

**North East**

Regional Transfusion Committee

**TRANSFUSION BITES**  
**RCI Case Study**

David Bruce 11/03/2021

# New referral from JCUH received 02/10/2020

- 39 year old male
- Diagnosis: pre-op (TKR)
- Medical history of anxiety and depression
- Hb 149
- ? Group
- **No previous transfusion / BMT**

# JCUH Grifols automated ABO group results:

- Long group: Dual Cell Population (mixed field reactions) against anti-A, group not interpretable
- Short group: O RhD pos

Next slides show images of the automated Grifols ABO groups (supplied by Sue Barnes, JCUH).

USER  
jcheve

LOGGED DATE / TIME  
MON 05 OCT 2020 / 13:47

PROFILE INFORMATION

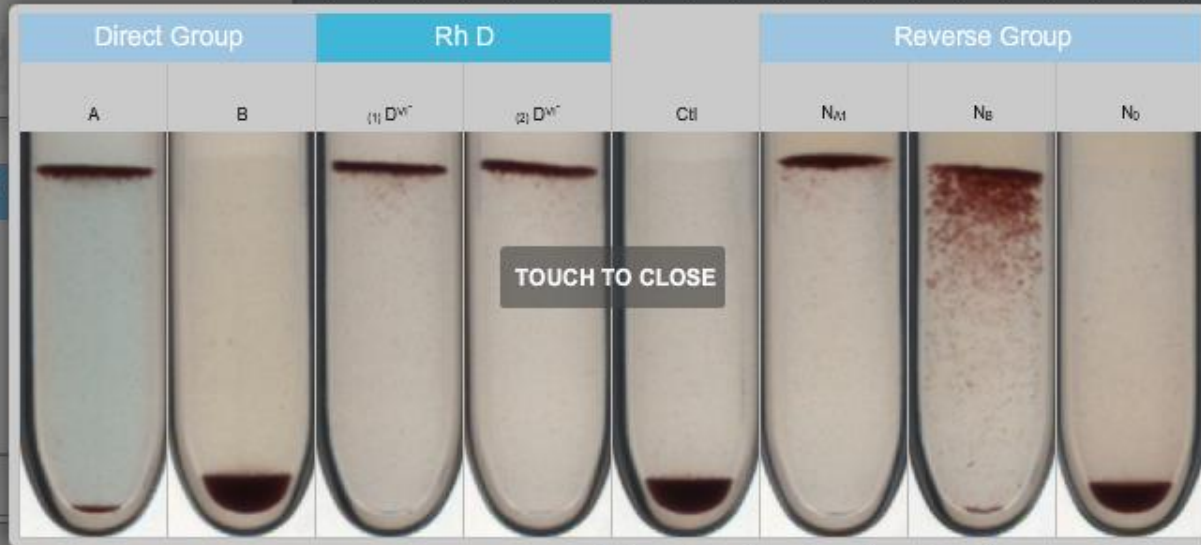
017398

(1) Full Group

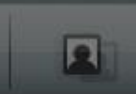
ABO-Rh (CR)(A1-B-O)

RESULTS

Group Rh:	NI
Group:	NI
Direct Group:	NI
Reverse Group:	O
Rh D:	Pos

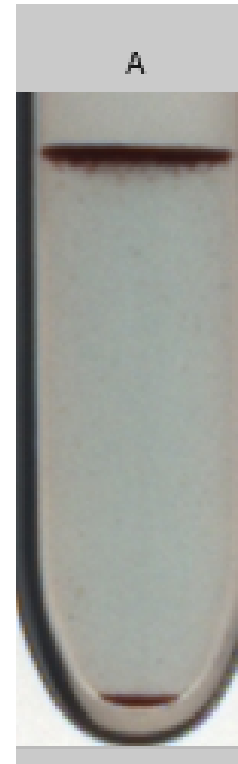


NI	Pos	O	RESULT
DP -	4+ 4+	- 4+ 3+	CURRENT
DP -	4+ 4+	- 4+ 3+	ORIGINAL



# Possible reasons for a mixed field phenotype:

- Recent transfusion
- Recent transplant
- Antigen shedding
- Weak sub-group
- Chimerism
- DAT positive (cold auto)



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PROFILE INFORMATION

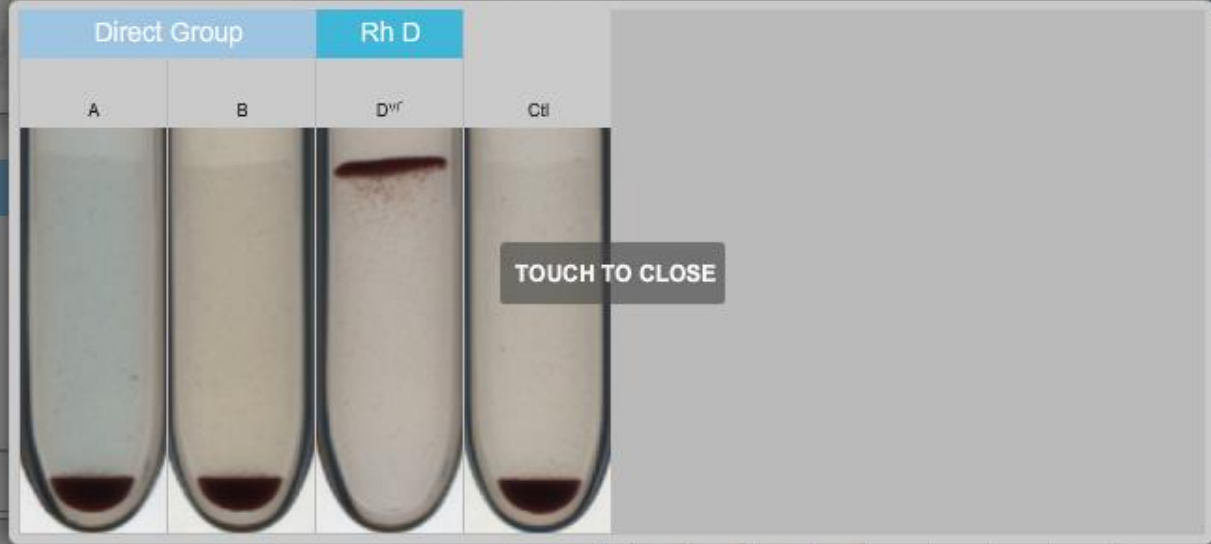
017508

(3) Confirm Group

Confirm P

RESULTS

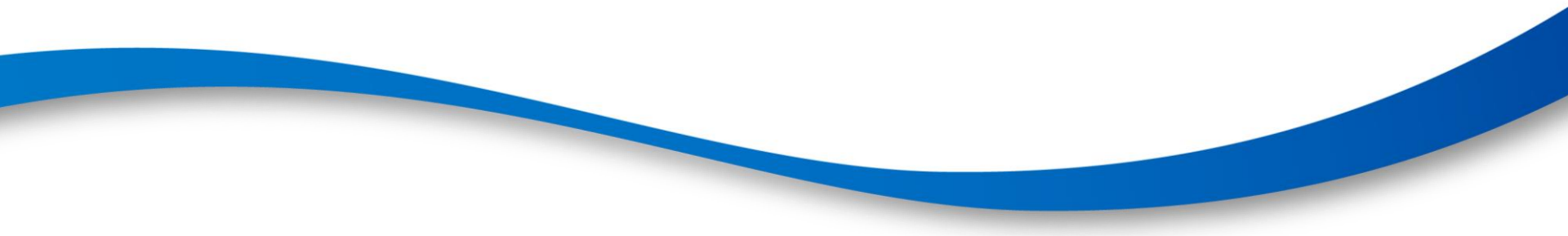
Group Rh:	O+
Group:	O
Direct Group:	O
Rh D:	Pos



<input type="radio"/>	<input checked="" type="radio"/>	RESULT
-	-	CURRENT
-	4+	ORIGINAL



# RCI results:

- Automated ABO group (Bio-Rad IH-1000): O RhD pos
  - Manual ABO group (tube): O RhD pos
  - NAAD by IAT or enzyme IAT (Bio-Rad IH-1000)
  - Auto: Weakly pos
  - DAT: IgG 1+ (very weak).
- 

Patient / sample data

Sample: d099482000305763  
 IH-1000 1100109:  
 NBS Full Group (5074)

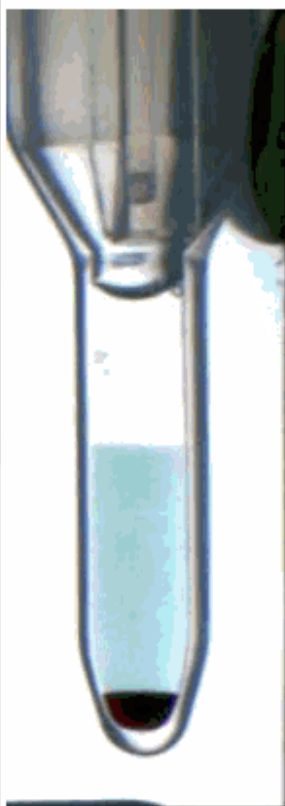
Test time: 02/10/2020 13:31  
 Processed by: Chris  
 Verified by: Chris



Anti-A

50742.75.21/21.04/340126

Reaction comment:



✓ Current results
✓ Antibody panels

1	2	3	4	5	6	1	2	3	4	5	6
Anti-A	Anti-B	Anti-D VI	Anti-D VI	A1	B	Anti-C	Anti-c	Anti-E	Anti-e	Anti-K	Ctl
-	-	++++	++++	++++	++	++++	++++	-	++++	-	-

**Result**

**0 Rh D positive CcD.ee Kell negative AC positive**

ABO:	<input type="text" value="0"/>	ABS:	<input type="text"/>
Phenotype:	<input type="text" value="Cc"/> <input type="text" value="ee"/>	DAT:	<input type="text"/>
Rhesus-D:	<input type="text" value="Rh D positive"/>	Auto ctrl.:	<input type="text" value="AC positive"/>
Kell:	<input type="text" value="Kell negative"/>	Antibodies / other antigens:	<input type="text"/>



## ABO grouping using Tube Agglutination

Batch Numbers	80016193	7491801D	7411951A					2012 2721	2022 3321	2013 7321	2014 3721
Expiry	06/07/21	06/07/21	02/07/21					29/06/21	29/06/21	29/10/21	29/10/21
	Anti-A	Anti-B	Anti-A,B	Anti-D	Anti-D	Auto	Inert control	A1 cells	A2 cells	B cells	O cells
7657 30573	0	9	0			0	0	5	5	5	0
Controls											
A1 rr	5	0	5								
A2 rr	5	0	5								
B rr	0	5	5								
O R1r	0	0	0								
	Batch Numbers.			Expiry Dates							

**IMPORTANT:** The patient's red cells were also tested against anti-A1 and were found to be strongly reactive (DA 4+)



Patient / sample data

Sample: d099482000305763  
 IH-1000 1100109:  
 GB19CA IAT 10 Cell Panel +AC

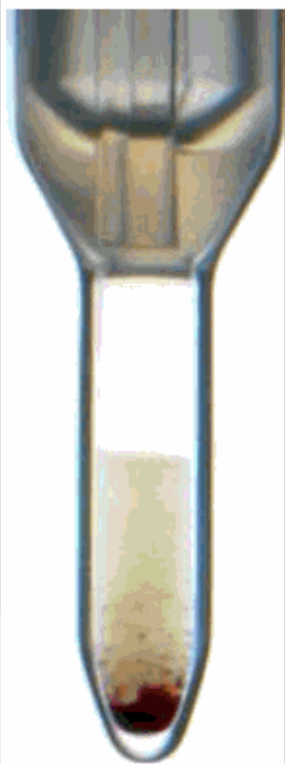
Test time: 02/10/2020 13:31  
 Processed by: Chris  
 Verified by: Chris



AC

50531.56.18/21.10/305454

Reaction comment:



Current results  Antibody panels

<input checked="" type="checkbox"/> 1	<input checked="" type="checkbox"/> 2	<input checked="" type="checkbox"/> 3	<input checked="" type="checkbox"/> 4	<input checked="" type="checkbox"/> 5	<input checked="" type="checkbox"/> 6	<input checked="" type="checkbox"/> 1	<input checked="" type="checkbox"/> 2	<input checked="" type="checkbox"/> 3	<input checked="" type="checkbox"/> 4	<input checked="" type="checkbox"/> 5
1	2	3	4	5	6	7	8	9	10	AC
-	-	-	-	-	-	-	-	-	-	+
<input checked="" type="checkbox"/> 1	<input checked="" type="checkbox"/> 2	<input checked="" type="checkbox"/> 3	<input checked="" type="checkbox"/> 4	<input checked="" type="checkbox"/> 5	<input checked="" type="checkbox"/> 6					

Result

**0 Rh D positive CcD.ee Kell negative AC positive**

Recalculate Interpretation

ABO:  ABS:

Phenotype:   DAT:

Rhesus-D:  Auto ctrl.:

Kell:

Antibodies / other antigens:

Details ...

Accept all

Save

Cancel

# What to do next?

## Case referred to DB for consideration (Friday am 5 pm)

- DB had just days earlier completed Karen DeSay's new digital modules available on Shine Academy which included Polyagglutination
- With this fresh in his head he remembered Anti-A1 (alias *Dolichols biflorus*) is one of the lectins in the routine polyagglutination lectin panel

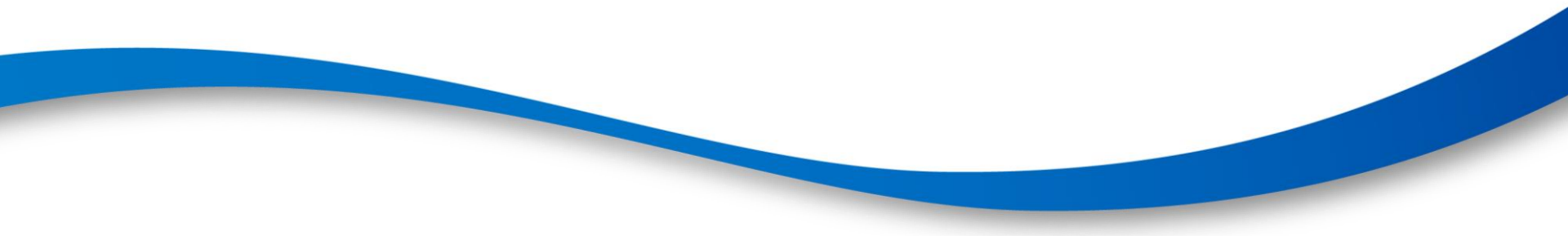


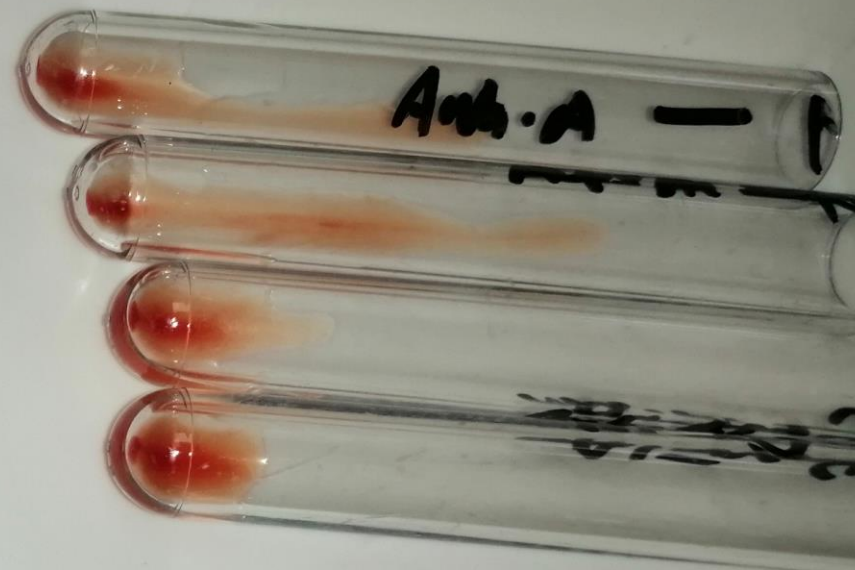
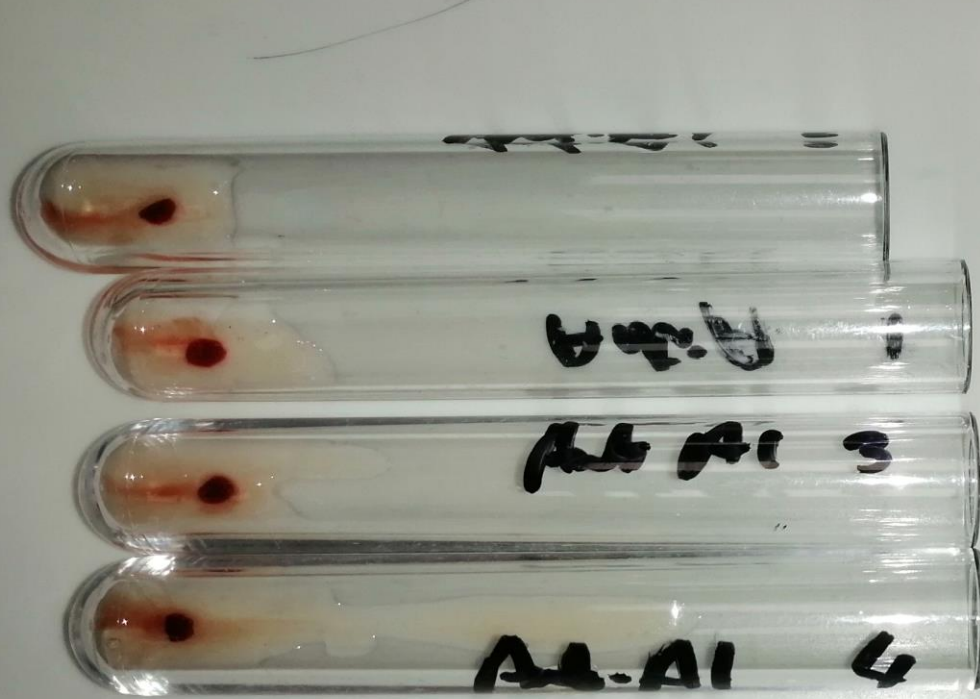
# What is polyagglutination?

A state in which red cells are agglutinated by all or most normal sera from adults.

- Phenomenon involves a change in the red cells by which a latent or hidden cryptic receptor is exposed or where red cells are released from the marrow with such a receptor accessible

**Firstly the manual tests with anti-A and anti-A1 were repeated with different batches of reagent**

A thick, blue, wavy line that curves across the bottom of the slide, starting from the left edge and ending at the right edge.



Tests using 4 different anti-A1 (*Dolichos biflorus*) reagents (on the left) and 4 different monoclonal anti-A typing reagents (on the right).

# These results do not suggest the patient has a weak sub-group of A

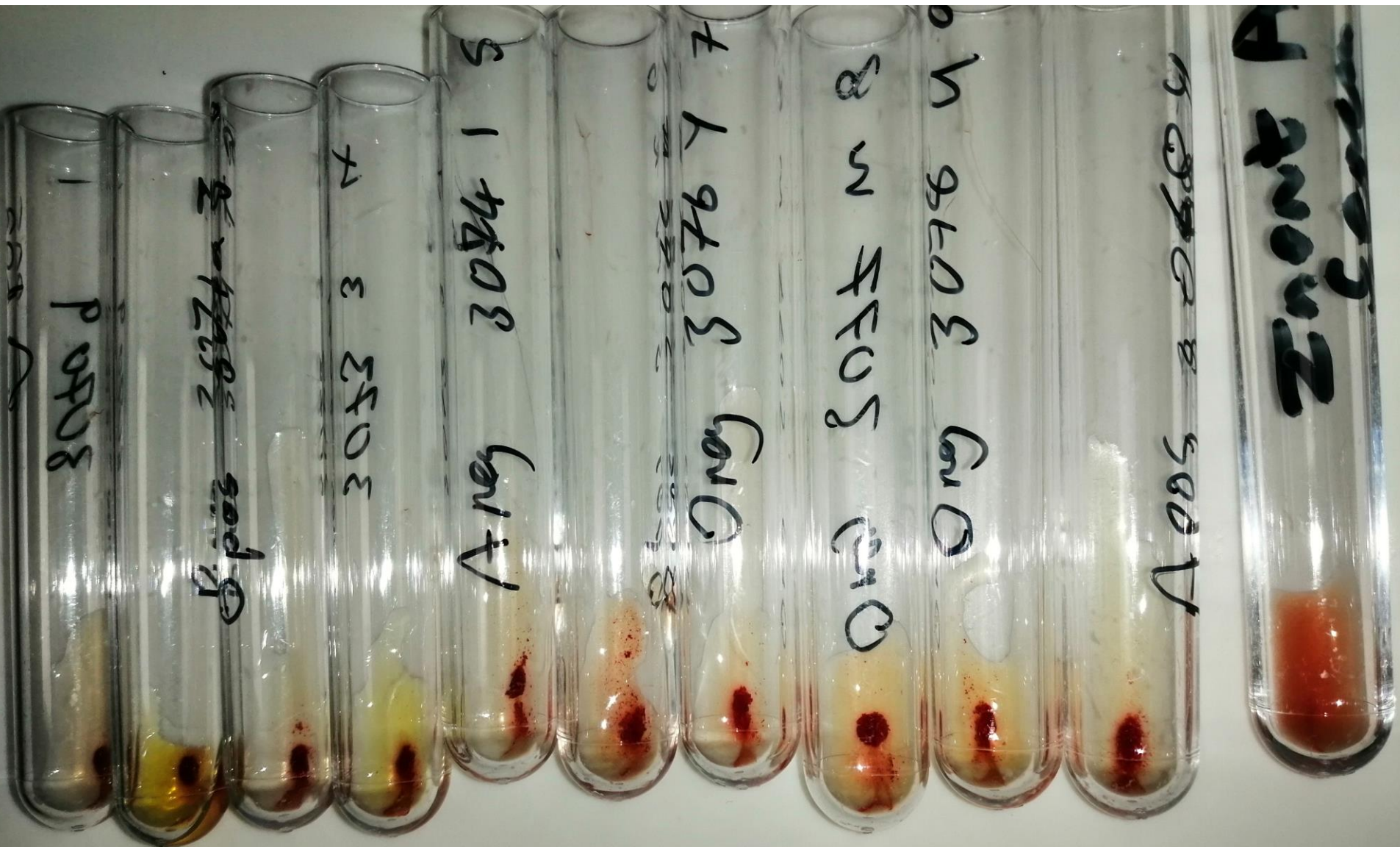
Weak sub-groups react more strongly with anti-A and are normally negative when tested with anti-A1 (*Dolichos biflorus*).

# Newcastle don't have a lectin panel so what next ?

Red cells are said to be polyagglutinable when they are agglutinated by almost all normal human plasma although not by the patient's own plasma






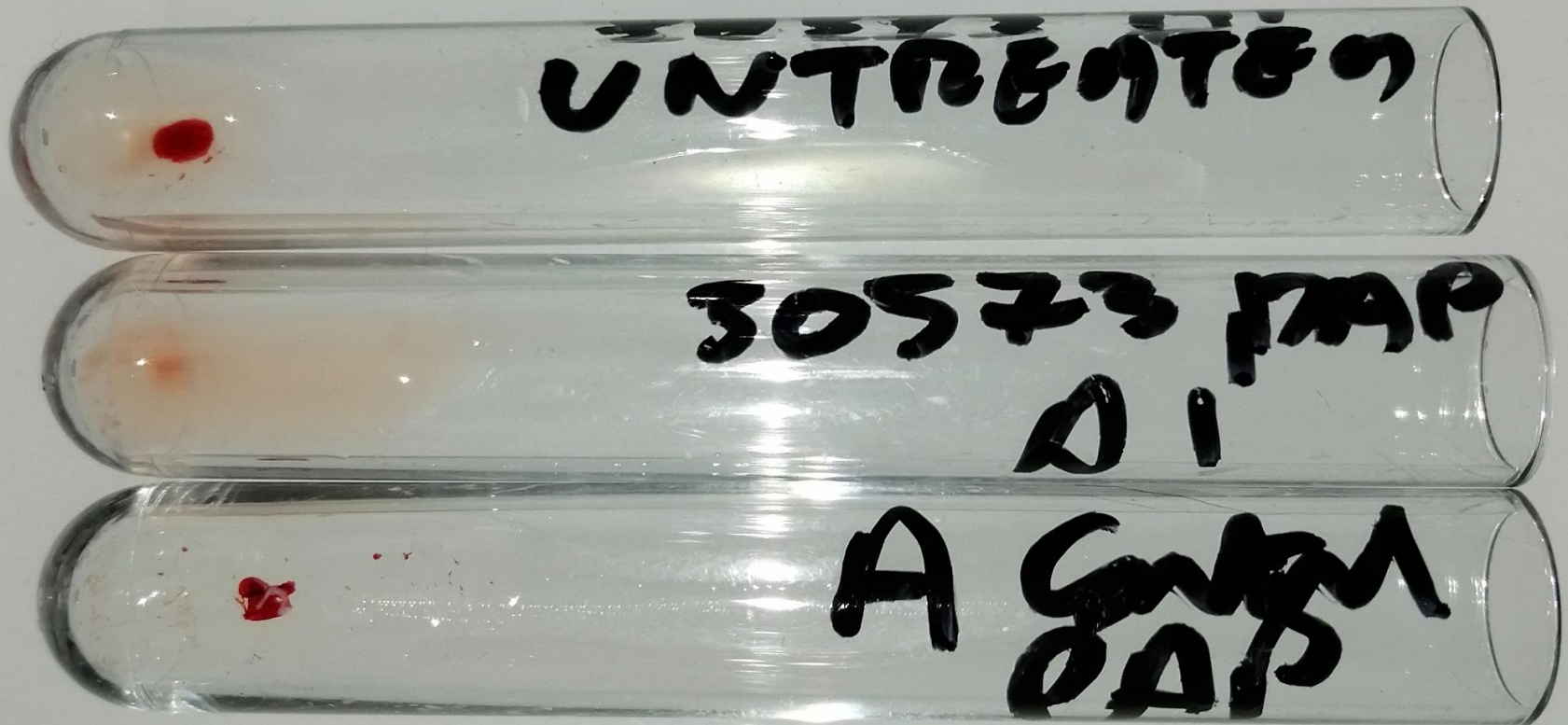


Patient red cells against different examples of normal human plasma and inert AB serum. All normal human plasma (including group A) was agglutinated to various degrees and mixed field reactions observed. Note the inert AB serum was unreactive and the patient's auto (see ABO manual group) is known to be neg.

# The results so far suggest polyagglutination and most probably Tn polyagglutination

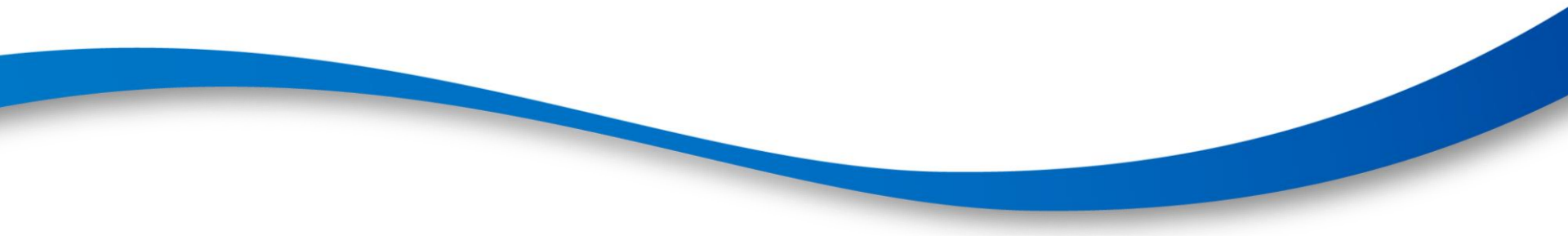
- In Tn agglutination subjects have two populations of red cells, one normal and one showing the Tn change.
  - Explains the mixed field reactions observed and (probably) the Grifols long group results which show a clear dual cell population against anti-A.
  - Other types of polyagglutination (with the exception of Cad) do not react with *Dolichos biflorus*.
- 

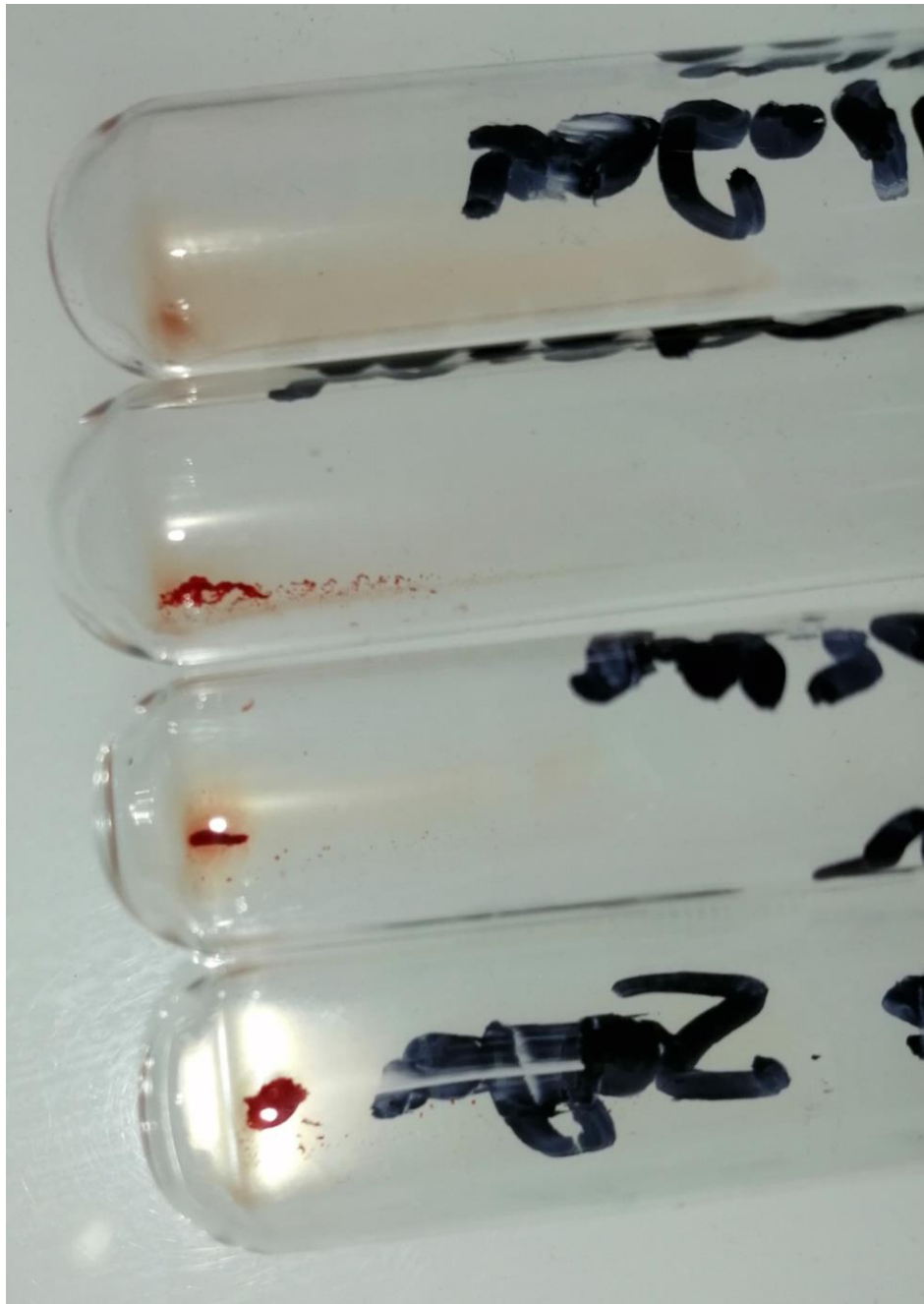
# Additional test:



Patient's red cells were papainised and retested with *Dolichos biflorus*. The cells failed to react (see middle tube). Papain treatment does not alter the A antigen (it enhances it) but does weaken the Tn antigen.

## Next steps:

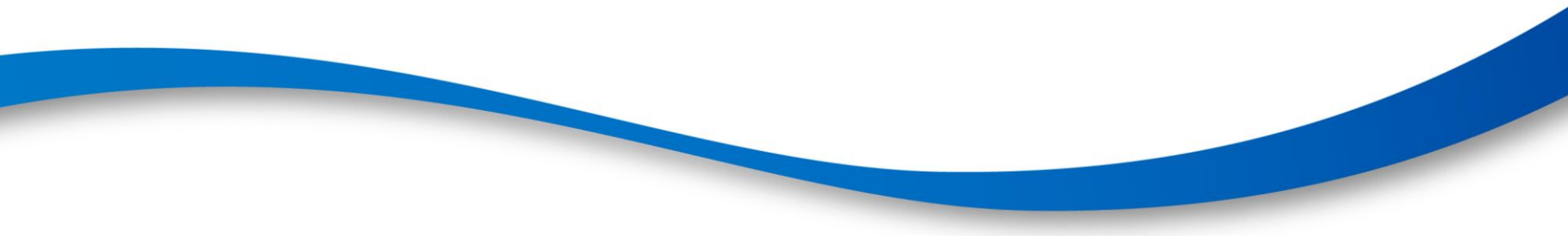
- Repeat sample requested from JCUH for referral to Sheffield RCI for confirmation using their ployagglutination lectin panel
  - In the interim Sheffield sent some aliquots of lectins to DB to test against the initial sample:
- 



Tests against *Arachis hypogaea* were negative (top tube).

Tests against *Salvia sclarea*, *Dolichos Biflorus*, and *Glycine soya* were all positive which would again suggest Tn activation.

**NOTE: most of these tests were not done under controlled conditions and additional testing by Sheffield RCI was required to confirm the results**

A thick, blue, wavy line that curves across the bottom of the slide, starting from the left edge and ending at the right edge.

Patient red cells against plasma from 10 different patients.

patient treated patient cells against into A1  
 untreated treated A1 pap A pap  
 PA 4 0 4 4

Plasma	DA
O <sub>1</sub> 3076 Y	2+ ? weak MF
B <sub>1</sub> 3071 Z	4+ slight MF
O <sub>2</sub> 3072 S	2+ MF
A <sub>1</sub> 3073 B	5+ MF
B <sub>2</sub> 3074 I	4+ v.v. weak MF
O <sub>3</sub> 3075 *	4+ MF
O <sub>4</sub> 3076 Y	4+ MF
O <sub>5</sub> 3077 W*	4+ MF
O <sub>6</sub> 3078 U	4+ MF
A <sub>2</sub> 3068 Y	1+ MF
Treat A:B cross	None

\* Plasma from 3077 W selected with Treated cells and reg control and have re-tested against cells from patient  
 plasma selected 15/11/2020  
 1W 2 00024 4 15/11/2020 4+ MF  
 plasma selected reg control 15/11/2020 4+ MF

Patient red cells against	Dalman Biflorus	30573 Patient group O cells
Anti-A <sub>1</sub> 116120 - A3	expiry 29/11/2020	DA 4+MF
Anti-A <sub>1</sub> V151920	expiry 13/29/2016	4+MF
Anti-A <sub>1</sub> 12400993	expiry 07/01/2011	4+MF
Anti-A <sub>1</sub> 130504 B	expiry 11/22/11	4+MF

Control group O cells 011 POCG 3371 29/10/2020  
 DA 0 0 0 0

Against nonstandard A<sub>1</sub>B:  
 TM 11501 C  
 4 02/11/10 0

Against nonstandard A<sub>1</sub>:

P. Penn	05/12/2020	1	2	3
210510	27/01/13	0	8006142206	60446
0	0	0	17/11/13	05/01/13
			0	0

The infrequency with which Tn polyagglutination syndrome is encountered, its clinical features and its pathophysiology make it a **formidable** diagnostic challenge.



Lectin Kit Batch No 79190514 Expiry 15/04/21

Lectin Reagents	Arachis hypogaea	Salvia sclarea	Dolichos Biflorus***	Glycine soja		Polybrene (if required)
Test Cells <i>Patient</i>	0	S	S	S		
Patient						
Controls						
T+ control (if required)	S	0	0	S		
Neg Control	0	0	0	0		
Typical Findings						
T active	Pos	Neg	Neg	Pos		
Tk active*	Pos	Neg	Neg	Neg		
Tn	Neg	Pos	Pos	Pos		
Th	Pos	Neg	Neg	Neg		
Tx	Pos	Neg	Neg	Neg		
Cad	Neg	Neg	Pos**	Pos/Neg**		

\*It is possible to differentiate Tk from Tx using papain –Tk will be enhanced Tx will be weakened  
 \*\* Varying strengths of agglutination have been reported with Cad  
 \*\*\*Dolichos biflorus will agglutinate A+ red cells – patients of this ABO group with suspected polyagglutination cannot be tested with this reagent. Dolichos biflorus also contains Anti-Sda

Performed by JN

Date 07/04/20

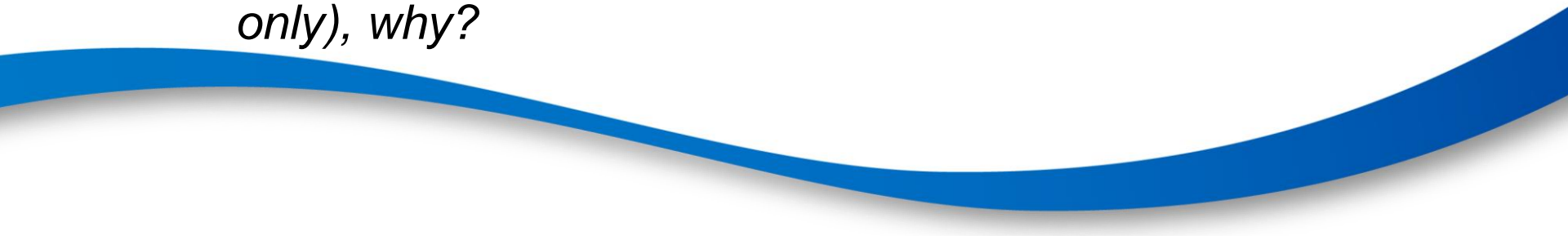
Checked by [Signature]

Date 01/10/2020

(Temporary version 01/10/2020)

Repeat sample received and tested by Sheffield (thanks James Naseem)

# Unanswered questions:

- Monoclonal antibodies used for ABO typing don't contain the polyagglutinins in normal human plasma so you would not normally expect polyagglutinable cells to react with monoclonal anti-A.
    - *Why did the anti-A in the Grifols long group react with the Tn activated cells?*
  - The auto-control is most often negative in polyagglutination.
    - *Although the auto-control by direct agglutination is negative the patient does have a weak 1+ DAT (IgG only), why?*
- 

# Some potential answers



# Case investigated by Fiona Lisle the Grifols UK Ltd Technical Application Specialist

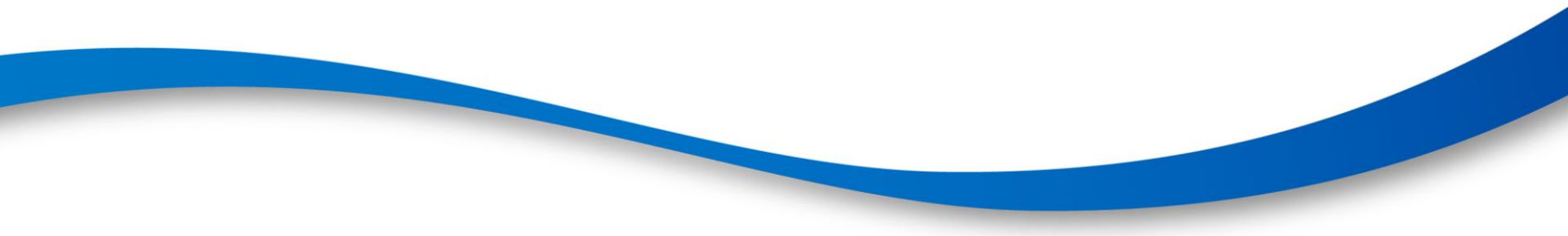
Each microtube of the DG Gel ABO/Rh (CR) card contains a gel in buffered medium with preservatives. The different microtubes are identified by the front label of the card.

- Microtube A: monoclonal anti-A (mixture of IgM and IgG antibodies of murine origin, clones 16243G2 + 16247E6).

Each microtube of the DG Gel Confirm P card contains a gel in buffered medium with preservative. The microtubes are identified by the front label of the card.

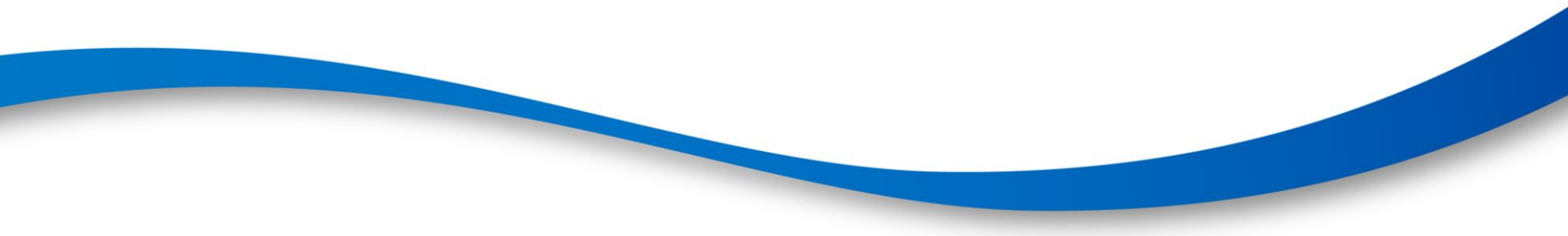
- Microtube A: monoclonal anti-A (IgM antibodies of murine origin, clone Birma-1).

# Limitations section of the DG Gel ABO/Rh (CR) card

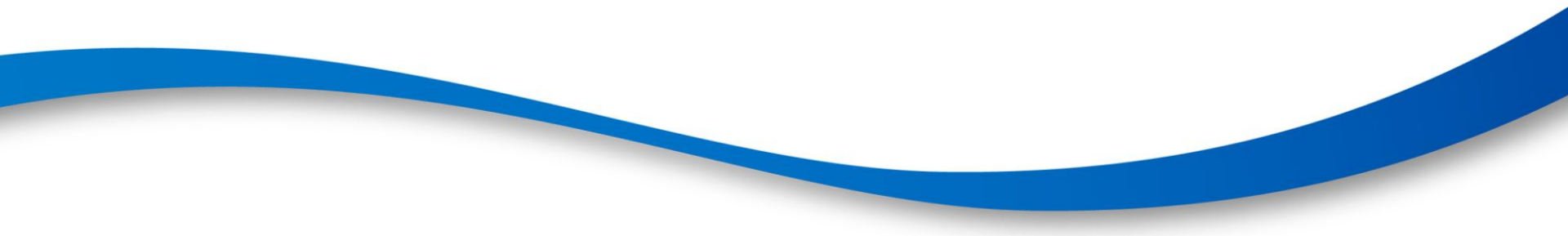
8. Discrepancies between forward and reverse groups may be observed in patients with low or non-existent levels of isoagglutinins: newborns up to the age of 4 - 6 months, elderly persons, patients with immunodeficiency or with very diluted antibodies due to plasma exchange procedures<sup>5</sup>.
  9. A very weak expression or variants of the D antigens may not be detected.
  10. The anti-A reagent contained in this card could react with Tn cryptantigens.
  11. On occasions, unagglutinated red blood cells may be retained somewhere in the gel column with the appearance of very minute red dot or fleck. However, this nonspecific retention should not interfere with the interpretation of the result.
- 

# Additional info from Grifols

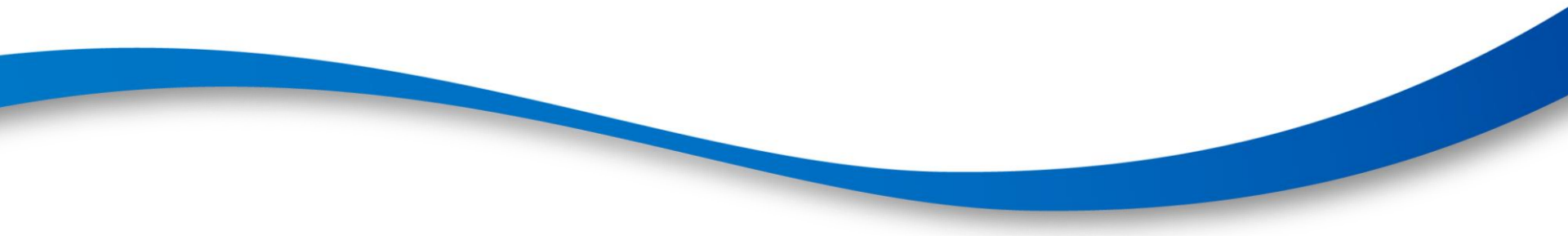
Grifols have previously investigated only one such case:

- Tn polyagglutination in Turkey 2019
  - Experienced the same results:
    - Negative result in the anti-A well of the confirm group card
    - Positive result in the anti-A well of the forward group
- 

# What about the patient's positive DAT?

- Plasma of patients with polyagglutinable red cells often (but not quite always) lack serologically demonstrable levels of the particular polyagglutinin that reacts with the type of polyagglutinable red cells involved.
  - In polyagglutination has been associated AIHA.
- 

# How common is Tn polyagglutination?

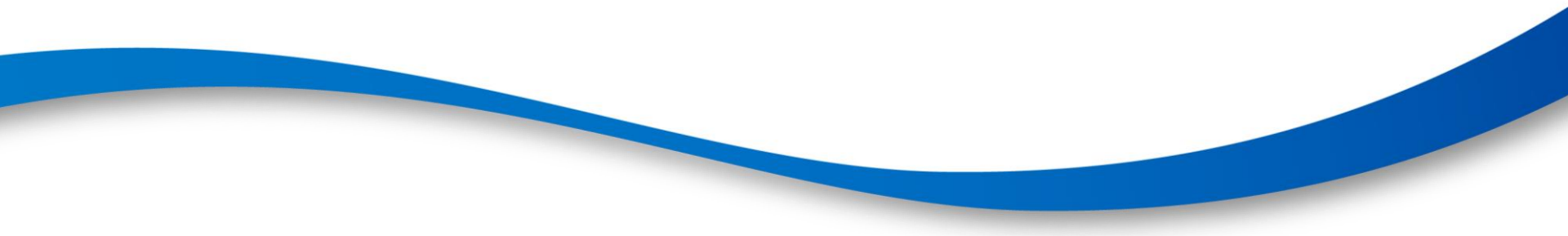
- When 150 000 donors were screened for Tn polyagglutination there was not one positive reaction.
  - Prevalence extremely low
  - Impossible to give a reliable figure
  - **Usually found by alert technician** investigating blood samples for an unrelated problem and observing the phenomena of polyagglutination (Eric Berger 1999 "*Biochemin et Biophysica*" : 255-268).
- 



# Some interesting Tn Polyagglutination info:

- Not a direct action of bacteria or viral enzyme but is due to a somatic mutation
- Nearly always mixed field
- Tn polyagglutination has been associated with MDS, AIHA (? Explains the patient being DAT positive for IgG), cytopenia, and leukaemia
- Affects red cells, white cells and platelets
- Nearly always a permanent condition
- Immunodominant carbohydrate is GalNac (but with alpha linkage to serine or threonine)


# Biochemistry

- The Tn antigen is an incompletely glycosylated membrane glycoprotein with an exposed N-acetylgalactosamine residue.
  - The Tn antigen results from inactivation of C1GALT1C1, which encodes a chaperone required for the correct functioning of T-synthetase (C1GALT1; 610555), an enzyme essential for the correct biosynthesis of O-glycans.
  - Absence of active T-synthetase results in exposure of GalNAc residues, with a proportion of these residues becoming sialylated and forming a sialyl-Tn antigen.
- 

# Transfusion considerations

- Almost all normal human plasma although not the patient's own plasma will all contain anti-Tn
- RBCs transfusions should not be an issue

**BUT: when a small dose of Tn-polyagglutinable red cells were injected into a normal individual the cells were rapidly destroyed! (Issitt 1999 "*Applied Blood Group Serology*" : 1105-1109).**

- So what about plasma products (Platelets, FFP etc.)  
???????
  - And lastly what about Donors who's red cells are unbeknown to be Tn polyagglutinable ????????????
- 

# Patient follow up

- Post TKR the patient experienced some unexpected weeping at the surgery wound
- Slight thrombocytopenia and leukopenia noted pre-op
  - Plts  $116 \times 10^9/L$  and WBC  $4.15 \times 10^9/L$
- Post op Hb 129 g/L Plts  $182 \times 10^9/L$  and WBC  $12.2 \times 10^9/L$
- Patient has a history of anxiety and depression so should he be informed of his Tn polyagglutinable state?

# But then lightning strikes twice


- Second patient from the same hospital referred for ?ABO group on 06/11/2020
- Male
- DoB 06/07/1935
- Diagnosis MDS
- Depending on the grouping card used the patient is either group O or group not determined.



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PROFILE INFORMATION

021557  

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18:22





(1) Full Group  
ABO-Rh (CR)(A1-B-O)









RESULTS

Group Rh:	NI
Group:	Dis
Direct Group:	A
Reverse Group:	O
Rh D:	Pos

TECHNIQUE DETAILS

719001210010141580  

DG Gel ABO/Rh (CR)

Direct Group		Rh D		Reverse Group			
A	B	(1) D <sup>Wt</sup>	(2) D <sup>Wt</sup>	Ctl	N <sub>A1</sub>	N <sub>B</sub>	N <sub>O</sub>
							

<b>A</b>	<b>Pos</b>	<b>O</b>	RESULT					
4+	-	4+	4+	-	4+	4+	-	CURRENT
4+	-	4+	4+	-	4+	4+	-	ORIGINAL



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MON 09 NOV 2020 / 14:16

PROFILE INFORMATION

021557



05/11/20  
19:10



(3) Confirm Group

Confirm P

TECHNIQUE DETAILS



718302210070107054

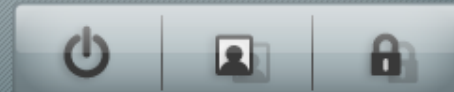
DG Gel Confirm P

Direct Group		Rh D	
A	B	D <sup>vr</sup>	Ctrl

RESULTS

Group Rh:	O+
Group:	O
Direct Group:	O
Rh D:	Pos

	O	Pos	RESULT
CURRENT	-	4+	-
ORIGINAL	-	4+	-

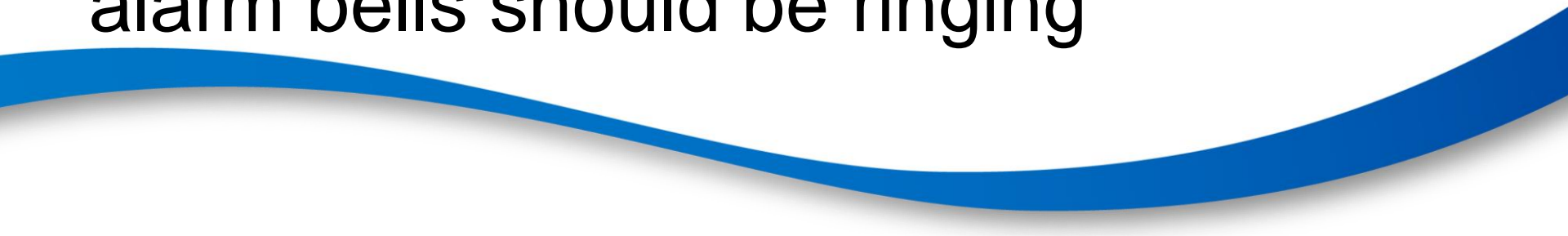


# 2<sup>nd</sup> patient referral results

- The IAT and enzyme IAT panels were negative with a weak IAT auto and the is DAT IgG 3+.
- The lectin kit was positive against all 4 lectins, however the result was significantly weaker (2+ vs 5+) against *Arachis hypogaea*.
- But saline spin auto also gave a 2+ reaction.
- The result therefore is strongly suggestive of Tn activation (neither T, Tn, Tk, Th, Tx or Cad are reactive against all of the lectins), Tn is typically negative x *A. hypogaea* and positive against the others.



# Serological lessons learnt

- If no obvious explanation for an unresolved ABO forward group then consider the very rare possibility of polyagglutination
  - If you have reactions with anti-A1 and tests with anti-A are negative then alarm bells should be ringing
- 

## Other lessons learnt:

- Complete the digital modules available on Shine Academy
  - Get a fresh pair of eyes to review results
  - Read the small print
- 