a cord search was carried out identifying the following units:

Donor	Cord bank	HLA-A*	HLA-B*	HLA-C*	HLA-DRB1*	HLA-DQB1*	TNC (x10^7)	CD34 (x10^5)	Vol (ml)	Blood group	AABB/FACT accredited
1	SE - Cord	02; 02	44; 57		04:01; 07:01		314	90	25	B RhD pos	Yes
2	RU - Samara	02:06/10; 25	56; 57		07:01; 14:03		236	79	23	A RhD pos	No
3	ES - Malaga	01; 02	56:01; 57		01; 07		234	117	162	A RhD pos	Yes
4	BE - Leuven	02; 68	56:01; 57		01:01; 07:01		173	48	25		Yes
5	US- Durham	02; 25	18:01; 57		04:01; 07:01		151	63	25	A RhD pos	Yes

Patient HLA Type:

	HLA-A*		HLA-B*		HLA-C*		HLA-DRB1*		HLA-DQB1*		HLA-DPB1*	
Patient	02:01	25:01	56:01	57:01	01:02	06:02	04:01	07:01	03:01	03:03	04:01	13:01

Patient is 80Kg

Selection of Cord Donors

HLA matching and donor selection for haematopoietic progenitor cell transplantation
Hough et al., 2016


bjh guideline

Recommendations for a standard UK approach to incorporating umbilical cord blood into clinical transplantation practice: an update on cord blood unit selection, donor selection algorithms and conditioning protocols

- 8/8 at HLA-A, B, C and DRB1
- Nucleated cell dose (for malignant disorders)
  TNC >3 x 10^7/kg, CD34 1.0–1.7 x 10^5/kg
- Consider 5 or 6/8 HLA-matched units
  Avoid HLA-DRB1 mismatches
  TNC dose should be >5 x 10^7/kg
- Use double cord when insufficient cells in single
  Sum TNC >3.5 x 10^7/kg, CD34 1.8 x 10^5/kg
  Each TNC >1.5 x 10^7/kg
- Use an accredited blood bank

BSHI Guidelines: Little et al, 2021

American Guidelines: Politikos et al., 2020

Q4: Cord Selection

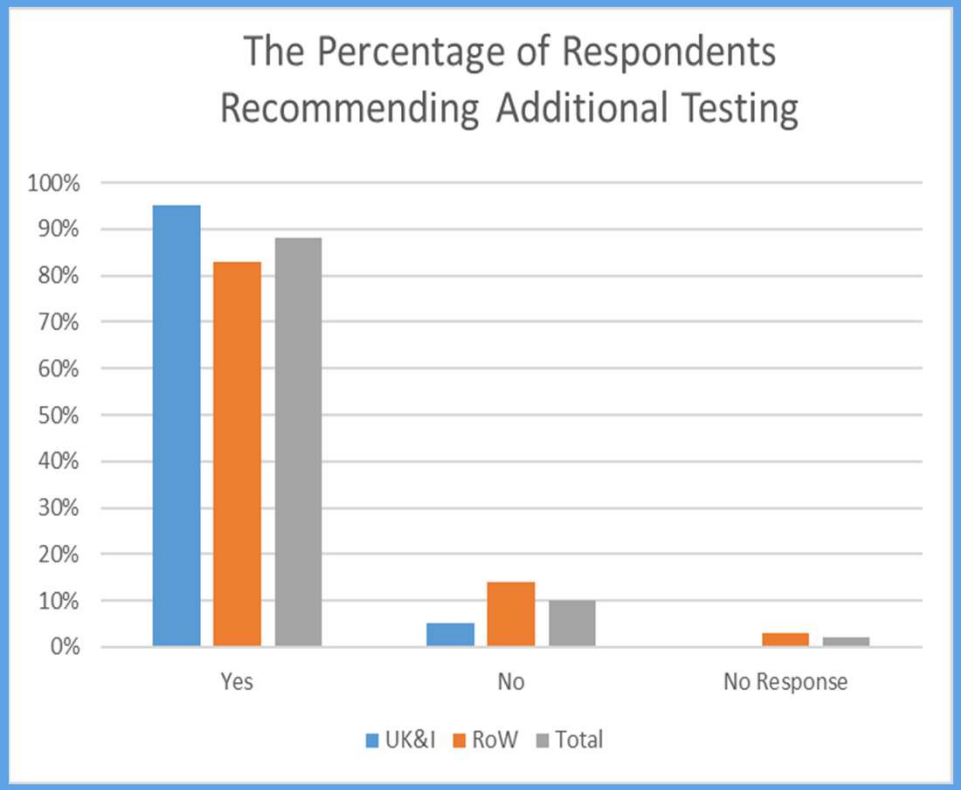
	UK&I%	Row %	Total %	Summary of Reasons for Selection
Donor 1	28%	39%	28%	Accredited cord bank Potential 4/6 match 5/8 in GvH. 6/8 in HvG direction DRB1 match High TNC dose Relatively high CD34 count Low volume Suitable for single unit cord transplant
Donor 2	7%	3%	5%	No DSA Potential 6/8 A*02:06 an unacceptable mismatch DR mismatch Potential 5/6 match DRB1 match Low volume Low CD34 count Low TNC dose US based units often expensive
Donors 1+5	17%	16%	18%	Double unit required due to patient weight DRB1 matched Good combined TNC and CD34 dose
Donors 1+2	3%	0%	2%	High TNC dose High CD34 count
Donors 2+3	0%	3%	2%	Good cell dose
None	14%	14%	15%	All units have HLA matching grade of 4/6 to 5/6 Units do not provide the minimum recommended dose of TNC or CD34 for the adult patient in this case Require further typing of the units HLA antibody testing of the patient required Haploidentical donor preferable

NEQAS comments

- **None of these cord units are desirable.**
- **Potentially use a double cord transplant to achieve recommended cell dose.**

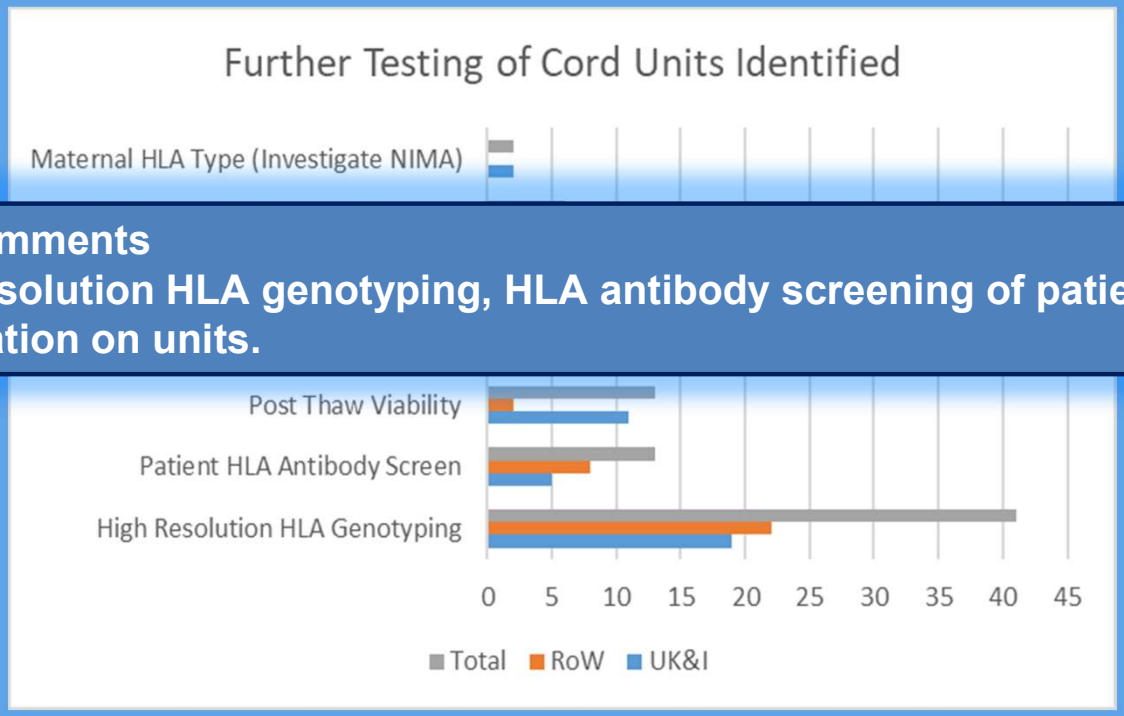
Q5: Additional Testing

Would you recommend any additional testing of these cord units?



Q5: Additional Testing

What additional testing of these cord units would you recommend?



NEQAS comments

- **High resolution HLA genotyping, HLA antibody screening of patient, further information on units.**

Q6: Haploidentical Donor Selection

The transplant consultant decides not to use an unrelated donor or cord unit; a haplo-identical donor is considered. HLA typing of family members and HLA Class I screening results for the patient are provided.

	HLA-A*		HLA-B*		HLA-C*		HLA-DRB1*		HLA-DQB1*		HLA-DPB1*	
Patient	02:01	25:01	56:01	57:01	01:02	06:02	04:01	07:01	03:01	03:03	04:01	13:01
	HLA-A*		**HLA-B***		**HLA-C***		**HLA-DRB1***		**HLA-DQB1***		**HLA-DPB1***	
Sibling	02:01	#	56:01	58:01	01:02	03:02	04:01	13:02	03:01	06:09	04:01	104:01
Child 1	02:01	24:02	13:02	56:01	01:02	06:02	04:01	10:01	03:01	05:01	04:01	#
Child 2	02:01	24:02	27:05	56:01	01:02	02:02	01:01	04:01	03:01	05:01	02:01	04:01
Child 3	24:02	25:01	13:02	57:01	06:02	#	07:01	10:01	03:03	05:01	04:01	13:01
Child 4	24:02	25:01	27:05	57:01	02:02	06:02	01:01	07:01	03:03	05:01	02:01	13:01

HLA Class I Potential Donor Specific Antibodies	**Date of Sample and MFI**	
Specificity	**26/06/2020**	**28/07/2020**
A*24:02	17,510	18,018
B*13:02	25,004	24,791
B*27:05	19,675	19,387
B*58:01	Negative	Negative
C*02:02	3445	3064
C*03:02	4036	3962

Q6: Haploidentical Donor Selection

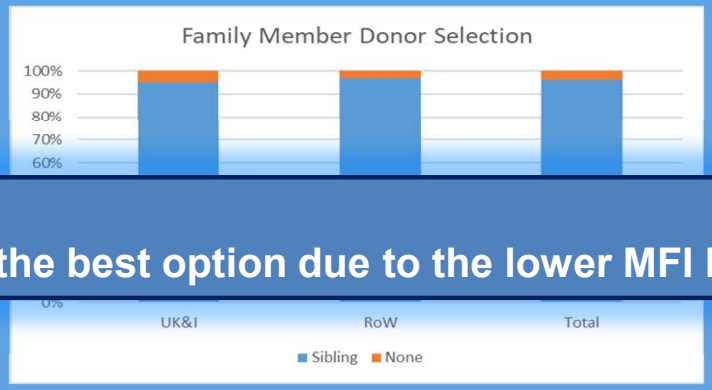
	HLA-A*		HLA-B*		HLA-C*		HLA-DRB1*		HLA-DQB1*		HLA-DPB1*	
Patient	02:01	25:01	56:01	57:01	01:02	06:02	04:01	07:01	03:01	03:03	04:01	13:01
Sibling	02:01	02:01	56:01	58:01	01:02	03:02	04:01	13:02	03:01	06:09	04:01	104:01
Child 1	02:01	24:02	13:02	56:01	01:02	06:02	04:01	10:01	03:01	05:01	04:01	-
Child 2	02:01	24:02	27:05	56:01	01:02	02:02	01:01	04:01	03:01	05:01	02:01	04:01
Child 3	24:02	25:01	13:02	57:01	06:02	06:02	07:01	10:01	03:03	05:01	04:01	13:01
Child 4	24:02	25:01	27:05	57:01	02:02	06:02	01:01	07:01	03:03	05:01	02:01	13:01

A*24:02 17510, 18018 B*27:05 19675, 19387 C*03:02 4342, 3064

B*13:02 25004, 24791 C*02:02 3445, 3064

Q6: Haploidentical Donor Selection

Which donor would you suggest as being the favourable option and give your reasons for selection?



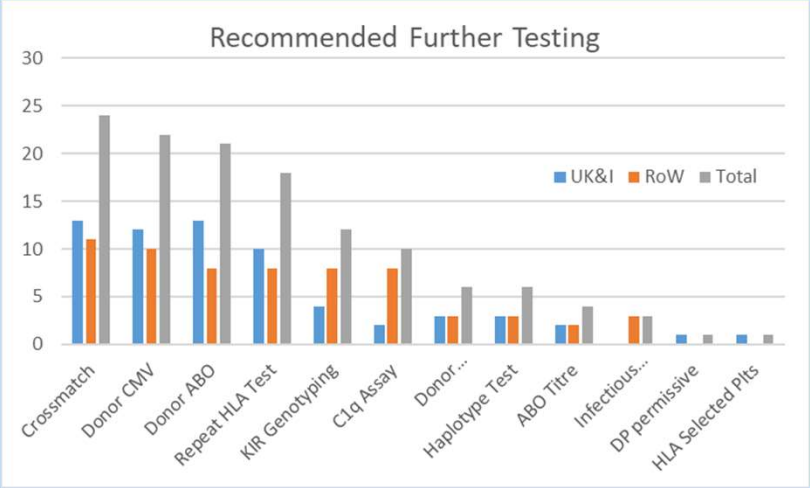
NEQAS comments

- **The sibling would be the best option due to the lower MFI DSA**

Most Common Reasons for Selection	
Sibling	Shares one haplotype - 6/12 match (GvH direction) and 7/12 match (HvG direction)
	Lowest cumulative DSAs of options available (C*03:02)
	DSA against HLA-C which has low expression
	HLA-DPB1 mismatch is non-permissive
	Potential issue around GVH direction homozygous HLA A2
	Desensitisation would likely be effective against a DSA at this level
	More likely to be matched at minor histocompatibility alleles
None of the Donors	Very high, paternal origin DSA against children. Intermediate DSA against sibling.
	Unknown whether children are above 18 years old and age of sibling unknown.

Q6: Haploidentical Donor Selection

What, if any, further testing would you recommend to assess the risk of transplantation?



Testing Identified	UK&I	RoW	Total
Crossmatch	13 (20%)	11 (17%)	24 (19%)
Donor CMV	12 (19%)	10 (15%)	22 (17%)
Donor ABO	13 (20%)	8 (12.5%)	21 (16%)
Repeat HLA Antibody Screen	10 (15%)	8 (12.5%)	18 (14%)
KIR Genotyping	4 (7%)	8 (12.5%)	12 (9%)
C1q Assay	2 (3%)	8 (12.5%)	10 (8%)
Donor Age/Gender	3 (5%)	3 (5%)	6 (5%)
Haplotype Determination	3 (5%)	3 (5%)	6 (5%)
ABO Titre	2 (3%)	2 (3%)	4 (3%)
Infectious Markers	0 (0%)	3 (5%)	3 (2%)
DP permissive/non-permissive	1 (1.5%)	0 (0%)	1 (1%)
HLA selected platelets if required	1 (1.5%)	0 (0%)	1 (1%)

Further Comments…

- If the patient is receiving HLA selected products a request could be made that HLA-B*58 and -C*03:02 should be avoided in the selected units to avoid sensitisation.
- Size of the patient relative to the donor is considered, we wouldn't use a donor less than 2/3 the weight of the recipient. The donor would need to have a full health check.
- Expected CDC and FC crossmatch to be negative. However, if it were to be positive, we would recommend 2 rounds of plasma exchange, followed by post-transplant antibody monitoring and early chimerism monitoring.
- We would need to consider the siblings age and fitness to transplant. We would still prefer to transplant using a 9/10 DSA negative VUD donor.
- Always important to discuss the clinical urgency as part of the MDT so that HR typing of sibling and children could potentially be initiated early if required.
- Concern about likelihood of disease relapse with haploidentical donor source.
- If the sibling is unsuitable we would crossmatch the children and perform antibody removal if required.
- Desensitization of HLA antibodies against HLA-C*03:02 (MFI 3962) before transplantation process.
- Monitor antibody post graft for prompt treatment if antibody continues prior to full chimerism.

Follow-up & Discussion…

- Based on a real case. The unrelated donor options in question 2 were a true reflection of those available
  - Both donors were investigated, both were unavailable.
- Unrelated donor search identified potential 9/10 mismatched donor (HLA-A* homozygote, 9/10 in GvH direction only)
  - The clinical team were unwilling to perform a mismatched transplant for this patient
  - Alternative transplant options were pursued.
- Upon review of the cord search, the only units put forwards for consideration were Donor 2 and Donor 4
  - Dismissed due to the cord bank not being accredited and cell dose respectively.
  - The other cord units listed in this question were fictitious.
- The clinical team decided to proceed with a haploidentical transplant
  - Due to the strength of DSAs, the sibling was chosen as the best option.
- A wet crossmatch was considered but the sibling living in another country and logistical difficulties in getting fresh cells to the laboratory, a virtual crossmatch was used.
    - The patient is now 4 months post-transplant and has been reported at 100% donor chimaerism in the whole blood sample, myeloid and T-lymphocyte subsets.

03

Educational Scheme (iED) Scenario 3: Transfusion Related Acute Lung Injury (TRALI)

Platelet Scenarios

Year	Transfusion/Platelet	Returns
2015	Matched platelet selection	27
2016	Platelet Refractoriness	23
2017	TRALI	27
2018	NAIT	24
2019	Platelet Refractoriness then HSCT	37

- Dispatched on 19th January 2021
- 33 Responses
  - 16 from UK and Ireland (UK&I)
  - 17 from the Rest of the World (RoW)

Definition of TRALI

Vlaar APJ, Kleinman S. An update of the transfusion-related acute lung injury (TRALI) definition. Transfus Apher Sci. 2019 Oct;58(5):632-633. doi: 10.1016/j.transci.2019.07.011. Epub 2019 Sep 5. PMID: 31522921.

One simple definition of TRALI is provided by the UK Haemovigilance Serious Hazards of Transfusion (SHOT) Scheme as:

"Acute dyspnoea with hypoxia and bilateral pulmonary infiltrates during or within 6 hours of transfusion, in the absence of circulatory overload or other likely causes, or in the presence of human leucocyte antigen (HLA) or human neutrophil antigen (HNA) antibodies cognate with the recipient."

Can be confused with transfusion-associated dyspnoea (TAD) or transfusion associated circulatory overload (TACO) which are more common.

Table 1b
New consensus TRALI definition [2].

TRALI type I - Patients who have no risk factors for ARDS and meet the following criteria:

a.	i.	Acute onset	
	ii.	Hypoxemia	$PaO_2/F_iO_2 \leq 300$[a] or $SpO_2 < 90\%$ on room air
	iii.	Clear evidence of bilateral pulmonary edema on imaging (e.g. chest radiograph, chest CT, or ultrasound)	
	iv.	No evidence of left atrial hypertension (LAH)[b] or, if LAH is present, it is judged to not be the main contributor to the hypoxemia	
b.		Onset during or within 6 hours of transfusion[c]	
c.		No temporal relationship to an alternative risk factor for ARDS	

TRALI type II - Patients who have risk factors for ARDS (but who have not been diagnosed with ARDS) or who have pre-existing mild ARDS (PaO_2/F_iO_2 of 200-300), but whose respiratory status deteriorates[d] and is judged to be due to transfusion based on:

a. Findings as described in categories *a and b* of TRALI type I, and
b. Stable respiratory status in the 12 hours prior to transfusion

[a] If altitude is higher than 1000 m, the correction factor should be calculated as follows: $[(PaO_2/FiO_2)x(\text{barometric pressure}/760)]$.

[b] Use objective evaluation when LAH is suspected (imaging e.g. echocardiography, or invasive measurement using e.g. pulmonary artery catheter).

[c] Onset of pulmonary symptoms (e.g. hypoxemia – lower P/F ratio or SpO_2) should be within 6 h of end of transfusion. The additional findings needed to diagnose TRALI (pulmonary edema on a lung imaging study and determination of lack of substantial LAH) would ideally be available at the same time but could be documented up to 24 h after TRALI onset.

[d] Use PaO_2/F_iO_2 ratio deterioration along with other respiratory parameters and clinical judgement to determine progression from mild to moderate or severe ARDS. See conversion table in appendix to convert nasal O_2 supplementation to F_iO_2.

Causes and Mechanism of TRALI

Classical TRALI is caused by antibodies in the donor blood reacting with the patient's neutrophils, monocytes or pulmonary endothelium. Inflammatory cells are sequestered in the lungs, causing leakage of plasma into the alveolar spaces (non-cardiogenic pulmonary oedema).

Caused by HLA and/or HNA patient specific antibodies in the donor

Mechanism for the development of TRALI: Two Hit Hypothesis
1 – Predisposing Clinical Condition: trauma, surgery, infection, malignancy, disease – activate vascular endothelium, pulmonary neutrophil priming and adherence
2 – Transfusion: stimulate primed neutrophils – causes endothelial cell damage, capillary leakage

	Immune TRALI	Non-immune TRALI
Trigger	Leucocyte antibodies	Biologically active lipids
Main blood components implicated	Fresh-frozen plasma > platelet concentrates	Stored platelet concentrates > stored red blood cells
Occurrence	Can even occur in healthy individuals	Occurs predominantly in critically ill patients
Clinical course	Severe, often life-threatening, TRALI (70% mechanical ventilation)	Mild TRALI (oxygen support is usually sufficient)

Lab Investigations for TRALI

- Testing to confirm TRALI should be performed on fresh donor samples and pre- and post- transfusion samples from the recipient.

- Test donors for HNA and HLA specific antibodies
  - If multiple donors involved start investigation with female & transfused male donors
  - An individual may have both auto and allo HNA antibodies (unlikely in a healthy donor)

- HLA and HNA Type DONORS
  - Used to aid antibody investigation
  - HNA type used to confirm auto or allo antibodies

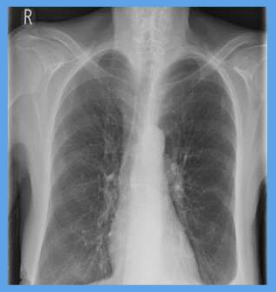
- HLA and HNA Type PATIENT
  - Used to identify the presence of any cognate antigens to donor antibodies

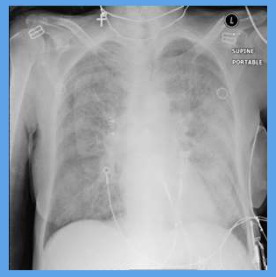
- HLA and HNA crossmatching
  - Rarely performed as need viable granulocytes from the patient for HNA XM

TRALI confirmed if donor has patient specific antibodies

In 65% to 90% of TRALI cases, HLA or HNA antibodies identified in the plasma of the implicated donor.

UK NEQAS Scenario #3





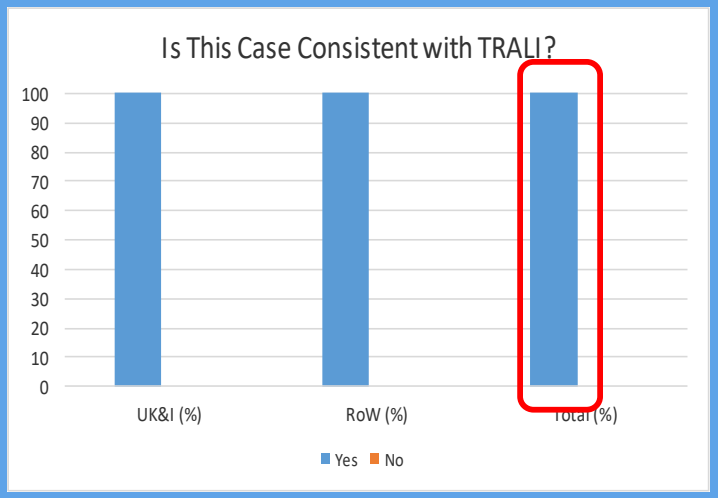
A patient case report is received in your laboratory

- Female
- 69 years old
- Myelofibrosis
- Transfused 2 units of red cells for anaemia
- Patient found unresponsive, hypotensive and wheezing 15 minutes after 2nd unit transfused
- Patient intubated and ventilated, improved after 48 hours ITU care
- CT scan showed bilateral infiltrates

Q1: Diagnosis of TRALI

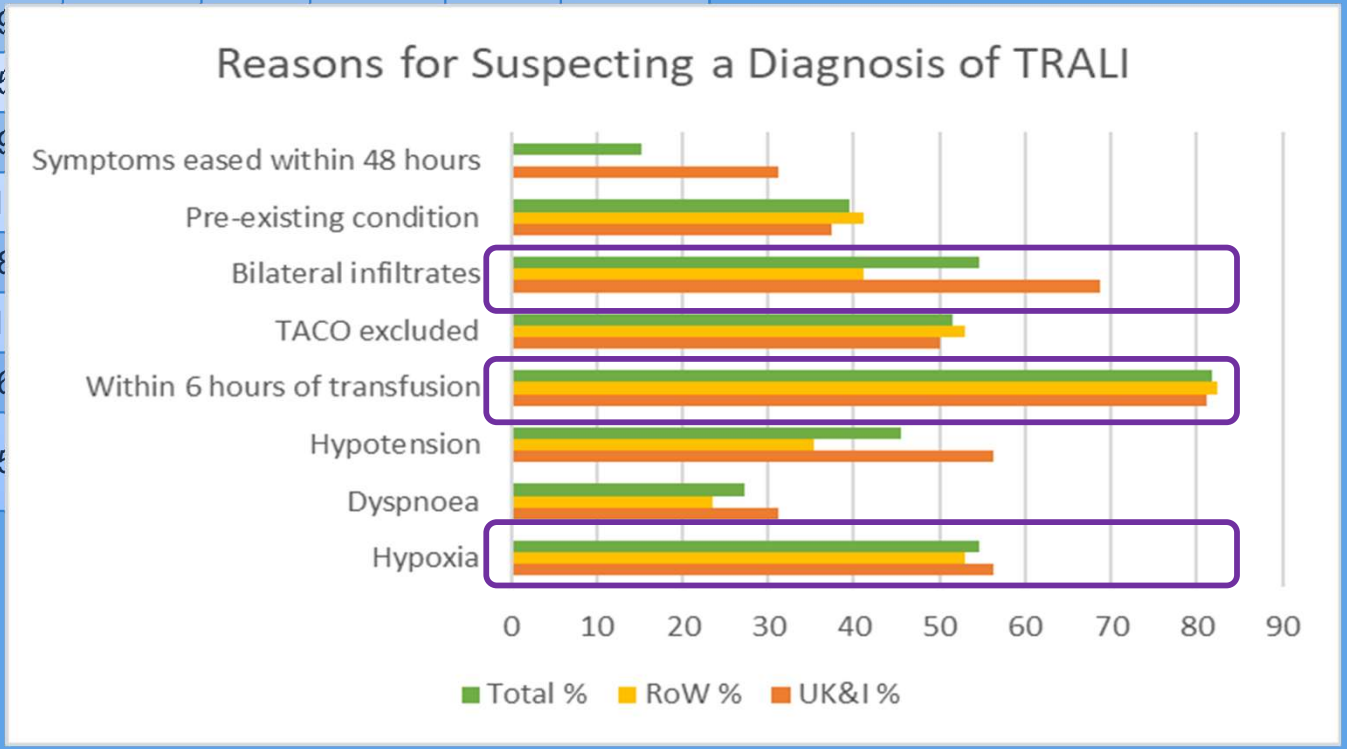
- Based on the information provided in this initial patient case report, would you suspect this case is consistent with TRALI?

	UK&I	UK&I (%)	RoW	RoW (%)	Total	Total (%)
Yes	16	100	17	100	33	100
No	0	0	0	0	0	0



Q1: Give reasons for your answer

Reasons	UK&I	UK&I %	RoW	RoW %	Total	Total %
Hypoxia	9					
Dyspnoea	5					
Hypotension	9					
Within 6 hours of transfusion	1					
TACO excluded	8					
Bilateral infiltrates	1					
Pre-existing condition	6					
Symptoms eased within 48 hours	5					



Q2: Translate the patient HLA genotype to the serological equivalent

HLA Allele	Serological Equivalent		UK&I %	RoW %	Total %	Errors
	Split	Broad				
A*32:01:01	**A32**	**A19**	100	100	100	N/A
A*34:02:01	**A34**	**A10**	100	100	100	N/A
B*40:01:02	**B60**	**B40**	100	100	100	N/A
B*40:01:02	**B60**	**B40**	100	100	100	N/A
C*03:04:01	**Cw10**	**Cw3**	100	100	100	N/A
C*03:04:01	**Cw10**	**Cw3**	100	100	100	N/A
DRB1*04:01	**DR4**		100	100	100	N/A
DRB1*15:01:01	**DR15**	**DR2**	100	100	100	N/A
DRB4*01:03:01	**DR53**		100	76	94	DR52 Not defined
DRB5*01:01:01	**DR51**		100	88	88	Not defined
DQB1*03:02:01	**DQ8**	**DQ3**	100	94	97	DQ7
DQB1*06:02:01	**DQ6**	**DQ1**	100	94	97	Not defined

Summary of Results

	Patient	Donor 1	Donor 2
HLA Type	A*32:01, A*34:02; B*40:01, -; C*03:04, - DRB1*04:01, DRB1*15:01; DRB4*01:03; DRB5*01:01; DQB1*03:02, DQB1*06:02	A*02:20, A*29:02; B*13:02, B*44:03; C*06:02, C*16:01 DRB1*07:01, -; DRB4*01:01, DRB4*01:03; DQB1*02:02, -	A*02:01, A*03:01; B*07:02, B*08:01; C*07:01, C*07:02 DRB1*03:01, DRB1*04:07; DRB3*01:01; DRB4*01:03; DQB1* 02:01, DQB1*03:01
HNA Type	1b1c 3a3b 4a4b 5a5bw	1b1c 3a3b 4a4b 5a5a	1a1b 3a3a 4a4a 5a5b
Patient Specific HLA Antibodies (>1,000 MFI)		**B60 - 20502**	**DR51 – 1107** **DQ8 – 1576** **DQ6 – 2930**
Patient Specific HNA Antibodies		**Negative** (Indirect GCLT, GIFT and LIFT Positive, ?specificity)	**Negative**
Comments		**Autoreactivity:** B13 – 21770 B44 – 22577 Cw6 – 8981	

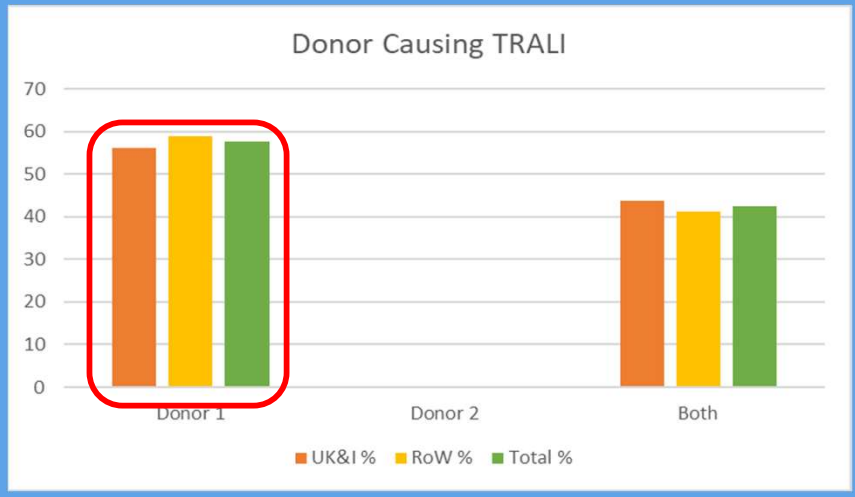
Q2: Do the results provided support a diagnosis of antibody mediated TRALI?

	UK&I	UK&I (%)	RoW	RoW (%)	Total	Total (%)
Yes	16	100	17	100	33	**100**

The presence of HLA specific antibodies to the recipient's cognate HLA antigen/s did support a diagnosis of TRALI.

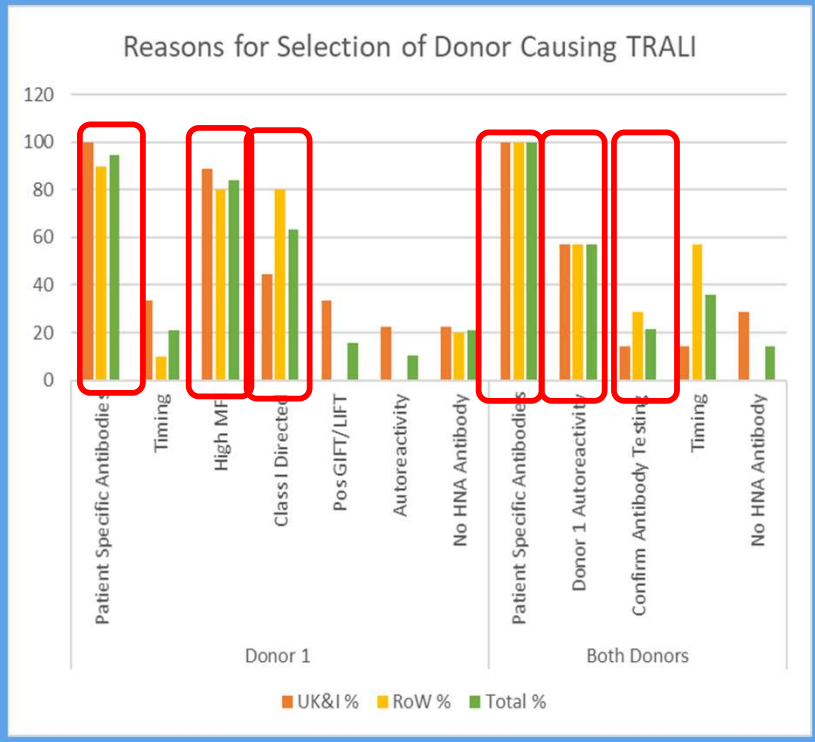
Which donor(s) are likely to be the cause?

Donor Causing TRALI	UK&I	UK&I %	RoW	RoW %	Total	Total %
Donor 1	9	56	10	59	19	**58**
Donor 2	0	0	0	0	0	**0**
Both	7	44	7	41	14	**42**



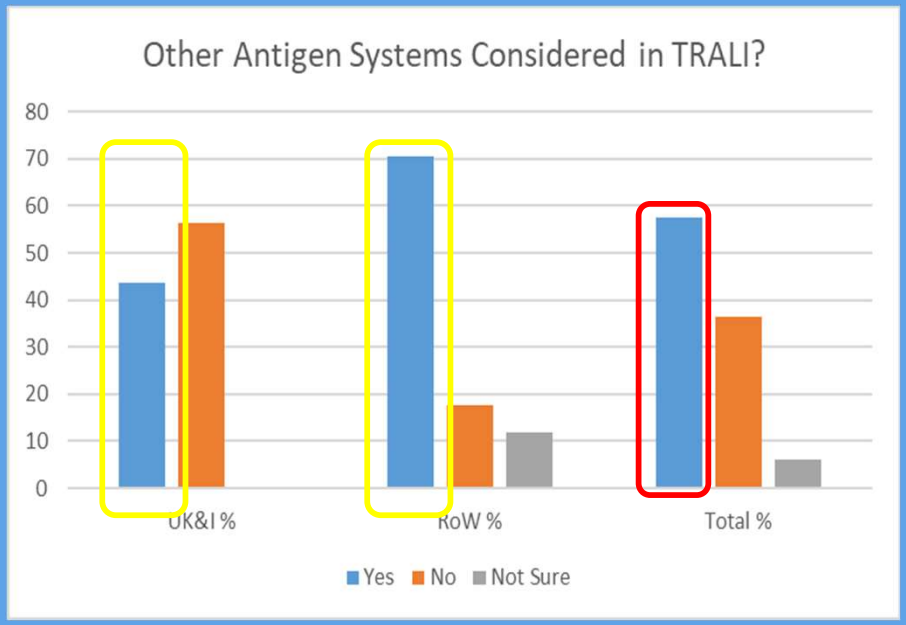
Reasons for selecting donor

Donor	Reasons	UK&I	UK&I %	RoW	RoW %	Total	Total %
Donor 1	Patient Specific Antibodies	9	100	9	90	18	**95**
	Timing	3	33	1	10	4	**21**
	High MFI	8	89	8	80	16	**84**
	Class I Directed	4	44	8	80	12	**63**
	Pos GIFT/LIFT	3	33	0	0	3	**16**
	Autoreactivity	2	22	0	0	2	**11**
	No HNA Antibody	2	22	2	20	4	**21**
Both Donors	Patient Specific Antibodies	7	100	7	100	14	**100**
	Donor 1 Autoreactivity	4	57	4	57	8	**57**
	Confirm Antibody Testing	1	14	2	29	3	**21**
	Timing	1	14	4	57	5	**36**
	No HNA Antibody	2	29	0	0	2	**14**



Q3: Do you consider any other antigen systems when considering a diagnosis of TRALI?

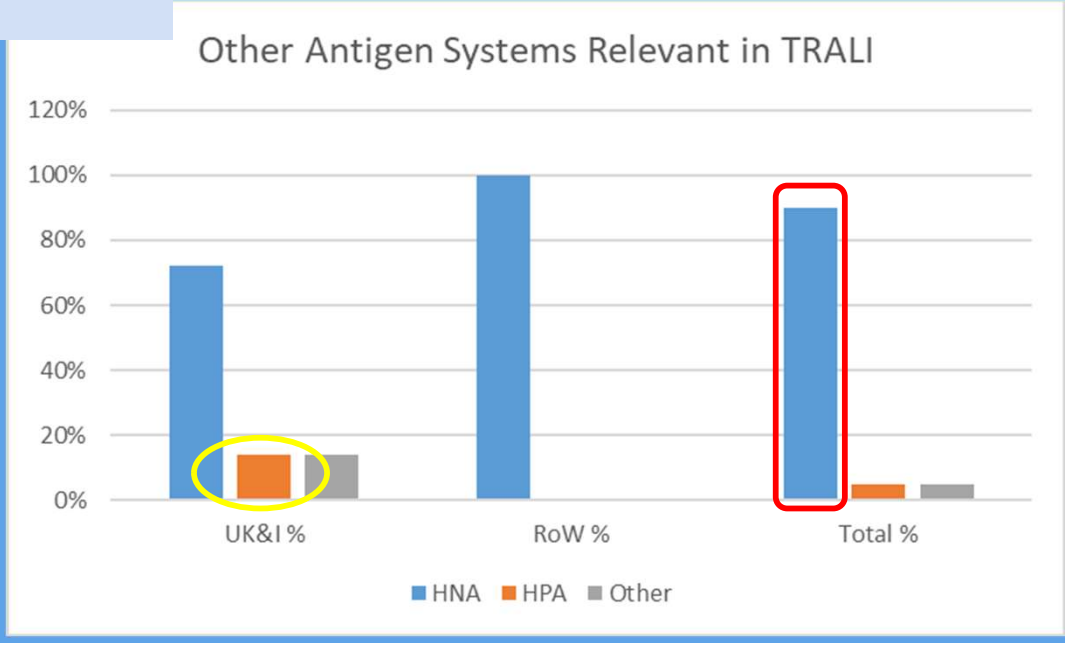
	UK&I	UK&I %	RoW	RoW %	Total	Total %
Yes	7	44	12	71	19	**58**
No	9	56	3	18	12	**36**
Not Sure	0	0	2	12	2	**6**



If yes, please provide further details

Other Antigen Systems	UK&I	UK&I %	RoW	RoW %	Total	Total %
HNA	5	72%	12	100%	17	**90%**
HPA	1	14%	0	0%	1	**5%**
Other factors*	1	14%	0	0%	1	**5%**

**Included IgA antibodies, bacterial contamination and allergy*



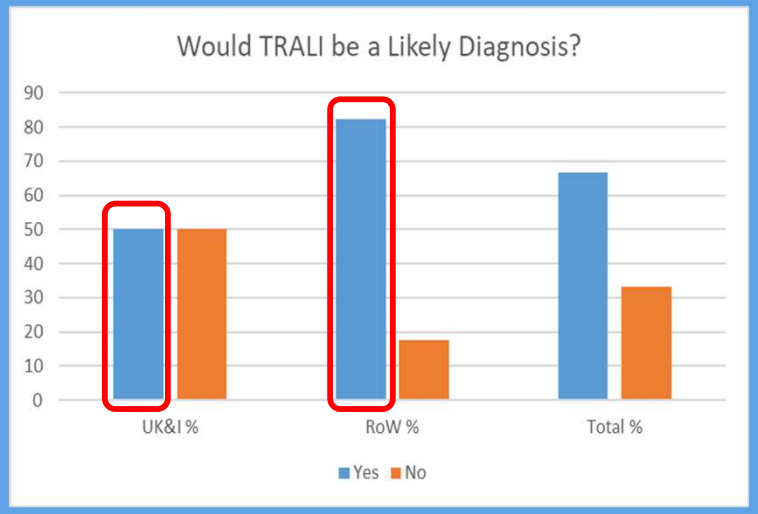
Q4: Second Referral

A second case if referred to your laboratory

- 1 unit of red cells from Donor X was transfused to Patient X
- 4 hours later Patient X experienced TRALI-like symptoms
- Upon testing Donor X had a potential patient specific antibody to DPB1*04:01 MFI-2564
- No HNA antibodies were detected

Do these results support a diagnosis of TRALI?

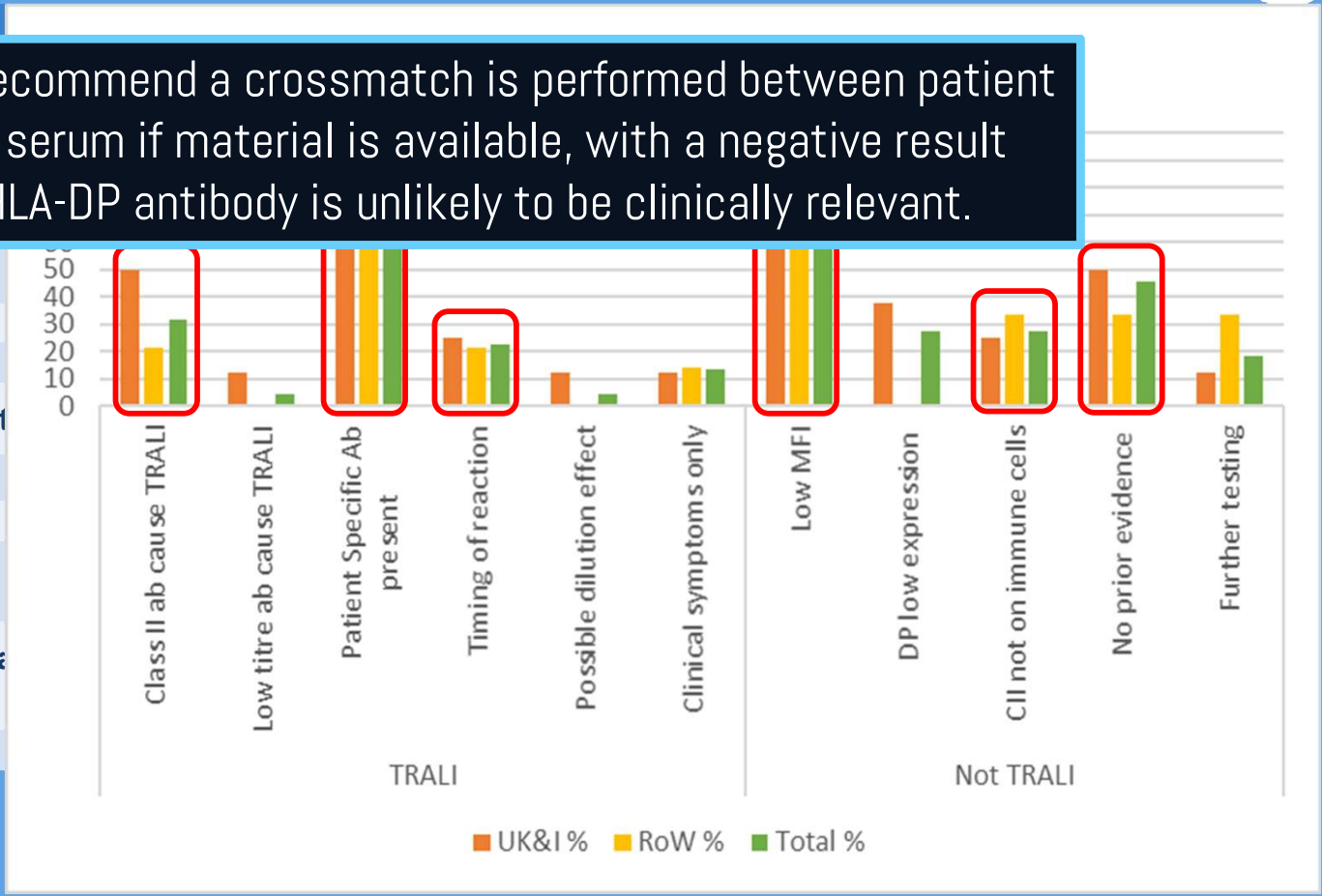
	UK&I	UK&I %	RoW	RoW %	Total	Total %
Yes	8	50	14	82	22	**67**
No	8	50	3	18	11	**33**



Reasons for your Decision

	Reasons
	Class II anti
	Low titre an
Yes	Patient Specific Antibody present
	Timing of reaction
	Possible dilution effect
	Diagnosis based on clinical sympt
	Low MFI
	DP low expression
No	CII low/no expression on immune
	No documented cases of TRALI ca by DP antibodies
	Further testing

NEQAS would recommend a crossmatch is performed between patient cells and donor serum if material is available, with a negative result indicating the HLA-DP antibody is unlikely to be clinically relevant.

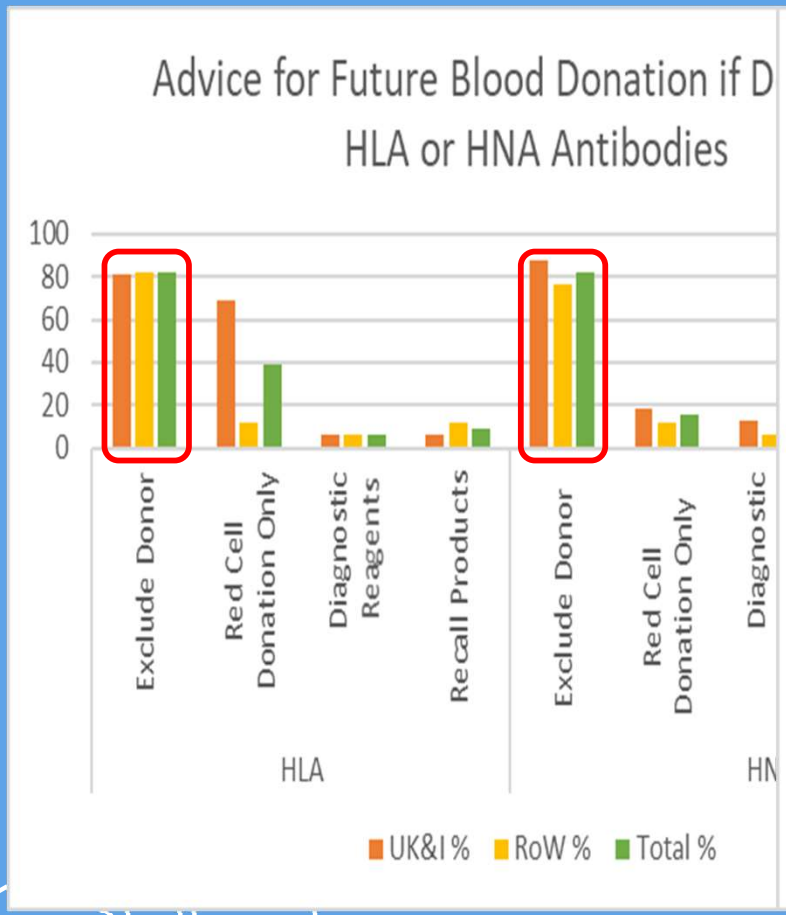


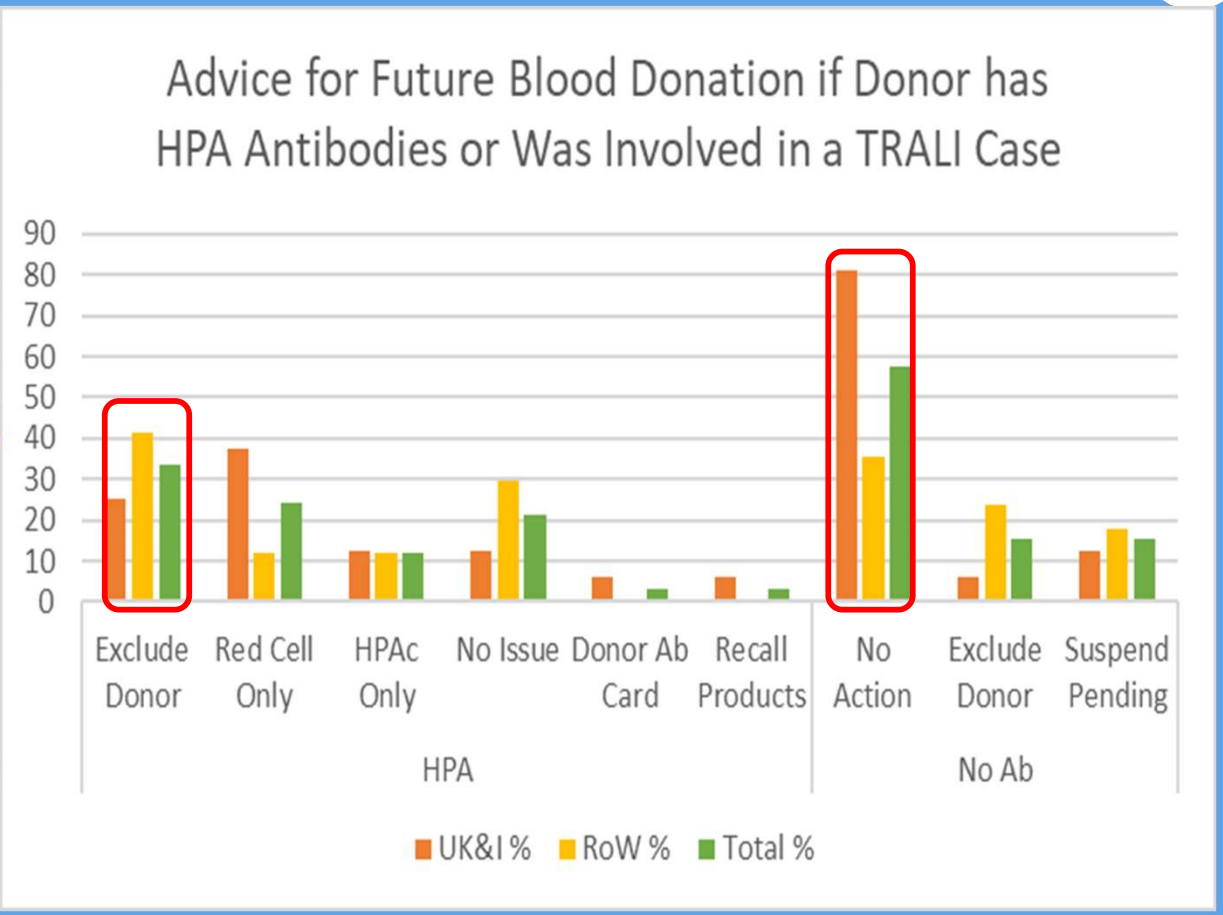
Q5: Advice regarding future blood component production if a donor with the following antibodies involved in TRALI

Donor Ab	Reasons	UK&I	UK&I %	RoW	RoW %	Total	Total %
HLA	Exclude Donor (if PSA)	13	81	14	82	27	**82**
	Red Cell Donation Only (non-PSA HLA ab)	11	69	2	12	13	**39**
	Use for QA/Diagnostic Reagents	1	6	1	6	2	**6**
	Red Cell Donation Only (no Plt Donation)	6	38	2	12	8	**24**
	Use in HPA compatible Patients Only	2	13	2	12	4	**12**
	No Issue	2	13	5	29	7	**21**
	Produce Donor Ab Card	1	6	0	0	1	**3**
	Recall Products	1	6	0	0	1	**3**
No Ab	No Action	13	81	6	35	19	**58**
	Exclude Donor	1	6	4	24	5	**15**
	Suspend Donor Pending Investigation	2	13	3	18	5	**15**

NEQAS would recommend, in line with UK practice, that if a donor is identified as possessing HNA-3a that because of the association of this antibody with more severe cases of TRALI, the donor is excluded from donation of all blood products for clinical use.

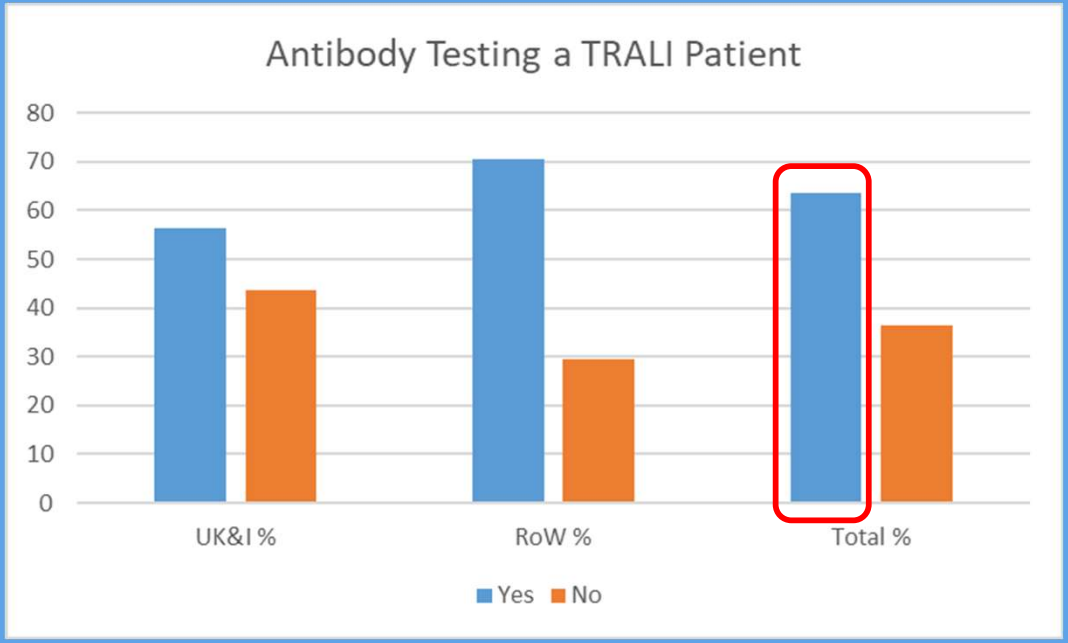
Future Blood Donation





Q6: Would you Consider Antibody Testing a Suspected TRALI Patient

	UK&I	UK&I %	RoW	RoW %	Total	Total %
Yes	9	56	12	71	21	**64**
No	7	44	5	29	12	**36**

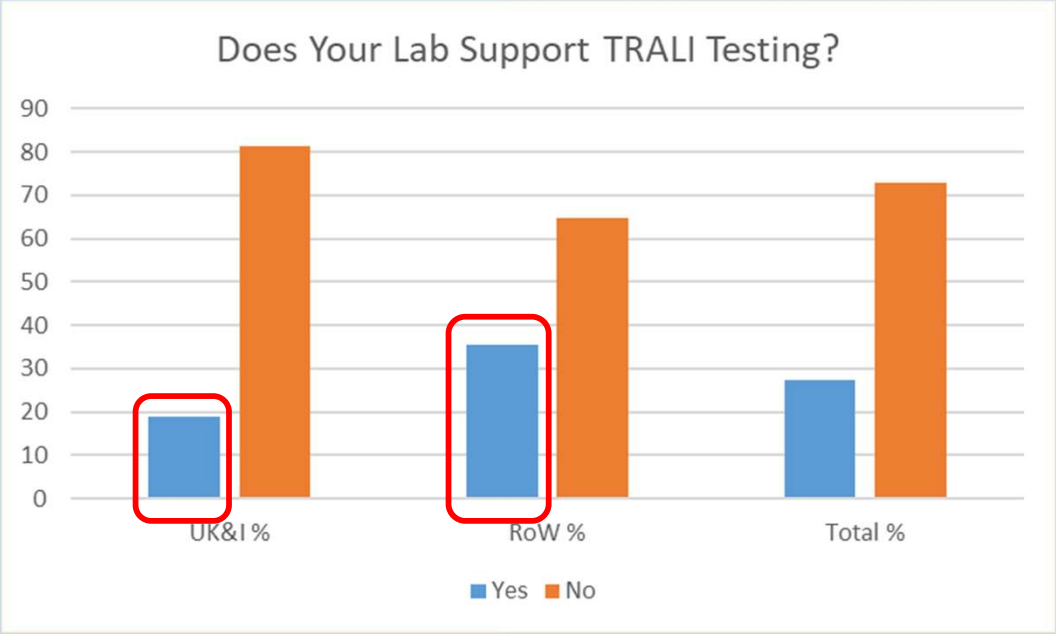


Most Common Reasons Given

Yes	Investigate for HLA/HNA antibodies if indicated, e.g. if associated donors are antibody negative or antibodies are non-donor directed. Reaction of patient antibodies with donor leukocytes is feasible.
	There is documentation of donor leukocytes reacting with recipient derived antibodies in TRALI
	In rare cases TRALI can be caused by patient antibodies. Once donors have been tested and excluded from investigation, patient antibodies can be investigated.
	Approximately 80% of TRALI cases are due to HLA/HNA antibodies in the donor, but 20% are cause unknown and could be caused by antibodies in the patient directed towards cells in the blood product, especially with granulocyte infusions.
	Cases of TRALI due to patient antibody reacting with transfused donor cells have been reported. Although UK blood products are leucodepleted they are not leucocyte free. If no donor antibodies reacting with the patient or other donor antigens are detected antibodies in the patient may be responsible for a TRALI reaction. Three cases of TRALI apparently due to patient HLA antibodies reacting with donor cells in leucodepleted products have been described. (de Clippel, Emonds and Compernolle, Transfusion, 2019, 59, 2788-2793).
	There are reports of TRALI occurring after transfusion of donor leucocytes, which have interacted with patient derived antibodies (apheresis or buffer coat granulocytes).
	Transfusion recipient data would allow assessment of the safety of blood component modifications, in addition to additional mitigation strategies.
	Some cases of TRALI (reverse/inverted TRALI) are triggered by anti-HLA or anti-HNA antibodies in the patient's plasma.
	To support the diagnosis of TRALI and to prevent reoccurrence of TRALI in future.
No	Recipient antibodies not thought to be relevant due to low risk of passenger lymphocytes after implementation of Leucodepletion in the UK in 1999.
	Not in the Guidelines to test for antibodies in the patient.
	It could be useful to know the patients antibody profile in order to explain any further reactions while the patient is being supported in the recovery from TRALI – for instance if the patient receives further blood units and experiences a fever due to a febrile non-haemolytic transfusion reaction (FNHTR).
	Would consider if all other potential causes have been ruled out.
	No proven link between patient antibodies against donors and TRALI.
	Not unless the patient has received a granulocyte transfusion, which is exceptional.

Does Your Laboratory Support Testing for the Diagnosis of TRALI?

Yes
27%



No
73%

	UK&I	UK&I %	RoW	RoW %	Total	Total %
Yes	3	19	6	35	9	27
No	13	81	11	65	24	73

Further Comments

- Additional information on the blood donors would have been useful in this case, e.g. gender, sensitising events (pregnancies). Also, storage time/age of the blood products would have been helpful.
- Answered as if the antibody and HLA types of the donors had been swapped around. Otherwise the HLA antibody profile of donor one would be invalid as would be to themselves as well.
- B60 MFI lower than "self" MFI which would call all results into question.
- Q4: Answer should be "potentially", as there is insufficient clinical and laboratory detail to make a definitive diagnosis.
- Useful to see lots of clinical information. We noticed that Donor 1 is probably the real donor 2, and vice versa, which affects what one learns from this scenario about onset of transfusion reactions.
- Donor 1 has autoantibodies in the class I panel, which are not explained. To discriminate DQB and DQA antibodies in donor 2 class II panel, the results on negative beads should be provided, as well as DQA typing of the donor and patient.
- Using only male blood donors might mitigate the risk of TRALI. Female blood donors with pregnancy history should have HLA antibody testing performed if going to be used as plasma donors. HLA antibody testing in platelet donors. Use of PAS (platelet additive solution).

Follow Up and Discussion

This scenario was based on a real-life TRALI investigation. The patient case report provided at the beginning of this scenario was reviewed by an expert panel of Anaesthetists who approved the case for laboratory investigation.

For this scenario the HLA serology raw data was swapped between the two donors resulting in high level "self" antigen reactivity in the luminex SAB results. NEQAS were hoping this unusual reactivity should have prompted a comment of concern and request for repeat samples.

Interestingly, only a total of 5 UK&I and 3 RoW based labs (8/33, 24%) commented on the usual self-reactivity seen in Donor 1, with an additional 3 UK&I and 2 RoW labs (5/33, 15%) questioning whether samples had been swapped.

One of the many purposes of performing EQA testing is to highlight potential discrepancies at the pre-analytical, analytical and post-analytical phases. In this scenario we were hoping labs might question, as they should in a clinical situation, where unusual results are found whether samples had been mixed up at one of the analytical phases.

UK NEQAS Histocompatibility & Immunogenetics

UK NEQAS H&I

Educational Crossmatch Scenario (EDXM)

Dr Tracey Rees

@UKneqasHI
@UK_NEQAS

UK NEQAS
Histocompatibility
& Immunogenetics

"Schemes should relate more closely to clinical scenarios rather than testing individual test assays."

Whole Process ‘EQA’

Assessed Schemes

- 1A, 4A1, 4A2 – HLA Typing
- 6 – HLA Antibody Detection
- 3 – HLA Antibody Specification
- 2A, 2B – Crossmatching

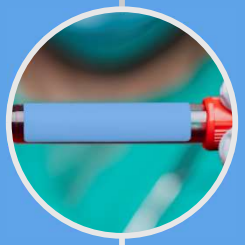
Educational Schemes

- Interpretative Educational Scenarios
- Educational Crossmatch Scheme
  - Clinical decision making based on results from multiple assays
  - Each assay only gives part of the picture
  - Results from one assay can influence the interpretation of another
  - Variation between centres (repertoires, cut-offs)

Educational Scheme Distribution

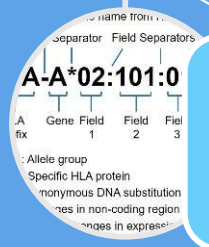
Educational Scheme Distribution

'Donor' Sample

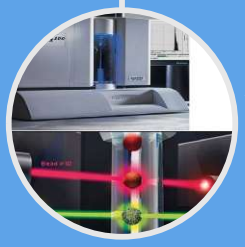


'Patient' Samples

3 x Serum Samples



Clinical Interpretation
Transplant Risk Stratification



Antibody Detection / Specification

(Schemes 3 & 6)

2020 Submissions

- 36 participants submitted results
- Not all labs reported results for all tests

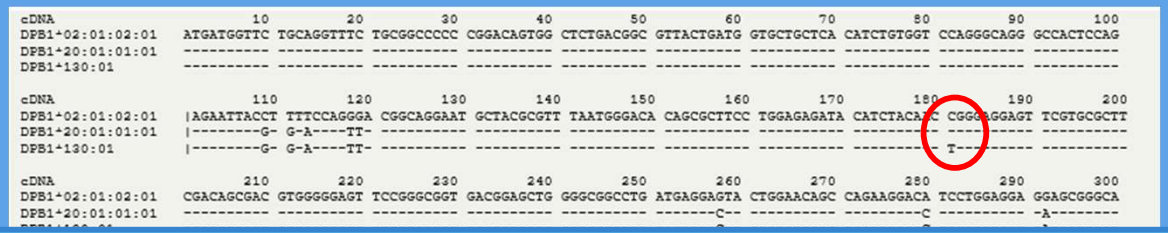
- 100% agreement on HLA type except DQA
- No consensus reached on DPB1 type

	A*	B*	C*	DRB1*	DRB4*	DRB5*	DQA1*	DQB1*	DPA1*	DPB1*
	29	40(60)	03(10)	07	01	01	01	03 (9)	01	02:01
	31	44	16	15	-	-	02	06	-	20:01/ 130:01
Number of reports	35	35	35	35	21	21	21	35	22	28
% Labs in consensus	100%	100%	100%	100%	100%	100%	95%	100%	100%	N/A

- 28 participants submitted a DP type
- All reported DPB1*02:01
- 39% (11/28) reported DPB1*130:01 and 61% (17/28) reported DPB1*20:01

2020 Submissions

- 39% (11/28) reported DPB1*130:01 and 61% (17/28) reported DPB1*20:01
- The polymorphism used to differentiate DPB1*20:01 from DPB1*130:01 is in **exon 2**:



Comments included:

- LABType calls DPB1*02:01 & DPB1*20:01. NGS calls DPB1*02:01 & DPB1*130:01. Cannot exclude DPB1*130:01.
- Ambiguous DPB1: DPB1*02/20 + 130/191.
- Most probable allelic equivalent for DP alleles: DP*02:01, DP*130:01.
- DPB1*130 was confirmed by SSP kit.

We do not capture method used for HLA typing in EDXM

01 Serum 1

Results

Serum 1 Results

	Result	% Consensus	Comments
HLA Class I Antibodies	Positive	97% (34/35)	
HLA Class II Antibodies	Positive	100% (35/35)	
DSA	Yes	100% (35/35)	Some labs also reported antibodies that were not donor specific
CDC XM	PBL Not Assessed T cell Negative B cell Positive	50% (3/6) 94% (16/17) 100% (16/16)	
FCXM T Cell	Positive	100% (26/26)	
FCXM B Cell	Positive	100% (24/24)	
Transplant Risk	Contraindication	77% (27/35)	20% (7/35) reported High risk, 1% (1/35) reported Medium
Recommendations	N/A	N/A	Possible antibody removal prior to transplant Investigate alternative donor options e.g. exchange scheme

02 Serum 2

Results

Serum 2 Results

	Result	% Consensus	Comments
HLA Class I Antibodies	Positive	100% (35/35)	
HLA Class II Antibodies	Not Assessed	66% (23/35)	66% reported negative
DSA	Yes	100% (35/35)	Huge range in MFI reported e.g. B44 (detected by 100% participants) from 611-10,576 A31 (detected by 97% participants) from 1477-14,481
CDC XM	PBL Not Assessed T cell Negative B cell Negative	57% (4/7) 100% (17/17) 94% (16/17)	57% of participants reported PBL crossmatch as negative
FCXM T Cell	Positive	96% (23/26)	
FCXM B Cell	Positive	79% (19/24)	
Transplant Risk	High	37% (13/35)	31% (11/35) reported contraindication 29% (10/35) reported medium risk
Recommendations	N/A	N/A	Seek alternative donor HLAi use appropriate desensitisation Investigate of antibodies are complement fixing

Serum 2 Further Analysis

	Consensus Result	% Consensus	Comments
FCXM T Cell	Positive	96% (23/26)	**Labs reporting Neg n=1 (Lab 14)** **Labs reporting Equivocal n=2 (Labs 142, 238)**
FCXM B Cell	Positive	79% (19/24)	**Labs reporting Neg n=4 (Labs 14, 15, 54, 122)** **Labs reporting Equivocal n=1 (Lab 238)**

- 3 labs reported negative or equivocal T cell XM
- 5 labs reported negative or equivocal B cell XM

We analysed the DSA and MFI ranges reported by these labs:

Lab ID	T cell	B cell	DSA <2,000	2001-5,000	5001-10,000	Cumulative MFI	Interpreted Risk
14	Neg	Neg		A31	B44	11,094	High risk
15	Pos	Neg			A31 B44	13,309	High risk
54	Pos	Neg		B44	A31	9,774	High risk
122	Pos	Neg	A29 DR51 DQ6	B44		8,162	High risk
142	Equ	Pos	A29		A31 B44	19,877	High risk
238	Equ	Equ	A29 B44	A31		5,018	Low risk

03 Serum 3

Results

Serum 3 Results

	Result	% Consensus	Comments
HLA Class I Antibodies	Negative	94% (33/35)	
HLA Class II Antibodies	Not Assessed	74% (26/35)	74% reported negative
DSA	No	100% (35/35)	
CDC XM	PBL Negative T cell Negative B cell Negative	100% (7/7) 100% (18/18) 100% (17/17)	
FCXM T Cell	Negative	96% (25/26)	
FCXM B Cell	Negative	92% (22/24)	
Transplant Risk	Low	97% (34/35)	3% (1/35) reported medium
Recommendations	N/A	N/A	Proceed to transplant

Summary of Crossmatch and DSA Detection Results

2020 Results		Serum 1		Serum 2		Serum 3	
DSA Defined by Luminex		Class I	Class II	Class I	Class II	Class I	Class II
MFI >10,000		A31 (97%)	N/A	A31 (100%)	DR7 (100%) DR53 (86%) DQ9 (100%)	N/A	N/A
MFI 5,001-9,999		B44 (100%) A30 (3%)	N/A	N/A	DQA1*02 (17%)	N/A	N/A
MFI 2,501-5,000		N/A	N/A	N/A	DP2 (57%) DP20 (31%)	N/A	N/A
MFI <2,500		A29 (63%) B60 (3%)	DR51 (3%) DR53 (3%) DQ6 (3%)	A29 (3%) B60 (11%) B44 (6%) Cw10 (3%) Cw16 (3%)	DQA1*01 (3%) DPA1*01 (3%)	N/A	N/A
CDCXM B CELL	No DTT	Positive		Negative		Negative	
	DTT	Positive		Negative		Negative	
FCXM	T Cell	Positive		Positive		Negative	
	B Cell	Positive		Positive		Negative	
Risk		Contraindication/High (97%)		Contraindication/High (68%)		Low (97%)	

The table shows the percentage of participants identifying a DSA and the most common MFI range it was reported in.

Benefits

Benchmarking

Monitor performance of multiple techniques
Make clinical interpretations on own results
Compare local policies for clinical assessment

Education

Monitor concordances
Review variations
Staff training

Competency

Labortory staff
Clinical staff

Future Considerations

Formal Assessment

Basis of future scheme design

Complexity

How to assess the correct clinical interpretation

Workload

Participants
NEQAS team

Competency

Individual competency assessment

Thanks!

Do you have any questions?

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