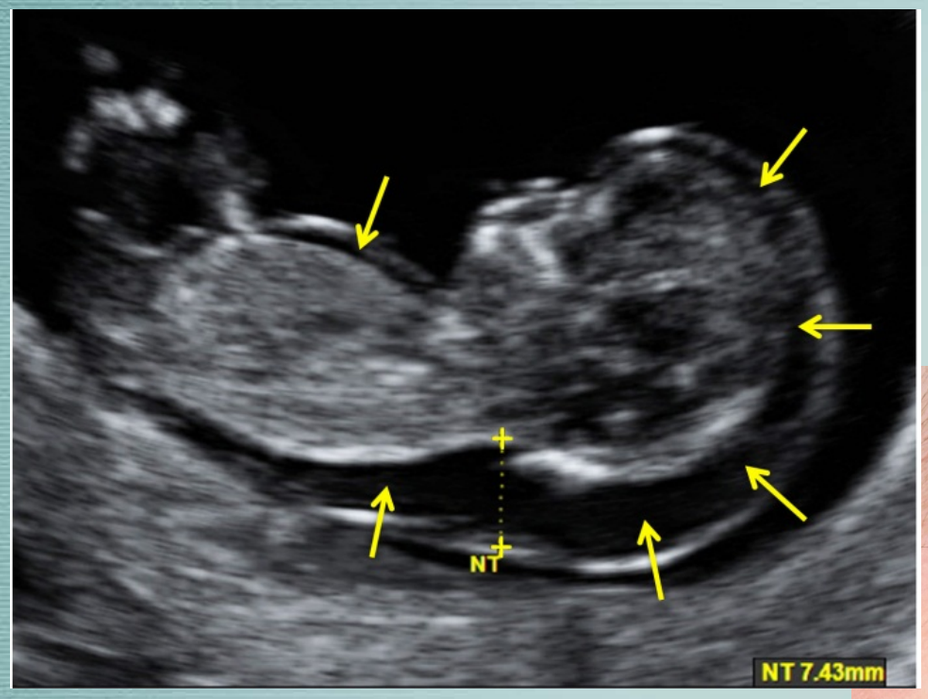
# 1:16 - what does it mean?



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# Antibody titration

- Antibody titration is performed on patients' samples to support:
  - The prediction and management of HDFN
  - Determining cold agglutinins in CHAD
  - The management of ABO mismatched organ transplant

# The prediction and management of HDFN

- HDFN occurs when maternal IgG antibodies cross the placenta and bind to the corresponding antigen on the fetal red cells
- The resultant IgG-coated cells interact with fetal macrophages; this facilitates the removal of these cells in the fetal spleen
- The severity of the disease ranges from mild anaemia to stillbirth
- Titration of an alloantibody to a red cell antigen is used to measure the level of maternal antibody during pregnancy
- Generally a titre of <32 is unlikely to result in significant disease; a titre of >32 is considered significant

# Cold agglutinins

- Cold agglutinins occur naturally in nearly everyone
  - Natural cold autoantibodies occur at low titres, usually <64 at 4ºC and have no activity at higher temperatures
  - Pathological cold agglutinins (such as with CHAD) occur at titres >64 and react at 28-31ºC
  - Pathological cold agglutinins can cause agglutination and occlusion in the micro-vasculature and initiate haemolysis and eventually chronic anaemia

# Organ transplantation

- According to the NHSBT organ donation website, as of yesterday, 2229 people have received transplants since 1st April 2013 however 7134 people are still waiting

- A significant proportion will die before a suitable organ becomes available

- In principle solid organ transplants must be performed from a donor of an ABO compatible blood group

  - ABO antigens are also expressed on endothelial cells of the transplanted organ

- However, last year 1068 living donor kidney transplants were carried out, many of them from ABO incompatible donors

# ABOi renal transplantation

- Low levels of antibody appear not to be harmful to transplanted organs
- Transplantation is safe if anti-donor A or B antibody is, or can be reduced to low levels at the time of transplantation
- The most important issue to determine is the quantity of anti-donor A or B antibody in the recipients circulation
- An ABOi transplant can be performed if the titre of anti-donor A or B antibody is <16, or can be reduced to <16 by antibody removal (plasma exchange or immunoabsorption)
- ABOi renal transplantation is not normally considered for patients with a starting antibody titre >256

# Reminder

- A titre of <32 is unlikely to result in significant HDFN; a titre of >32 is considered significant

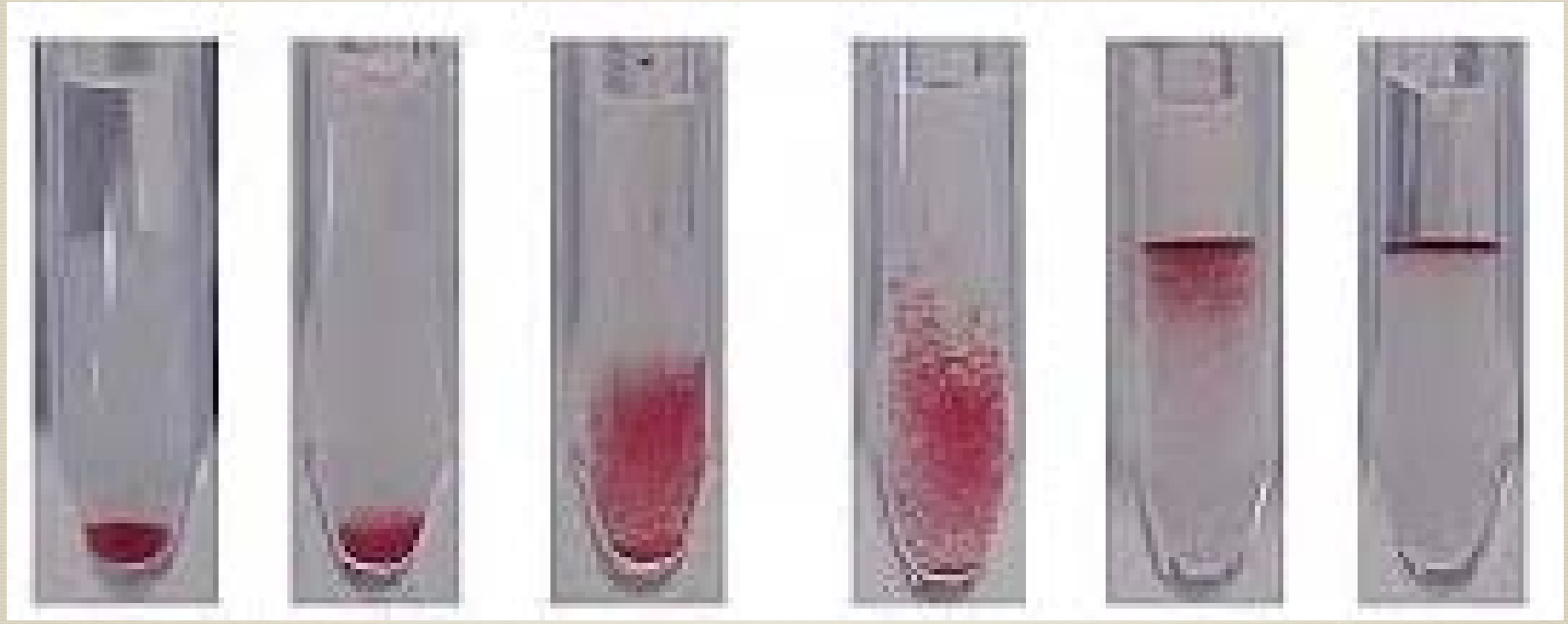
- Pathological cold agglutinins (such as with CHAD) occur at titres >64 and react at 28-31$^{o}$C

- An ABOi transplant can be performed if the titre of anti-donor A or B antibody is <16; ABOi renal transplantation is not normally considered for patients with a starting antibody titre >256

# The technique

- Prepare patients plasma in saline using a doubling dilution method
- Prepare 0.8% red cell suspension
- Perform a LISS IAT and a direct agglutination at RT
- The endpoint is considered as the highest dilution to give a (1+) positive reaction

# Which is the 1+ reaction?



# Which is the 1+ reaction?

# The problem

- Titrations produce a large disparity of values amongst individuals

| Technique | P1 | P2 |
|---|---|---|
| DRT in-house Diamed | 64 (32-256) | 2 (2-4) |
| DRT in-house Ortho | 128 (32-128) | 4 (2-4) |
| DRT in-house tube | 64 (8-256) | 4 (2-16) |
| DRT in-house Immucor | 32 (32-32) | 4 (4-4) |
| **DRT in-house all (37)** | **64 (2-256)** | **4 (2-16)** |
| IAT in-house Diamed | 96 (64-256) | 8 (4-16) |
| IAT in-house Ortho | 1024 (128-1024) | 64 (16-64) |
| IAT in-house tube | 128 (32-256) | 6 (4-8) |
| IAT in-house Immucor | 64 (64-128) | 8 (8-8) |
| **IAT in-house all (16)** | **128 (32-1024)** | **8 (4-64)** |

# Why?

- Antibody titrations have been notoriously difficult to standardise
  - Vast inconsistencies in technique and interpretation amongst laboratories and technologists
- Determining the endpoint
- Optimal incubation time, temperature and centrifugation speed
- Zygosity, phenotype, age and concentration of reagent red cells

# The consequences

- HDFN - inaccuracies in testing may lead to the mother missing vital investigations or being subjected to unnecessary interventions
- Mis-diagnosis of CHAD and subsequent management and/or treatment may be inappropriate
- ABOi renal transplants - inconsistencies in testing could mean the patient is under or over-treated or not at all

# What needs to be done?

- Standardisation and uniform practice should be emphasised
  - Initial samples should be tested by 2 workers
  - Subsequent samples should be tested alongside the previously tested sample
    - Pipetting technique, changing tips between dilutions to prevent carryover
- Gel card technique shows less variance than tube testing
- Automation

# Summary

- Antibody titrations are important in antenatal testing, antibody investigations and performing and monitoring ABOi organ transplants
- NEQAS results have demonstrated significant variation in results between laboratories
- Standardisation of technique continues to be elusive
- Automation has to be the way forward