Laboratory aspects of Anti-D and other antibodies

Presented by
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Red Cell Immunohaematology

Haemolytic Disease of the Fetus or Newborn

- Not exclusive to anti-D
- Any IgG red cell antibody (IgG1 / IgG3)
- Sufficiently 'strong' (titer)
- Antigen (group) is developed and expressed on the fetal red cells
- Potential for disease

- Anti-D (Rh1) recognised as the most likely to cause significant disease
- Anti-c (Rh4) well documented
- Anti-K (Kell1) has equal potential – as a result of transfusion rather than pregnancy
2004 BCSH – Guidelines for blood grouping and red antibody testing in pregnancy

- Aim is to prevent HDN
- ABO and RhD type – identify RhD-negatives for Ig prophylaxis
- To detect clinically significant red cell antibodies – may affect the fetus
  - may highlight transfusion problems
- Test at booking and 28 weeks (RhD-positives) and at 34 weeks if RhD-negative
- Measure/monitor strength of clinically significant antibodies
- Test every 4 weeks up to 28 weeks; then every 2 weeks to delivery
- At delivery perform a DAT, if positive monitor Hb and bilirubin

Measuring the strength of antibody

- Titration (doubling dilutions) – simple but only semi-quantitative
  - (Neat ~ 512) - used for anti-K and other clonal anti-other than anti-D/c
  - poor intra and inter laboratory reproducibility (CV ≥ 100%)
  - dependent on the equilibrium constant of the antibody, concentration of red cells, inherent error of a manual method
  - test previous sample in parallel
  - endpoint ≥ 20 high risk

- Radioisotope methods – direct labelling of the test anti-D
  - labelling of a purified IgG anti-D
  - competitive radioimmunoassay
  - provide "true" estimates, without the use of standards
  - sensitivity is poor

- Enzyme linked immunosorbant assay (ELISA) – sensitive
  - time consuming, labour intensive

- Immunoprecipitation – radial diffusion
  - electrophoresis
  - insensitive
  - time consuming

- Any quantitation needs to carried out with reference to a known standard
Automated Antibody Quantitation
Continuous Flow Analyser

Advantages:
• Rapid results possible
• Reliable in good hands
• Reproducible
• Very inexpensive
• High volume throughput possible

Disadvantages:
• Dated technology
• Very operator dependent
• The ^^!?&@!! factor

WARNING !!!
This machine subject to Breakdown during periods of Critical Need

A special circuit in the machine called a Crisis Detector senses the operator's emotional state in terms of how desperate he or she is to use the machine. The Crisis Detector then creates a malfunction proportional to the desperation of the operator. Threatening the machine with violence only aggravates the situation. Likewise, attempts to use the other machine may cause it to malfunction too - they belong to the same union. Keep cool and say nice things to the machine. Nothing else seems to work.
AutoAnalyzer

History

1964: Rosenfield et al developed Bromelin-PVP method for Technicon AAI

1968: Lalezari - modified LISP method

1968: Marsh et al - modified Bromelin-Methyl Cellulose

• Method hasn't changed significantly since then
• Technology has
Cells

Bromelin treated red cells
• 20% suspension
• R1R1 for anti-D
• rr for anti-c
Samples

• Need to be diluted to fit within standard curve

Standards

NIBSC Standards

• Anti-D – 73/515
• Anti-c – 84/628
• Calibrated against the WHO International standard

Standard Curve
Dilutors

Microlab 500c
• Process, Analysis and Automation software
  – 1/25 to 1/2,000 single stage dilutions
  – 1/2,000 to 1/2,000,000 two stage dilutions

Methyl Cellulose
Aggregating/rouleaux inducing agent
• 0.12% solution
• Freeze/thaw method
XYZ Sampler

- Up to 90 unknown samples a day
- Standards run every 15 samples

Pump

- Peristaltic pump
- 65 rpm
- Specific flow rated tubing
Pump

Manifold

- Jacketed mixing coils at 37°C
- Red cells, sample and methyl cellulose incubated for 14 minutes
Cold Phase

• Temperature dropped to 15°C
• Albumin saline added to break up non-specific aggregation/rouleaux
• Agglutinates removed
• Remaining unbound red cells haemolysed with Triton
Red Cell Immunohaematology

Standard Curves

QA Scheme
Run by Colindale and Manchester
- International scheme – samples sent worldwide
- Anti-D - 4 samples 4 times a year
- Anti-c - 2 samples 4 times a year
**Significant Levels**

**For anti-D:**
- <4 IU/ml HDN unlikely
- 4 - 15 IU/ml Moderate risk
- >15 IU/ml High risk

- monitor in obst. unit
- alert Obstetrician
- consider refer to FMU

**Significant Levels 2**

**For anti-c:**
- <7.5 IU/ml HDN unlikely
- 7.5 - 20 IU/ml Moderate risk
- >20 IU/ml High risk

- monitor in obst. unit
- alert Obstetrician
- consider refer to FMU

**Fetal monitoring**

- **Invasive**
  - amniocentesis, measure bilirubin
  - fetal sampling, group & Hb
  - re-immunise the mother, more antibody
  - risk of miscarriage

- **Non-invasive**
  - MCA Doppler / ultrasound
  - blood velocity as a measure of anaemia
  - evidence of oedema due to heart failure
  - slight risk

- **Intervention**
  - IUT, early delivery, exchange Tx
Origin of the antibody

- Samples referred for confirmation and quantitation
- May be booking sample or later
- May have previous and current history
- May have received
  - Ig prophylaxis for miscarriage, abortion, invasive procedure, abdominal trauma, RAADP, at delivery
  - a dose of 250, 500, 1000, 1250, 1500, 2500 IU/ml
- If we receive no history, how do we tell if the anti-D detected is prophylactic or immune in origin?

With no history we cannot say
- Could we exclude from quantitation on the basis of strength of reaction? If < grade 2 accept history of prophylaxis and not quant.
- Each RCI laboratory reviewed their cases, not able to exclude with confidence
- Case: low anti-D at 28 weeks, record of prophylaxis
  - Repeat samples requested but did not refer again
  - At delivery the baby was jaundiced, required exchange Tx
  - Post delivery quantitation >20 IU/ml
  - History of prophylaxis was for previous pregnancy!

Below 0.3 IU/ml with no definite history RCI can only advise on both options
- If no prophylaxis has been given, then continue to test as per BCSH Guidelines
- If prophylaxis has been given, no further testing is required after 28 weeks and continue to give prophylaxis
- With a statement for the HTL to review the case
What’s in a level?

- Samples referred to RCI Colindale for quantitation
- Anti-D level reported at between 90 and 110 IU/ml over several weeks
- In between, patient was referred to FMU at King’s, fetus had IUT.
- Samples to RCI Tooting for quantitation
- Reported levels of ~135 IU/ml
- Referring HTL was not happy about the discrepancy
- Wanted to know what to report to Midwives / Obstetrician?

What’s in a level?

- The CV for anti-D quantitation by AA is 20%
- For a sample tested on two runs that allows +/- 10%
- Colindale’s result of 110 IU/ml (range is 99 – 121 IU/ml)
- Tooting’s result of 135 IU/ml (range is 121.5 – 148.5 IU/ml)
- Any anti-D of >15 IU/ml is seen as high risk
- Quantitation by AA is optomised to detect the trigger levels of 4 and 15 IU/ml for anti-D and 7.5 and 20 IU/ml for anti-c
- The important message is that the high level has been recognised and the appropriate action / management of the pregnancy taken

Transfusion Update Meeting

anti-D Day

Royal Society of Medicine
Saturday, 6th December 2008