UK NEQAS Histocompatibility & Immunogenetics

UK NEQAS H&I

Annual Participant's Meeting 2021-22

Ask a question:
Go to www.menti.com and use code 4384 0711

WIFI: _Conference WIFI Password: Springlamb

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@UK_NEQAS

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

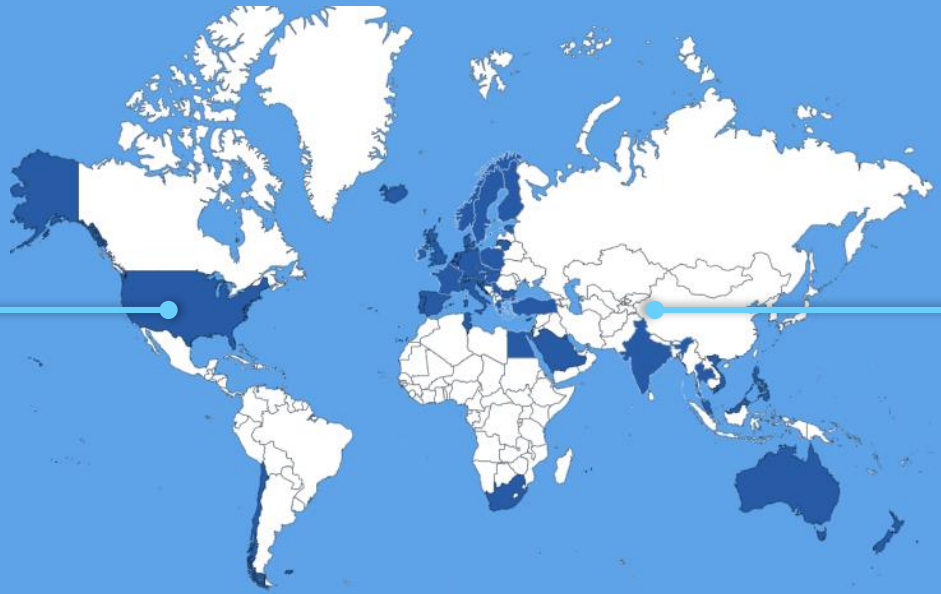
Healthcare Scientist Practitioner: Geraint Clarke

QA Technical Officer: Jack Jefferies

MLA: Owain Seldon

UK NEQAS for H&I: An Overview

Over 320 participants...



In >50 countries.

UK NEQAS International Quality Expertise Histocompatibility & Immunogenetics

UK NEQAS for H&I Steering Committee 2021

Helena Lee (Chair)
Arthi Anand
Katy Derbyshire
Sylvia McConnell
Katherine Mounsey
Anthony Poles
Sunil Daga (Clinical Representative)
Elizabeth Wroe (BSHI Representative to UK NQAAP)

Kathryn Robson (Expert Advisor Scheme 5B)
Barbara McNamara (Expert Advisor Scheme 5B)
Tim Clench (Expert Advisor Scheme 5B)

Welcome and Introduction

Dr Helena Lee

Chair of UK NEQAS for H&I Steering Committee

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 2022 changes

Further Details...

The presentation will be available to view on our website.

Lab Locations...

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

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 2022-23

Steering Committee

New Expert Advisor required

Webinars

iED feedback continuing

Participant's Portal

Continuing improvements

Scheme Changes

Increased sample frequency Schemes 3, 6, 4A1 and 4A2

Scheme 2A

Cytotoxic Crossmatching

Ask a question:
Go to www.menti.com and use code 4384 0711

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	2017	2018	2019	2020	2021
Number of Participants (UK&I)	75 (19)	71 (18)	71 (22)	66 (16)	63 (15)
Number with Unsatisfactory Performance (< 85%) (UK&I)	16 (6)	16 (7)	5 (1)	7 (0)	4 (0)
% Unsatisfactory Performance (UK&I)	21.3% (31.6%)	22.5% (38.8%)	7.0% (4.5%)	10.6% (0)	6.3% (0)

2021: 4 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)	33	34	39	38	40	39
% NT	20.3%	24.7%	13.1%	12.0%	16.6%	18.8%
NT	73	79	68	77	93	128
% incorrect assignments	3.6%	4.7%	1.2%	2.3%	2.9%	2.8%
False Positive	9	9	3	8	6	9
False Negative	4	6	3	7	10	10

Scheme 2A: Unacceptable Performers 2021

Lab ID	PBL -DTT	T -DTT	B -DTT	PBL + DTT	T + DTT	B + DTT	Lab Identified Error
116	N/A	74%		N/A	81%		Contamination in T cell prep
149	83%	N/A	N/A	83%	N/A	N/A	Technical issue/ low cell viability
292	N/A			N/A	83%		Delivery delays
1349						75%	Low cell volume/viability

Scheme 2B

Crossmatching by Flow Cytometry

Ask a question:
Go to www.menti.com and use code 4384 0711

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	2017	2018	2019	2020	2021
Number of Participants (UK&I)	85 (22)	83 (22)	84 (23)	80 (21)	80 (22)
Number with Unsatisfactory Performance (< 85%) (UK&I)	8 (1)	15 (2)	12 (1)	11 (0)	5 (0)
% Unsatisfactory Performance (UK&I)	8.7% (4.5%)	18.1% (9.1%)	14.2% (4.3%)	13.8% (0)	6.3% (0)

2021: 5 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	22	28	30	21	26	29
Number of XM assessed (>75% consensus)	33/40	37/40	30/40	38/40	39/40	31/40
Number of Positive XM	20	19	19	33	33	29
Number of Negative XM	13	18	11	5	6	2
Number of incorrect assignments	20 (2.3%)	30 (2.7%)	38 (3.2%)	28 (3.3%)	47 (2.5%)	16 (1.4%)
Number of False Pos	9	13	4	8	3	1
Number of False Neg	11	17	34	20	44	15
Number of equivocal assignments	4 (0.5%)	3 (0.3%)	3 (0.3%)	3 (0.4%)	4 (0.4%)	1 (0.1%)
Number of NT assignments	36 (4.1%)	90 (8.0%)	143 (11.9%)	45 (5.4%)	57 (5.5%)	188 (16.2%)

UK&I and RoW receive different blood samples

Scheme 2B: Unacceptable Performers 2021

Lab	T Cell	No. of results submitted	B Cell	No. of results submitted	Issue
139	90.0%	32/40	48.4%	32/40	No reply
142	80.0%	40/40	100%	40/40	Sensitivity issues in T-cells – protocol changed
191	90.0%	40/40	80.6%	40/40	Transcription / Not testing same as clinical sample
260	81.1%	40/40	59.0%	40/40	No reply
1360	76.7%	40/40	94.0%	40/40	T cell pos cut off too high

5 labs with UP (<85%)

Scheme 2B: Equivocal Results

- In 2021 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

- 2021 Summary
  - 14 T cell equivocal results (from 3168 = 0.4%)
  - 8 B cell equivocal results (from 3032 = 0.3%)
  - 14 T cell equivocal results assessed as unacceptable (0.4%)
  - 8 B cell equivocal results assessed as unacceptable (0.3%)

2021	No of Labs Reporting Equivocal	No. of Labs Reporting >1 Equivocal Result
UK (n=22)	2 (9.1%)	1 (4.5%)
OS (n= 58)	11 (18.9%)	4 (6.9%)
Total (n=80)	13 (16.3%)	5 (6.3%)

2021	T cell Equivocal Results	Total Results	B cell Equivocal Results	Total Results	Equivocal Assessed as Unacceptable Result	
					T cell	B cell
1+2	1	624	1	592	1	0
3+4	2	632	2	608	1	1
5+6	4	632	1	608	3	0
7+8	1	640	1	616	1	1
9+10	6	640	3	608	8	6
Totals	14	3168	8	3032	14	8

Scheme 6

HLA Antibody Detection

Ask a question:
Go to www.menti.com and use code 4384 0711

Scheme 6: HLA Antibody Detection

Purpose

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

Satisfactory Performance

80% reports agree with consensus in distribution year

Consensus

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

12 serum samples over 3 distributions

Scheme 6: Performance

0 Unsatisfactory Performers (0 UK&I)

	2017	2018	2019	2020	2021
Number of Participants (UK&I)	101 (24)	88 (25)	82 (25)	74 (25)	71 (23)
Number with Unsatisfactory Performance (< 80%) (UK&I)	21 (0)	5 (0)	8 (0)	2 (0)	0 (0)
% Unsatisfactory Performance	20.8% (0%)	5.7% (0%)	9.7% (0%)	2.7% (0%)	0% (0%)

29% negative

63% positive

8% samples not assessed (1 Class I, 54% pos)

Scheme 3

HLA Antibody Specificty Analysis

Ask a question:
Go to www.menti.com and use code 4384 0711

Scheme 3: HLA Antibody Specificity Analysis

Purpose

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

Satisfactory Performance

75% reports agree with consensus in distribution year

Consensus

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

10 serum samples over 3 distributions

Scheme 3: Performance

Class I		2017	2018	2019	2020	2021
Number of Participants (UK&I)		72 (24)	73 (25)	70 (25)	64 (24)	65 (24)
Number with Unsatisfactory Performance (UK&I)	Presence	10 (0)	15 (1)	3 (0)	1 (0)	1 (0)
	Absence	3 (0)	5 (0)	2 (0)	1 (0)	1 (0)
% Unsatisfactory Performance	Presence	13.8%	20.5%	4.2%	1.6%	1.5%
	Absence	4.2%	6.8%	2.6%	1.6%	1.5%

Overall 3 labs with UP (0 UK&I)

Class II		2017	2018	2019	2020	2021
Number of Participants (UK&I)		72 (24)	75 (25)	69 (25)	63 (24)	64(24)
Number with Unsatisfactory Performance (UK&I)	Presence	5 (0)	12 (0)	5 (0)	2 (0)	3 (0)
	Absence	2 (0)	3 (0)	2 (0)	1 (0)	1 (0)
% Unsatisfactory Performance	Presence	6.9%	16.0%	7.2%	3.2%	4.7%
	Absence	2.8%	4.0 %	2.8%	1.6%	1.6%

Scheme 3: Unacceptable Performers 2021

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

Lab	Class I		Class II		CAPA	Kit
	Presence	Absence	Presence	Absence		
169	91%	100%	66%	100%	No reply	LABScreen
302	77%	37%	67%	82%	No reply	No info
260	58%	81%	60%	66%	No reply	LABScreen

Scheme 3: Class I Assessment

	Number of HLA Class I Specificities (n=65)										
	301	302	303	304	305	306	307	308	309	310	Total
Present (≥75%)	9	36	25	24	0	10	33	19	8	11	174
Absent (<5%)	3	31	27	32	39	8	31	10	21	12	214
Negative 0%	72	10	28	17	43	67	14	35	36	63	385
Not Assessed (5-74%)	4	12	9	14	7	5	11	25	34	3	124

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

- 14.6% reached consensus presence
- 41.7% reached consensus absence
- 24.2% specificities were not assessed

Scheme 3: Class II Assessment

DPB included in assessment in 2021

	Number of HLA Class II Specificities (DR, DQ, DP) (n=64)										
	301	302	303	304	305	306	307	308	309	310	Total
Present (≥75%)	4	13	7	10	18	5	12	0	15	4	88
Absent (<5%)	6	4	17	14	11	19	4	6	8	6	95
Negative 0%	34	22	18	15	11	20	21	29	14	34	218
Not Assessed (5-74%)	2	7	4	7	6	2	9	11	9	2	59

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=62)										
	301	302	303	304	305	306	307	308	309	310	Total
Present (≥75%)	0	0	0	0	13	0	0	0	12	0	25
Absent (<5%)	2	0	0	7	0	8	0	3	0	1	21
Negative 0%	15	19	15	11	4	11	18	14	7	17	131
Not Assessed (5-74%)	1	0	4	1	2	0	1	2	0	1	12

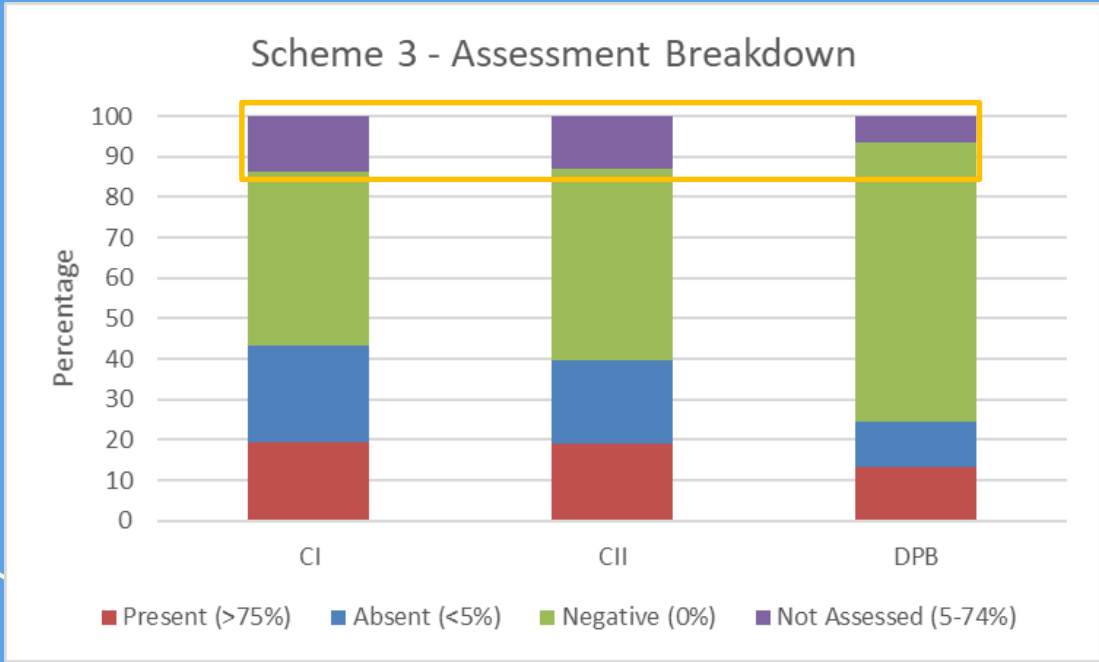
2 samples had DPB1 specificities that reached consensus

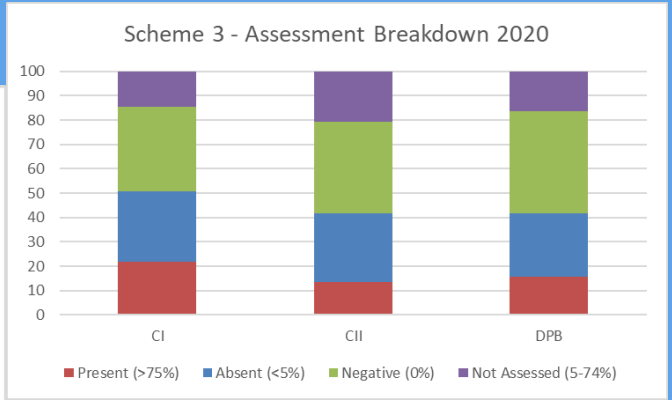
58 specificities reported over 10 samples

- 43.1% reached consensus presence
- 36.2% reached consensus absence
- 20.7% specificities were not assessed

Scheme 3: Assessment Breakdown

Specificities reported over 10 samples





Scheme 3: Reporting of DQA and DPA Antibodies

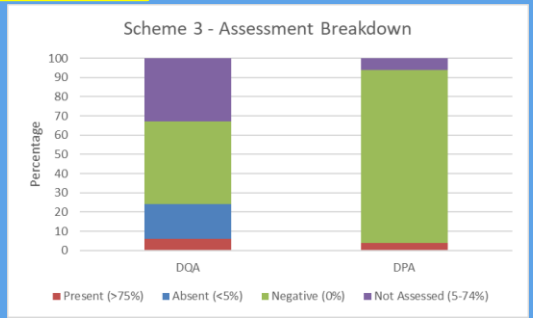
- 24% participants would like to be assessed for DQA antibodies only
  - UK&I - 21%
  - RoW - 27%
- 48% would like to be assessed for DQA and DPA antibodies
  - UK&I - 54%
  - RoW - 41%
- 25% UK&I labs and 32% RoW would not like to be assessed for DQA/DPA antibodies
- 80% labs have a cut off for defining 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%
- bs consider DPA antibodies when assessing potential tability
  - UK&I - 67%
  - RoW - 18%

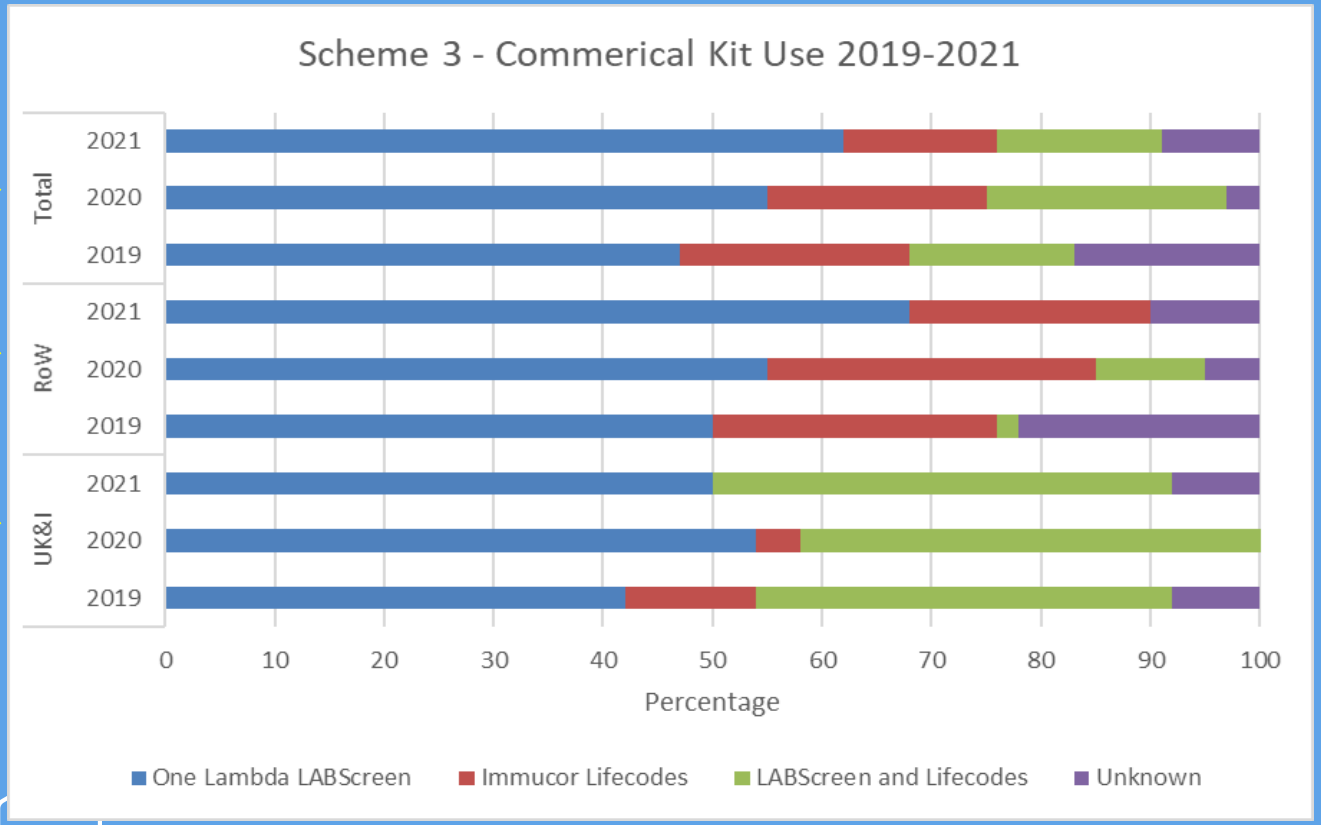
DQA 2021-22 (25 labs)	01:01	01:02	03	05:05	06:01	Total (n=130)	Percentage
Present (>75% Pos)				1	2	8	6%
Absent (>95% Neg)	4	2		2		23	18%
Negative (100% Neg)	4	6			2	4	56	43%
No Consensus	2	2			5	4	43	33%

DPA 2021-22 (19 labs)	01:03	Percentage
Present (>75% Pos)		4%
Absent (>95% Neg)		0%
Negative (100% Neg)	10	90%
No Consensus		6%

Q - Should we formally assess?



Scheme 3: Kit Use 2019-2021



Overall LABScreen kits are the most widely used

UK&I labs are more likely to use a combination of kits

Immucor only kit use more prevalent in RoW labs

Scheme 3 and 6: Analysis

A hidden duplicate sample was used in Scheme 3 and Scheme 6.

Sample 612, 301 and 310 were all sourced from the same donor.

Sample 612: CI POS (70/70), CII POS (66/66)

Scheme 3	Sample	Absent (>95%)	Present (>75%)	Not Assessed	Negative (100%)	Comments
Class I	301	12	11	3	63	Pos - A66 B7 B13 B27 B60 B61 B48 B73 B81 Absent - B52, B77, Cw2 NA - A34, B703, B2708, B47
	310	3	9	4	73	Pos - A66 B7 B13 B27 B2708 B60 B61 B47 B48 B73 B81 Absent - A32 B52 B77 B38 B39 B55 B56 B41 B42 B46 B72 Cw2 NA - A34 B703 B67
Class II	301	6	4	2	34	Pos - DR4, DQ7, DQ8, DQ9 Absent - DR11, DR8, DR9, DR51, DP5, DP10 NA - DR7, DP14
	310	6	4	2	34	Pos - DR4, DQ7, DQ8, DQ9 Absent - DR11, DR8, DR9, DR51, DQ5, DP10 NA - DR7, DP14

B2708 - 301 70.3% NA

310 98.4% Present

B47 - 301 61% NA

310 75% Present

CI	A34	A66	A32	B52	B7	B703	B13	B77	B38	B39	B55	B56	B27	B2708	B60	B61	B41	B42	B46	B47	B48	B67	B72	B73	B81	Cw2
301	NA	Pres	Neg	Abse	Pres	NA	Pres	Abse	Neg	Neg	Neg	Neg	Pres	NA	Pres	Pres	Neg	Neg	Neg	NA	Pres	Neg	Neg	Pres	Pres	Abse
310	NA	Pres	Abse	Abse	Pres	NA	Pres	Abse	Abse	Abse	Abse	Abse	Pres	Present	Pres	Pres	Abse	Abse	Abse	Pres	Pres	NA	Abse	Pres	Pres	Abse

Definition of Unacceptable Antigens (UA)

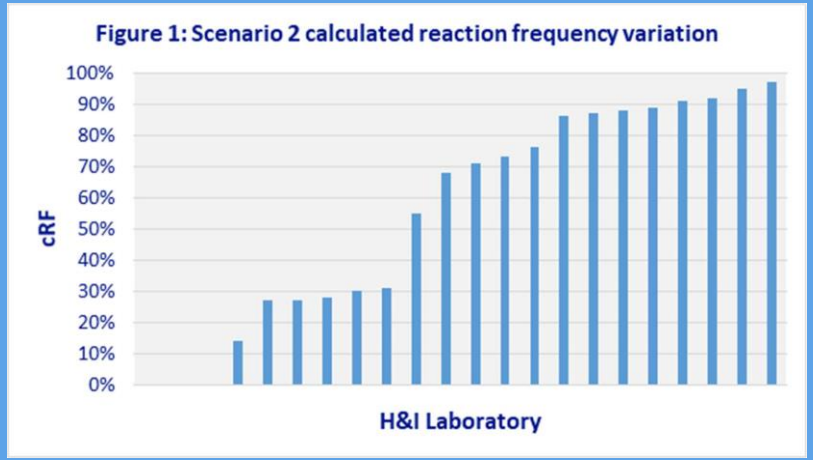
A survey was sent to all UK&I labs in collaboration with the BSHI Research Executive to investigate how labs define UA and inform discussions on how differences in practice could impact equity of access to transplant.

iED1-2019 asked labs to define UA for a sensitised male with a complex antibody profile.

The number of UA listed by each of the UK&I labs varied from 0-21 (median 5).

There was 18 different UA profiles.

cRF for this patient varied from 0-97%.



Definition of Unacceptable Antigens (UA)

The results will be discussed at an upcoming BSHI RE SIG but some of the interesting points were:

- 50% labs use a defined cut off to define the presence of HLA antibodies when using One Lambda kits, 23% for Immucor kits
- 81% use a defined cut off determine UA

  Cut-off defined by aligning to clinical outcome (53%), pos FCXM (47%), other analysis (40%)
- 69% need to detect an antibody twice before listing as UA, 19% only once, 12% other (review in terms of sensitising events, etc.)
- 44% perform additional testing to assist definition of UA (modified SAB, 3rd party FCXM, epitope analysis, etc.)
- 25% will list a mm from a previous graft even if no antibodies defined (44% will list if ab present but below standard pos cut off)
- 56% will adjust positivity threshold if auto-reactivity present
- 50% review UA after every sample tested
- 88% will de-list UA

Scheme 11

HPA Antibody Detection/Specification

Ask a question:
Go to www.menti.com and use code 4384 0711

Scheme 11: HPA Antibody Detection/Specification

Purpose

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

Satisfactory Performance

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

Consensus

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

8 serum/plasma samples over 2 distributions

Scheme 11: Performance

- 6 Unsatisfactory Performers (0 UK&I)

	2017 Pilot	2018	2019	2020	2021
Number of Participants (UK&I)	13 (3)	35 (4)	39 (5)	42 (4)	43 (4)
Number with Unsatisfactory Performance (< 75%) (UK&I)	N/A	1 (0)	1 (0)	3 (0)	6 (0)
% Unsatisfactory Performance	N/A	2.9%	2.6%	7.1%	13.9%

Scheme 11: HPA Antibody Detection/Specification

Sample	Consensus Present	Not Assessed	Expected Results
1	**1a**	GP IIb/IIIa (88% absent)	1a (NIBSC)
2	**3a**	GP IIb/IIIa (91% absent)	3a (NIBSC)
3	**5b**	None	5b (NIBSC)
4	*Not assessed*	15b (86% absent)	15b (NIBSC)
5	**1b 5b**	3b (65% present) 15b (94% absent) GPIIb/IIIa (78% absent) GPIb (87% absent)	1b 5b
6	**Negative**	HPA-5a (94% absent)	HLA pos only
7	**1a**	3a (87% absent) GPIIb/IIIa (94% absent) GPIa/IIa (94% absent)	1a
8	**None**	1b (60% absent) 3b (73% absent) 5a (92% absent) 5b (54% absent) GPIIb/IIIa (65% present) GPIa/IIa (57% present) GPIb (94% absent)	Weak 1b 5b

Scheme 11: Analysis of Samples 1-4

- NIBSC Standards used

ID	Sample	Dilution	Result Positive	Unacceptable Positives
1101/2021	NIBSC Standard HPA-1a (05/106)	1:4	HPA-1a (90.7%)	HPA-3a (n=1 (267)), HPA-3b (n=1 (387)), HPA-4b (n=1(1346))
1102/2021	NIBSC Standard HPA-3a (03/190)	1:8	HPA-3a (76.7%)	HPA-1a (n=1 (180)), HPA-4b (n=1 (1346))
1103/2021	NIBSC Standard HPA-5b (99/666)	1:2	HPA-5b (100%)	GP Ia/IIa (n=2 (130, 394))
1104/2021	NIBSC Standard HPA-15b (18/220)	1:16	HPA-15b (14%)	

- Methods Used

Method(s) 2021	Users	Percentage	Detection Issues
MAIPA	9	21	Depends on monocloncals used
Luminex PAK-Lx	14	33	Cannot detect HPA-6 or HPA-15
ELISA PAKPlus	4	9	Cannot detect HPA-15
MAIPA and Luminex PAKLX	7	16	
MAIPA and ELISA PAKPlus	3	7	
Luminex PAK-Lx and PIFT	2	5	Cannot detect HPA-15
Luminex PAK-Lx and ELISA PAK-Plus	3	7	Cannot detect HPA-15
MAIPA and PIFT	1	2	
TOTALS	*43*	*100*	

In sample 11-04
6 labs detected HPA-15 all used MAIPA (with or without another technique).
37 labs reported HPA-15 absent,

- 4 (38%) of these labs used techniques that had the potential to detect HPA-15,
- 23 (62% would not be able to detect HPA-15).

Scheme 11: Unacceptable Performers 2021

Lab	HPA Presence	HPA Absence	Samples reported	Method	Error
130	67%	83%	8/8	PAK-Lx	Interpretation/kit issues
180	67%	96%	8/8	PAK-Plus	Interpretation/kit issues
388	67%	91%	8/8	PAK-Plus	Procedural issues
389	50%	100%	8/8	MAIPA In-house	Delivery delay/ interpretation issues
390	33%	100%	8/8	MAIPA Commercial	Interpretation/ procedural issues
410	67%	100%	8/8	PAK-Plus	Interpretation issues

6 labs with UP (<75%)

Scheme 11: Factors Affecting Performance

- Limitations of commercial kits
- Scheme Design
    - lack of genotype
- Sample quality
    - volume of sample – increasing to 1.5ml in 2022-23
    - complexity of sera
- Individual testing strategy
    - ability to detect certain antibodies e.g. HPA-15

Key Data from the Schemes

Deborah Pritchard
UK NEQAS for H&I Deputy Director

Scheme 1A

HLA Phenotyping

Ask a question:
Go to www.menti.com and use code 4384 0711

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

- 2 labs with unsatisfactory performance (0 UK&I).

	2017	2018	2019	2020	2021
Number of Participants (UK&I)	38 (6)	38 (6)	38 (5)	34 (4)	33 (2)
Number with Unsatisfactory Performance (< 90%) (UK&I)	1 (0)	6 (1)	8 (1)	3 (1)	2 (0)
% Unsatisfactory Performance	2.6%	15.8%	21.1%	8.8%	6.1%

Scheme 1A: 2021 Incorrect Assignments

9/330 (2.7%) incorrect HLA types in 2021 reported by 7 labs:

4 reports that contained broad not split specificity (e.g. DQ3 v DQ7)
3 reports that contained an incorrect specificity (e.g. DR4 v DR13)
2 reports with molecular based nomenclature (e.g. A01 v A1)

CAPA responses

- Reported using molecular nomenclature not phenotype nomenclature – procedural error

1/2 labs with unsatisfactory performance completed CAPA

Scheme 1A: 2021 Incorrect Assignments Resulting in UPs

Sample	Lab Number	Consensus	Report
1A 01	142	A3, A66; B41, B51	A3, A10; B41, B51
1A 01	181 & 206	A3, A66; B41, B51	A3, A26; B41, B51
1A 01	194	A3, A66; B41, B51; Cw1, Cw17; DR1, DR8; DQ4, DQ5	A03, A66; B41, B51; Cw01, Cw17; DR01, DR08; DQ04, DQ05
1A 02	194	A2, A2; B44, B60; Cw5, -; DR10, DR11; DQ5, DQ7	A02, A02; B44, B60; Cw05, -; DR10, DR11; DQ05, DQ07
1A 05	299	A2, A29; B7, B37; DR10, DR15	A2, A29; B7, B37; DR10, DR16
1A 06	181	A24, A26; B45, B57; DR4, DR7; DQ8, DQ9	A24, A26; B45, B57; DR4, DR7; DQ3, DQ3
1A 08	159	A1, A24; B8, B60	A1, A24; B8, B40
1A 09	315	A68, A68; B18, B27	A28, A28; B18, B27

Scheme 4A1

DNA Typing at 1st Field Resolution

Ask a question:
Go to www.menti.com and use code 4384 0711

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 3 distributions

Scheme 4A1: Performance

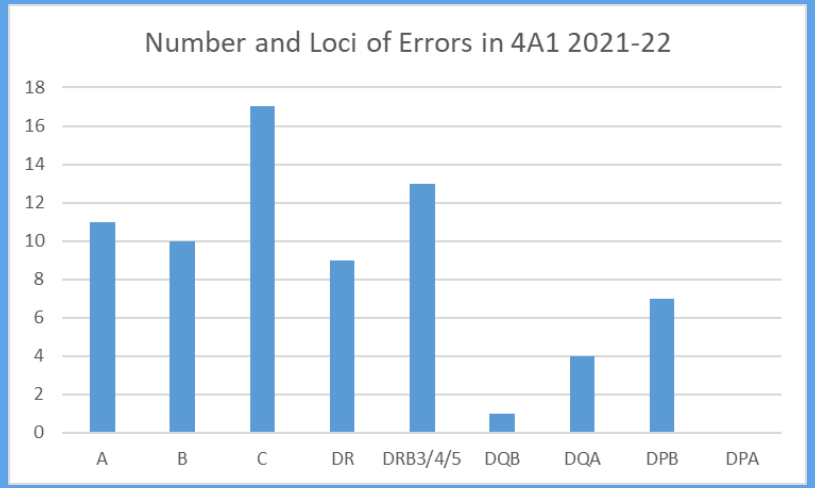
- 6 labs with unsatisfactory performance (1 UK&I)

	2017	2018	2019	2020	2021
Number of Participants (UK&I)	106 (28)	105 (28)	100 (28)	88 (26)	82 (25)
Number with Unsatisfactory Performance (< 90%) (UK&I)	11 (1)	15 (1)	4 (1)	8 (0)	6 (1)
% Unsatisfactory Performance	10.4%	14.3%	4%	9.1%	7.3%

Scheme 4A1: 2021-22 Incorrect Assignments

- 72/10855 (0.66%) incorrect alleles reported by 16 different labs (5 UK&I)
  - 10 incorrect assignments (e.g. A*02 instead of A*68) (1 UK&I)
  - 6 incorrect uses of nomenclature (e.g. C*5 instead of C*05) (2 UK&I)
  - 4 missed the presence of DRB3/4/5
  - 8 missed assignment (e.g. reported homozygous when heterozygous)
  - 5 HLA types completely incorrect (1 lab)
  - 5 Data entry errors (EQA specific)

6 HLA types with multiple errors
25 HLA types with one error



Scheme 4A1: Unacceptable Performers 2021

Lab	Sample	Error	CAPA Response
132	01-05	Sample mix up	Reporting mix up
141	01-10	No results returned	No reply
309	01-05 + 06	Multiple reporting errors	Ambiguities not reported
51	06 + 09	Incorrect DPB1* / DQA1* type	Interpretation/reporting errors
260	06-10	No results submitted for DRB*3/4/5	No reply
374	07 + 09	Incorrect A* type	Interpretation issues

Scheme 4A1: Result Analysis

- Sample 08/2021 – was not assessed – used as educational on this ocassion
  - 32 labs submitted results
  - The first allele had high concordance DPB1*02 (32/32)
  - The second allele varied;
    - 14/32 labs reported DPB1*02 or DPB1*02:011
    - 1/32 labs reported DPB1*02:01/416:01
    - 17/32 labs reported DPB1*416 or DPB1*416:01

DPB1*02 and *416:01 differ in exon 3 - 1 base (codon 178) C-A

	Kit Used					
Allele	**Combination**	**One Lambda**	**Immucor**	**LinkSeq**	**CareDx**	**Unknown**
DPB1*02	4 (29%)	4 (29%)	1 (7%)	1 (7%)	1 (7%)	3 (21%)
DPB1*416	6 (35%)	3 (18%)	2 (12%)	2 (12%)	1 (6%)	3 (18%)
Both	0	1 (100%)	0	0	0	0

Scheme 4A1i

Interpretive HLA Genotype

Ask a question:
Go to www.menti.com and use code 4384 0711

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

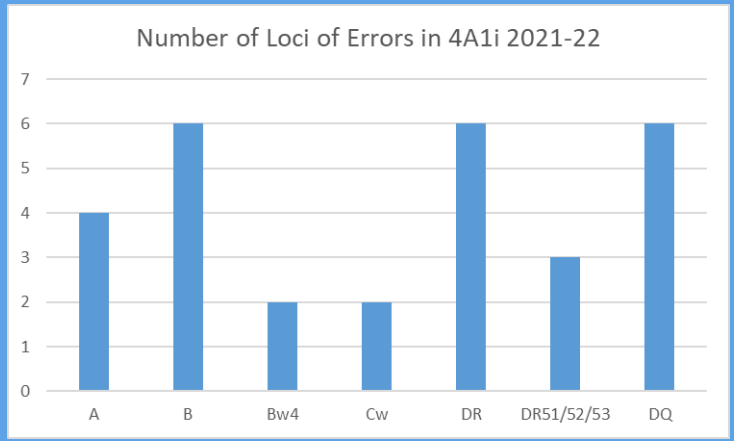
- 6 labs with unsatisfactory performance (2 UK&I)

	2017	2018	2019	2020	2021
Number of Participants (UK&I)	36 (20)	40 (21)	44 (22)	44 (22)	42 (21)
Number with Unsatisfactory Performance (< 90%) (UK&I)	6 (1)	6 (0)	8 (1)	6 (2)	5 (1)
% Unsatisfactory Performance	16.7%	15.0%	18.1%	13.6%	11.9%

Scheme 4A1i: 2021-22 Incorrect Assignments

- 29/5405 (0.54%) incorrect results reported by 8 different labs (2 UK&I)
  - 10 reporting at broad not split specificity level
  - 2 incorrect assignments (e.g. DR1 instead of DR103)
  - 4 incorrect uses of nomenclature (e.g. DQB05 instead of DQ5) (2 UK&I)
  - 3 reporting the presence of DR53 when null allele identified
  - 1 HLA type completely incorrect

5 HLA types with multiple errors
9 HLA types with single errors



Scheme 4A1i: Unacceptable Performers 2021

Lab	Sample	Error	CAPA Response
9	03 + 05	DR53 reporting error / reported broad not split	Post analytical/reporting error
128	01 + 05	In correct DRB1* type / reported broad not split	Interpretation/reporting error
141	01-10	No results returned	No reply
226	01-04	Reported broad not split	Reporting error
1352	01 + 10	Reported broad not split / incorrect type	Transcription error/mix up

Scheme 4A2

DNA Typing to 2nd or 3rd Field Resolution

Ask a question:
Go to www.menti.com and use code 4384 0711

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 3 distributions

Scheme 4A2: Performance

- 44/63 participants registered for 2nd field
- 15/63 participants registered for 3rd field

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

4/6 labs with unsatisfactory performance completed CAPA

	2017	2018	2019	2020	2021
Number of Participants (UK&I)	66 (21)	63 (20)	62 (20)	64 (20)	63 (22)
Number with Unsatisfactory Performance (< 90%) (UK&I)	4 (0)	9 (2)	9 (1)	7 (0)	6 (0)
% Unsatisfactory Performance	6.1%	14.3%	14.5%	11.0%	11.1%

Scheme 4A2: Incorrect Assignments: 2nd Field

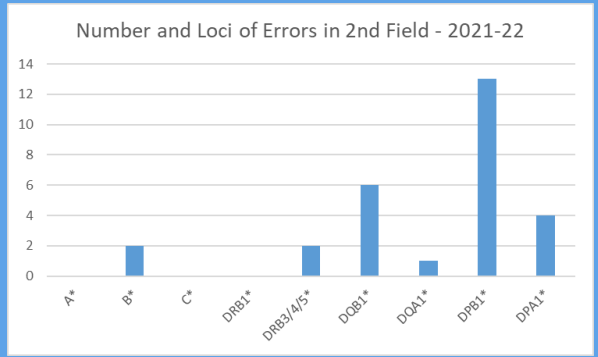
28/840 (3.3%) incorrect HLA alleles reported by 14 labs (5 UK&I)

6 HLA types with multiple errors
14 HA types with single errors

- 15 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 errors at the 2nd field (e.g. B*42:01 rather than B*42:02)
- 3 reports of not defining a null (e.g. DPB1*03:01/276 rather than DPB1*03:01/276N)
- 2 reports of incorrect allele (e.g. DPA*03:01 rather than DPA*01:03)
- 2 reports of homozygous type when heterozygous (e.g. DQA1*01:01, - rather than DQA1*01:01, 01:02)
- 1 report of heterozygous type when homozygous (e.g. B*15:01, 47:66 rather than B*15:01, -)

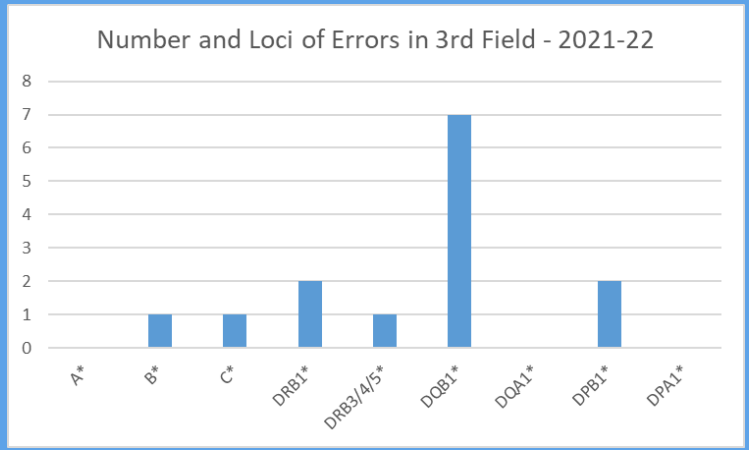


Scheme 4A2: Incorrect Assignments: 3rd Field

14/212 (6.6%) incorrect HLA alleles reported by 4 labs (0 UK&I)

- 9 reports of unresolved ambiguities (e.g. DQB1*06:02:01/06:02:49)
- 2 data entry errors (e.g. C*07:01:0)
- 1 incorrect assignment (e.g. DPB1*15:01:01 rather than DPB1*05:01:01)
- 1 error at 3rd field (e.g. DPB1*02:01:01 rather than DPB1*02:01:02)
- 1 reports of additional allele at homozygous loci

4 HLA types with multiple errors
5 HLA types with single errors



Scheme 4A2: Unacceptable Performers 2021

Lab	Sample	Error	2nd/3rd Field	CAPA Response
127	01 + 03	Unacceptable ambiguities for DQB1* (03:276 not reported as Null)	2nd	Resolution/kit issues
141	01-10	No results returned	3rd	No reply
267	01 + 02 + 04 + 05	Multiple unacceptable ambiguities reported for DPB1*	2nd	Clerical/transcription errors
165	01 + 10	4A2 01 – DRB4*01:103 unacceptable ambiguity; 4A2 10 – reported DPA1*02:02, consensus DPA1*02:06	2nd	No reply
309	09 +10	4A2 09 – reported DPB1*02:01:01, consensus DPB1*02:01:02 4A2 10 – reported DPB1*15:01:01, consensus DPB1*05:01:01	3rd	Transcription/reporting error
380	06-10	Multiple ambiguities reported	3rd	New kit/interpretation issues

Scheme 9

KIR Genotyping

Ask a question:
Go to www.menti.com and use code 4384 0711

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

- 1 lab with unsatisfactory performance (multiple errors)

	2016 (Pilot)	2017	2018	2019	2020	2021
Number of Participants (UK&I)	11 (2)	8 (3)	9 (1)	12 (1)	12 (1)	15 (1)
Number with Unsatisfactory Performance (UK&I)	*N/A*	0 (0)	1 (0)	3 (0)	0 (0)	1 (0)
% Unsatisfactory Performance	*N/A*	0%	11.1%	25%	0%	6.7%

Scheme 10

HPA Genotyping

Ask a question:
Go to www.menti.com and use code 4384 0711

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*
  - 32/38 reported HPA-1, 2 , 3, 4, 5 and 15
  - 31/38 labs reported HPA-4
  - 27/38 labs reported HPA-6
- Also able to report any other HPA polymorphisms detected, *for information*

Scheme 10: HPA Genotyping

- 1 error
- 0 labs with unsatisfactory performance

	2016 Pilot	2017	2018	2019	2020	2021
Number of Participants (UK&I)	12 (4)	15 (5)	37 (6)	38 (6)	40 (0)	38 (6)
Number with Unsatisfactory Performance (< 100%) (UK&I)	N/A	1 (0)	1 (0)	3 (0)	0 (0)	0 (0)
% Unsatisfactory Performance	N/A	6.7%	2.7%	7.9%	0%	0%

Scheme 1B

HLA-B27 Testing

Ask a question:
Go to www.menti.com and use code 4384 0711

Scheme 1B: HLA-B27 Testing

Purpose

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

Satisfactory Performance

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

Consensus

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

10 donor samples sent over 5 distributions

Scheme 1B: Performance

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

	2017	2018	2019	2020	2021
Number of Participants (UK&I)	127 (52)	133 (54)	133 (53)	141 (52)	141 (50)
Number with Unsatisfactory Performance (< 100%) (UK&I)	7 (2)	10 (3)	4 (1)	12 (2)	3 (0)
% Unsatisfactory Performance (UK&I)	5.5%	7.5%	3.0%	8.5%	2.1%

Scheme 1B: 2021 Incorrect Assignments

Sample	Result	Lab Number	Technique	HLA Type	Lab Identified Cause
1B 03	False neg	373	Molecular	B8 B27	Transcription error
1B 03&04	False neg	1376	Molecular	B8 B27 & B18 B27	No reply
1B08	False neg	357	Serological	B8 B27	No reply

- 5/10 samples distributed were HLA-B27 positive
- 3 errors: 3 false neg
- 2/3 errors involved molecular technique
- 1 transcription error

1/3 labs with unsatisfactory performance completed CAPA

Scheme 5A

HFE Typing

Ask a question:
Go to www.menti.com and use code 4384 0711

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

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

	2017	2018	2019	2020	2021
Number of Participants (UK&I)	56 (42)	58 (44)	51 (38)	49 (36)	45 (32)
Number with Unsatisfactory Performance (< 100%) (UK&I)	3 (2)	0 (0)	2 (1)	1 (1)	1 (1)
% Unsatisfactory Performance	5.3%	0%	3.9%	2.0%	2.2%

CAPA responses

- Transcription error during reporting

Scheme 5B

Interpretive HFE genotype and Hereditary Haemochromatosis

Ask a question:
Go to www.menti.com and use code 4384 0711

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

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

	2017	2018	2019	2020	2021
Number of Participants (UK&I)	20	21 (18)	21 (17)	19 (15)	16 (12)
Number with Unsatisfactory Performance (UK&I)	0	1 (1)	3 (1)	1 (0)	0 (0)
% Unsatisfactory Performance	0%	4.8%	14%	5.3%	0%

Scheme 5B: Performance

- 2021 – All 4 scenarios:

  maximum 6 penalty points per scenario, 24 in total.

2	labs got	0 penalty points
0	got	0.5 penalty points
2	labs got	1 penalty point
0	got	1.5 penalty points
2	labs got	2 penalty points
0	got	2.5 penalty points
4	labs got	3.0 penalty points
4	labs got	4 penalty points
2	labs got	5 penalty points

Scheme 7

HLA-B*57:01 Typing for Drug Hypersensitivity

Ask a question:
Go to www.menti.com and use code 4384 0711

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
- 1 labs with unacceptable performance

	2017	2018	2019	2020	2021
Number of Participants (UK&I)	64 (26)	67 (27)	67 (27)	67 (27)	64 (25)
Number with Unacceptable Performance (< 100%) (UK&I)	4 (1)	2 (0)	0 (0)	2 (0)	1 (1)
% Unsatisfactory Performance	6.3%	3.0%	0.0%	3.1%	1.6%

CAPA responses

- Lab 41 - Human error - sample mix up

Scheme 8

HLA Genotyping for Coeliac and other HLA Associated Disease

Ask a question:
Go to www.menti.com and use code 4384 0711

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

Purpose

Assess participants ability to correctly determine HLA type associated with various 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

- 12 Unsatisfactory Performers (2 UK&I)

	2017	2018	2019	2020	2021
Number of Participants (UK&I)	45 (9)	52 (10)	50 (11)	55 (12)	55 (10)
Number with Unsatisfactory Performance (< 100%) (UK&I)	15 (2)	14 (4)	13 (2)	17 (5)	12 (2)
% Unsatisfactory Performance	33% (22%)	27% (40%)	26% (18%)	31% (42%)	22% (20%)

CAPA responses

- Human error – not following checking procedures
- Transcription errors
- Kit interpretation error
- Reporting error

5/12 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	54	6
Narcolepsy	DQB1*06:02	22	2
Actinic Prurigo	DRB1*04:07	4	1
Birdshot Retinopathy	A*29	9	0
Behçet's	B*51	14	0
Rheumatoid Arthritis	DRB1*04	4	1
Diabetes	DR3, DR4	7	2
Psoriasis	C*06	3	0
Allopurinol Hypersensitivity	B*58	1	0

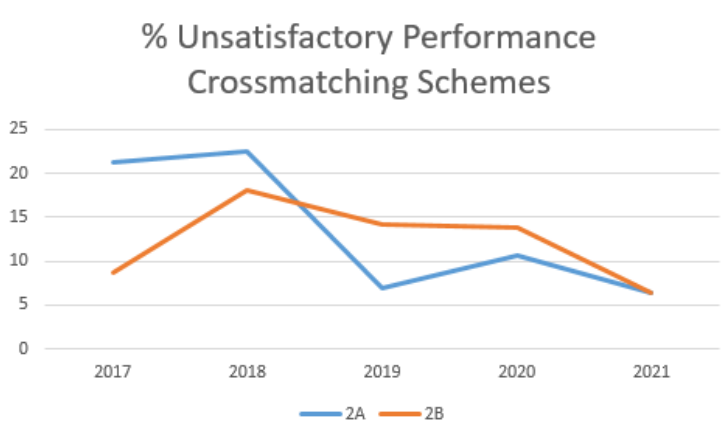
Scheme 8: Unacceptable Performers 2021

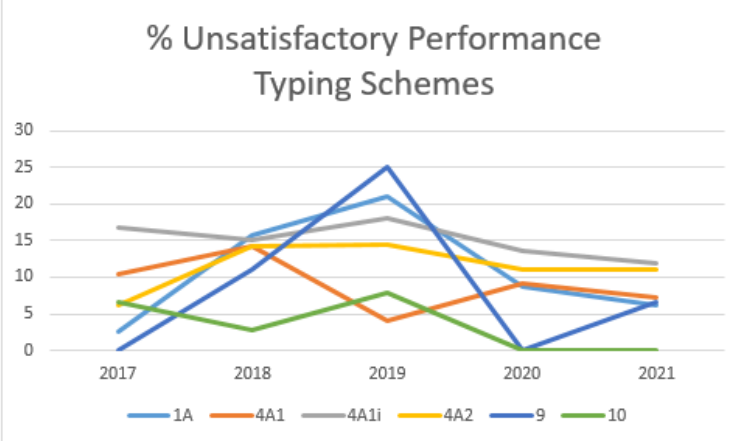
Lab	Sample	Error	CAPA Response
12	01-03	No results submitted for Behcets	Clerical error/registration error
113	01 + 02	Multiple errors	Interpretation/transcription/reporting errors
142	03	Interpretable haplotypes	Interpretation/resolution/technical issues
281	03 + 04 + 09	Incorrect Positive association with Coeliac Disease	Clerical/reporting/transcription error
54	07	Incorrect DQA*03 reported	Error reporting allele strings
278	04 + 06 + 07	Multiple reporting errors	No reply
317	04	Interpretation issues with DQ*02	No reply
354	06 + 07	Interpretation issues	No reply
226	08 + 10	Incorrect association with Narcolepsy	No reply
355	08-10	No results returned	No reply

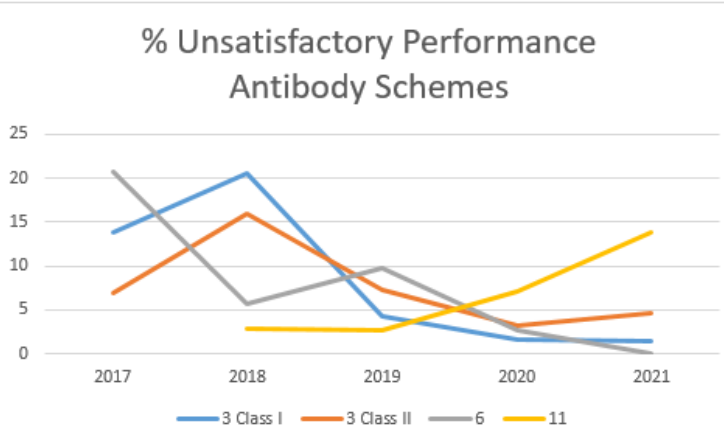
Scheme Summary

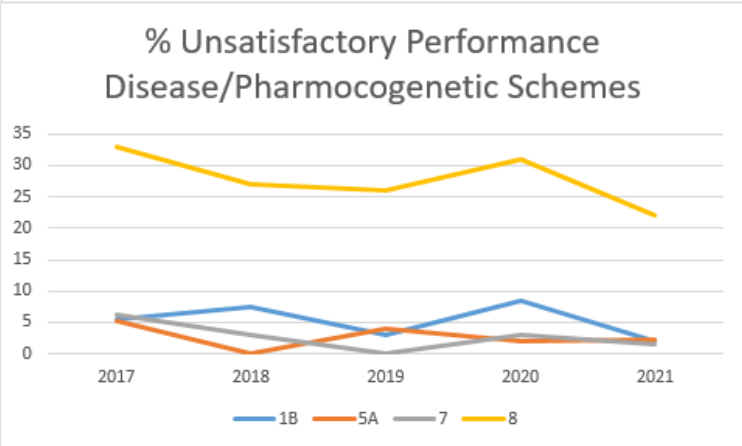
Performance Summary for all Schemes

5 Year Trends in Unsatisfactory Performance









Feedback Summary 2021

Participant Survey Results

Ask a question:
Go to www.menti.com and use code 4384 0711

Participant Survey

- Biannual survey of all participants
- Aim to inform service development
- Online questionnaire sent in October 2021
- Cover Service Provision, Communication and Engagement, Portal, Manual, Website, Finance, Unacceptable Performance, Steering Committee, Scheme Design, Sample Quality
- 91 responses were received (27% response rate)
- Not all respondents answered all questions

Participant Survey Summary

Service Provision - **87%** rated our service as good or excellent.

"Very good organization, good analysis of the results, excellent personalized support if there is a technical problem or request for additional aliquots"

Communication - **90%** rated communication with us as good or excellent. **77%** of participants have corresponded with us

"Correspondence has always been of very high standards. We have no issues in communicating and the response time is usually within 24hrs."

Participants' Portal - **80%** rated the Portal good or excellent and **79%** rated it easy to use. **75%** rated functionality of the Portal good or excellent.

"It is easy to log in and user friendly."

Participant Manual – **71%** have accessed the Participant Manual, **95.6%** of those that had accessed it rated it as good or excellent.

"Very comprehensive and informative. Professional and slick appearance, inspiring confidence in the quality of the service."

Website – **67%** had accessed the new website, **80%** rated it good or excellent.

"Looks good at the outset but yet to really make use of the site"

Finance – **70%** stated the service was good or excellent in terms of value of money. **75%** rated the invoicing process as good or excellent.

"Good service."

Unacceptable Performance Process – **35%** had experienced the UP process in the last 2 years with **73%** of those that had rating it as good or excellent.

"Rapid responses from NEQAS in both informing us of unacceptable performance and responding to our corrective actions."

Feedback Survey Summary

Steering Committee – **43%** aware of the role of Steering Committee. Only **4%** of Participants had interacted with Steering Committee but **100%** of those that had rated the response as good or excellent.

"They are doing a great and valuable job."

Sample Quality – **84%** rated blood samples as good or excellent, **75%** Isolated Cell**s, 97%** Sera and **94%** DNA.

"The sample quality has improved over recent years and is as we require."

Scheme Design – **89%** rated the design of schemes are good or excellent. **71%** rated the sample frequency as just right (**27%** would like more samples less frequently). **91%** rated sample selection as good or excellent. **82%** rated reporting times as good or excellent. **78%** rated result entry as good or excellent. **85%** rated reports as good or excellent. **83%** rated end of year reports as good or excellent. **53%** would like a virtual handling slip.

"Good distribution frequency."

"Given the resources I think we do receive a good spread of antibody and genotype specificities for the schemes we are enrolled in. It would be nice to have more rare antibodies but we know how difficult it is to source anti-sera and to have enough to distribute globally can be challenging. Your efforts are appreciated."

Educational Schemes – **92%** stated they considered the educational schemes worthwhile.

"Very helpful for use with trainees and also excellent to generate wider discussion amongst staff."

Participant Engagement – **88%** stated the Annual Participants Meeting was worthwhile. **26%** had attended NEQAS Webinars.

"Good to see overview of other laboratories as well as own. Opportunity to discuss other viewpoints and also (when face to face) enables open discussions with other H&I services to discuss test strategies and alternative methods/testing approaches."

Expansion of services – **14%** would be interested in an individual competency assessment service.

Feedback Summary

Summary (% rating good or excellent)

What is Going Well	What Requires Improvement
• Overall service rated 87% • Communication rated 90% • Portal rated 80% • Quality of Participant Manual 96% • Responses from SC 100% • Sample quality (blood 84%, sera 97%, DNA 94%) • Selection of samples rated 91% • Scheme design rated 89% • Reports rated 82% • Educational schemes rated 92% • Participants Meeting rated 88% • 26% Participants attended a webinar • 67% accessed website in first month	• Value for money 70% • Awareness of Steering Committee 43% • Quality of isolated cells 75% • Scheme data entry 78% (especially Scheme 3 and 8) • Sample frequency rated just right by 71% • CAPA process 73% • 71% accessed Participant Manual

Action Plan

Area for Improvement	Issue	Action Taken
Participant Manual	Accessibility	Content now available on the website
Portal	Notifications	Reduced frequency of system generated notifications – shipping notice and one 24 hours prior to result submission deadline
	Scheme 3 Result Entry	Fields reduced and simplified
Frequency of Samples	Inconsistency	Schemes 3, 6, 4A1 and 4A2 shipped 3 times per year
Quality of Isolated Cells	Long term viability	Cell quality study
Value for Money	Cost of schemes	Not for profit organisation
Steering Committee	Awareness	Website, webinars, annual meeting
Sample Delivery	Delays	Changed customs paperwork, working with NEQAS Logistics Group

UK NEQAS for H&I
Educational Crossmatch Scenario (EDXM)

Dr Tracey Rees
UK NEQAS for H&I Director

Ask a question:
Go to www.menti.com and use code 4384 0711

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

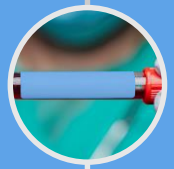
Educational Schemes

- 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

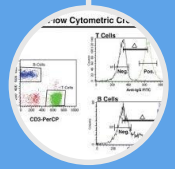


‘Patient’ Samples

3 x Serum Samples

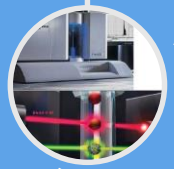
HLA Typing

(Schemes 4A1 & 4A2)



Crossmatching

(Schemes 2A & 2B)



Antibody Detection / Specification

(Schemes 3 & 6)

Clinical Interpretation
Transplant Risk Stratification

2021 Submissions

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

- 100% agreement on HLA type:

Consensus HLA Type	A*	B*	C*	DRB1*	DRB3*	DRB4*	DQA1*	DQB1*	DPA1*	DPB1*
	01	27	01	04	02	01	01	03 (7)	01	04:01
	31	44	03(9)	14	-	-	03	05	02	14:01
Number of reports	34	34	34	34	24	25	32	32	24	29
% Labs in consensus	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%

01

Serum 1

Results

Serum 1 Results

	Result	% Consensus	Comments
HLA Class I Antibodies	Positive	100% (31/31)	
HLA Class II Antibodies	Positive	100% (31/31)	
DSA	Yes	100% (33/33)	
CDC XM	PBL Positive T cell Positive B cell Positive	87.5% (7/8) 83% (10/12) 100% (12/12)	
FCXM T Cell	Positive	100% (29/29)	
FCXM B Cell	Positive	100% (27/27)	
Transplant Risk	Contraindication/ High	100% (33/33)	
Recommendations	N/A	N/A	Not suitable for transplantation, seek alternative donor. Review listing of unacceptable antigens.

02 Serum 2

Results

Serum 2 Results

	Result	% Consensus	Comments
HLA Class I Antibodies	No consensus	71% (22/31)	Only one specificity reached consensus present (A25 in 2-5,000 MFI range)
HLA Class II Antibodies	Negative	94% (27/31)	
DSA	No consensus	12% (4/33)	12% labs reported the presence of Cw1 at an MFI range of 1-2,000
CDC XM	PBL Negative T cell Negative B cell Negative	100% (8/8) 100% (12/12) 100% (12/12)	
FCXM T Cell	Negative	100% (29/29)	
FCXM B Cell	Negative	79% (27/27)	
Transplant Risk	Standard Medium	94% (31/33) 6% (2/33)	Interestingly 2 labs defined this as a medium risk transplant. One was likely an error as they stated Standard risk in the associated comments. The other lab defined potential DSA (<2,000).
Recommendations	N/A	N/A	Process to transplant. Standard Immunosuppression. Regular post-transplant monitoring.

03 Serum 3

Results

Serum 3 Results

	Result	% Consensus	Comments
HLA Class I Antibodies	Positive	94% (29/31)	B57 (5-10,000 MFI) and B58 (2-5,000) were defined as present
HLA Class II Antibodies	Negative	74% (29/31)	74% reported negative
DSA	No	3% (1/33)	One lab reported DSA against Cw1 (1048 MFI) and DQ5 (14,724)
CDC XM	PBL No consensus T cell Negative B cell No consensus	80% (4/8) 92% (11/12) 50% (6/12)	Unexplained positive crossmatch.
FCXM T Cell	Positive	93% (27/29)	
FCXM B Cell	Positive	81% (22/27)	
Transplant Risk	Standard Medium High/ Contraindication	56% (18/32) 16% (5/32) 28% (9/32)	Split between proceeding to transplant with increase immunosuppression in discussion with clinical team and delaying transplant until unexplained positive crossmatch investigated.
Recommendations	N/A	N/A	Repeat testing, perform autologous crossmatch, densensitisation, investigate other transplant options.

Serum 3 Further Discussion

- This serum contained Human Neutrophil Antigen (HNA) 3a antibodies.
- Supplied by a female patient with 3 pregnancies and multiple blood transfusions.
- This patient had a strong positive flow cytometry crossmatch with a deceased donor kidney offer. Autologous flow cytometry crossmatch was negative.
- No donor specific antibodies had been identified by Luminex Single Antigen Bead testing.
- The transplant did not proceed.
- Two further third party crossmatches were also strong T & B cell positive in the absence of donor specific antibodies.

Samples were sent to the specialist reference laboratory in the UK for granulocyte immunology testing.
The reference lab confirmed the patient's HNA type as HNA-3b/3b and the presence of HNA-3a antibodies.

Serum 3 Further Discussion

HNA antibodies are likely to be rare in transplant waiting list patients (Key *et al*, 2020 estimated to be approx. 1%).

Laboratories should be aware of the potential for these non-HLA antibodies to cause strong T and B cell flow cytometry crossmatches.

UK NEQAS for H&I distributed this serum to highlight this, especially for laboratories that may not have previously seen sera containing HNA-3a antibodies.

It was interesting to note that the majority of laboratories (but not all) reported a positive flow cytometry crossmatch in the absence of donor specific antibodies. However, there was wide variation in the clinical risk associated with these results.

Key T, Carter V, Day S, Goodwin J, Goodwin P, Knight A, Mather F, Poles A, Shaw O, Rigg K, McKane W Human Neutrophil Antibodies are Associated with Early and Chronic Antibody Mediated Rejection in Kidney Transplant Recipients.2019, J Renal Transplant Sci, 2(2), 81.
https://www.scitcentral.com/documents/16f32d2225ef8c1ca869bf29397171b4.pdf

Summary of Crossmatch and DSA Detection Results

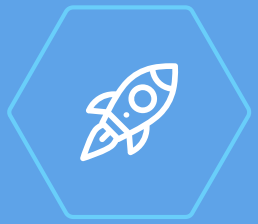
2021 Results		Serum 1		Serum 2		Serum 3	
DSA Defined by Luminex		Class I	Class II	Class I	Class II	Class I	Class II
MFI >10,000		B27 (100%) B44 (94%)	DR4 (88%) DR14 (76%) DR52 (3%) DR53 (70%)	N/A	N/A	N/A	N/A
MFI 5,001-9,999		N/A	DQ5 (52%)	N/A	N/A	N/A	N/A
MFI 2,501-5,000		Cw1 (55%)	N/A	N/A	N/A	N/A	N/A
MFI <2,500		N/A	DQA1*01 (3%)	Cw1 (12%) Cw9 (3%)	DQ7 (6%)	Cw1 (3%)	DQ5 (3%)
CDCXM B CELL	No DTT	Positive		Negative		No consensus	
	DTT	Positive		Negative		No consensus	
FCXM	T Cell	Positive		Negative		Positive	
	B Cell	Positive		Negative		Positive	
Risk		Contraindication/High (100%)		Standard (94%) Medium (6%)		Standard (56%) Medium (16%) High (28%)	

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

Benefits

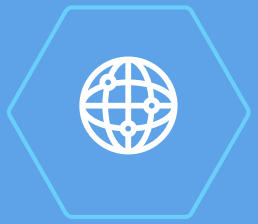
Benchmarking

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

Laboratory 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

Any ideas?

Thanks!

Do you have any questions?

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