NHS
Blood and Transplant

Passive or Immune anti-D

One Born Every Minute

The Durham Centre, Belmont Industrial Estate, Durham, DH1 1TN

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Content

- Use of prophylactic anti-D
- Why try to distinguish if immune or passive?
- Current practices used to help distinguish immune from passive
- NEQAS Questionnaire
- Newcastle RCI study
- Conclusion

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Use of prophylactic anti-D

Prevent haemolytic disease of the foetus and newborn (HDFN) due to maternal alloanti-D

Alloanti-D (immune anti-D) is the red cell antibody most commonly associated with HDFN

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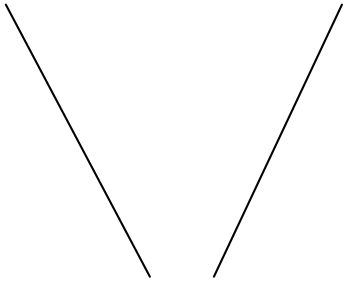
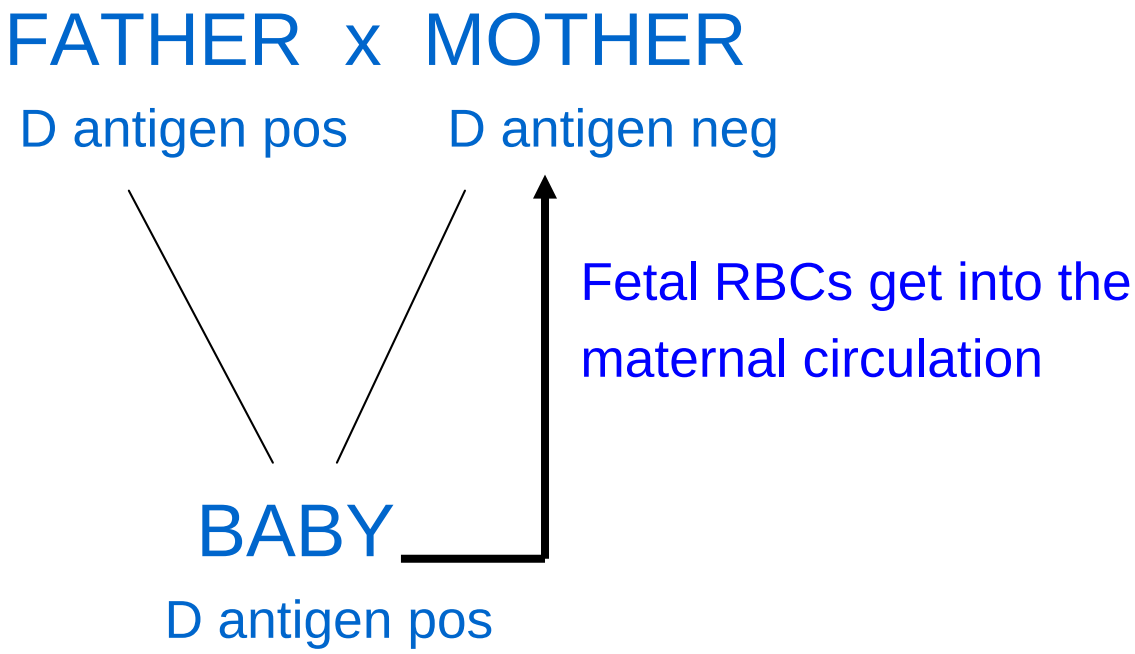
FATHER x MOTHER

D antigen pos D antigen neg



BABY

D antigen pos


FATHER x MOTHER
D antigen pos
D antigen neg
Fetal RBCs get into the maternal circulation
BABY
D antigen pos

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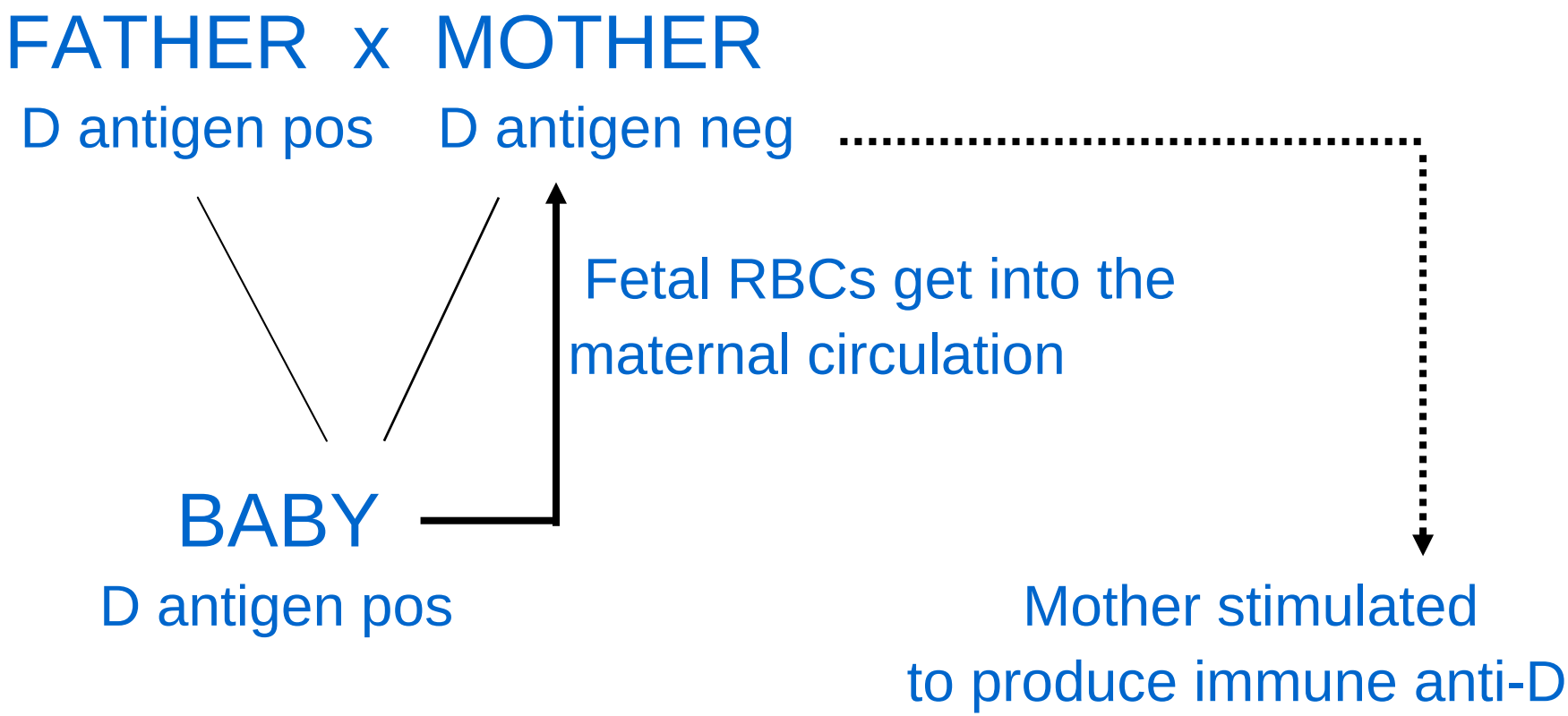

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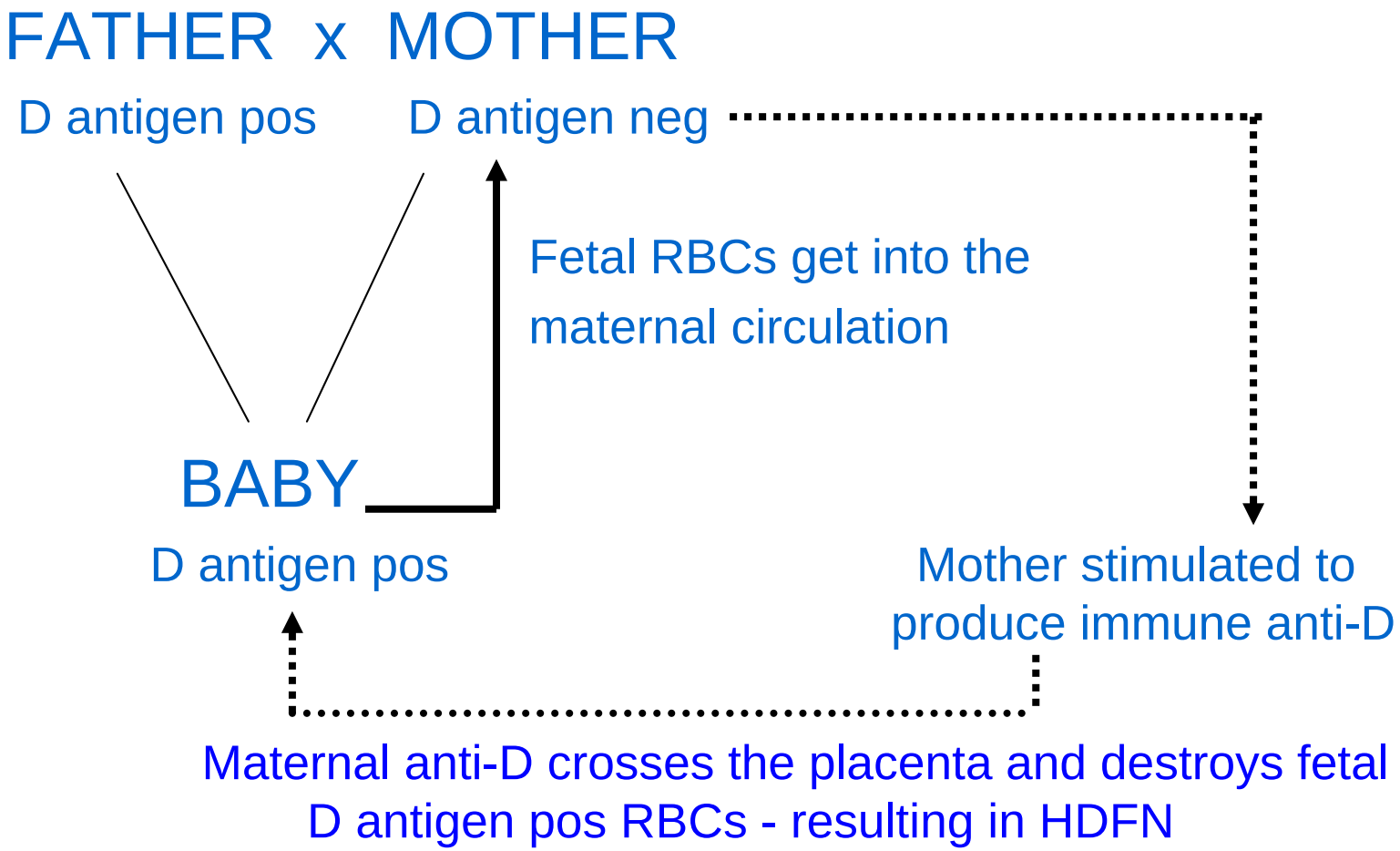

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FATHER x MOTHER

D antigen pos D antigen neg

Prophylactic anti-D immunoglobulin

Fetal RBCs get into the maternal circulation

BABY

D antigen pos

Prevents the mother producing antibodies against fetal D positive red cells

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Use of prophylactic anti-D

Anti-D prophylaxis is given to unimmunised D negative women following the known causes of sensitisation (125 IU is recommended per mL of RhD positive fetal red cells.)

Anti-D Ig may also be given as routine antenatal prophylaxis (RAADP) at 28 and 34 weeks or 28 weeks alone.

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Prophylaxis Works

– Has resulted in a 100-fold reduction in haemolytic disease of the fetus and neonate (HDFN)

– Despite this, sensitisations to RhD still occur in 500 pregnancies per year in England and Wales

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NHS Blood and Transplant Memo

Please note that from Jan 2013, SHOT would like reports of any new case of immune alloanti-D in order to look into how sensitisation is still arising

(see SHOT website http://www.shotuk.org/wp-content/uploads/2013/01/SHOTNewsletterDec20121.pdf)

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NEQAS Survey Results From 164 Hospitals (2010):

98.5% of labs in England and Wales have implemented RAAPD following the 2008 reviewed NICE guidance.

Most hospitals have changed to a single (higher) dose since implementing RAADP

- 73% use Rhophylac 1500 IU x 1
- 11% use D - Gam 1500 IU x 1
- 15% use D - Gam 500 IU x 2

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Impact of this Change in RAADP Regimes

Whilst the incidence of immune anti-D has declined the incidence of positive antibody screens due to prophylactic anti-D has increased

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Why try to Distinguish if Immune or Passive?

The risk of misinterpreting passive and immune anti-D is clear:

- if passive anti-D is misinterpreted anti-D prophylaxis may be omitted leading to sensitisation.
- If immune anti-D is misinterpreted appropriate follow-up may be curtailed putting the fetus at risk.

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2011:

SHOT reported 7 cases where the detection of immune anti-D during pregnancy was misinterpreted as prophylaxis, with resulting HDFN in 6/7 cases

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Infectious or potentially infectious Anti-D:

- Between 1977 and 1994 over 20,000 vials of infectious / potentially infectious Anti-D were manufactured. Plasma pools were infected with Hepatitis C

- Over 500 recipients of these vials confirmed to have been infected with Hepatitis C

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Could we reduce the problem?

Mass fetal genotyping for *RHD*

Benefits:

- 35-45% of RhD neg women unnecessarily given anti-D
- frequency and cost of administration (product, staff time etc)
- reduced need for cord blood testing
- reduction in antenatal visits
- reduced exposure to pathogens (hep C and CJD)

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Current practice used to help distinguish immune from passive

Based on:

Experience / knowledge

Requires:

Patient sample testing

Utilises:

Guidelines / Protocols

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Patient Sample Testing

Red Cell Serological Techniques used to help distinguish immune from passive:

- Continuous Flow Analyser
- CAT IAT
  - Antibody screen (Strength of reaction)
  - Panel (exclude presence of other antibodies)
  - Antibody Titre / Titre Score

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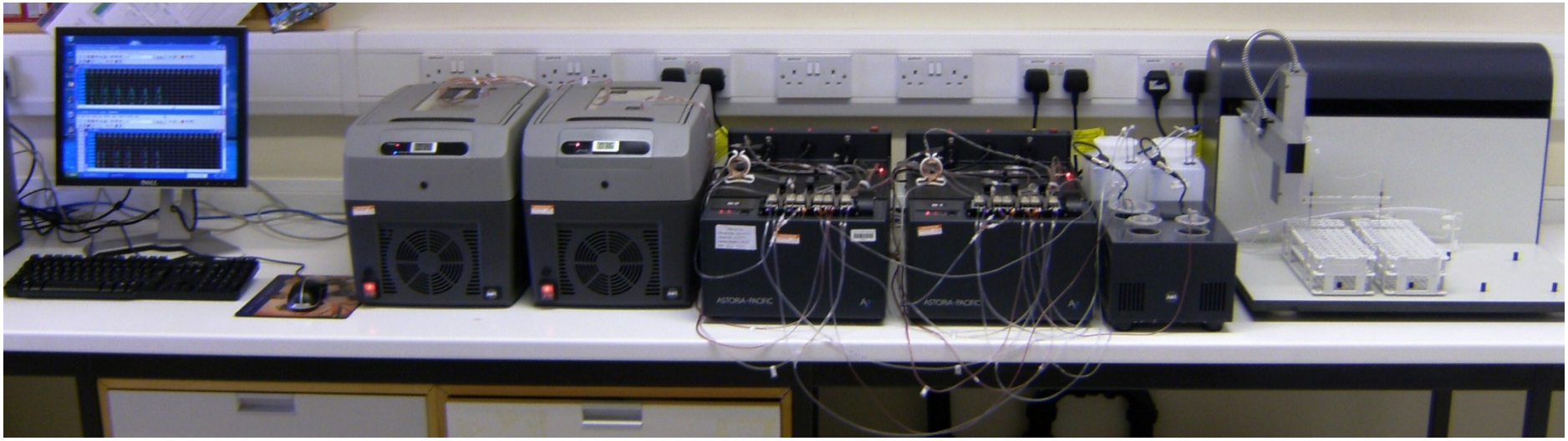
CFA: The Astoria 2 AutoAnalyser



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Disadvantages of using the CFA:

1. Intralaboratory reproducibility has a CV of approximately 10%

2. Interlaboratory reproducibility has been found to have a CV of about 20% (Fleetwood and McNeill 1990).

3. Difficult to standardise between laboratories with a multitude of variables and machines.

Experience / Knowledge

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Following an intramuscular injection of anti-D Ig:

- Serologically detectable levels are reached within minutes and peak within 72–84 hours.

- The half-life is approximately 3 weeks, but anti-D may be detectable
  - up to 8 weeks by IAT
  - up to 12 weeks (in exceptional cases for several months) by continuous flow analysis for anti-D quantification

When doses in excess of 2500IU are given passive anti-D may be detectable by IAT up to 6 months later (BCSH guidelines).

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Immune anti-D is produced following Sensitisation

Immune anti-D

- may become detectable 4 weeks after exposure to D-positive red cells
- Generally reaches a peak after 6–8 weeks.

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Distinguishing between Passive and Immune anti-D

- Passive anti-D levels will fall with time.

- Immune anti-D levels will usually remain stable or rise if there is re-stimulation of the antibody.

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Guidelines / Protocols

Applicable BCSH Guidelines:

1. Blood Grouping And Antibody Testing In Pregnancy, 2007 (section 4.3.1.1)

2. Use of prophylactic anti-D immunoglobulin, 2006 (section 4.2)

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BSCH Guidelines Summarised:

"If there is a record of administration of anti-D within 8 weeks and the antibody reaction is weak, testing should be as for non-sensitised women."

"If there is no record of administration or information regarding prophylaxis is not available the antibody should be monitored as by both IAT and quantification as for immunised women."

Royal College of Obstetricians and Gynaecologists

Bringing to life the best in women's health care

The Use of Anti-D Immunoglobulin for Rhesus D Prophylaxis, (Green-top 22, 2011 guidelines)

"Passive anti-D can be detected for up to 8 weeks following administration and levels are generally < 1 IU/mL"

"Accurate documentation of anti-D Ig administrattion will help differentiate between passive and immune anti-D"

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RCI Advice when <1iu/ml anti-D is detected

If anti-D immunoglobulin ***has*** been given:

- anti-D is most likely to be passive
- follow-up tests and Rh prophylaxis should be advised as for unimmunised pregnant women

If confirmed that prophylaxis **has not** been given in the preceding 8 weeks or the information is unreliable:

- tests should be undertaken 4 weekly to 28 weeks and 2 weekly after 28 weeks

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If the anti-D becomes undetectable by IAT:

- it is safe to assume that the anti-D previously detected was passive; follow-up as for unimmunised women

If anti-D remains detectable by IAT longer than 8 weeks after the last injection:

- the antibody is likely to be immune; follow-up as for immunised women.

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RCI Advice when >1iu/ml anti-D is detected

- **If the anti-D level is 1iu/ml or greater, it is likely (but not certain) that it is immune.**
  - **Check if the woman has been given a recent high dose of prophylactic anti-D.**
  - **Recommend repeat testing by IAT and quantitation as per routine protocol**
    - **to identify fetuses and infants at risk of HDFN**

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Caution!

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The previous guidance applies when only one standard dose of anti-D Ig (500iu or 1250iu) has been given.

- Passive anti-D may be detected for up to 12 weeks following a standard dose of anti-D Ig.
- Following a >1250iu dose, passive anti-D may persist for even longer

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UK NEQAS Questionnaire

SCENARIO: The lab receives a routine pre-RAADP sample from a RhD neg woman at 28/40

- Request form indicates that standard dose of prophylactic anti-D was administered in hospital at 24 weeks following an APH
- Antibody screen positive
- Panel confirms anti-D

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UK NEQAS Questionnaire cont.

Check anti-D given?

- 66% check issued and administered
- 20% check issued only
- 11% check administered
- 3% NO CHECK

  (It is a mandatory DOH requirement to maintain an audit trail of anti-D Ig)

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UK NEQAS Questionnaire cont.

Check previous results:

- 93% Yes
- 7% No

UK NEQAS Questionnaire cont.

Refer for Quantification?

- No confirmation anti-D given:

  71% Yes

  29% NO

- Positive confirmation that anti-D was given:

  91% No

  9% YES

UK NEQAS Questionnaire cont.

If could confirm anti-D given

How would you make an in-house judgement about origin of anti-D before deciding on course of action?:

- 66% based on reaction strength
- 3% on titre
- 8% on titre and reaction strength
- 29% something else (??)

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88% of labs use a anti-D level of 2+ or weaker by IAT to help decide on the origin of the anti-D / need for referral for quantification

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Newcastle RCI study

We compared the results of 42 samples containing passive anti-D with 24 samples with known low levels of immune anti-D (< 4 IU/mL):

We assessed

- Antibody screen strength of reaction
- Quantification value (IU/mL)
- Titre / Titre Score

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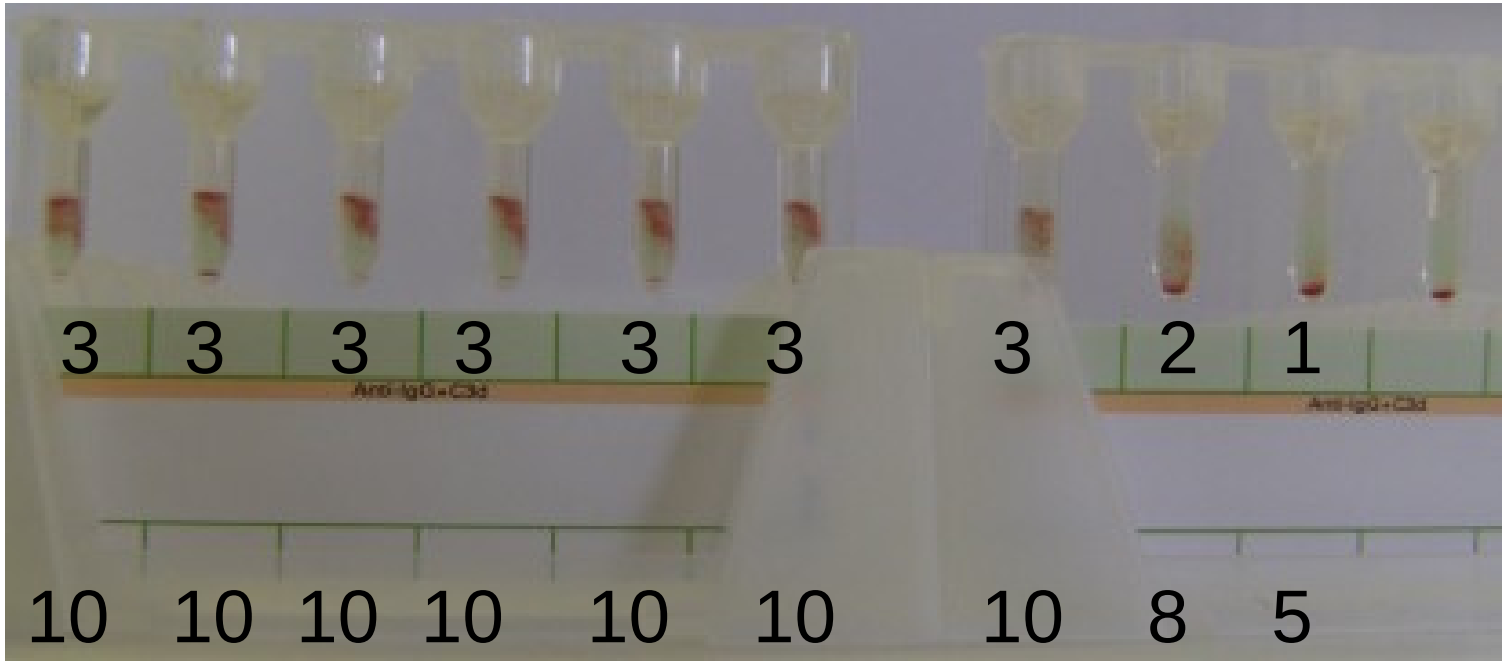
Example of a Titre Score

Dilution:	Neat	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512
Grade	3	3	3	3	3	3	3	2	1	
Score	10	10	10	10	10	10	10	8	5	



TOTAL TITRE SCORE: 83

IU Given	Quantification value IU/m	Antibody screen grade of reaction (1-4+)	Titre End Point	Titre Score
250 (n=4)	0.04 – 0.05	0 - 1	0	0
500 (n=15)	0.03 – 0.1	WK - 1	0 - 2	0 - 13
1250 (n=5)	0.03 – 0.14	1 - 2	1 - 4	5 – 18
1500 (n=18)	0.04 – 0.26	WK - 2	0 – 8	0 – 24
Immune anti-D (n=24)	0.3 - 1.0	1 - 3	2 - 16	5 – 44
	1.0 – 4 .0	2 - 4	32 - 256	42 – 72

Results

Of the 42 samples from patients known to have received anti-D immunoglobulin :

- Mean quantification result was 0.08 IU/mL (SD 0.04)
- Mean titre score was 8.2 (SD 9)
- Maximum values were 0.26 and 24 respectively.

No patient in the study group with immune anti-D had a quantification result this low but three patients had titres scores in this range.

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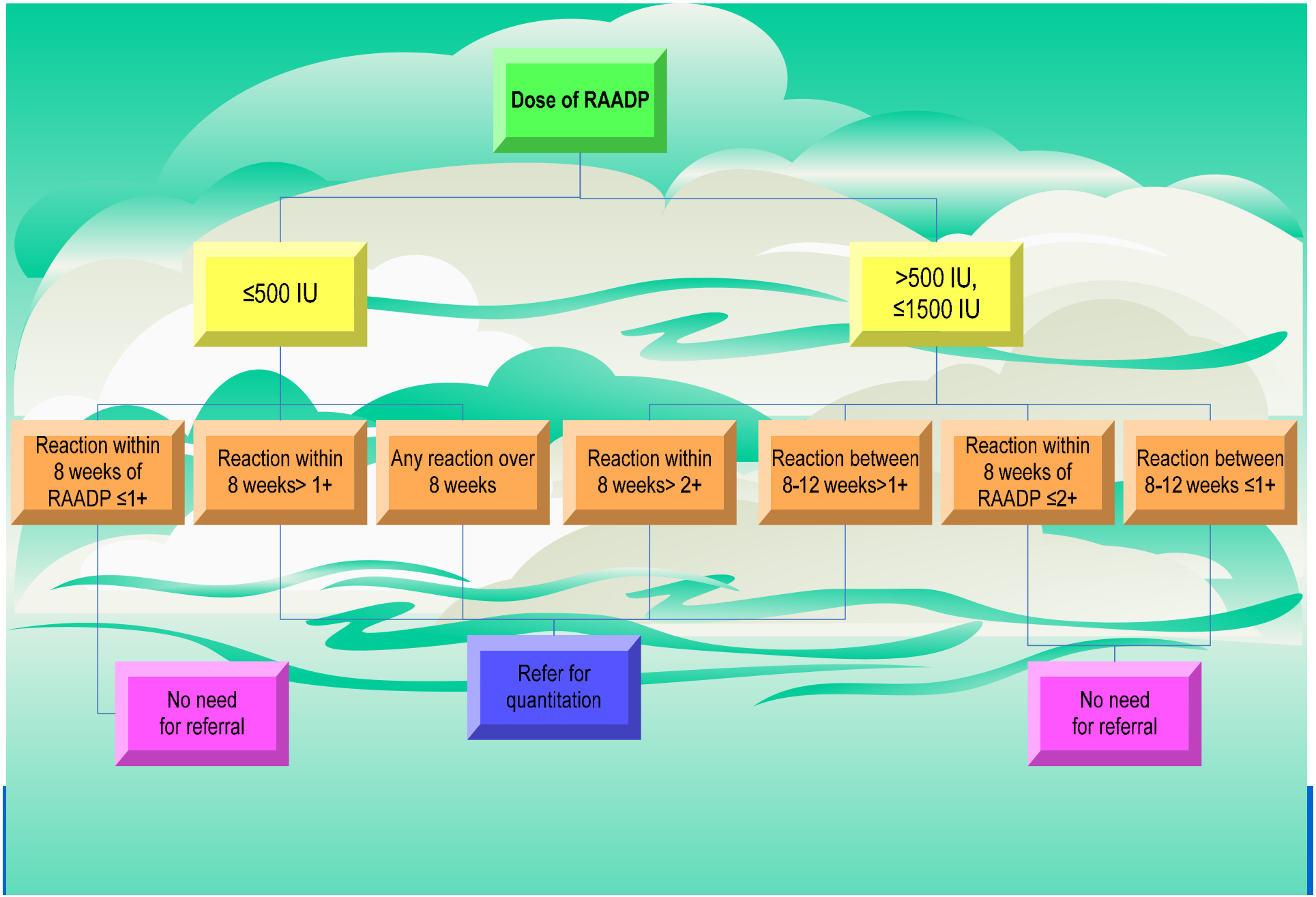
Titre end points/scores in the region of **≤ 8** and a titre score of **< 26** may help to distinguish passive anti-D from immune.

None of the samples known to contain prophylactic anti-D were seen to exceed these values and none of these samples had an antibody screen grade of reaction **> 2+**.

When these values are exceeded the sample should be referred to an RCI laboratory for quantification

Suggested Testing Protocol



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Current Protocol:

– A CFA level <1 IU/mL, when prophylaxis has been given within 8 weeks of sampling, is likely to be passive.

Newcastle Study:

– Could the CFA level of <1 IU/mL be replaced by a value of <0.3 IU/mL?

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Further work required:

- The “cut off” value of 0.3 IU/mL needs to be confirmed by testing a larger cohort of samples
- Need to determine the effects of larger doses of passive anti-D (>1500 IU/mL) on the patients serology
- Further correlate titre scores with CFA results whilst assessing interlaboratory reproducibility

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Conclusion

When trying to distinguish passive anti-D from immune you must consider:

- Dose given
- When it was given
- Serology
- In vivo decay of IgG (monitor level)

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Conclusion

To determine with 100% accuracy if anti-D detected is immune or prohylactic is:

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Passive and immune anti-D are indistinguishable by serological tests and critical scientific / clinical judgement must be exercised in all cases and at all times before advice is provided.

While there is doubt about the origin of the anti-D, anti-D prophylaxis should be advised.

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