# ABID: The weird & the wonderful

## WEBINAR 3/3

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NHSBT

# Antibody Identification

## Session 3/3

## The weird and the wonderful

Helen Thom

# Today’s session overview

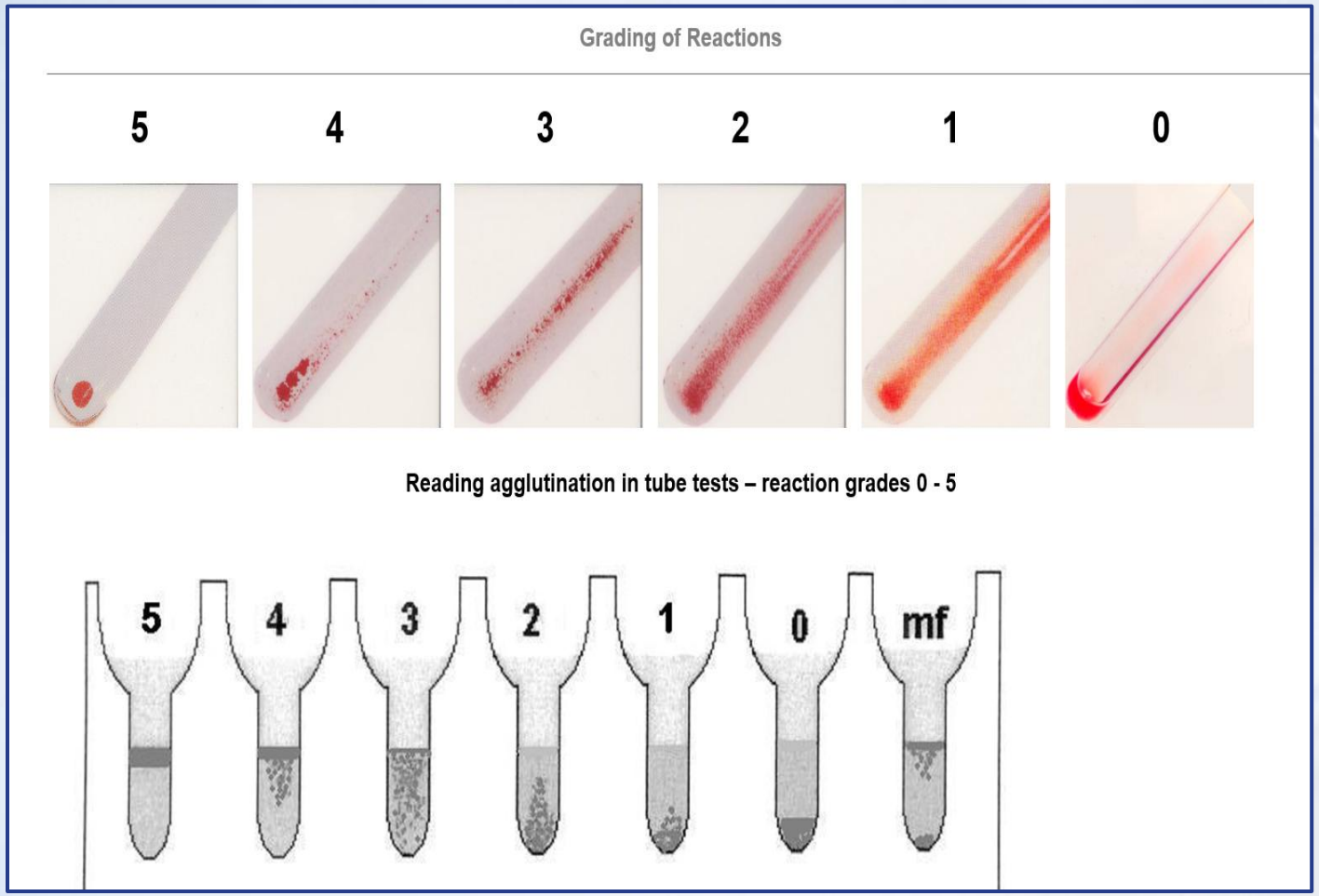
- Brief recap of some tools from previous sessions
- Overview of high incidence antibodies
- Advanced techniques: DTT treatment, neutralisation, inhibition, alloadsorption
- Cases examples demonstrating practice

# The basic principles of ABID


- Unknown antibody with known antigen – ABID
- Or known antibody with unknown antigen – grouping/phenotyping
- Antibody will bind with its corresponding antigen

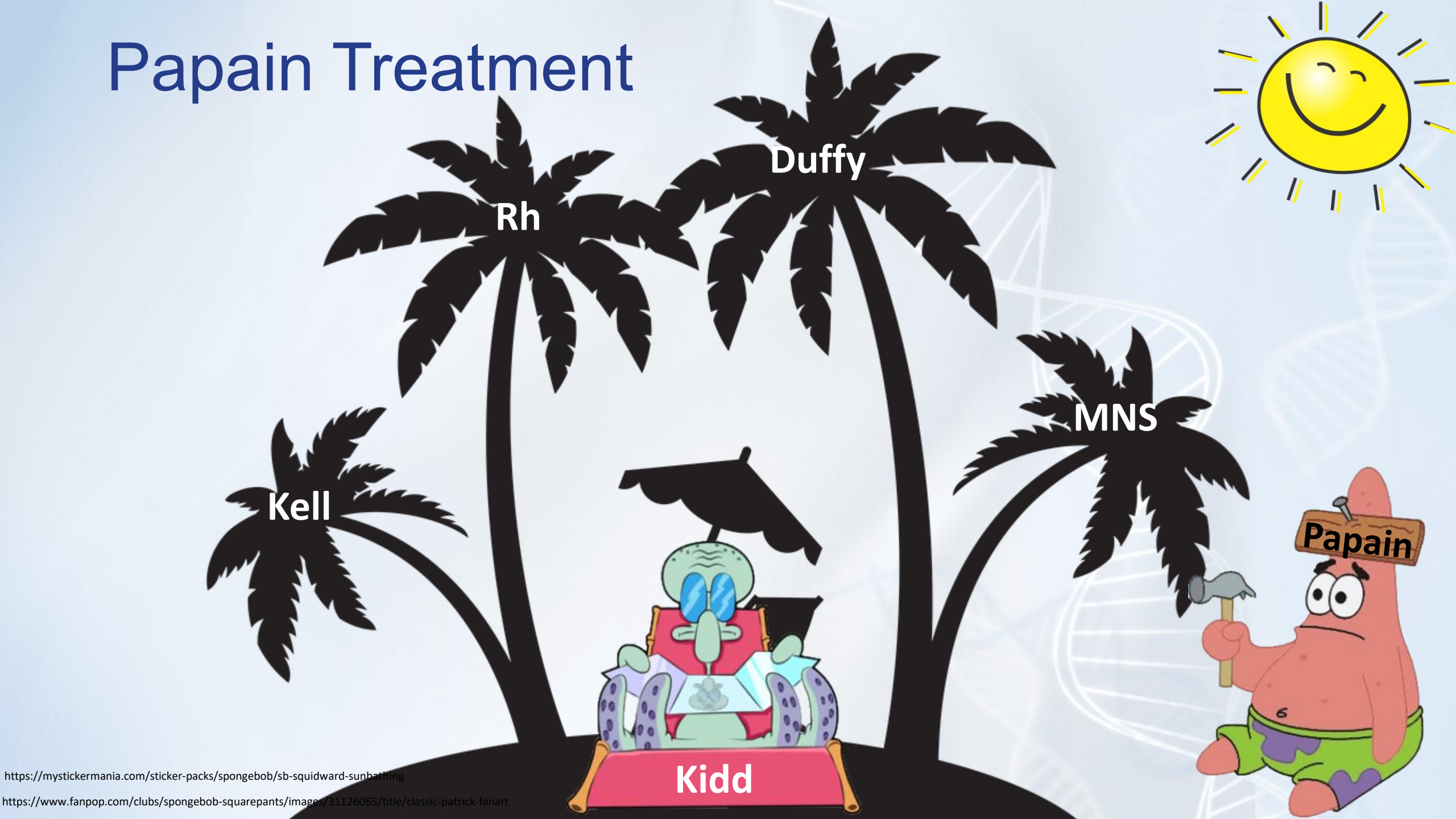
https://www.google.com/url?sa=i&url=https%3A%2F%2Fspongebob.fandom.com%2Fwiki%2FChum_Bucket&psig

# Grading




- Can assist with ABID
- Multiple antibodies
- Enhanced reactions
- Dosage

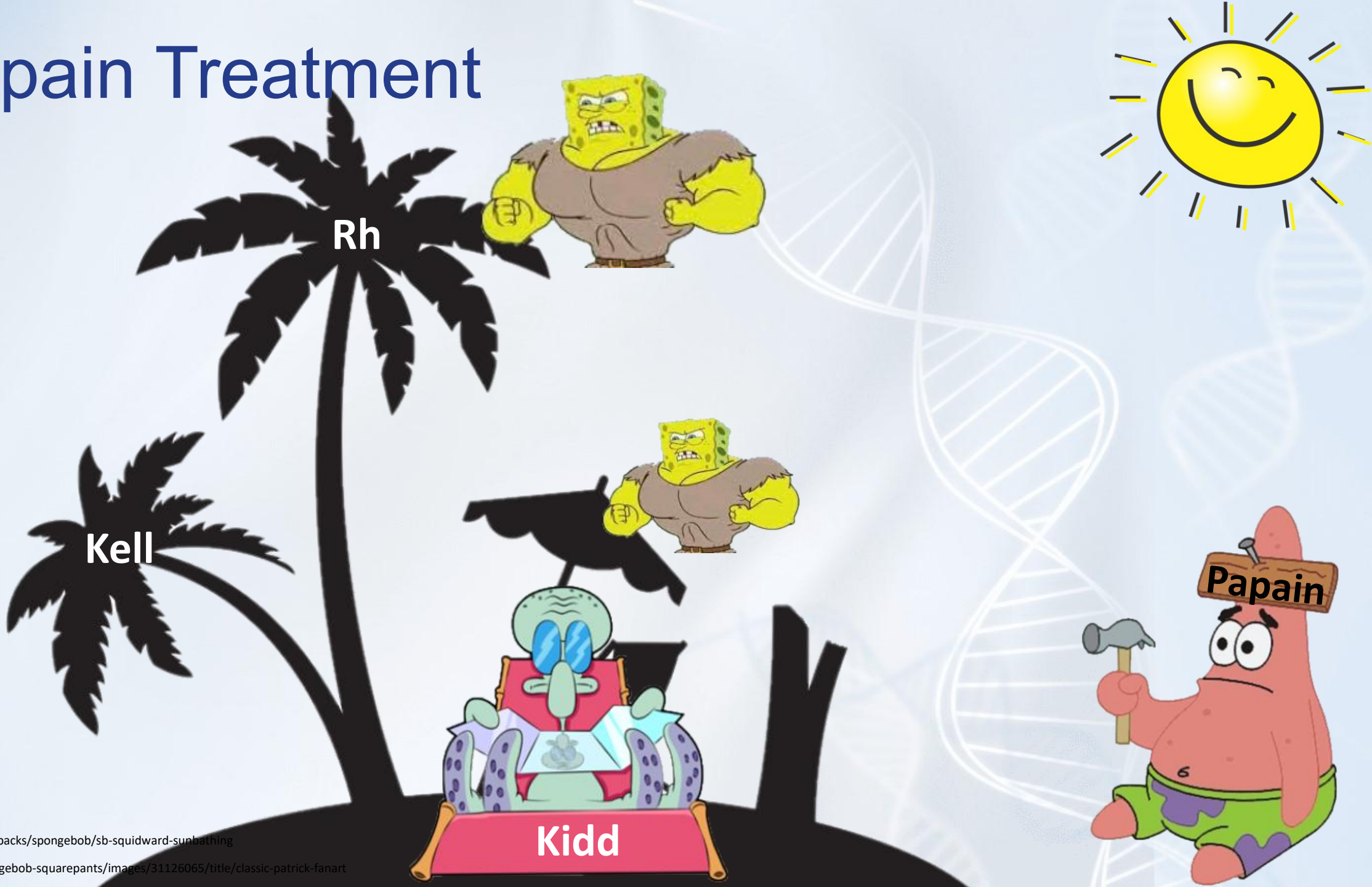
# Papain Treatment




https://mystickermania.com/sticker-packs/spongebob/sb-squidward-sunbathing

https://www.fanpop.com/clubs/spongebob-squarepants/images/31126065/title/classic-patrick-fanart

# Papain Treatment




https://mystickermania.com/sticker-packs/spongebob/sb-squidward-sunbathing

https://www.fanpop.com/clubs/spongebob-squarepants/images/31126065/title/classic-patrick-fanart

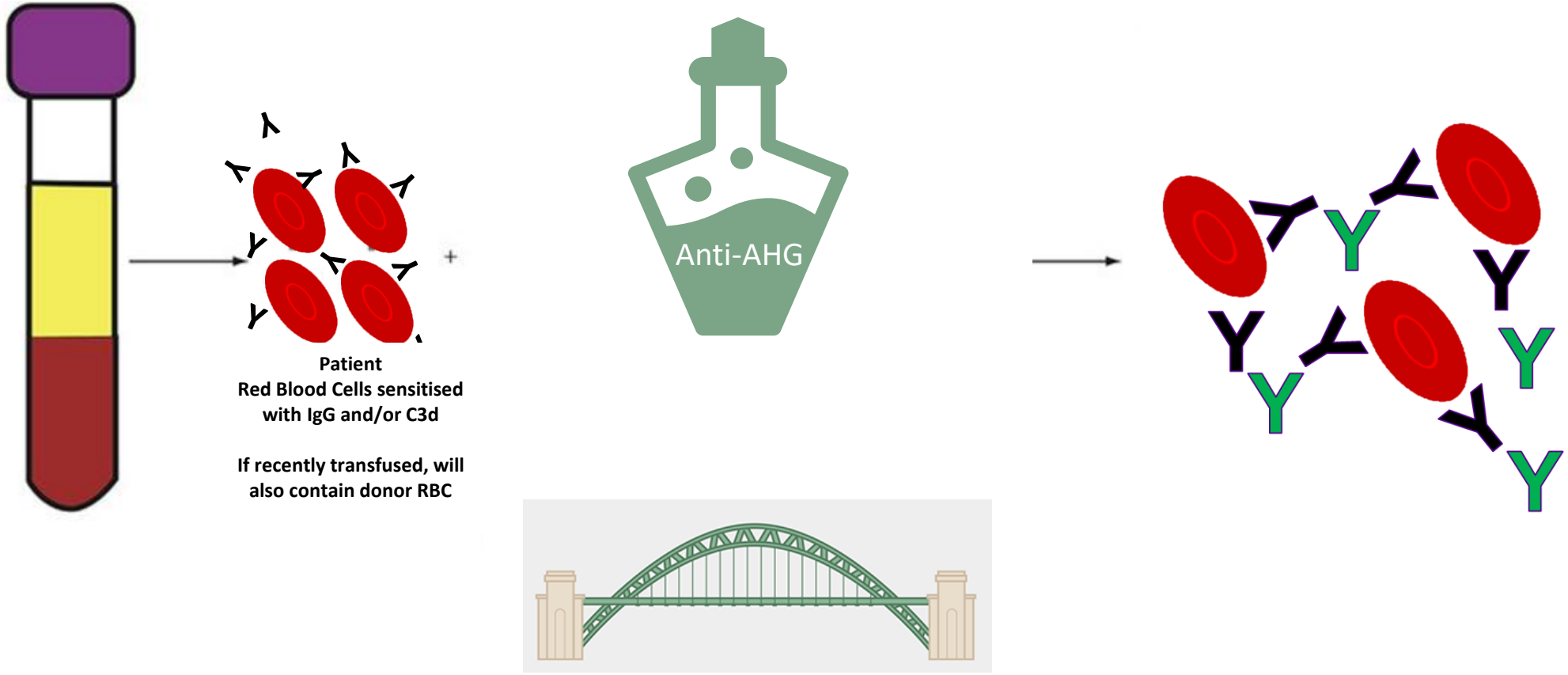
# Knowing the clinically significance of antibodies

ISBT:

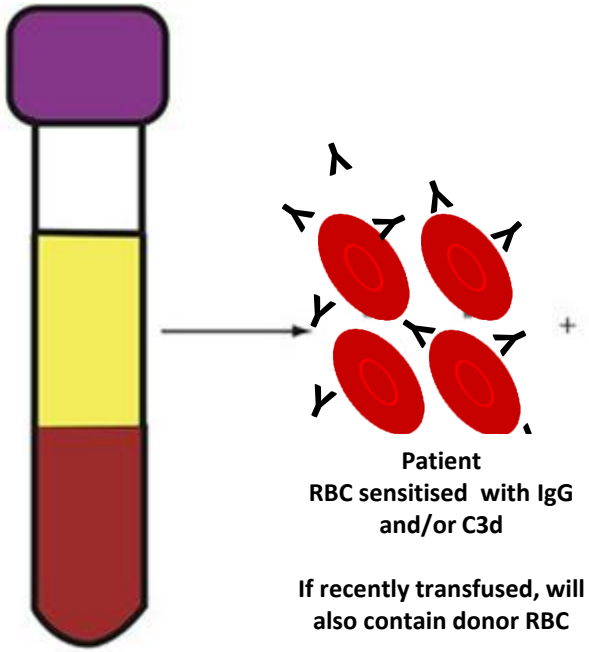
*“A clinically significant antibody can be defined as one capable of causing accelerated destruction of a significant proportion of transfused cells, or one capable of crossing the placenta and causing haemolytic disease of the fetus and newborn”*


https://onlinelibrary.wiley.com/doi/abs/10.1111/voxs.12325#:~:text=A%20clinically%20significant%20antibody%20can,of%20the%20fetus%20and%20newborn.

# Positive DAT

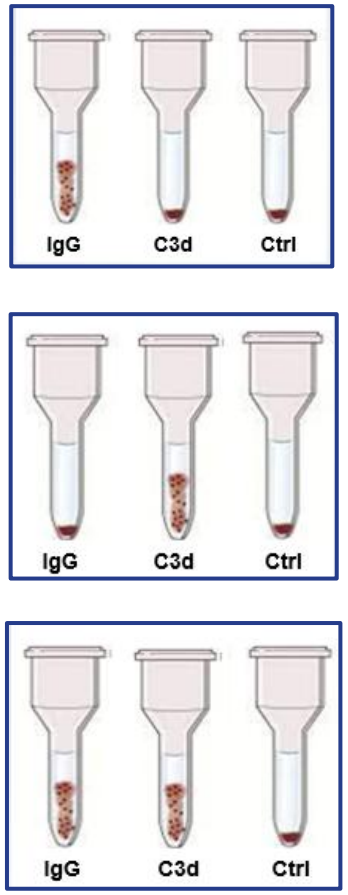


# Positive DAT



Patient
RBC sensitised with IgG
and/or C3d

If recently transfused, will
also contain donor RBC


# ‘Pan-reactivity’ and auto control/DAT

*Pan from the Latin word-forming element meaning “all, every, whole, all-inclusive”*

- High incidence antibodies most often appear as pan-reactivity with negative auto control/DAT (not always the case though)
- The antibody binds to its corresponding antigen – which happens to be >90 or >99% of reagent (donor) cells
- The patients’ auto control is negative as their own cells are antigen negative, so the antibody has no antigen to bind to (not always the case though)
- Pan reactive antibodies most often appear as pan-reactivity with positive auto control/DAT
  - IgG, IgM, C3d or a mixture
- The patient’s autoantibody will bind with *all* red cell antigens, including their own, hence pan-reactive

# High incidence - what you'd typically see

| Cell | Rh | D | C | E | c | e | M | N | S | s | P1 | Lu$^a$ | K | k | Kp$^a$ | Le$^a$ | Le$^b$ | Fy$^a$ | Fy$^b$ | Jk$^a$ | Jk$^b$ | Other | IAT | EIAT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | $R_1^wR_1$ | + | + | 0 | 0 | + | + | 0 | + | 0 | 0 | 0 | 0 | + | 0 | 0 | + | + | 0 | + | 0 | | 4 | 5 |
| 2 | $R_1R_1$ | + | + | 0 | 0 | + | 0 | + | 0 | + | 0 | 0 | + | + | 0 | + | 0 | 0 | + | 0 | + | | 4 | 5 |
| 3 | $R_2R_2$ | + | 0 | + | + | 0 | + | 0 | + | 0 | 0 | 0 | 0 | + | 0 | + | 0 | 0 | + | + | + | | 4 | 5 |
| 4 | r'r | 0 | + | 0 | + | + | 0 | + | + | 0 | 0 | 0 | 0 | + | 0 | 0 | + | 0 | + | + | 0 | | 4 | 5 |
| 5 | r''r | 0 | 0 | + | + | + | 0 | + | 0 | + | 4 | 0 | 0 | + | 0 | 0 | + | + | 0 | 0 | + | | 4 | 5 |
| 6 | rr | 0 | 0 | 0 | + | + | | | | | | | | 0 | 0 | 0 | + | + | 0 | 0 | + | | 4 | 5 |
| 7 | rr | 0 | 0 | 0 | + | + | | | | | | | | + | 0 | + | 0 | 0 | + | + | 0 | | 4 | 5 |
| 8 | rr | 0 | 0 | 0 | + | + | | | | | | | | + | + | 0 | + | 0 | + | 0 | + | | 4 | 5 |
| 9 | rr | 0 | 0 | 0 | + | + | + | 0 | 0 | + | 2 | 0 | 0 | + | 0 | + | 0 | + | 0 | 0 | + | | 4 | 5 |
| 10 | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 3 | 0 | 0 | + | 0 | + | 0 | + | 0 | + | 0 | | 4 | 5 |
| | | | | | | | | | | | | | | | | | | | | | | Auto | 0 | / |
| | | | | | | | | | | | | | | | | | | | | | | + control | 2 | / |

Are there any underlying alloantibodies?

# Or it could be like this…

| Cell | Rh | D | C | E | c | e | M | N | S | s | P1 | Lu$^a$ | K | k | Kp$^a$ | Le$^a$ | Le$^b$ | Fy$^a$ | Fy$^b$ | Jk$^a$ | Jk$^b$ | Other | IAT | EIAT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | $R_1^wR_1$ | + | + | 0 | 0 | + | + | 0 | + | 0 | 0 | 0 | 0 | + | 0 | 0 | + | + | 0 | + | 0 | | 2 | 0 |
| 2 | $R_1R_1$ | + | + | 0 | 0 | + | 0 | + | 0 | + | 0 | 0 | + | + | 0 | + | 0 | 0 | + | 0 | + | | 2 | 0 |
| 3 | $R_2R_2$ | + | 0 | + | + | 0 | + | 0 | + | 0 | 0 | 0 | 0 | + | 0 | + | 0 | 0 | + | + | + | | 2 | 0 |
| 4 | r'r | 0 | + | 0 | + | + | 0 | + | + | 0 | 0 | 0 | 0 | + | 0 | 0 | + | 0 | + | + | 0 | | 2 | 0 |
| 5 | r''r | 0 | 0 | + | + | + | 0 | + | 0 | + | 4 | 0 | 0 | + | 0 | 0 | + | + | 0 | 0 | + | | 2 | 0 |
| 6 | rr | 0 | 0 | 0 | + | + | | | | | | | | 0 | 0 | 0 | + | + | 0 | 0 | + | | 2 | 0 |
| 7 | rr | 0 | 0 | 0 | + | + | | | | | | | | + | 0 | + | 0 | 0 | + | + | 0 | | 2 | 0 |
| 8 | rr | 0 | 0 | 0 | + | + | | | | | | | | + | + | 0 | + | 0 | + | 0 | + | | 2 | 0 |
| 9 | rr | 0 | 0 | 0 | + | + | + | 0 | 0 | + | 2 | 0 | 0 | + | 0 | + | 0 | + | 0 | 0 | + | | 2 | 0 |
| 10 | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 3 | 0 | 0 | + | 0 | + | 0 | + | 0 | + | 0 | | 2 | 0 |
| | | | | | | | | | | | | | | | | | | | | | | Auto | 0 | / |
| | | | | | | | | | | | | | | | | | | | | | | + control | 2 | / |

Are there any underlying alloantibodies?

# Some ‘common’ examples

**MNS**
- Anti-U
- Antigen negative RBC

**Kidd**
- Anti-Jk3
- Antigen negative RBC

**Duffy**
- Anti-Fy3
- Antigen negative RBC

**Chido/ Rogers**
- Anti-Ch/Rg
- Least serologically incompatible RBC

**Kell**
- Anti-k
- Antigen negative RBC

**Knops**
- Knops-McCoy antibodies
- Least serologically incompatible RBC

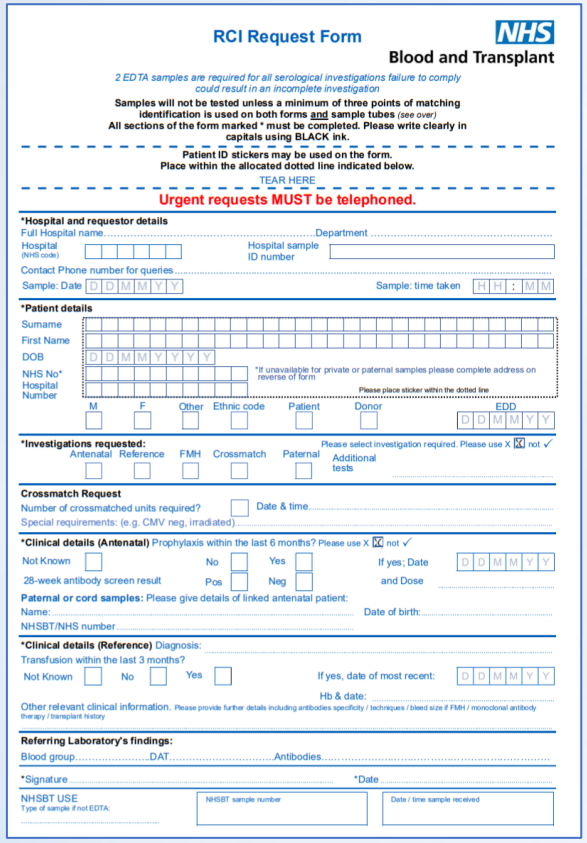
# Identifying high incidence antibodies

- Apparent pan-reactivity with negative auto control – but not always
- Ethnicity
- Rare cell panel can be performed – similar to primary panel but negative for high incidence antigens
- May need referral to International Blood Grouping Reference Laboratory (IBGRL)
- Serological clues
  - How the antibody behaves
  - Papain enhanced/sensitive
  - Grading – strength of reactions
  - Patient's phenotype - e.g. S-s-, Fy(a-b-), Jk(a-b-), K+

# Blood selection

- High frequency antigens may create difficulties in obtaining compatible blood
- Red cells negative for high frequency antigens are not readily available, and availability should be discussed with the National Frozen Blood Bank (NFBB)
- It may be that there are multiple antibodies to consider, making it even harder
- Some require antigen negative blood
- Some are serologically least incompatible

# Case 1:
# History – 06/06/2019



RCI Request Form

NHS Blood and Transplant

*2 EDTA samples are required for all serological investigations failure to comply could result in an incomplete investigation*

**Samples will not be tested unless a minimum of three points of matching identification is used on both forms and sample tubes** *(see over)*
**All sections of the form marked * must be completed. Please write clearly in capitals using BLACK ink.**

**Patient ID stickers may be used on the form.**
**Place within the allocated dotted line indicated below.**

TEAR HERE

**Urgent requests MUST be telephoned.**

***Hospital and requestor details**
Full Hospital name ..... Department .....
Hospital (NHS code) | Hospital sample ID number
Contact Phone number for queries .....
Sample: Date D D M M Y Y | Sample: time taken H H : M M

***Patient details**
Surname
First Name
DOB D D M M Y Y Y Y
NHS No* / Hospital Number
*If unavailable for private or paternal samples please complete address on reverse of form
Please place sticker within the dotted line
M | F | Other | Ethnic code | Patient | Donor | EDD D D M M Y Y

***Investigations requested:** Please select investigation required. Please use X ☒ not ✓
Antenatal | Reference | FMH | Crossmatch | Paternal | Additional tests

**Crossmatch Request**
Number of crossmatched units required? | Date & time .....
Special requirements: (e.g. CMV neg, irradiated) .....

***Clinical details (Antenatal)** Prophylaxis within the last 6 months? Please use X ☒ not ✓
Not Known | No | Yes | If yes; Date and Dose D D M M Y Y
28-week antibody screen result: Pos | Neg
**Paternal or cord samples:** Please give details of linked antenatal patient:
Name: ..... Date of birth: .....
NHSBT/NHS number .....

***Clinical details (Reference)** Diagnosis: .....
Transfusion within the last 3 months?
Not Known | No | Yes | If yes, date of most recent: D D M M Y Y
Hb & date: .....
Other relevant clinical information. Please provide further details including antibodies specificity / techniques / bleed size if FMH / monoclonal antibody therapy / transplant history

**Referring Laboratory's findings:**
Blood group ..... DAT ..... Antibodies .....

*Signature ..... *Date .....

NHSBT USE
Type of sample if not EDTA: .....
NHSBT sample number | Date / time sample received

- Patient demographics – 85 y/o male
- Investigation requested:
  - Crossmatch (ABID and 2 XM units)
- Clinical details:
  - Diagnosis: ‘Anaemia’
  - Hb - 70
  - Transfusion history: tx 04/04/2019
- Referring laboratory findings
  - Historical ABO O+ C-, DAT pos, Pos Screen

# 1st Investigation – NHSBT Panel 1

| Cell | Rh | D | C | E | c | e | M | N | S | s | P1 | Lu$^a$ | K | k | Kp$^a$ | Le$^a$ | Le$^b$ | Fy$^a$ | Fy$^b$ | Jk$^a$ | Jk$^b$ | Other | IAT | EIAT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | $R_1^wR_1$ | + | + | 0 | | | | | 0 | + | 0 | 0 | + | + | 0 | 0 | + | + | 0 | + | 0 | | Wk | 4 |
| 2 | $R_1R_1$ | + | + | 0 | | | | | + | 0 | 4 | 0 | 0 | + | 0 | + | 0 | 0 | + | 0 | + | | Wk | 4 |
| 3 | $R_2R_2$ | + | 0 | + | + | 0 | 0 | + | 0 | + | 3 | 0 | 0 | + | 0 | + | 0 | 0 | + | + | 0 | | 0 | 2 |
| 4 | r'r | 0 | + | 0 | + | + | 0 | + | 0 | + | 2 | 0 | 0 | + | 0 | 0 | + | 0 | + | + | 0 | | Wk | 4 |
| 5 | r''r | 0 | 0 | + | + | + | + | 0 | + | 0 | 1 | 0 | 0 | + | 0 | 0 | + | + | 0 | 0 | + | | 0 | Wk |
| 6 | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 3 | 0 | + | 0 | 0 | 0 | + | + | + | 0 | + | | 0 | 2 |
| 7 | rr | 0 | 0 | 0 | + | + | + | 0 | + | 0 | 0 | + | + | + | 0 | 0 | + | + | + | + | 0 | | 0 | 3 |
| 8 | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 3 | 0 | 0 | + | + | + | + | + | + | 0 | + | | 0 | 2 |
| 9 | rr | 0 | 0 | 0 | + | + | + | 0 | 0 | + | 4 | + | 0 | + | 0 | 0 | + | + | + | + | 0 | | 0 | 3 |
| 10 | rr | 0 | 0 | 0 | + | + | 0 | + | + | 0 | 1 | 0 | 0 | + | 0 | + | 0 | 0 | + | + | + | | 0 | 2 |
| | | | | | | | | | | | | | | | | | | | | | | Auto | 1 | / |
| | | | | | | | | | | | | | | | | | | | | | | K control | 2 | / |

Anti-C

Looks like Anti-e

Looks like a bit Jka-ish

Requires DAT

# 1st Investigation – NHSBT Panel 2

| Cell | Rh | D | C | E | c | e | M | N | S | s | P1 | Lu$^a$ | K | k | Kp$^a$ | Le$^a$ | Le$^b$ | Fy$^a$ | Fy$^b$ | Jk$^a$ | Jk$^b$ | Other | IAT | EIAT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | $R_1^wR_1$ | + | + | 0 | | | | | + | 0 | 0 | 0 | 0 | + | 0 | 0 | + | + | 0 | + | 0 | | Wk | 4 |
| 2 | $R_1R_1$ | + | + | 0 | | | | | 0 | + | 2 | 0 | + | + | 0 | 0 | + | 0 | + | 0 | + | | Wk | 4 |
| 3 | $R_2R_2$ | + | 0 | + | + | 0 | + | 0 | + | 0 | 2 | 0 | 0 | + | 0 | + | 0 | + | 0 | 0 | + | | 0 | 0 |
| 4 | r'r | 0 | + | 0 | + | + | + | 0 | + | 0 | 2 | 0 | 0 | + | 0 | 0 | + | 0 | + | 0 | + | | Wk | 4 |
| 5 | r''r | 0 | 0 | + | + | + | 0 | + | 0 | + | 3 | 0 | 0 | + | 0 | 0 | + | 0 | + | + | 0 | | 0 | 3 |
| 6 | rr | 0 | 0 | 0 | + | + | + | 0 | + | 0 | 4 | 0 | + | 0 | 0 | 0 | + | + | 0 | 0 | + | | 0 | 3 |
| 7 | rr | 0 | 0 | 0 | + | + | + | 0 | 0 | + | 1 | + | + | + | 0 | + | + | + | 0 | + | 0 | | 0 | 3 |
| 8 | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 4 | 0 | 0 | + | + | + | + | + | 0 | + | 0 | | 0 | 2 |
| 9 | rr | 0 | 0 | 0 | + | + | 0 | + | + | 0 | 0 | 0 | 0 | + | 0 | + | + | + | 0 | 0 | + | | 0 | 2 |
| 10 | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 2 | + | 0 | + | 0 | + | 0 | + | 0 | 0 | + | | 0 | 2 |
| | | | | | | | | | | | | | | | | | | | | | | Auto | / | / |
| | | | | | | | | | | | | | | | | | | | | | | K control | 2 | / |

Anti-C

Looks like Anti-e

Still looks like a bit Jka-ish

# What we have so far

- Identified antibodies
  - Anti-C
  - Anti-e
  - Possible anti-Jka
- Are these allo or auto antibodies? Are they clinically significant?
- What is the phenotype? Can we phenotype? Tx 9 weeks earlier
- Positive auto control – needs DAT
- Patient not tx within last 6 weeks – but has made antibodies – go with your gut!

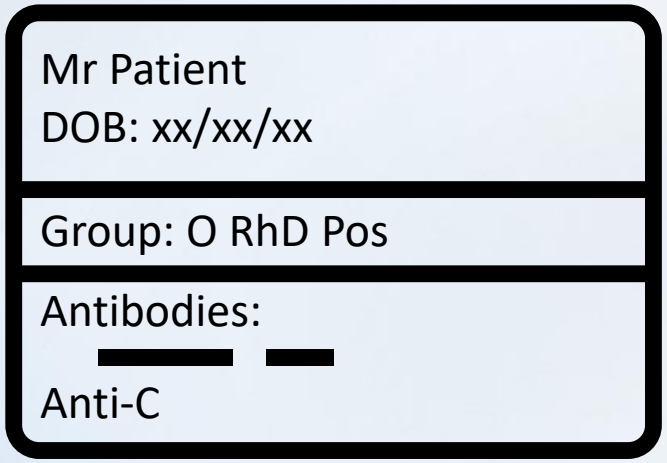
# 1st Investigation – Eluate NHBST Panel 1

| Cell | Rh | D | C | E | c | e | M | N | S | s | P1 | Lu$^a$ | K | k | Kp$^a$ | Le$^a$ | Le$^b$ | Fy$^a$ | Fy$^b$ | Jk$^a$ | Jk$^b$ | Other | IAT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | $R_1^wR_1$ | + | + | 0 | 0 | + | + | + | 0 | + | 0 | 0 | + | + | 0 | 0 | + | + | 0 | + | 0 | | 3 |
| 2 | $R_1R_1$ | + | + | 0 | 0 | + | + | 0 | + | 0 | 4 | 0 | 0 | + | 0 | + | 0 | 0 | + | 0 | + | | 3 |
| 3 | $R_2R_2$ | + | 0 | + | + | 0 | 0 | + | 0 | + | 3 | 0 | 0 | + | 0 | + | 0 | 0 | + | + | 0 | | 3 |
| 4 | r'r | 0 | + | 0 | + | + | 0 | + | 0 | + | 2 | 0 | 0 | + | 0 | 0 | + | 0 | + | + | 0 | | 3 |
| 5 | r''r | 0 | 0 | + | + | + | + | 0 | + | 0 | 1 | 0 | 0 | + | 0 | 0 | + | + | 0 | | | | 3 |
| 6 | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 3 | 0 | + | 0 | 0 | 0 | + | + | + | | | | 3 |
| 7 | rr | 0 | 0 | 0 | + | + | + | 0 | + | 0 | 0 | + | + | + | 0 | 0 | + | 0 | + | | | | 3 |
| 8 | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 3 | 0 | 0 | + | + | + | 0 | + | 0 | 0 | + | | 3 |
| 9 | rr | 0 | 0 | 0 | + | + | + | 0 | 0 | + | 4 | + | 0 | + | 0 | 0 | + | + | 0 | + | 0 | | 3 |
| 10 | rr | 0 | 0 | 0 | + | + | 0 | + | + | 0 | 1 | 0 | 0 | + | 0 | + | 0 | 0 | + | 0 | + | | 3 |
| | | | | | | | | | | | | | | | | | | | | | | Auto | 1 |
| | | | | | | | | | | | | | | | | | | | | | | K control | 2 |

Pan-reactive

# Results 06/06/19

- RhK phenotype:
  - C- c+ E+ e+ K+
  - R2r
  - Jk(a-b+)




- Alloanti-C – clinically significant
- Autoanti-e – unlikely to be clinically significant
- ? Alloanti-Jka ‘poking out’
  - Couldn’t confirm due to autoanti-e
  - Regard as allo and clinically significant
- DAT 1+ IgG – eluate: 3+ pan
- 2 units O+ C- Jka- issued

# Results 11/07/19

- Historical phenotypes:
  - C- c+ E+ e+ K+
  - R2r
  - Jk(a-b+)

Mr Patient
DOB: xx/xx/xx

Group: O RhD Pos

Antibodies:

Anti-C, anti-Jka

- Alloanti-C
- Alloanti-Jka
- Pan reactive enzyme antibody
- DAT 2+ IgG – eluate: 3+ pan
- 2 units O+ C- Jka- issued
- Updated antibody card provided

# Results 12/08/19

- Historical phenotypes:
  - C- c+ E+ e+ K+
  - R2r
  - Jk(a-b+)
- Alloanti-C
- Alloanti-Jka
- DAT 2+ IgG – eluate not performed as no change in DAT strength
- 2 units O RhD+, C- Jka- issued

# October - 4th Investigation – NHSBT Panel 1

| Cell | Rh | D | C | E | c | e | M | N | S | s | P1 | Lu$^a$ | K | k | Kp$^a$ | Le$^a$ | Le$^b$ | Fy$^a$ | Fy$^b$ | Jk$^a$ | Jk$^b$ | Other | IAT | EIAT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | $R_1^wR_1$ | + | + | 0 | | | | | 0 | + | 3 | 0 | 0 | + | 0 | 0 | + | 0 | + | 0 | + | | 0 | wk |
| 2 | $R_1R_1$ | + | + | 0 | | | | | + | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 0 | + | 0 | | 0 | wk |
| 3 | $R_2R_2$ | + | 0 | + | | | | | 0 | + | 2 | 0 | 0 | + | 0 | 0 | + | 0 | + | + | 0 | | 0 | 0 |
| 4 | r'r | 0 | + | 0 | | | | | 0 | + | 2 | 0 | 0 | + | 0 | 0 | + | + | 0 | + | 0 | | 0 | wk |
| 5 | r''r | 0 | 0 | + | + | + | + | 0 | + | 0 | 0 | 0 | 0 | + | 0 | 0 | + | + | 0 | 0 | + | | 0 | 0 |
| 6 | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 4 | 0 | + | 0 | 0 | 0 | + | 0 | + | 0 | + | | 0 | 0 |
| 7 | rr | 0 | 0 | 0 | + | + | 0 | + | + | 0 | 4 | + | 0 | + | 0 | + | | | | + | 0 | | 0 | 0 |
| 8 | rr | 0 | 0 | 0 | + | + | + | 0 | + | 0 | 0 | 0 | 0 | + | + | | | | | 0 | + | | 0 | 0 |
| 9 | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 0 | 0 | 0 | + | 0 | + | | | | 0 | + | | 0 | 0 |
| 10 | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 2 | + | 0 | + | 0 | | | | | | | | 0 | 0 |
| | | | | | | | | | | | | | | | | | | | | | | Auto | 3 | / |
| | | | | | | | | | | | | | | | | | | | | | | K control | 2 | / |

Enzyme only alloanti-C

Previously reported alloanti-Jka

Requires DAT:
DAT results 3+ IgG
Increase in strength – eluate required

# 4th Investigation – Eluate NHSBT Panel 1

| Cell | Rh | D | C | E | c | e | M | N | S | s | P1 | Lu$^a$ | K | k | Kp$^a$ | Le$^a$ | Le$^b$ | Fy$^a$ | Fy$^b$ | Jk$^a$ | Jk$^b$ | Other | IAT | EIAT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | $R_1^wR_1$ | + | + | 0 | 0 | + | 0 | + | 0 | + | 3 | 0 | 0 | + | 0 | 0 | + | 0 | + | 0 | + | | 2 | 3 |
| 2 | $R_1R_1$ | + | + | 0 | 0 | + | + | 0 | + | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 0 | + | 0 | | Wk | 3 |
| 3 | $R_2R_2$ | + | 0 | + | + | 0 | 0 | + | 0 | + | 2 | 0 | 0 | + | 0 | 0 | + | 0 | + | + | 0 | | 2 | 3 |
| 4 | r'r | 0 | + | 0 | + | + | + | 0 | 0 | + | | | 0 | + | 0 | 0 | + | + | 0 | + | 0 | | 2 | 3 |
| 5 | r''r | 0 | 0 | + | + | + | + | 0 | + | 0 | | | 0 | + | 0 | 0 | + | + | 0 | 0 | + | | 2 | 3 |
| 6 | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 4 | 0 | + | 0 | 0 | 0 | + | 0 | + | 0 | + | | 0 | 1 |
| 7 | rr | 0 | 0 | 0 | + | + | 0 | + | + | 0 | 4 | + | 0 | + | 0 | + | 0 | 0 | + | + | 0 | | Wk | 3 |
| 8 | rr | 0 | 0 | 0 | + | + | + | 0 | + | 0 | 0 | 0 | 0 | + | + | 0 | 0 | 0 | + | 0 | + | | Wk | 3 |
| 9 | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 0 | 0 | 0 | + | 0 | + | 0 | + | 0 | 0 | + | | Wk | 3 |
| 10 | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 2 | + | 0 | + | 0 | 0 | + | + | 0 | + | 0 | | Wk | 3 |
| | | | | | | | | | | | | | | | | | | | | | | Auto | 3 | / |
| | | | | | | | | | | | | | | | | | | | | | | K control | 2 | / |

Anti-k

# Antibody inclusions/exclusions

- 6.2.4.Antibody specificity should only be assigned when the plasma is reactive with at least two examples of reagent red cells expressing the antigen and non-reactive with at least two examples of reagent red cells lacking the antigen
- 6.2.5.When one antibody specificity has been identified, it is essential that the presence or absence of additional clinically significant antibodies is established
- 6.2.6.Failure to recognise all of the antibody specificities within a sample may lead to a haemolytic transfusion reaction (SHOT, 1996–2010). In particular, the presence of anti-Jk$^{a}$, anti-Jk$^{b}$, anti-S, anti-s, anti-Fy$^{a}$ and anti-Fy$^{b}$ should be excluded using red cells having homozygous expression of the relevant antigen
- A single example only of each phenotype is sufficient for exclusion

I have a feeling of impending doom!

https://b-s-h.org.uk/guidelines/guidelines/pre-transfusion-compatibility-procedures-in-blood-transfusion-laboratories/

https://tenor.com/view/sleep-roll-alone-bed-squidward-gif-15576803

# 4th Investigation – Eluate NHSBT Panel 2

| Cell | Rh | D | C | E | c | e | M | N | S | s | P1 | Lu$^a$ | K | k | Kp$^a$ | Le$^a$ | Le$^b$ | Fy$^a$ | Fy$^b$ | Jk$^a$ | Jk$^b$ | Other | IAT | EIAT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | $R_1^wR_1$ | + | + | 0 | 0 | + | + | 0 | + | 0 | 2 | 0 | 0 | + | 0 | 0 | + | + | 0 | + | 0 | | Wk | 3 |
| 2 | $R_1R_1$ | + | + | 0 | 0 | + | 0 | + | 0 | + | 0 | 0 | + | + | 0 | + | 0 | 0 | + | 0 | + | | Wk | 3 |
| 3 | $R_2R_2$ | + | 0 | + | + | 0 | + | 0 | + | 0 | 0 | 0 | 0 | + | 0 | + | 0 | 0 | + | + | 0 | | Wk | 3 |
| 4 | r'r | 0 | + | 0 | + | + | 0 | + | + | 0 | 0 | 0 | 0 | + | 0 | 0 | + | 0 | + | + | 0 | | Wk | 3 |
| 5 | r''r | 0 | 0 | + | + | + | 0 | + | 0 | + | 4 | 0 | 0 | + | 0 | 0 | + | + | 0 | 0 | + | | 1 | 3 |
| 6 | rr | 0 | 0 | 0 | + | + | + | 0 | + | 0 | 3 | 0 | + | 0 | 0 | 0 | + | + | 0 | 0 | + | | 0 | 2 |
| 7 | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 1 | 0 | + | + | 0 | + | 0 | 0 | + | + | 0 | | Wk | 3 |
| 8 | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 0 | + | | 1 | 3 |
| 9 | rr | 0 | 0 | 0 | + | + | + | 0 | 0 | + | 1 | + | 0 | + | 0 | + | 0 | + | 0 | 0 | + | | 1 | 3 |
| 10 | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 3 | 0 | 0 | + | 0 | + | 0 | + | 0 | + | 0 | | 1 | 3 |
| | | | | | | | | | | | | | | | | | | | | | | Auto | / | / |
| | | | | | | | | | | | | | | | | | | | | | | K control | 2 | / |

# 4th Investigation – Eluate Additional Cells

## NHSBT R1R1 panel

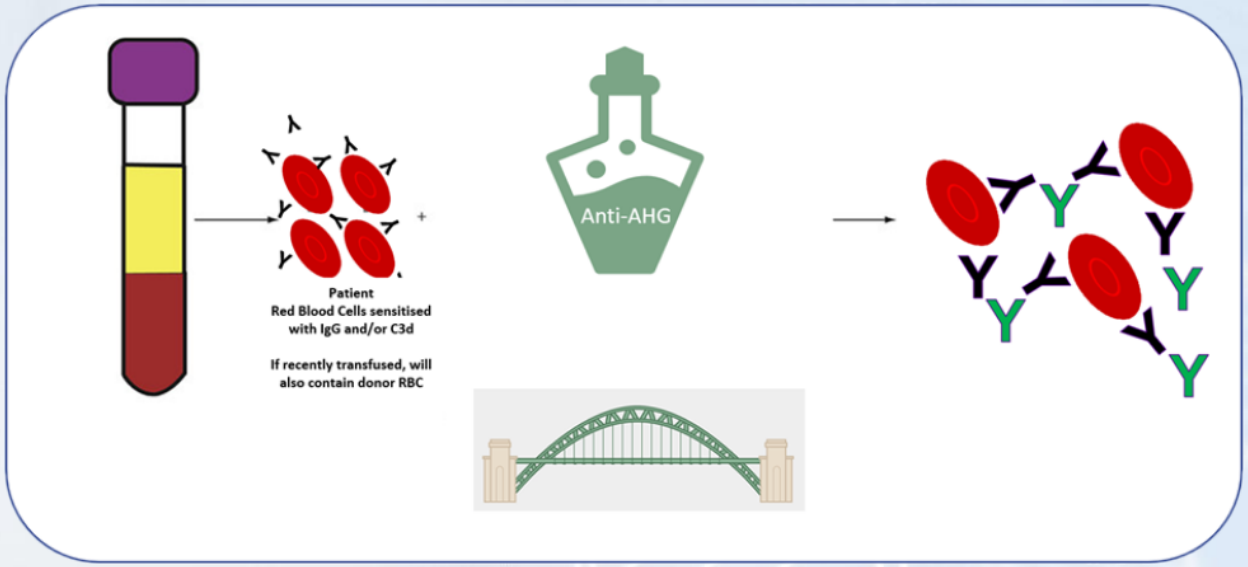
| Cell | Rh | D | C | E | c | e | M | N | S | s | P1 | Lu$^a$ | K | k | Kp$^a$ | Le$^a$ | Le$^b$ | Fy$^a$ | Fy$^b$ | Jk$^a$ | Jk$^b$ | Other | IAT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 5 | RoRo | + | 0 | 0 | + | + | 0 | + | 0 | + | 4 | 0 | 0 | + | 0 | 0 | + | + | 0 | 0 | + | | 2 |
| 6 | R1R1 | + | + | 0 | 0 | + | + | 0 | + | 0 | 4 | 0 | + | 0 | 0 | 0 | + | + | 0 | 0 | + | | 0 |

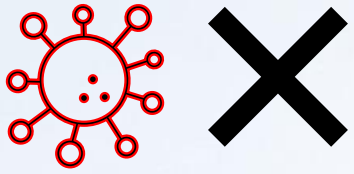
## NHSBT R2R2 panel

| Cell | Rh | D | C | E | c | e | M | N | S | s | P1 | Lu$^a$ | K | k | Kp$^a$ | Le$^a$ | Le$^b$ | Fy$^a$ | Fy$^b$ | Jk$^a$ | Jk$^b$ | Other | IAT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 5 | R2R2 | + | 0 | + | + | 0 | 0 | + | 0 | + | 4 | 0 | 0 | + | 0 | 0 | + | + | 0 | 0 | + | | 2 |
| 6 | R2R2 | + | 0 | + | + | 0 | + | 0 | + | 0 | 4 | 0 | + | 0 | 0 | 0 | + | + | 0 | 0 | + | | 0 |

# k Phenotype

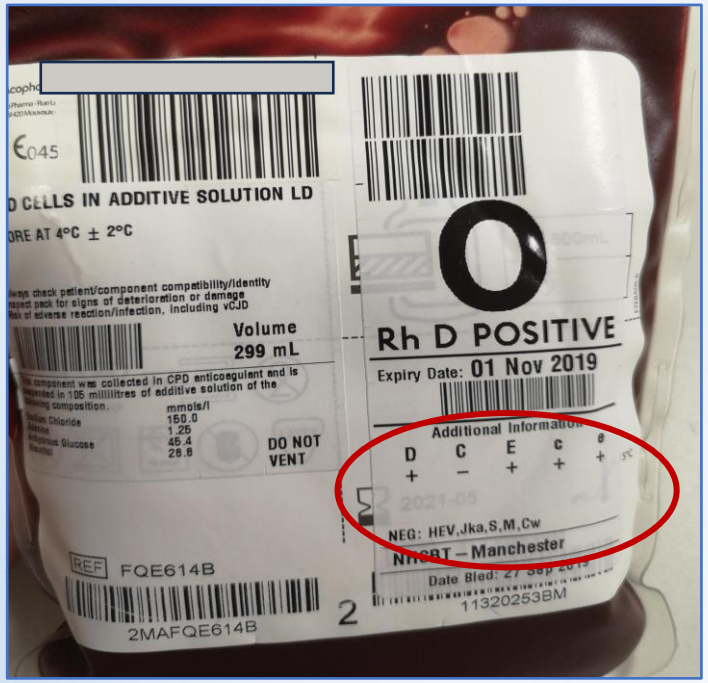
- Patient's phenotype:
  - C- c+ E+ e+ K+ k?
  - Jk(a-b+)
- Anti-k antisera is IgG
- Patient has 3+ IgG pos DAT – false positive
- Patient is also recently transfused – dual population of cells



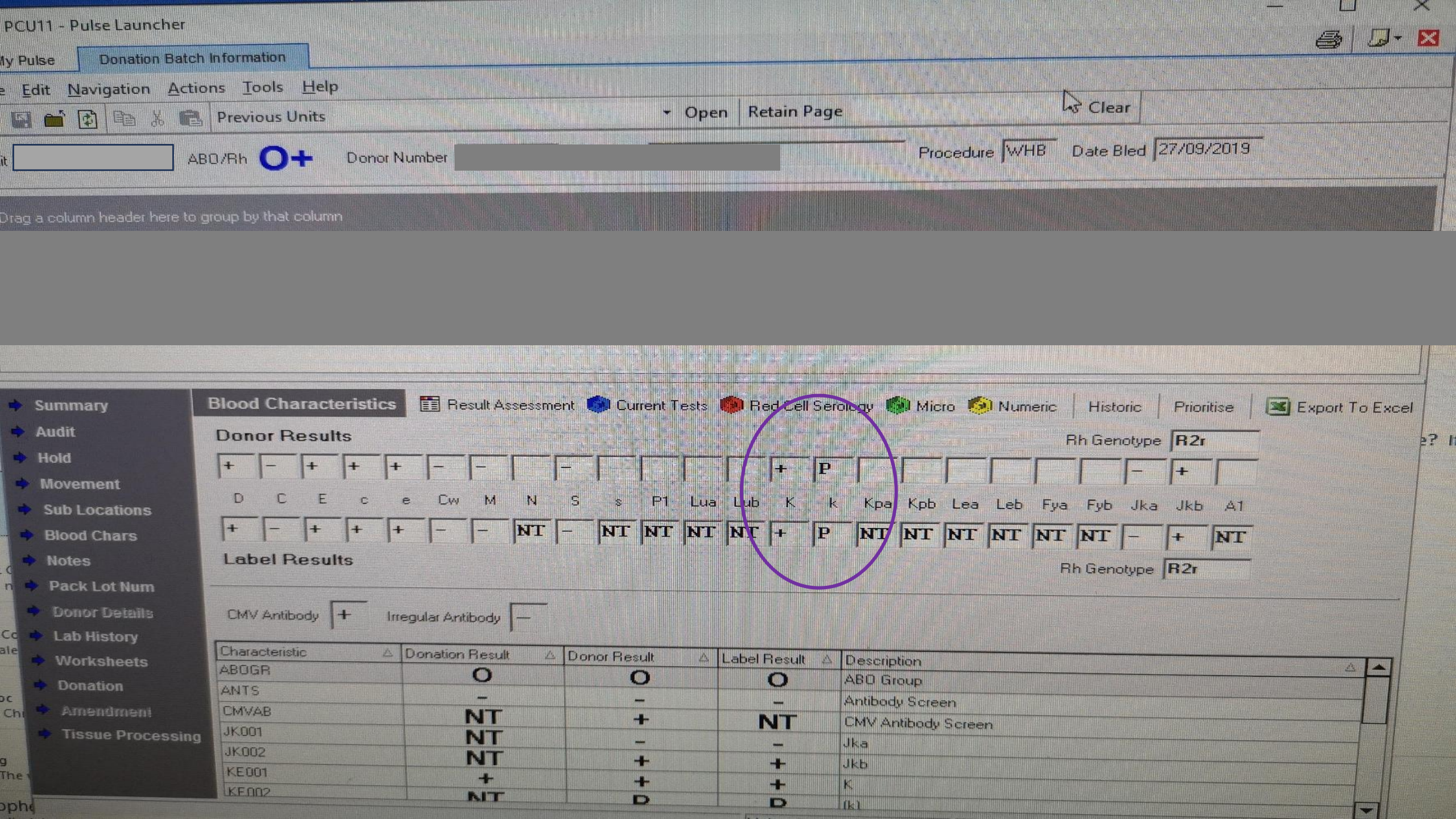
# Frequency Calculations (UK donors)

- Frequency of group O ~ 45%
- Frequency of C antigen ~ 68%
- Frequency of Jka antigen ~ 76%
- Frequency of k antigen ~ 9%




- C- RBC 32%
- Jk(a-) RBC 24%
- k- RBC 0.2%
- 2 units as requested:
- Random selection: 22,604
- Group O random selection: 13,020

- This was one of the units I was crossmatching
- Was it k-?


PCU11 - Pulse Launcher
My Pulse
Donation Batch Information
Edit Navigation Actions Tools Help
Previous Units
Open
Retain Page
Clear
ABO/Rh O+
Donor Number
Procedure WHB
Date Bled 27/09/2019
Drag a column header here to group by that column
Summary
Audit
Hold
Movement
Sub Locations
Blood Chars
Notes
Pack Lot Num
Donor Details
Lab History
Worksheets
Donation
Amendment
Tissue Processing
Blood Characteristics
Result Assessment
Current Tests
Red Cell Serology
Micro
Numeric
Historic
Prioritise
Export To Excel
Donor Results
Rh Genotype R2r
D C E c e Cw M N S s P1 Lua Lub K k Kpa Kpb Lea Leb Fya Fyb Jka Jkb A1
Label Results
Rh Genotype R2r
CMV Antibody +
Irregular Antibody –
Characteristic
Donation Result
Donor Result
Label Result
Description
ABOGR O O O ABO Group
ANTS – – – Antibody Screen
CMVAB NT + NT CMV Antibody Screen
JK001 NT – – Jka
JK002 NT + + Jkb
KE001 + + + K
KE002 NT D D (k)

Selection criteria locked whilst enquiry is active. To unlock you must close the enquiry results page using the close button on the enquiry

## Serology Search Products + -

Action | View Details | Print | Export To Excel | Option

<> 4 units found / 0 Selected

NT = Testing

Site / Location /

Exclude Blood Chars

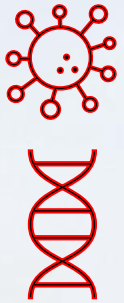
Show additional

Show test results

| Pack Details | | | Additional Information | | | Test Results | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | ABO/Rh | Donation | Product | Bled Date | Status | Preferred Loc | Cat. / C | Jka | (k) | Test Value |
| Site : T7 | | | | | | | | | | |
| Location : 0013 | | | | | | | | | | |
| | O- | [redacted] | 0054 | 06/11/2019 | V - Reserved | | H | - | - | - | 0 |
| | O- | [redacted] | 0054 | 05/11/2019 | V - Reserved | | H | - | - | - | 0 |
| Location : 0029 | | | | | | | | | | |
| | O- | [redacted] | 0054 | 12/11/2019 | S - Validated | | H | - | - | - | 0 |
| Site : W1 | | | | | | | | | | |
| Location : 0013 | | | | | | | | | | |
| | O- | [redacted] | 0054 | 08/11/2019 | V - Reserved | | M | - | - | N | 0 |

# Results 03/10/19

- Enzyme only Alloanti-C
- Previously reported Alloanti-Jka
- DAT 3+ IgG – eluate: anti-k and pan enzyme
- 2 units O, C- Jka- k- ordered and issued following day
- Updated antibody card issued



- Couldn't perform k phenotyping due to IgG pos DAT and recent transfusion
- Genotype performed on subsequent sample
- Confirmed as K+ k-

Mr Patient
DOB: xx/xx/xx

Group: O RhD Pos

Antibodies:

Anti-C, anti-Jka, anti-k

# Key Testing Tools – session 1

- Patient history
- Antibody screen
- Grading
- Antibody identification
- Inclusions and exclusions
- Antibody panel cells (IAT and enzyme)
- Antigram (panel sheet)
- Clinical significance of antibodies
- Phenotyping/genotyping
- Direct antiglobulin test
- Elution

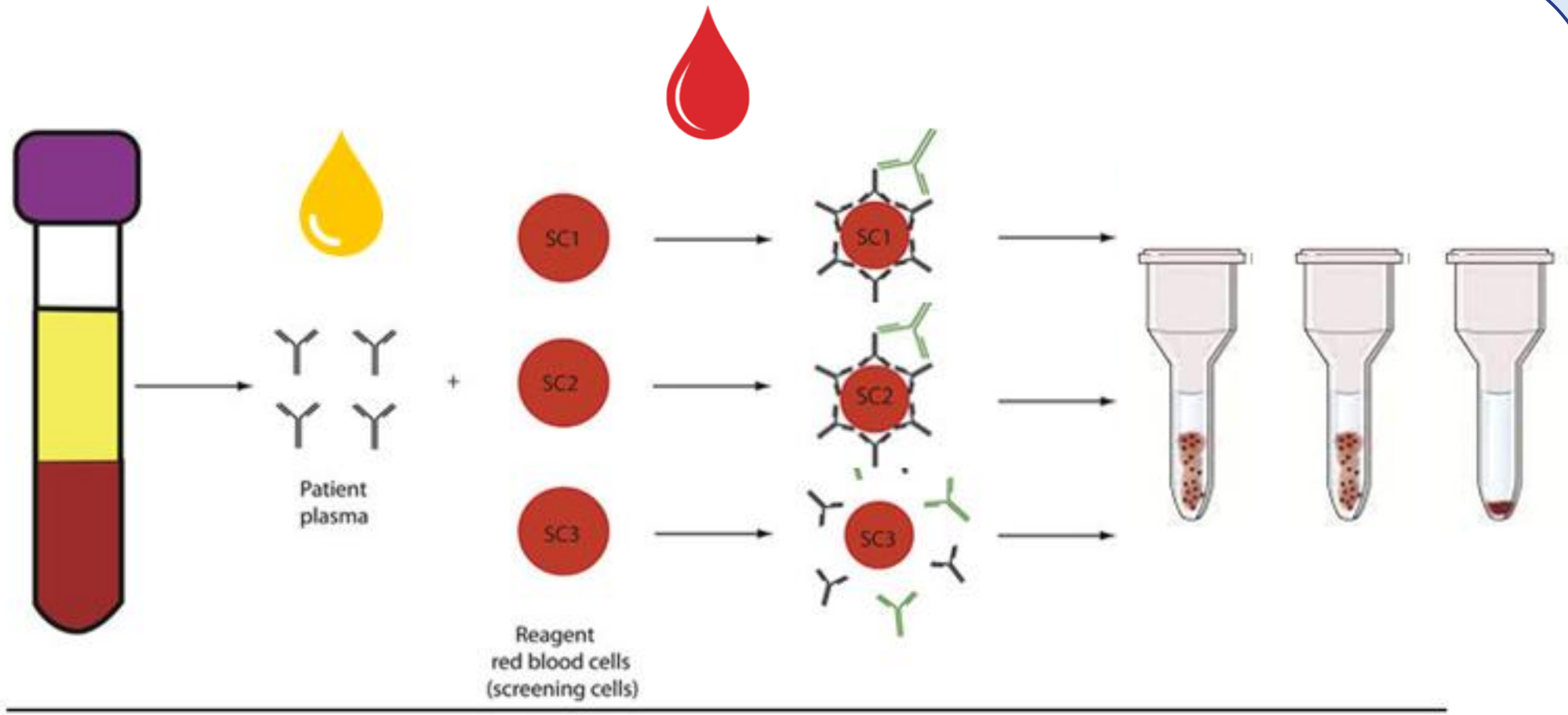
# Modification

- As well as using rare cells to identify high incidence antibodies, the red cells or plasma can be modified to assist ABID – can be used to stop interference from certain antibodies
- DTT – treated red cells - anti-CD38
- Neutralisation with AB serum – Chido/Rogers
- Inhibition with soluble recombinant proteins – Knops, anti-CD38
- Alloadsorption – pan-reactive autoantibodies, complex mixtures, separation

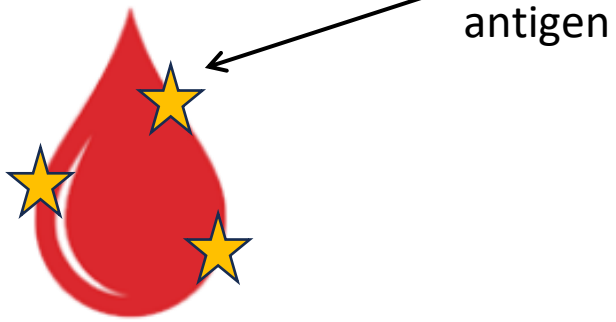
# DTT Treatment

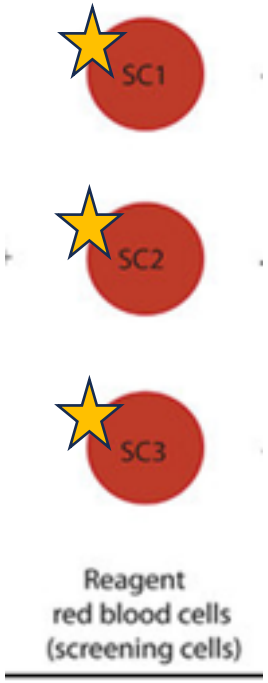
- Dithiothreitol (DTT)
- Reducing agent that breaks disulphide bridges
- Really useful in Blood Banking for ABID for patient's receiving anti-CD38 therapy
- When reagent red cells are treated with DTT, it disrupts the CD38 antigen structure
- Also denatures DTT sensitive antigens, such as Kell system

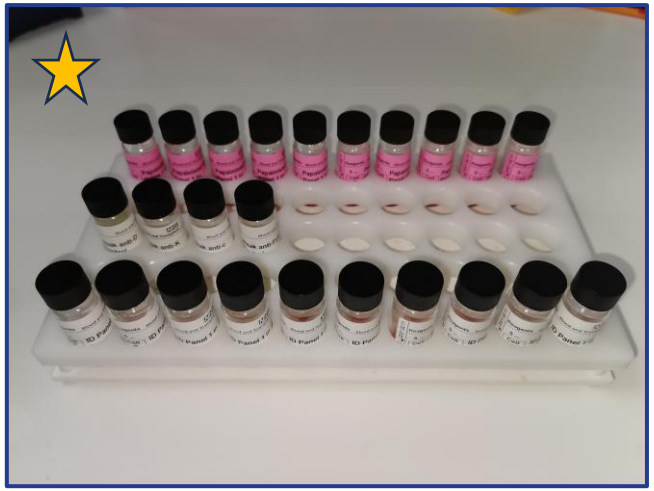
# Antibody Identification



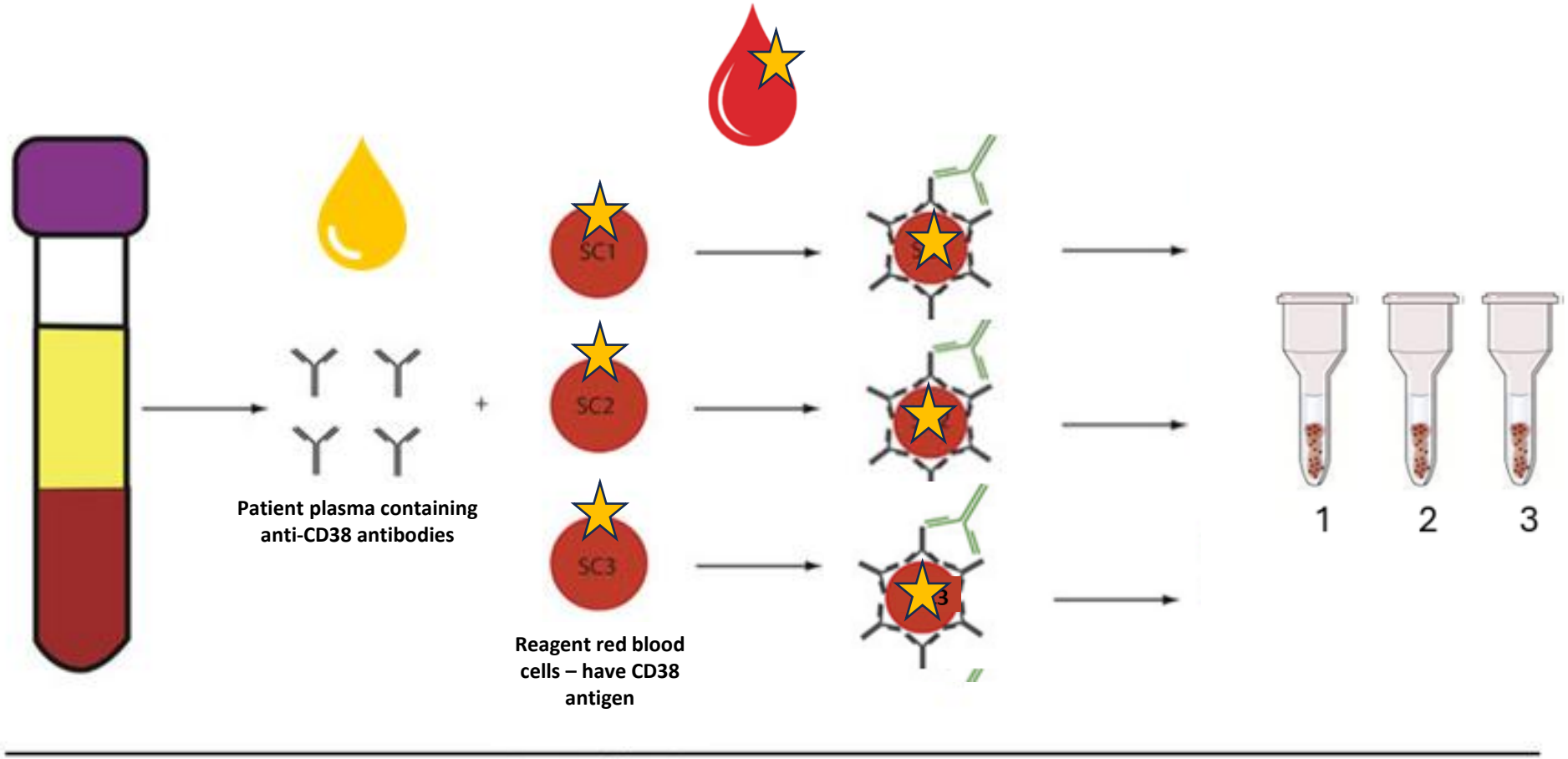
# Reagent red cells



# Anti-CD38 patient

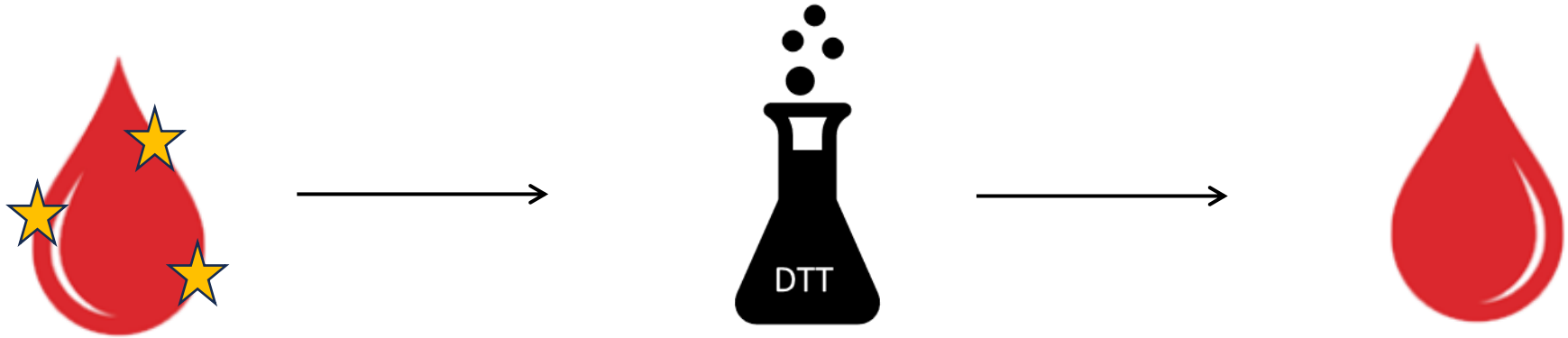



# Anti-CD38 - what you'd typically see

| Cell | Rh | D | C | E | c | e | M | N | S | s | P1 | $Lu^a$ | K | k | $Kp^a$ | $Le^a$ | $Le^b$ | $Fy^a$ | $Fy^b$ | $Jk^a$ | $Jk^b$ | Other | IAT | EIAT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | $R_1^wR_1$ | + | + | 0 | 0 | + | 0 | + | 0 | + | **3** | **0** | **0** | **+** | **0** | **0** | **+** | **0** | **+** | **0** | **+** | | 2 | 2 |
| **2** | $R_1R_1$ | + | + | 0 | 0 | + | + | 0 | + | 0 | **0** | **0** | **+** | **+** | **0** | **+** | **0** | **+** | **0** | **+** | **0** | | 2 | 2 |
| **3** | $R_2R_2$ | + | 0 | + | + | 0 | 0 | + | 0 | + | **2** | **0** | **0** | **+** | **0** | **0** | **+** | **0** | **+** | **+** | **0** | | 2 | 2 |
| **4** | r'r | 0 | + | 0 | + | + | + | 0 | 0 | + | **2** | **0** | **0** | **+** | **0** | **0** | **+** | **+** | **0** | **+** | **0** | | 2 | 2 |
| **5** | r''r | 0 | 0 | + | + | + | + | 0 | + | 0 | **0** | **0** | **0** | **+** | **0** | **0** | **+** | **+** | **0** | **0** | **+** | | 2 | 2 |
| **6** | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | + | **4** | **0** | **+** | **0** | **0** | **0** | **+** | **0** | **+** | **0** | **+** | | 2 | 2 |
| **7** | rr | 0 | 0 | 0 | + | + | 0 | + | + | 0 | **4** | **+** | **0** | **+** | **0** | **+** | **0** | **0** | **+** | **+** | **0** | | 2 | 2 |
| **8** | rr | 0 | 0 | 0 | + | + | + | 0 | + | 0 | **0** | **0** | **0** | **+** | **+** | **0** | **0** | **0** | **+** | **0** | **+** | | 2 | 2 |
| **9** | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | + | **0** | 0 | **0** | **+** | **0** | **+** | **0** | **+** | **0** | **0** | **+** | | 2 | 2 |
| **10** | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | + | **2** | | | | | | | | | | | | 2 | 2 |
| | | | | | | | | | | | | | | | | | | | | | | **Auto** | 0 | / |
| | | | | | | | | | | | | | | | | | | | | | | **K control** | **2** | / |

Pan-reactive antibody
Unable to see if there are underlying alloantibodies

# DTT treatment of RBC




Also removes DTT sensitive RBC antigens

# DTT panel - what you'd typically see

| Cell | Rh | D | C | E | c | e | M | N | S | s | P1 | Lu$^a$ | K | k | Kp$^a$ | Le$^a$ | Le$^b$ | Fy$^a$ | Fy$^b$ | Jk$^a$ | Jk$^b$ | Other | IAT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | $R_1^wR_1$ | + | + | 0 | 0 | + | 0 | + | 0 | + | **3** | **0** | **0** | **+** | **0** | **0** | **+** | **0** | **+** | **0** | **+** | | 0 |
| **2** | $R_1R_1$ | + | + | 0 | 0 | + | + | 0 | + | 0 | **0** | **0** | **+** | **+** | **0** | **+** | **0** | **+** | **0** | **+** | **0** | | 0 |
| **3** | $R_2R_2$ | + | 0 | + | + | 0 | 0 | + | 0 | + | **2** | **0** | **0** | **+** | **0** | **0** | **+** | **0** | **+** | **+** | **0** | | 0 |
| **4** | r'r | 0 | + | 0 | + | + | + | 0 | 0 | + | **2** | **0** | **0** | **+** | **0** | **0** | **+** | **+** | **0** | **+** | **0** | | 0 |
| **5** | r''r | 0 | 0 | + | + | + | + | 0 | + | 0 | **0** | **0** | **0** | **+** | **0** | **0** | **+** | **+** | **0** | **0** | **+** | | 0 |
| **6** | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | + | **4** | **0** | **+** | **0** | **0** | **0** | **+** | **0** | **+** | **0** | **+** | | 0 |
| **7** | rr | 0 | 0 | 0 | + | + | 0 | + | + | 0 | **4** | **+** | **0** | **+** | **0** | **+** | **0** | **0** | **+** | **+** | **0** | | 0 |
| **8** | rr | 0 | 0 | 0 | + | + | + | 0 | + | 0 | **0** | **0** | **0** | **+** | **+** | **0** | **0** | **0** | **+** | **0** | **+** | | 0 |
| **9** | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | + | **0** | 0 | **0** | **+** | **0** | **+** | **0** | **+** | **0** | **0** | **+** | | 0 |
| **10** | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | + | | | | | | | | | | | **0** | | 0 |
| | | | | | | | | | | | | | | | | | | | | | | **Auto** | 0 |
| | | | | | | | | | | | | | | | | | | | | | | **+ control** | **2** |

Pan-reactive antibody
No underlying alloantibodies
Cannot exclude Kell system antibodies

DTT

# Or it could be like this…

| Cell | Rh | D | C | E | c | e | M | N | S | s | P1 | $Lu^a$ | K | k | $Kp^a$ | $Le^a$ | $Le^b$ | $Fy^a$ | $Fy^b$ | $Jk^a$ | $Jk^b$ | Other | IAT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | $R_1^wR_1$ | + | + | 0 | 0 | + | 0 | + | 0 | + | **3** | **0** | **0** | **+** | **0** | **0** | **+** | **0** | **+** | **0** | **+** | | 2 |
| **2** | $R_1R_1$ | + | + | 0 | 0 | + | + | 0 | + | 0 | **0** | **0** | **+** | **+** | **0** | **+** | **0** | **+** | **0** | **+** | **0** | | 0 |
| **3** | $R_2R_2$ | + | 0 | + | + | 0 | 0 | + | 0 | + | **2** | **0** | **0** | **+** | **0** | **0** | **+** | **0** | **+** | **+** | **0** | | 2 |
| **4** | r'r | 0 | + | 0 | + | + | + | 0 | 0 | + | **2** | **0** | **0** | **+** | **0** | **0** | **+** | **+** | **0** | **+** | **0** | | 0 |
| **5** | r''r | 0 | 0 | + | + | + | + | 0 | + | 0 | **0** | **0** | **0** | **+** | **0** | **0** | **+** | **+** | **0** | **0** | **+** | | 0 |
| **6** | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | + | **4** | **0** | **+** | **0** | **0** | **0** | **+** | **0** | **+** | **0** | **+** | | 2 |
| **7** | rr | 0 | 0 | 0 | + | + | 0 | + | + | 0 | **4** | **+** | **0** | **+** | **0** | **+** | **0** | **0** | **+** | **+** | **0** | | 2 |
| **8** | rr | 0 | 0 | 0 | + | + | + | 0 | + | 0 | **0** | **0** | **0** | **+** | **+** | **0** | **0** | **0** | **+** | **0** | **+** | | 2 |
| **9** | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | + | **0** | 0 | **0** | **+** | **0** | **+** | **0** | **+** | **0** | **0** | **+** | | 0 |
| **10** | rr | 0 | 0 | 0 | + | | | | | | | | | | | | **+** | **+** | **0** | **+** | **0** | | 0 |
| | | | | | | | | | | | | | | | | | | | | | | **Auto** | 0 |
| | | | | | | | | | | | | | | | | | | | | | | **+ control** | **2** |

Pan-reactive antibody
Anti-$Fy^b$ underlying
Cannot exclude Kell system antibodies

# Blood selection

- BSH guidelines
- ABO, D and K compatible, extended Rh matched
- Antigen negative for any clinically significant alloantibodies
- Any special requirements for the patient

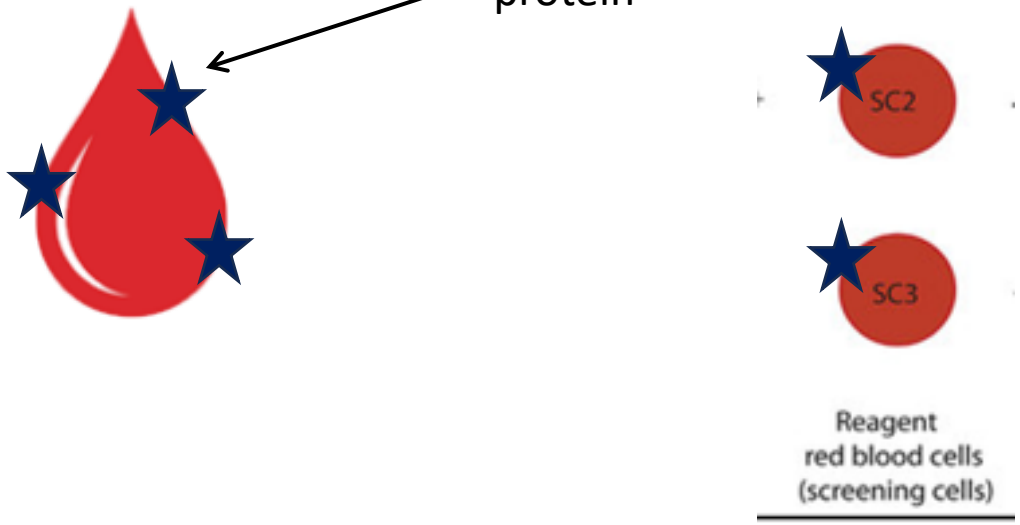
# Neutralisation/Inhibition

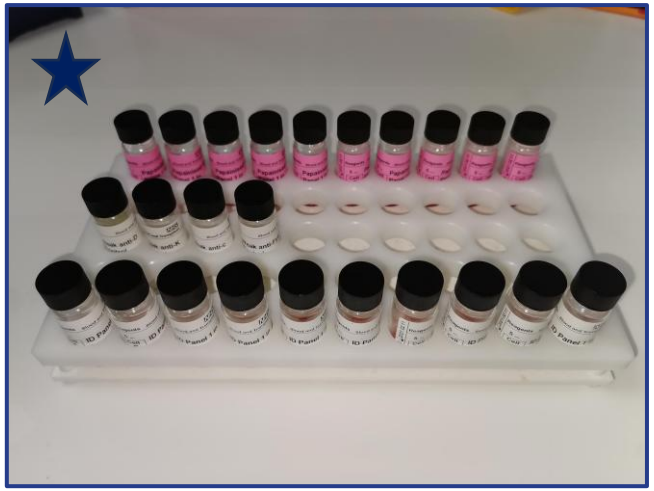
- Useful tool for ‘nuisance’ antibodies
- Patient serum or plasma is pre-incubated with reagent before the addition of the neutralised/inhibited patient sample to panel red blood cells
- Chido/Rogers (Ch/Rg)
  - Antibodies bind C4 complement proteins
  - Antibodies are neutralised by adding excess complement (AB serum)

# Neutralisation/Inhibition

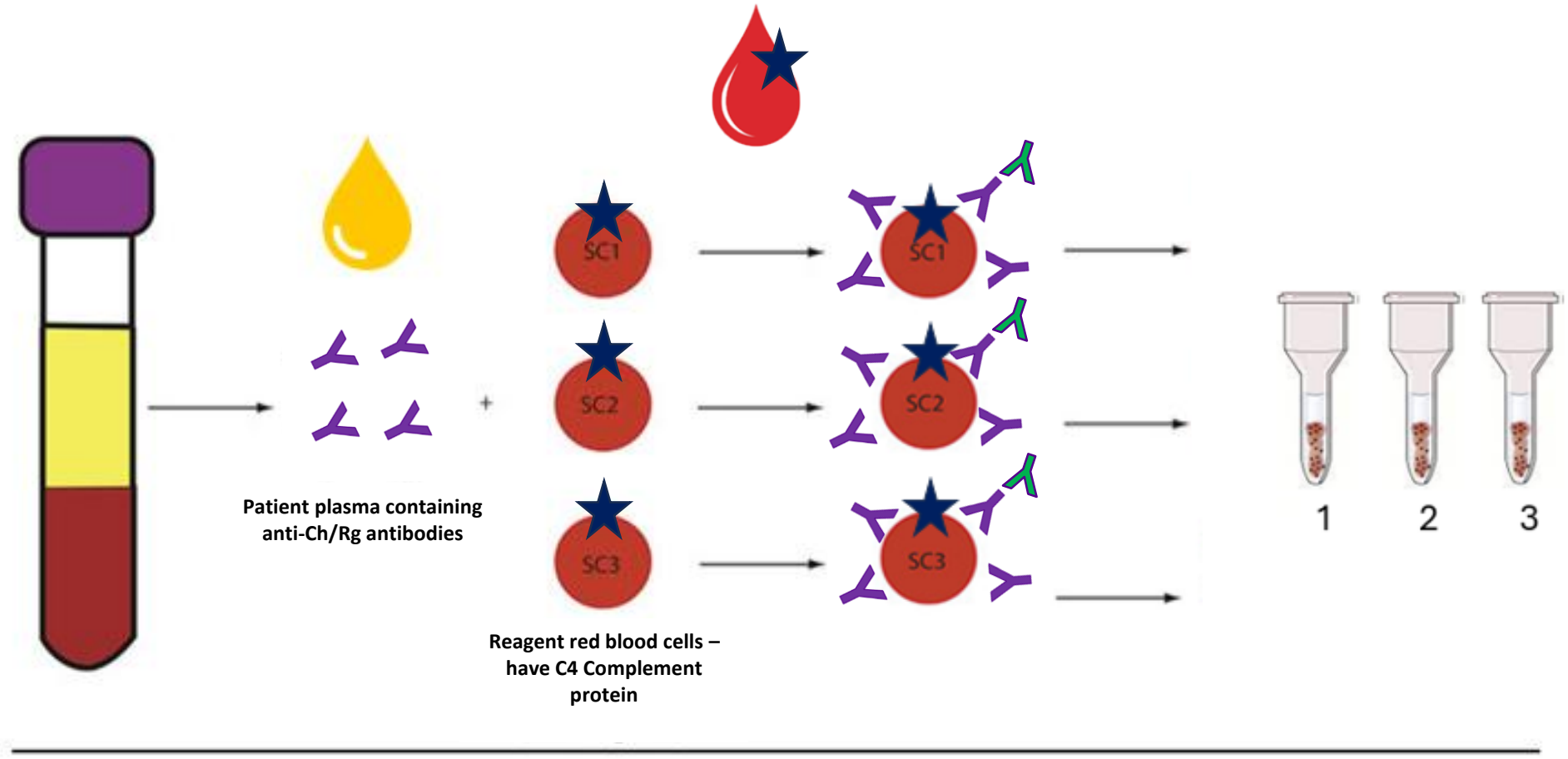
- Knops-McCoy
  - Antibodies bind complement receptor 1 (CR1)
  - Antibodies are inhibited by using soluble recombinant protein
  - Knops Inhibition Reagent (KNIR) - srCR1-LHR-C + srCR1-LHR-D
- CD38 monoclonal antibody therapy
  - Anti-CD38 binds CD38 antigen
  - Antibodies are inhibited by using soluble recombinant protein
  - srCD38
  - srCD38 has no reactivity with clinically significant red cell antibodies
  - Can therefore exclude DTT sensitive antigens (such as Kell system)

# Reagent red cells



# Anti-Ch/Rg


SC1
SC2
SC3
SC1
SC2
SC3
1
2
3
Patient plasma containing anti-Ch/Rg antibodies
Reagent red blood cells – have C4 Complement protein

# Anti-Ch/Rg - what you'd typically see

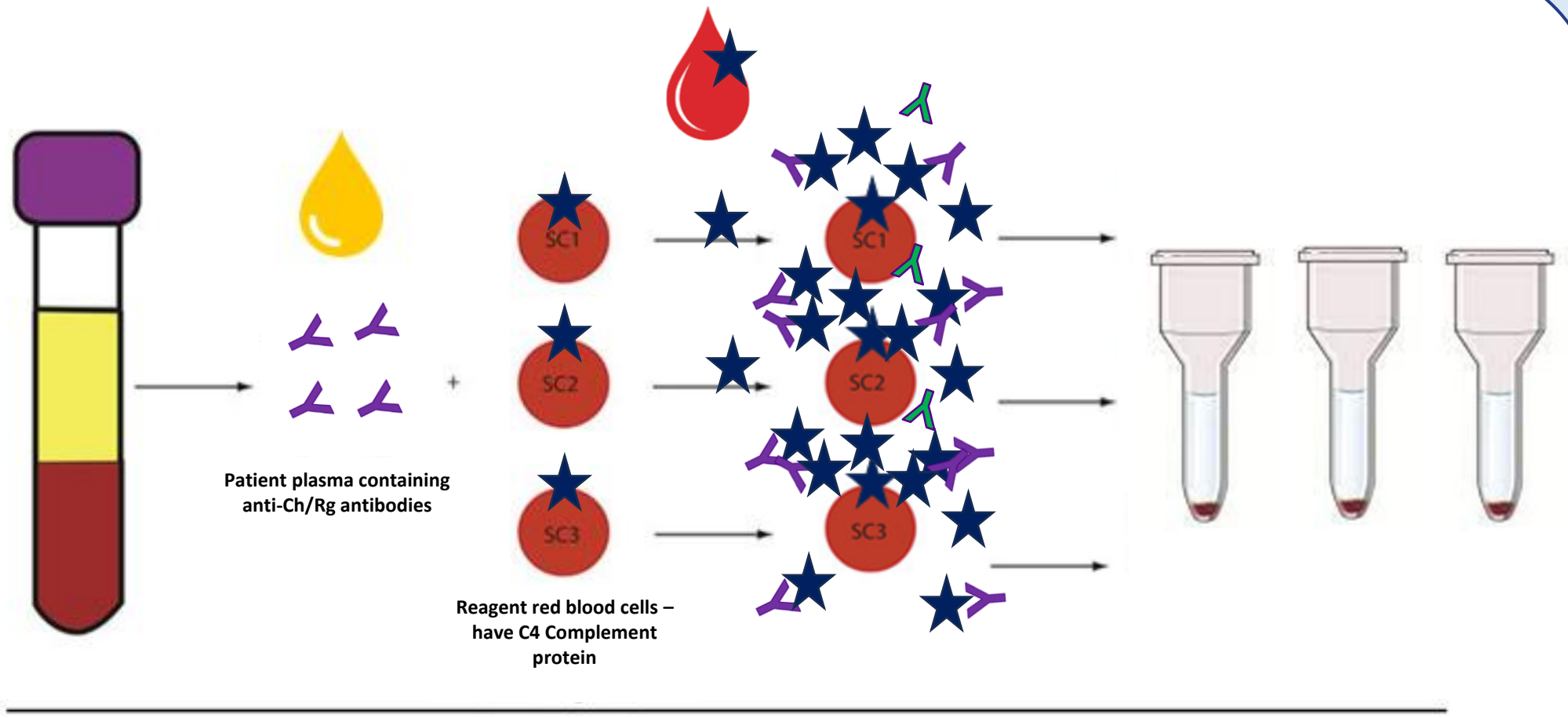
| Cell | Rh | D | C | E | c | e | M | N | S | s | P1 | Lu$^a$ | K | k | Kp$^a$ | Le$^a$ | Le$^b$ | Fy$^a$ | Fy$^b$ | Jk$^a$ | Jk$^b$ | Other | IAT | EIAT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | $R_1^wR_1$ | + | + | 0 | 0 | + | 0 | + | 0 | + | 3 | 0 | 0 | + | 0 | 0 | + | 0 | + | 0 | + | | 2 | 0 |
| 2 | $R_1R_1$ | + | + | 0 | 0 | + | + | 0 | + | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 0 | + | 0 | | 2 | 0 |
| 3 | $R_2R_2$ | + | 0 | + | + | 0 | 0 | + | 0 | + | 2 | 0 | 0 | + | 0 | 0 | + | 0 | + | + | 0 | | 2 | 0 |
| 4 | r'r | 0 | + | 0 | + | + | + | 0 | 0 | + | 2 | 0 | 0 | + | 0 | 0 | + | + | 0 | + | 0 | | 2 | 0 |
| 5 | r''r | 0 | 0 | + | + | + | + | 0 | + | 0 | 0 | 0 | 0 | + | 0 | 0 | + | + | 0 | 0 | + | | 2 | 0 |
| 6 | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 4 | 0 | + | 0 | 0 | 0 | + | 0 | + | 0 | + | | 2 | 0 |
| 7 | rr | 0 | 0 | 0 | + | + | 0 | + | + | 0 | 4 | + | 0 | + | 0 | + | 0 | 0 | + | + | 0 | | 2 | 0 |
| 8 | rr | 0 | 0 | 0 | + | + | + | 0 | + | 0 | 0 | 0 | 0 | + | + | 0 | 0 | 0 | + | 0 | + | | 2 | 0 |
| 9 | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 0 | 0 | 0 | + | 0 | + | 0 | + | 0 | 0 | + | | 2 | 0 |
| 10 | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 2 | | | | | | | | | | | | 2 | 0 |
| | | | | | | | | | | | | | | | | | | | | | | Auto | 0 | |
| | | | | | | | | | | | | | | | | | | | | | | K control | 2 | |

Pan-reactive antibody by IAT.
Unable to see if there are underlying alloantibodies

# AB Serum


Excess of C4 Complement protein neutralises Ch/Rg antibody

# Anti-Ch/Rg + AB serum



# AB Serum - what you'd typically see

| Cell | Rh | D | C | E | c | e | M | N | S | s | P1 | Lu$^a$ | K | k | Kp$^a$ | Le$^a$ | Le$^b$ | Fy$^a$ | Fy$^b$ | Jk$^a$ | Jk$^b$ | Other | IAT | AB serum | PBS | EIAT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | **$R_1^wR_1$** | + | + | 0 | 0 | + | 0 | + | 0 | + | **3** | **0** | **0** | **+** | **0** | **0** | **+** | **0** | **+** | **0** | **+** | | 2 | 0 | 2 | 0 |
| **2** | **$R_1R_1$** | + | + | 0 | 0 | + | + | 0 | + | 0 | **0** | **0** | **+** | **+** | **0** | **+** | **0** | **+** | **0** | **+** | **0** | | 2 | 0 | 2 | 0 |
| **3** | **$R_2R_2$** | + | 0 | + | + | 0 | 0 | + | 0 | + | **2** | **0** | **0** | **+** | **0** | **0** | **+** | **0** | **+** | **+** | **0** | | 2 | 0 | 2 | 0 |
| **4** | **r'r** | 0 | + | 0 | + | + | + | 0 | 0 | + | **2** | **0** | **0** | **+** | **0** | **0** | **+** | **+** | **0** | **+** | **0** | | 2 | 0 | 2 | 0 |
| **5** | **r''r** | 0 | 0 | + | + | + | + | 0 | + | 0 | **0** | **0** | **0** | **+** | **0** | **0** | **+** | **+** | **0** | **0** | **+** | | 2 | 0 | 2 | 0 |
| **6** | **rr** | 0 | 0 | 0 | + | + | 0 | + | 0 | + | **4** | **0** | **+** | **0** | **0** | **0** | **+** | **0** | **+** | **0** | **+** | | 2 | 0 | 2 | 0 |
| **7** | **rr** | 0 | 0 | 0 | + | + | 0 | + | + | 0 | **4** | **+** | **0** | **+** | **0** | **+** | **0** | **0** | **+** | **+** | **0** | | 2 | 0 | 2 | 0 |
| **8** | **rr** | 0 | 0 | 0 | + | + | + | 0 | + | 0 | **0** | **0** | **0** | **+** | **+** | **0** | **0** | **0** | **+** | **0** | **+** | | 2 | 0 | 2 | 0 |
| **9** | **rr** | 0 | 0 | 0 | + | + | 0 | + | 0 | + | **0** | 0 | **0** | **+** | **0** | **+** | **0** | **+** | **0** | **0** | **+** | | 2 | 0 | 2 | 0 |
| **10** | **rr** | 0 | 0 | 0 | + | + | 0 | + | 0 | + | **2** | **+** | **0** | **+** | **0** | **0** | **+** | **+** | **0** | **+** | **0** | | 2 | 0 | 2 | 0 |
| | | | | | | | | | | | | | | | | | | | | | | **Auto** | 0 | / | / | / |
| | | | | | | | | | | | | | | | | | | | | | | **K control** | **2** | / | / | / |

Anti-Ch/Rg is neutralised. PBS control used to show negativity is not due to dilution

AB Serum

# Soluble recombinant proteins

# Anti-CR1 (Knops) - what you'd typically see

| Cell | Rh | D | C | E | c | e | M | N | S | s | P1 | Lu$^a$ | K | k | Kp$^a$ | Le$^a$ | Le$^b$ | Fy$^a$ | Fy$^b$ | Jk$^a$ | Jk$^b$ | Other | IAT | EIAT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | $R_1^wR_1$ | + | + | 0 | 0 | + | 0 | + | 0 | + | 3 | 0 | 0 | + | 0 | 0 | + | 0 | + | 0 | + | | 2 | 3 |
| 2 | $R_1R_1$ | + | + | 0 | 0 | + | + | 0 | + | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 0 | + | 0 | | 2 | 3 |
| 3 | $R_2R_2$ | + | 0 | + | + | 0 | 0 | + | 0 | + | 2 | 0 | 0 | + | 0 | 0 | + | 0 | + | + | 0 | | 2 | 3 |
| 4 | r'r | 0 | + | 0 | + | + | + | 0 | 0 | + | 2 | 0 | 0 | + | 0 | 0 | + | + | 0 | + | 0 | | 2 | 3 |
| 5 | r''r | 0 | 0 | + | + | + | + | 0 | + | 0 | 0 | 0 | 0 | + | 0 | 0 | + | + | 0 | 0 | + | | 2 | 3 |
| 6 | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 4 | 0 | + | 0 | 0 | 0 | + | 0 | + | 0 | + | | 2 | 3 |
| 7 | rr | 0 | 0 | 0 | + | + | 0 | + | + | 0 | 4 | + | 0 | + | 0 | + | 0 | 0 | + | + | 0 | | 2 | 3 |
| 8 | rr | 0 | 0 | 0 | + | + | + | 0 | + | 0 | 0 | 0 | 0 | + | + | 0 | 0 | 0 | + | 0 | + | | 2 | 3 |
| 9 | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 0 | 0 | 0 | + | 0 | + | 0 | + | 0 | 0 | + | | 2 | 3 |
| 10 | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 2 | | | | | | | | | | | | 2 | 3 |
| | | | | | | | | | | | | | | | | | | | | | | **Auto** | 0 | |
| | | | | | | | | | | | | | | | | | | | | | | **K control** | **2** | |

Pan-reactive antibody
Unable to see if there are underlying alloantibodies

# KNIR- what you'd typically see

| Cell | Rh | D | C | E | c | e | M | N | S | s | P1 | Lu$^a$ | K | k | Kp$^a$ | Le$^a$ | Le$^b$ | Fy$^a$ | Fy$^b$ | Jk$^a$ | Jk$^b$ | Other | IAT | EIAT | KNIR | PBS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | **$R_1^wR_1$** | + | + | 0 | 0 | + | 0 | + | 0 | + | **3** | **0** | **0** | + | **0** | **0** | + | **0** | + | **0** | + | | 2 | 3 | 0 | 2 |
| **2** | **$R_1R_1$** | + | + | 0 | 0 | + | + | 0 | + | 0 | **0** | **0** | + | + | **0** | + | **0** | + | **0** | + | **0** | | 2 | 3 | 0 | 2 |
| **3** | **$R_2R_2$** | + | 0 | + | + | 0 | 0 | + | 0 | + | **2** | **0** | **0** | + | **0** | **0** | + | **0** | + | + | **0** | | 2 | 3 | 0 | 2 |
| **4** | **r'r** | 0 | + | 0 | + | + | + | 0 | 0 | + | **2** | **0** | **0** | + | **0** | **0** | + | + | **0** | + | **0** | | 2 | 3 | 0 | 2 |
| **5** | **r''r** | 0 | 0 | + | + | + | + | 0 | + | 0 | **0** | **0** | **0** | + | **0** | **0** | + | + | **0** | **0** | + | | 2 | 3 | 0 | 2 |
| **6** | **rr** | 0 | 0 | 0 | + | + | 0 | + | 0 | + | **4** | **0** | + | **0** | **0** | **0** | + | **0** | + | **0** | + | | 2 | 3 | 0 | 2 |
| **7** | **rr** | 0 | 0 | 0 | + | + | 0 | + | + | 0 | **4** | + | **0** | + | **0** | + | **0** | **0** | + | + | **0** | | 2 | 3 | 0 | 2 |
| **8** | **rr** | 0 | 0 | 0 | + | + | + | 0 | + | 0 | **0** | **0** | **0** | + | + | **0** | **0** | **0** | + | **0** | + | | 2 | 3 | 0 | 2 |
| **9** | **rr** | 0 | 0 | 0 | + | + | 0 | + | 0 | + | **0** | 0 | **0** | + | **0** | + | **0** | + | **0** | **0** | + | | 2 | 3 | 0 | 2 |
| **10** | **rr** | 0 | 0 | 0 | + | + | 0 | + | 0 | + | **2** | + | | | | | | | | | | | 2 | 3 | 0 | 2 |
| | | | | | | | | | | | | | | | | | | | | | | | 0 | / | / | / |
| | | | | | | | | | | | | | | | | | | | | | | | **2** | / | / | / |

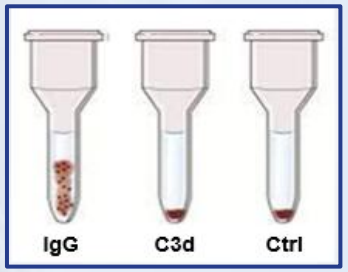
Anti-Knops-McCoy neutralised. PBS control used to show negativity is not due to dilution

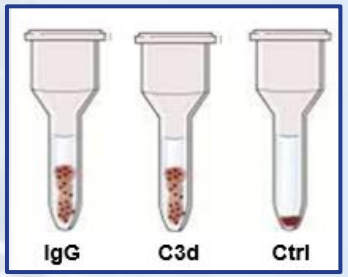
# Alloadsorption

- Time to introduce Spongebob alloadsorption pants!

# Alloadsorption

- Interpretation of positive DATs must include the patient's history, clinical data, and the results of other laboratory tests
- Auto Immune Haemolytic Anaemia (AIHA)
- Pan-reactive antibody
- Positive auto control
- DAT: pos IgG +/-C3d
- May need to perform an alloadsorption
- Must understand antibody/antigens interactions

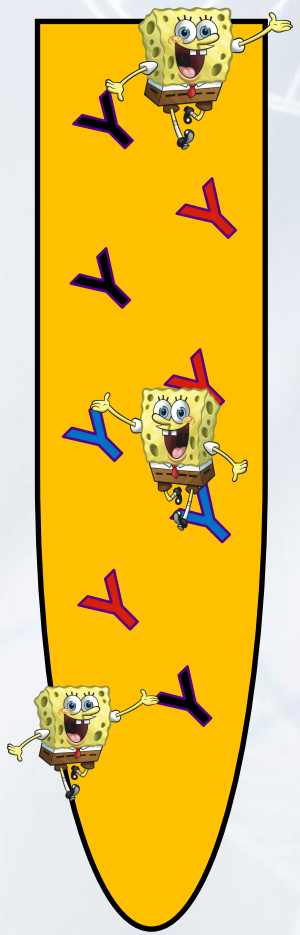



# Pan reactive - what you'd typically see

| Cell | Rh | D | C | E | c | e | M | N | S | s | P1 | Lu$^a$ | K | k | Kp$^a$ | Le$^a$ | Le$^b$ | Fy$^a$ | Fy$^b$ | Jk$^a$ | Jk$^b$ | Other | IAT | EIAT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | $R_1^wR_1$ | + | + | 0 | 0 | + | + | 0 | + | 0 | 0 | 0 | 0 | + | 0 | 0 | + | + | 0 | + | 0 | | 4 | 5 |
| 2 | $R_1R_1$ | + | + | 0 | 0 | + | 0 | + | 0 | + | 0 | 0 | + | + | 0 | + | 0 | 0 | + | 0 | + | | 4 | 5 |
| 3 | $R_2R_2$ | + | 0 | + | + | 0 | + | 0 | + | 0 | 0 | 0 | 0 | + | 0 | + | 0 | 0 | + | + | + | | 4 | 5 |
| 4 | r'r | 0 | + | 0 | + | + | 0 | + | + | 0 | 0 | 0 | 0 | + | 0 | 0 | + | 0 | + | + | 0 | | 4 | 5 |
| 5 | r''r | 0 | 0 | + | + | + | 0 | + | 0 | + | 4 | 0 | 0 | + | 0 | 0 | + | + | 0 | 0 | + | | 4 | 5 |
| 6 | rr | 0 | 0 | 0 | + | + | | | | | | | | 0 | 0 | 0 | + | + | 0 | 0 | + | | 4 | 5 |
| 7 | rr | 0 | 0 | 0 | + | + | | | | | | | | + | 0 | + | 0 | 0 | + | + | 0 | | 4 | 5 |
| 8 | rr | 0 | 0 | 0 | + | + | | | | | | | | + | + | 0 | + | 0 | + | 0 | + | | 4 | 5 |
| 9 | rr | 0 | 0 | 0 | + | + | + | 0 | 0 | + | 2 | 0 | 0 | + | 0 | + | 0 | + | 0 | 0 | + | | 4 | 5 |
| 10 | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 3 | 0 | 0 | + | 0 | + | 0 | + | 0 | + | 0 | | 4 | 5 |
| | | | | | | | | | | | | | | | | | | | | | | Auto | 4 | / |
| | | | | | | | | | | | | | | | | | | | | | | + control | 2 | / |

Are there any underlying alloantibodies?

# How does Alloadsorption work?

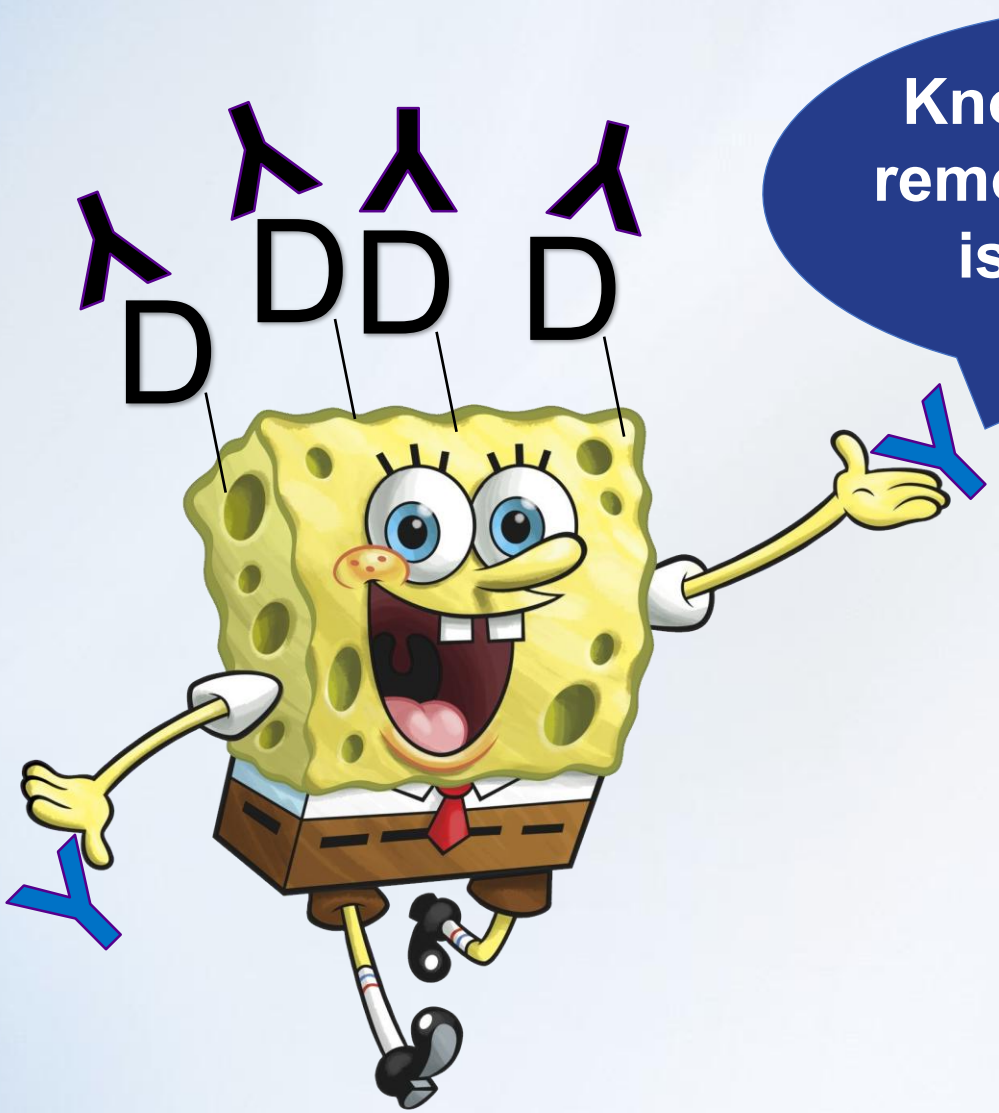




**Y – anti-D**

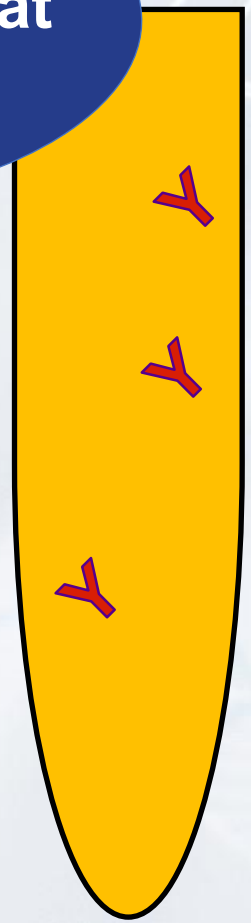
**Y – anti-Fy$^{a}$**

**Y – Autoantibody**

# How does Alloadsorption work?




**Knowing what is removed and what is left behind**




**Y – anti-D**

**Y – anti-Fy$^{a}$**

**Y – Autoantibody**

# Absorption Cell Profiles

| Rh | C | D | E | c | e | $C^w$ | K | $Jk^a$ | $Jk^b$ |
|---|---|---|---|---|---|---|---|---|---|
| $R_1R_1$ | + | + | 0 | 0 | + | 0 | 0 | + | 0 |
| rr | 0 | 0 | 0 | + | + | 0 | + | 0 | + |

MN Ss Fya Fyb?

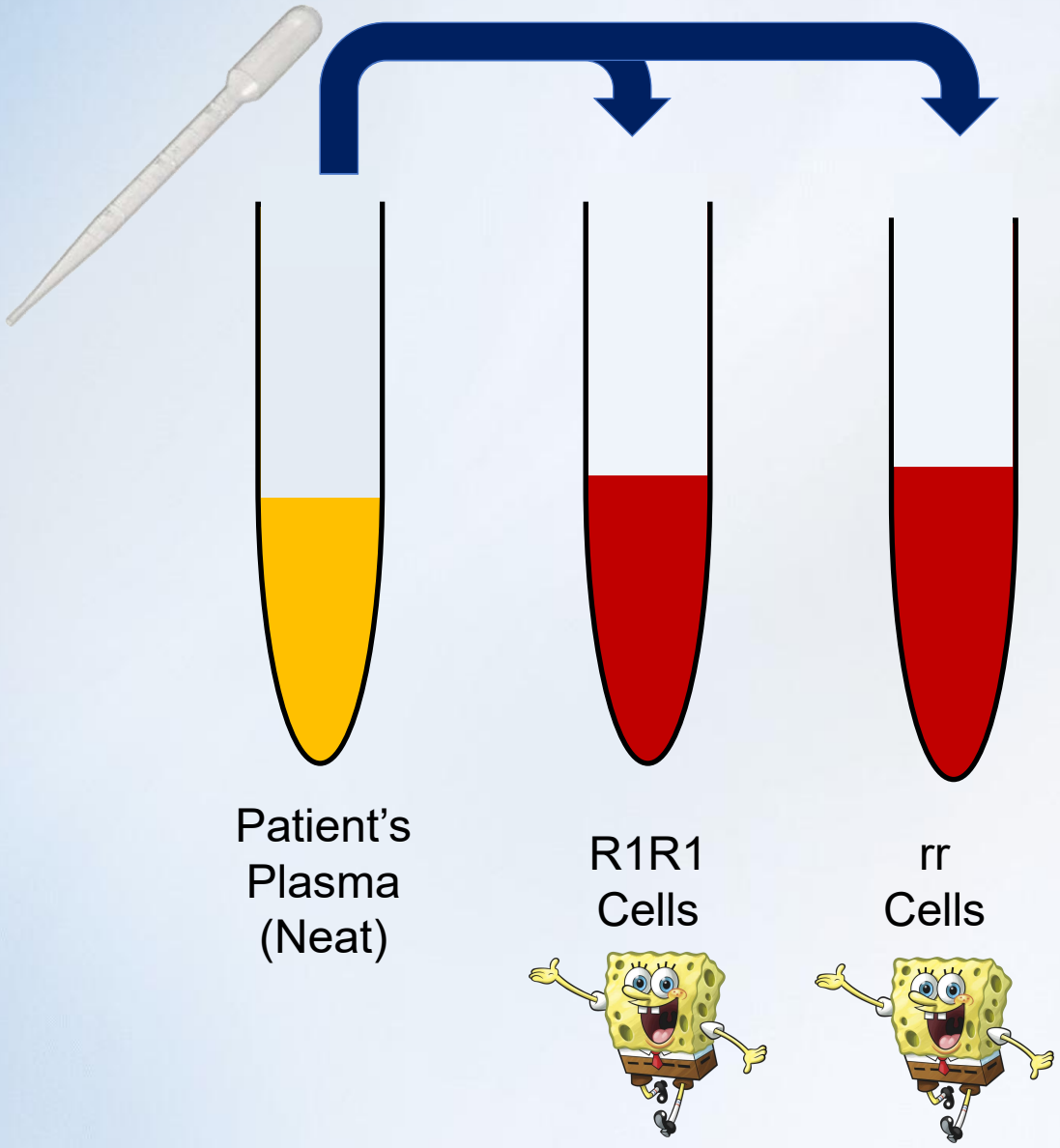
Destroyed by papain

Effectively antigen neg

Useful to know patient's extended Rh phenotype

R2R2 alloadsorption cell is available

# How to do it




Sequential double adsorptions with *papainised* cells

Mix patients plasma with R1R1 papainised RBCs (D+)

Mix patients plasma with rr papainised RBCs (D-)

Incubate for 10 mins at 37°C
Can add a cold step if required
Centrifuge
Remove plasma – save each aliquot
Repeat adsorptions using each of the absorbed plasma – R1R1 and rr

Antigen positive absorptions cells remove the corresponding antibody from the patient's plasma, as well as autoantibody

# What you end up with….

Neat Plasma

Haven't adsorbed

Contains pan-reactive autoantibody
Plus alloantibodies?

R1R1 (D+ C+ e+)
Adsorbed Plasma

Have adsorbed out pan-auto
Plus anti-D and -C and -e if they were present
Plus others depending on cell profile

Could contain alloantibodies

rr (D- c+ e+)
Adsorbed Plasma

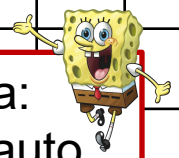
Have adsorbed out pan-auto
Plus anti-c and -e if they were present
Plus others depending on cell profile

Could contain alloantibodies

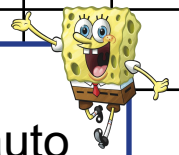
| Rh | C | D | E | c | e | $C^w$ | K | $Jk^a$ | $Jk^b$ |
|---|---|---|---|---|---|---|---|---|---|
| $R_1R_1$ | + | + | 0 | 0 | + | 0 | 0 | + | 0 |

# ABID 1

| Cell | Rh | D | C | E | c | e | M | N | S | s | P1 | Lu$^a$ | K | k | Kp$^a$ | Le$^a$ | Le$^b$ | Fy$^a$ | Fy$^b$ | Jk$^a$ | Jk$^b$ | Other | Neat | R1R1 | rr |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | $R_1^wR_1$ | + | + | 0 | 0 | + | + | 0 | + | 0 | 0 | 0 | 0 | + | 0 | | | | | | | | 4 | 0 | 0 |
| 2 | $R_1R_1$ | + | + | 0 | 0 | + | 0 | + | 0 | + | 0 | 0 | + | + | 0 | | | | | | | | 4 | 0 | 0 |
| 3 | $R_2R_2$ | + | 0 | + | + | 0 | + | 0 | + | 0 | 0 | 0 | 0 | + | 0 | | | | | | | | 4 | 0 | 0 |
| 4 | r'r | 0 | + | 0 | + | + | 0 | + | + | 0 | 0 | 0 | 0 | + | 0 | 0 | + | 0 | + | + | 0 | | 4 | 0 | 0 |
| 5 | r''r | 0 | 0 | + | + | + | 0 | + | 0 | + | 4 | 0 | 0 | + | 0 | | | | | | | | 4 | 0 | 0 |
| 6 | rr | 0 | 0 | 0 | + | + | + | 0 | + | 0 | 4 | 0 | + | 0 | 0 | | | | | | | | 4 | 0 | 0 |
| 7 | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 2 | + | + | + | 0 | | | | | | | | 4 | 0 | 0 |
| 8 | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 0 | + | | 4 | 0 | 0 |
| 9 | rr | 0 | 0 | 0 | + | + | + | 0 | 0 | + | 2 | 0 | 0 | + | 0 | + | 0 | + | 0 | 0 | + | | 4 | 0 | 0 |
| | | | | | | | | | | | | | 0 | + | 0 | + | 0 | + | 0 | + | 0 | | 4 | 0 | 0 |
| | | | | | | | | | | | | | | | | | | | | | | Auto | 4 | / | / |
| | | | | | | | | | | | | | | | | | | | | | | + control | 2 | / | / |



R1R1 plasma:
Removed pan-auto
Nothing left behind



rr plasma:
Removed pan-auto
Nothing left behind

Pan-reactive autoantibody
No Alloantibodies detected in the alloadsorbed plasma

# Blood Selection

BSH Guidelines state “Serological investigations in AIHA should focus on determination of the correct ABO, CcDEe and K status of the patient, and determination of the possible presence of an underlying alloantibody”

- ABO, D and extended RhK matched
  - To prevent alloimmunisation
- Antigen negative for alloantibodies
- Suitable – the neat crossmatch is positive due to the autoantibody
- May absorb out a high incidence antibody

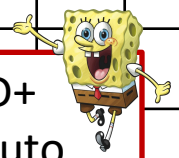
| | Neat Plasma | R1R1 plasma | rr plasma |
|---|---|---|---|
| Donation G072 421 111 111 2 | 4 | 0 | 0 |
| Auto | 4 | / | / |
| + control | 2 | / | / |

https://b-s-h.org.uk/guidelines/guidelines/pre-transfusion-compatibility-procedures-in-blood-transfusion-laboratories/

# ABID 2

| Cell | Rh | D | C | E | c | e | M | N | S | s | P1 | Lu$^a$ | K | k | Kp$^a$ | Le$^a$ | Le$^b$ | Fy$^a$ | Fy$^b$ | Jk$^a$ | Jk$^b$ | Other | Neat | R1R1 | rr |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | $R_1^wR_1$ | + | + | 0 | 0 | + | + | 0 | + | 0 | 0 | 0 | 0 | + | 0 | | | | | | | | 4 | 0 | 3 |
| 2 | $R_1R_1$ | + | + | 0 | 0 | + | 0 | + | 0 | + | 0 | 0 | + | + | 0 | | | | | | | | 4 | 0 | 3 |
| 3 | $R_2R_2$ | + | | | | | | | | | | | | | | | | | | | | | 4 | 0 | 3 |
| 4 | r'r | 0 | | | | | | | | | | | | | | | | | | | | | 4 | 0 | 0 |
| 5 | r''r | 0 | | | | | | | | | | | | | | | | | | | | | 4 | 0 | 0 |
| 6 | rr | 0 | | | | | | | | | | | | | | | | | | | | | 4 | 0 | 0 |
| 7 | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 2 | + | + | + | 0 | | | | | | | | 4 | 0 | 0 |
| 8 | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 0 | 0 | 0 | + | + | | | | | | | | 4 | 0 | 0 |
| 9 | rr | 0 | 0 | 0 | + | + | + | 0 | 0 | + | 2 | 0 | 0 | + | 0 | + | 0 | + | 0 | 0 | + | | 4 | 0 | 0 |
| | | | | | | | | | | | | | 0 | + | 0 | + | 0 | + | 0 | + | 0 | | 4 | 0 | 0 |
| | | | | | | | | | | | | | | | | | | | | | | Auto | 4 | / | / |
| | | | | | | | | | | | | | | | | | | | | | | + control | 2 | / | / |

| Rh | C | D | E | c | e | C$^w$ | K | Jk$^a$ | Jk$^b$ |
|---|---|---|---|---|---|---|---|---|---|
| $R_1R_1$ | + | + | 0 | 0 | + | 0 | 0 | + | 0 |
| rr | 0 | 0 | 0 | + | + | 0 | + | 0 | + |



R1R1 plasma D+
Removed pan-auto
removed Anti-D

Cannot remove anti-D
Anti-D left behind

Pan-reactive autoantibody
Anti-D detected in the alloadsorbed plasma
(allo/auto/non-spec?)

# ABID 3

| Cell | Rh | D | C | E | c | e | M | N | S | s | P1 | Lu$^a$ | K | k | Kp$^a$ | Le$^a$ | Le$^b$ | Fy$^a$ | Fy$^b$ | Jk$^a$ | Jk$^b$ | Other | Neat | R1R1 | rr |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | $R_1^wR_1$ | + | + | 0 | 0 | + | + | 0 | + | 0 | 0 | 0 | 0 | + | 0 | | | | | | | | 4 | 2 | 2 |
| 2 | $R_1R_1$ | + | + | 0 | 0 | + | 0 | + | 0 | + | 0 | 0 | + | + | 0 | | | | | | | | 4 | 0 | 0 |
| 3 | R R | + | 0 | + | + | 0 | + | 0 | + | 0 | 0 | 0 | 0 | + | 0 | | | | | | | | 4 | 0 | 0 |
| 4 | | | | | | | | | | | | | | | | | | | | | | | 4 | 0 | 0 |
| 5 | | | | | | | | | | | | | | | | | | | | | | | 4 | 2 | 2 |
| 6 | | | | | | | | | | | | | | | | | | | | | | | 4 | 2 | 2 |
| 7 | | | | | | | | | | | | | | | | | | | | | | | 4 | 0 | 0 |
| 8 | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 0 | 0 | 0 | + | + | | | | | | | | 4 | 0 | 0 |
| 9 | | | | | | | | | | | | | 0 | + | 0 | + | 0 | + | 0 | 0 | + | | 4 | 2 | 2 |
| 10 | | | | | | | | | | | | | 0 | + | 0 | + | 0 | + | 0 | + | 0 | | 4 | 2 | 2 |
| | | | | | | | | | | | | | | | | | | | | | | Auto | 4 | / | / |
| | | | | | | | | | | | | | | | | | | | | | | + control | 2 | / | / |

| Rh | C | D | E | c | e | C$^w$ |
|---|---|---|---|---|---|---|
| $R_1R_1$ | + | + | 0 | 0 | + | 0 |
| rr | 0 | 0 | 0 | + | + | 0 |

R1R1 plasma
Removed pan-auto
Cannot remove anti-Fy$^a$
Anti-Fy$^a$ left behind

rr plasma D-
Removed pan-auto
Cannot remove anti-Fy$^a$
Anti-Fy$^a$ left behind

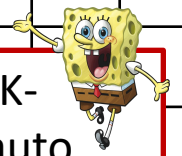
Pan-reactive autoantibody
Anti-Fy$^a$ detected in the alloadsorbed plasma
(allo/auto/non-spec?)

# ABID 4

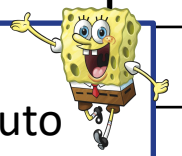
| Cell | Rh | D | C | E | c | e | M | N | S | s | P1 | Lu$^a$ | K | k | Kp$^a$ | Le$^a$ | Le$^b$ | Fy$^a$ | Fy$^b$ | Jk$^a$ | Jk$^b$ | Other | Neat | R1R1 | rr |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | $R_1^wR_1$ | + | + | 0 | 0 | + | + | 0 | + | 0 | 0 | 0 | 0 | + | 0 | | | | | | | | 4 | 2 | 2 |
| 2 | $R_1R_1$ | + | + | 0 | 0 | + | 0 | + | 0 | + | 0 | 0 | + | + | 0 | | | | | | | | 4 | 3 | 0 |
| 3 | $R_2R_2$ | + | 0 | + | + | 0 | + | 0 | + | 0 | 0 | 0 | 0 | + | 0 | | | | | | | | 4 | 2 | 2 |
| 4 | | | | | | | | | | | | | | | | | | | | | | | 4 | 0 | 0 |
| 5 | | | | | | | | | | | | | | | | | | | | | | | 4 | 0 | 0 |
| 6 | | | | | | | | | | | | | | | | | | | | | | | 4 | 3 | 2 |
| 7 | | | | | | | | | | | | | | | | | | | | | | | 4 | 3 | 0 |
| 8 | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 0 | 0 | 0 | + | + | | | | | | | | 4 | 0 | 0 |
| 9 | rr | 0 | 0 | 0 | + | + | + | 0 | 0 | + | 2 | 0 | 0 | + | 0 | | | | | | | | 4 | 2 | 2 |
| | | | | | | | | | | | | | 0 | + | 0 | | | | | | | | 4 | 0 | 0 |
| | | | | | | | | | | | | | | | | | | | | | | Auto | 4 | / | / |
| | | | | | | | | | | | | | | | | | | | | | | + control | 2 | / | / |

| Rh | C | D | E | c | e | $C^w$ |
|---|---|---|---|---|---|---|
| $R_1R_1$ | + | + | 0 | 0 | + | 0 |
| rr | 0 | 0 | 0 | + | + | 0 |

R1R1 plasma K-
Removed pan-auto
Cannot remove anti-M
Anti-M left behind
Cannot remove anti-K
Anti-K left behind



rr plasma K+
Removed pan-auto
Cannot remove anti-M
Anti-M left behind
Removed anti-K



Pan-reactive autoantibody
Anti-K and anti-M detected in the alloadsorbed plasma (allo/auto/non-spec?)

# Are autoantibodies clinically significant?

BSH Guidelines:

*"Many autoantibodies cause no clinical problems. Clinically benign autoantibodies often cause laboratory problems making determination of ABO and Rh groups problematic and preventing effective antibody screening due to cross-reacting antibody"*

*"Selection of blood for transfusion may be influenced by the presence of an autoantibody of simple or mimicking specificity (e.g. auto anti-e). However, specialist advice should be sought before selecting antigen-negative blood in these circumstances, as prevention of the development of alloantibodies is usually of more importance"*


https://onlinelibrary.wiley.com/doi/full/10.1111/j.1365-3148.2012.01199.x

# Alloadsorption – where else we can use it

- Multiple antibodies
  - Used to separate out complex mixtures of antibodies
- Anti-G investigations
  - Allows you to distinguish between anti-D, C and G
  - Blood selection
  - Prevent sensitisation
  - Eligibility for RAADP
  - Assess risk for HDFN

# You've got a friend in G

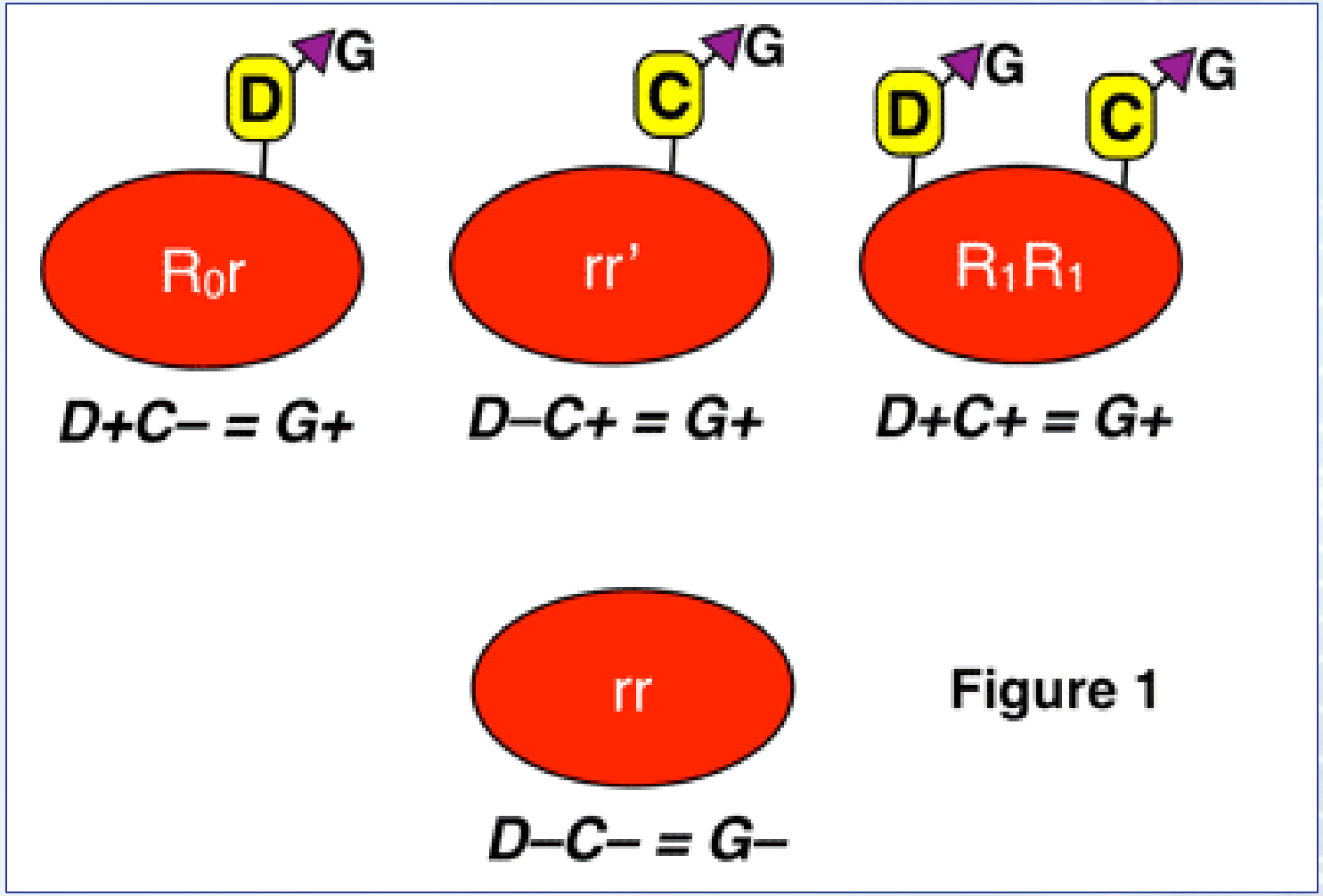



Figure 1

https://www.bbguy.org/2016/06/17/want-g-wiz/

# Some common G pos and G neg phenotypes

| Name | Pheno | G Pos | Name | Pheno | G neg |
|---|---|---|---|---|---|
| R1R1 | DCe/DCe | D+ C+ G+ | rr | dce/dce | D- C- G- |
| R2R2 | DcE/DcE | D+ C- G+ | r”r” | dcE/dcE | D- C- G- |
| r’r | dCe/dCe | D- C+ G+ | r”r | dcE/dce | D- C- G- |
| R1R2 | DCe/DcE | D+ C+ G+ | | | |
| RO | Dce/Dce | D+ C- G+ | | | |
| R1r | DCe/dce | D+ C+ G+ | | | |
| R2r | DcE/dce | D+ C- G+ | | | |

Not really ‘common’

# Anti-D

| Cell | Rh | D | C | E | c | e | M | N | S | s | P1 | $Lu^a$ | K | k | $Kp^a$ | $Le^a$ | $Le^b$ | $Fy^a$ | $Fy^b$ | $Jk^a$ | $Jk^b$ | Other | IAT | EIAT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | $R_1^wR_1$ | + | + | 0 | 0 | + | + | 0 | + | + | 0 | 0 | 0 | + | 0 | 0 | + | + | 0 | + | 0 | | 3 | 5 |
| 2 | $R_1R_1$ | + | + | 0 | 0 | + | 0 | + | 0 | + | 0 | 0 | + | + | 0 | + | 0 | 0 | + | 0 | + | | 3 | 5 |
| 3 | $R_2R_2$ | + | 0 | + | + | 0 | + | 0 | + | 0 | 0 | 0 | 0 | + | 0 | + | 0 | 0 | + | + | 0 | | 4 | 5 |
| 4 | r'r | 0 | + | 0 | + | + | 0 | + | + | 0 | 0 | 0 | 0 | + | 0 | 0 | + | 0 | + | + | 0 | | 0 | 0 |
| 5 | r''r | 0 | 0 | + | + | + | 0 | + | 0 | + | 4 | 0 | 0 | + | 0 | 0 | + | + | 0 | 0 | + | | 0 | 0 |
| 6 | rr | 0 | 0 | 0 | + | + | + | 0 | + | 0 | 4 | 0 | + | 0 | 0 | 0 | + | + | 0 | 0 | + | | 0 | 0 |
| 7 | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 2 | + | + | + | 0 | + | 0 | 0 | + | + | 0 | | 0 | 0 |
| 8 | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 0 | + | | 0 | 0 |
| 9 | rr | 0 | 0 | 0 | + | + | + | 0 | 0 | + | 2 | 0 | 0 | + | 0 | + | 0 | + | 0 | 0 | + | | 0 | 0 |
| 10 | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 3 | 0 | 0 | + | 0 | + | 0 | + | 0 | + | 0 | | 0 | 0 |
| | | | | | | | | | | | | | | | | | | | | | | Auto | 0 | / |
| | | | | | | | | | | | | | | | | | | | | | | K control | 2 | / |

# Anti-C

| Cell | Rh | D | C | E | c | e | M | N | S | s | P1 | Lu$^a$ | K | k | Kp$^a$ | Le$^a$ | Le$^b$ | Fy$^a$ | Fy$^b$ | Jk$^a$ | Jk$^b$ | Other | IAT | EIAT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | $R_1^wR_1$ | + | + | 0 | 0 | + | + | 0 | + | + | 0 | 0 | 0 | + | 0 | 0 | + | + | 0 | + | 0 | | 3 | 5 |
| 2 | $R_1R_1$ | + | + | 0 | 0 | + | 0 | + | 0 | + | 0 | 0 | + | + | 0 | + | 0 | 0 | + | 0 | + | | 3 | 5 |
| 3 | $R_2R_2$ | + | 0 | + | + | 0 | + | 0 | + | 0 | 0 | 0 | 0 | + | 0 | + | 0 | 0 | + | + | 0 | | 0 | 0 |
| 4 | r'r | 0 | + | 0 | + | + | 0 | + | + | 0 | 0 | 0 | 0 | + | 0 | 0 | + | 0 | + | + | 0 | | 3 | 5 |
| 5 | r''r | 0 | 0 | + | + | + | 0 | + | 0 | + | 4 | 0 | 0 | + | 0 | 0 | + | + | 0 | 0 | + | | 0 | 0 |
| 6 | rr | 0 | 0 | 0 | + | + | + | 0 | + | 0 | 4 | 0 | + | 0 | 0 | 0 | + | + | 0 | 0 | + | | 0 | 0 |
| 7 | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 2 | + | + | + | 0 | + | 0 | 0 | + | + | 0 | | 0 | 0 |
| 8 | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 0 | + | | 0 | 0 |
| 9 | rr | 0 | 0 | 0 | + | + | + | 0 | 0 | + | 2 | 0 | 0 | + | 0 | + | 0 | + | 0 | 0 | + | | 0 | 0 |
| 10 | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 3 | 0 | 0 | + | 0 | + | 0 | + | 0 | + | 0 | | 0 | 0 |
| | | | | | | | | | | | | | | | | | | | | | | Auto | 0 | / |
| | | | | | | | | | | | | | | | | | | | | | | K control | 2 | / |

# Anti-D + C

| Cell | Rh | D | C | E | c | e | M | N | S | s | P1 | Lu$^a$ | K | k | Kp$^a$ | Le$^a$ | Le$^b$ | Fy$^a$ | Fy$^b$ | Jk$^a$ | Jk$^b$ | Other | IAT | EIAT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | $R_1^wR_1$ | + | + | 0 | 0 | + | + | 0 | + | + | 0 | 0 | 0 | + | 0 | 0 | + | + | 0 | + | 0 | | 3 | 5 |
| 2 | $R_1R_1$ | + | + | 0 | 0 | + | 0 | + | 0 | + | 0 | 0 | + | + | 0 | + | 0 | 0 | + | 0 | + | | 3 | 5 |
| 3 | $R_2R_2$ | + | 0 | + | + | 0 | + | 0 | + | 0 | 0 | 0 | 0 | + | 0 | + | 0 | 0 | + | + | 0 | | 4 | 5 |
| 4 | r'r | 0 | + | 0 | + | + | 0 | + | + | 0 | 0 | 0 | 0 | + | 0 | 0 | + | 0 | + | + | 0 | | 3 | 5 |
| 5 | r''r | 0 | 0 | + | + | + | 0 | + | 0 | + | 4 | 0 | 0 | + | 0 | 0 | + | + | 0 | 0 | + | | 0 | 0 |
| 6 | rr | 0 | 0 | 0 | + | + | + | 0 | + | 0 | 4 | 0 | + | 0 | 0 | 0 | + | + | 0 | 0 | + | | 0 | 0 |
| 7 | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 2 | + | + | + | 0 | + | 0 | 0 | + | + | 0 | | 0 | 0 |
| 8 | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 0 | + | | 0 | 0 |
| 9 | rr | 0 | 0 | 0 | + | + | + | 0 | 0 | + | 2 | 0 | 0 | + | 0 | + | 0 | + | 0 | 0 | + | | 0 | 0 |
| 10 | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 3 | 0 | 0 | + | 0 | + | 0 | + | 0 | + | 0 | | 0 | 0 |
| | | | | | | | | | | | | | | | | | | | | | | Auto | 0 | / |
| | | | | | | | | | | | | | | | | | | | | | | K control | 2 | / |

# Anti-G (cannot excluded anti-D or C)

| Cell | Rh | D | C | E | c | e | M | N | S | s | P1 | Lu$^a$ | K | k | Kp$^a$ | Le$^a$ | Le$^b$ | Fy$^a$ | Fy$^b$ | Jk$^a$ | Jk$^b$ | Other | IAT | EIAT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | $R_1^wR_1$ | + | + | 0 | 0 | + | + | 0 | + | + | 0 | 0 | 0 | + | 0 | 0 | + | + | 0 | + | 0 | | 4 | 5 |
| 2 | $R_1R_1$ | + | + | 0 | 0 | + | 0 | + | 0 | + | 0 | 0 | + | + | 0 | + | 0 | 0 | + | 0 | + | | 4 | 5 |
| 3 | $R_2R_2$ | + | 0 | + | + | 0 | + | 0 | + | 0 | 0 | 0 | 0 | + | 0 | + | 0 | 0 | + | + | 0 | | 3 | 4 |
| 4 | r'r | 0 | + | 0 | + | + | 0 | + | + | 0 | 0 | 0 | 0 | + | 0 | 0 | + | 0 | + | + | 0 | | 3 | 4 |
| 5 | r''r | 0 | 0 | + | + | + | 0 | + | 0 | + | 4 | 0 | 0 | + | 0 | 0 | + | + | 0 | 0 | + | | 0 | 0 |
| 6 | rr | 0 | 0 | 0 | + | + | + | 0 | + | 0 | 4 | 0 | + | 0 | 0 | 0 | + | + | 0 | 0 | + | | 0 | 0 |
| 7 | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 2 | + | + | + | 0 | + | 0 | 0 | + | + | 0 | | 0 | 0 |
| 8 | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 0 | + | | 0 | 0 |
| 9 | rr | 0 | 0 | 0 | + | + | + | 0 | 0 | + | 2 | 0 | 0 | + | 0 | + | 0 | + | 0 | 0 | + | | 0 | 0 |
| 10 | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 3 | 0 | 0 | + | 0 | + | 0 | + | 0 | + | 0 | | 0 | 0 |
| | | | | | | | | | | | | | | | | | | | | | | Auto | 0 | / |
| | | | | | | | | | | | | | | | | | | | | | | K control | 2 | / |

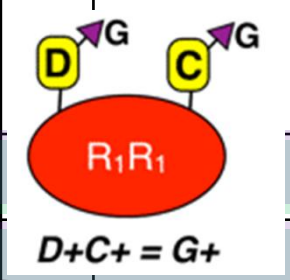
# Spot the difference!

There are other clues which the reference lab would use – but it can get very confusing

| Cell | Fy$^b$ | Jk$^a$ | Jk$^b$ | Other | IAT | EIAT |
|---|---|---|---|---|---|---|
| 1 | 0 | + | 0 | | 3 | 5 |
| 2 | + | 0 | + | | 3 | 5 |
| 3 | + | + | 0 | | 4 | 5 |
| 4 | + | + | 0 | | 3 | 5 |

R2R2 cells have more D antigen sites than R1R1 (steric hinderance) so with anti-D + C, you expect to see stronger reaction vs R2R2

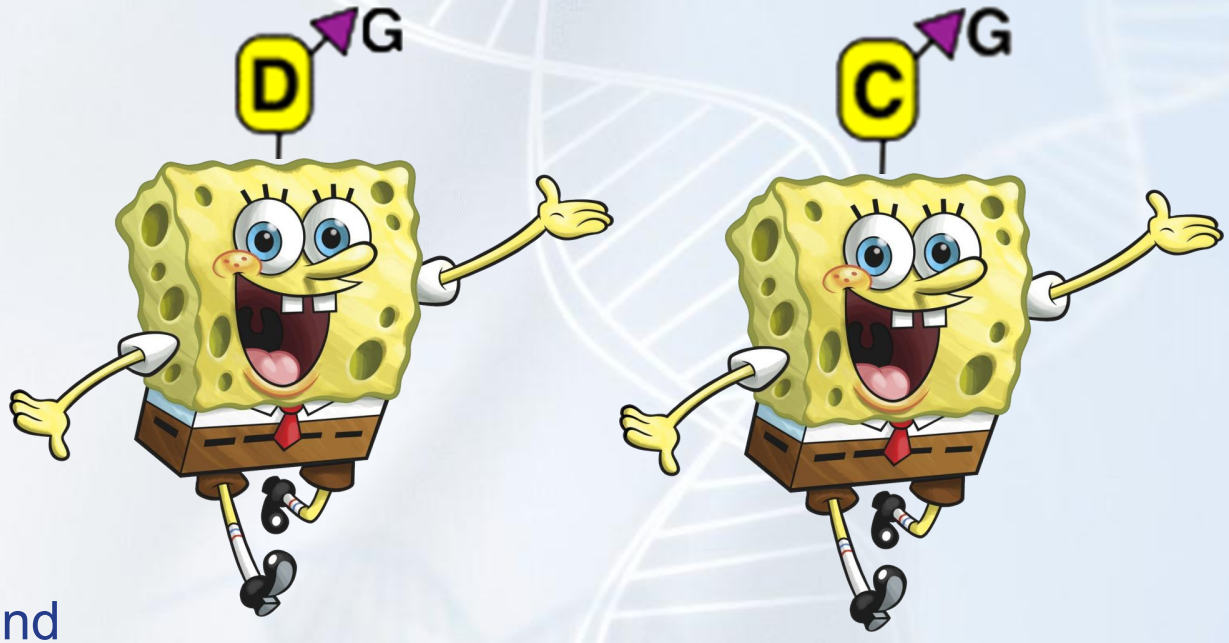
| Cell | Rh | D | C | E | c | e | M | N | S | s | P1 | Lu$^a$ | K | k | Kp$^a$ | Le$^a$ | Le$^b$ | Fy$^a$ | Fy$^b$ | Jk$^a$ | IAT | EIAT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | $R_1^wR_1$ | + | + | 0 | 0 | + | + | 0 | + | + | 0 | 0 | 0 | + | 0 | 0 | + | + | 0 | + | 4 | 5 |
| 2 | $R_1R_1$ | + | + | 0 | 0 | + | 0 | + | 0 | + | 0 | 0 | + | + | 0 | + | 0 | 0 | + | 0 | 4 | 5 |
| 3 | $R_2R_2$ | + | 0 | + | + | 0 | + | 0 | + | 0 | 0 | 0 | 0 | + | 0 | + | 0 | 0 | + | + | 0 | 3 | 4 |
| 4 | r'r | 0 | + | 0 | + | + | 0 | + | + | 0 | 0 | 0 | 0 | + | 0 | 0 | + | 0 | + | + | 0 | 3 | 4 |




Weaker reaction vs R2R2 cell *indicates* anti-G. It *does not* exclude anti-D and/or C

# G Alloadsorption

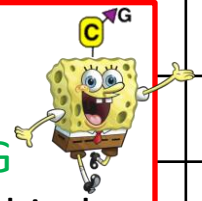
- Same procedure as for AIHA/pos DAT alloadsorptions
- Panel profiles are different
  - Ror (D+C-G+)
  - r'r (D-C+G+)
- Knowing what is removed and what is left behind



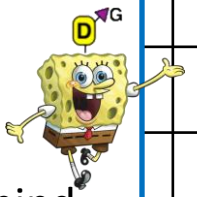
# If you have anti-D, C and G

| Cell | Rh | D | C | E | c | e | M | N | S | s | P1 | Lu$^a$ | K | k | Kp$^a$ | Le$^a$ | Le$^b$ | Fy$^a$ | Fy$^b$ | Jk$^a$ | Jk$^b$ | Neat IAT | r'r IAT | Ror IAT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | $R_1^wR_1$ | + | + | 0 | 0 | + | + | 0 | + | + | 0 | 0 | 0 | + | | | | | | | 0 | 4 | 2 | 3 |
| 2 | $R_1R_1$ | + | + | 0 | 0 | + | 0 | + | 0 | + | 0 | 0 | + | + | | | | | | | + | 4 | 2 | 3 |
| 3 | $R_2R_2$ | + | 0 | + | + | 0 | + | 0 | + | 0 | 0 | 0 | 0 | + | | | | | | | 0 | 3 | 2 | 0 |
| 4 | r'r | 0 | + | 0 | + | + | 0 | + | + | 0 | 0 | 0 | 0 | + | 0 | 0 | + | 0 | + | + | 0 | 3 | 0 | 3 |
| 5 | r''r | 0 | 0 | + | + | + | 0 | + | 0 | + | 4 | 0 | 0 | + | | | | | | | + | 0 | 0 | 0 |
| 6 | rr | 0 | 0 | 0 | + | + | + | 0 | + | 0 | 4 | 0 | + | 0 | | | | | | | + | 0 | 0 | 0 |
| 7 | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 2 | + | + | + | | | | | | | 0 | 0 | 0 | 0 |
| 8 | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 0 | + | 0 | 0 | 0 |
| 9 | rr | 0 | 0 | 0 | + | + | + | 0 | 0 | + | 2 | 0 | 0 | + | | | | | | | + | 0 | 0 | 0 |
| 10 | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 3 | 0 | 0 | + | | | | | | | 0 | 0 | 0 | 0 |
| | | | | | | | | | | | | | | | | | | | | | Auto | 0 | / | / |
| | | | | | | | | | | | | | | | | | | | | | K control | 2 | / | / |

r'r plasma:
removed
anti-C and G
leaves anti-D behind




Ror plasma:
removed
anti-D and G
leaves anti-C behind

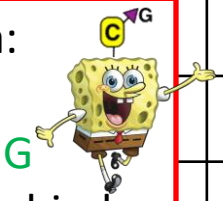



Neat plasma
Therefore contains
anti-D+C+G

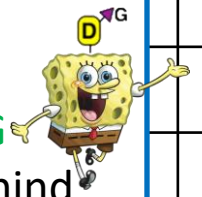
# If you have anti-D and G

| Cell | Rh | D | C | E | c | e | M | N | S | s | P1 | Lu$^a$ | K | k | Kp$^a$ | Le$^a$ | Le$^b$ | Fy$^a$ | Fy$^b$ | Jk$^a$ | Jk$^b$ | Neat IAT | r'r IAT | Ror IAT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | $R_1^wR_1$ | + | + | 0 | 0 | + | + | 0 | + | + | 0 | 0 | 0 | + | | | | | | | 0 | 4 | 4 | 0 |
| 2 | $R_1R_1$ | + | + | 0 | 0 | + | 0 | + | 0 | + | 0 | 0 | + | + | | | | | | | + | 4 | 4 | 0 |
| 3 | $R_2R_2$ | + | 0 | + | + | 0 | + | 0 | + | 0 | 0 | 0 | 0 | + | | | | | | | 0 | 3 | 3 | 0 |
| 4 | r'r | 0 | + | 0 | + | + | 0 | + | + | 0 | 0 | 0 | 0 | + | 0 | 0 | + | 0 | + | + | 0 | 3 | 0 | 0 |
| 5 | r''r | 0 | 0 | + | + | + | 0 | + | 0 | + | 4 | 0 | 0 | + | | | | | | | + | 0 | 0 | 0 |
| 6 | rr | 0 | 0 | 0 | + | + | + | 0 | + | 0 | 4 | 0 | + | 0 | | | | | | | + | 0 | 0 | 0 |
| 7 | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 2 | + | + | + | | | | | | | 0 | 0 | 0 | 0 |
| 8 | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 0 | 0 | 0 | + | | | | | | | + | 0 | 0 | 0 |
| 9 | rr | 0 | 0 | 0 | + | + | + | 0 | 0 | + | 2 | 0 | 0 | + | 0 | + | 0 | + | 0 | 0 | + | 0 | 0 | 0 |
| 10 | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 3 | 0 | 0 | + | | | | | | | 0 | 0 | 0 | 0 |
| | | | | | | | | | | | | | | | | | | | | | Auto | 0 | / | / |
| | | | | | | | | | | | | | | | | | | | | | K control | 2 | / | / |

r'r plasma:
removed
anti-C and G
leaves anti-D behind




Ror plasma:
removed
anti-D and G
Nothing left behind
Therefore does not
contain anti-C

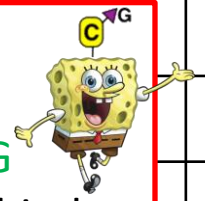



Neat plasma
Therefore contains
anti-D+G

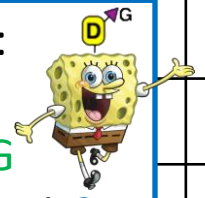
# If you have anti-C and G

| Cell | Rh | D | C | E | c | e | M | N | S | s | P1 | Lu$^a$ | K | k | Kp$^a$ | Le$^a$ | Le$^b$ | Fy$^a$ | Fy$^b$ | Jk$^a$ | Jk$^b$ | Neat IAT | r'r IAT | Ror IAT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | $R_1^wR_1$ | + | + | 0 | 0 | + | + | 0 | + | + | 0 | 0 | 0 | + | | | | | | | 0 | 4 | 0 | 3 |
| 2 | $R_1R_1$ | + | + | 0 | 0 | + | 0 | + | 0 | + | 0 | 0 | + | + | | | | | | | + | 4 | 0 | 3 |
| 3 | $R_2R_2$ | + | 0 | + | + | 0 | + | 0 | + | 0 | 0 | 0 | 0 | + | | | | | | | 0 | 3 | 0 | 0 |
| 4 | r'r | 0 | + | 0 | + | + | 0 | + | + | 0 | 0 | 0 | 0 | + | | | | | | | 0 | 3 | 0 | 3 |
| 5 | r''r | 0 | 0 | + | + | + | 0 | + | 0 | + | 4 | 0 | 0 | + | | | | | | | + | 0 | 0 | 0 |
| 6 | rr | 0 | 0 | 0 | + | + | + | 0 | + | 0 | 4 | 0 | + | 0 | | | | | | | + | 0 | 0 | 0 |
| 7 | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 2 | + | + | + | | | | | | | 0 | 0 | 0 | 0 |
| 8 | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 0 | 0 | 0 | + | | | | | | | + | 0 | 0 | 0 |
| 9 | rr | 0 | 0 | 0 | + | + | + | 0 | 0 | + | 2 | 0 | 0 | + | | | | | | | + | 0 | 0 | 0 |
| 10 | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 3 | 0 | 0 | + | | | | | | | 0 | 0 | 0 | 0 |
| | | | | | | | | | | | | | | | | | | | | | Auto | 0 | / | / |
| | | | | | | | | | | | | | | | | | | | | | K control | 2 | / | / |

r'r plasma:
removed
anti-C and G
Nothing left behind
Therefore does not
contain anti-D




Ror plasma:
removed
anti-D and G
Leaves behind anti-C




Neat plasma
Therefore contains
anti-C+G

# Blood selection

| Name | Pheno | G neg | Frequency (UK donor) |
|---|---|---|---|
| rr | dce/dce | D- C- G- | ~15% |
| r”r” | dcE/dcE | D- C- G- | <0.1% |
| r”r | dcE/dce | D- C- G- | 0.6% |

# Tools of the trade - Putting it into practice - The weird and the wonderful

National Blood Transfusion Committee

NHS Blood and Transplant

## Transfusion Training Hub

The one stop shop for transfusion education resources


**Learn at your own pace in your own time**

Transfusion 2024

Discover links to valuable transfusion resources
Visit nationalbloodtransfusion.co.uk/transfusion-training-hub
or **scan the QR code**

Thank you for listening

Any questions?

# Thank you

Certificates of attendance will be shared in the next few days

Register to the Only Cells Webinar mailing list (scan QR code)