Identifying antibodies to the constituents in diagnostic reagent red blood cells

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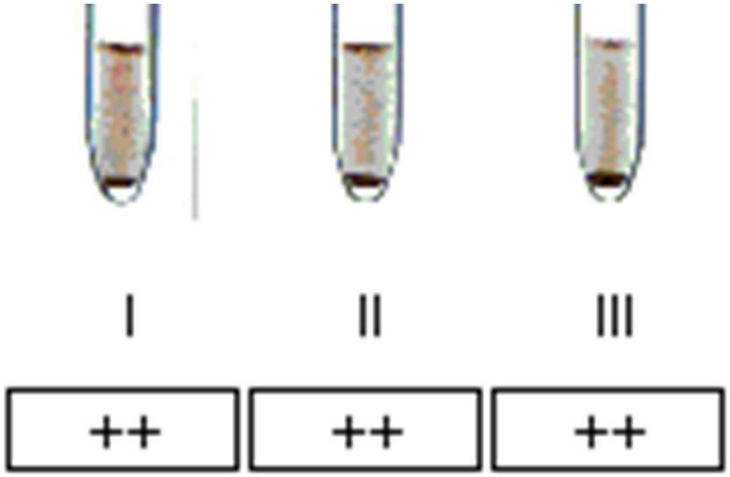
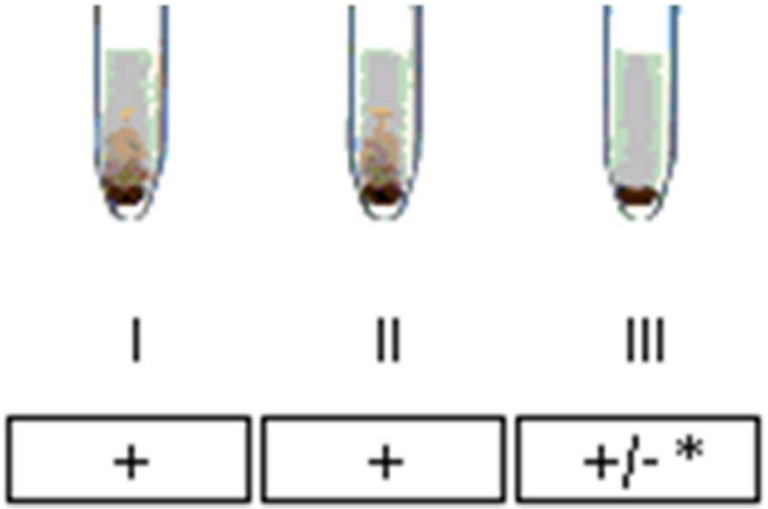
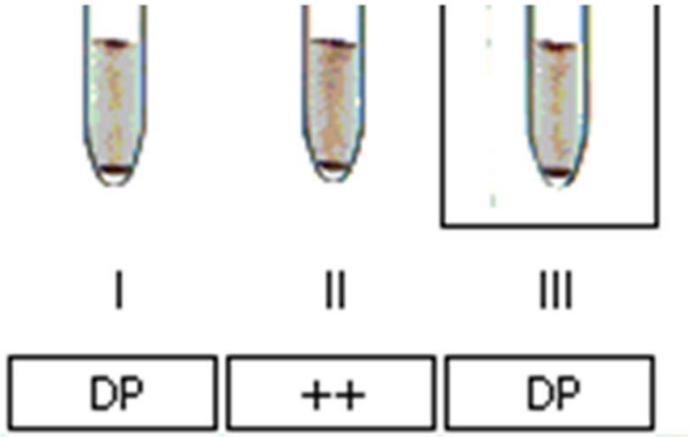
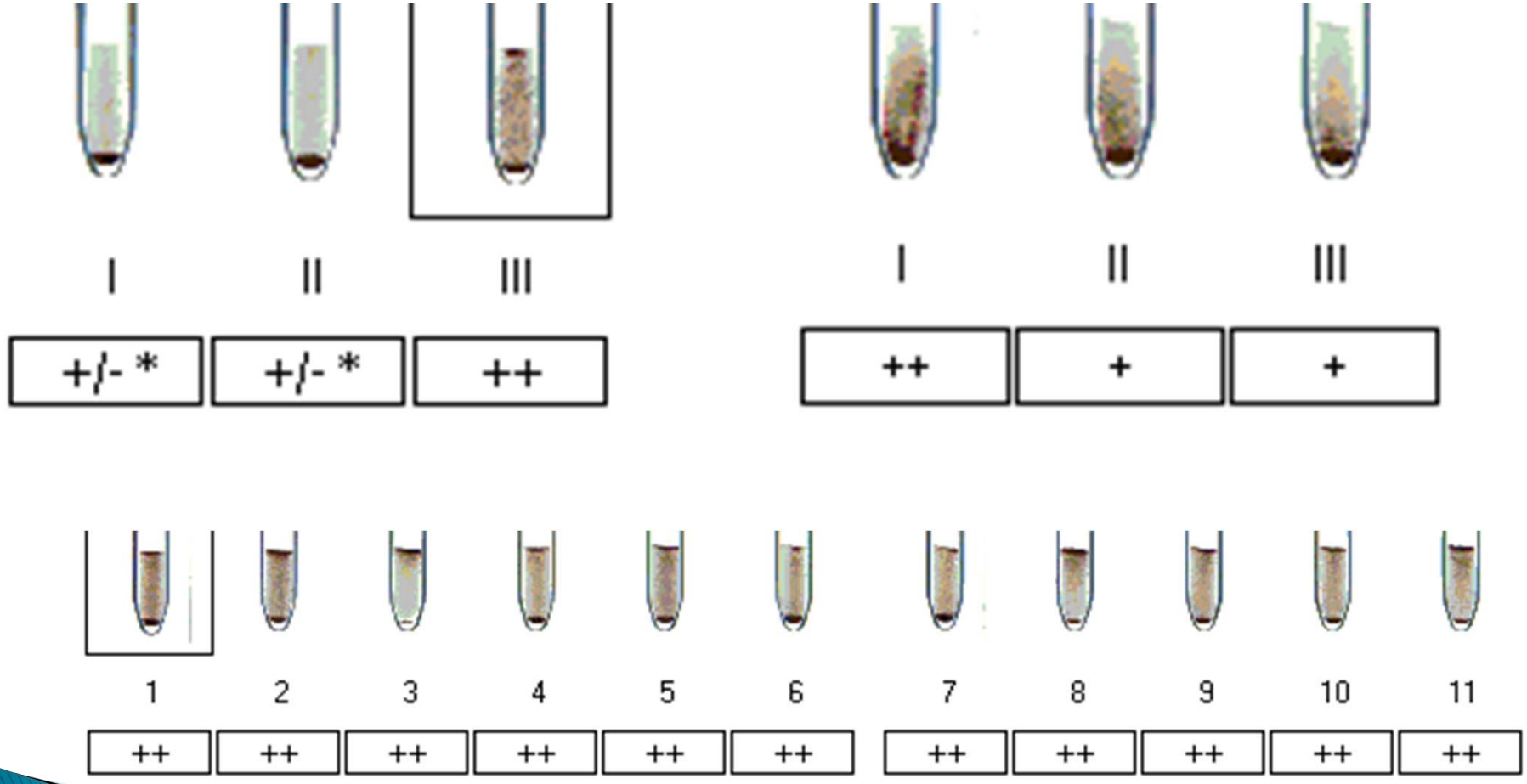
What do these reactions have in common?







What about these?



What is causing these reactions?

- These patients do not have any allo-antibodies.
- They do not have any auto-antibodies.
- The reactions are caused by one or more of the constituents in the diagnostic reagent red cells.

What are these constituents?

- Antibacterial Agents.
- Diluents.
- Preservatives.

Example- BioCSL 3% cells contain:

- Chloramphenicol and Neomycin Sulphate.
- Celpresol, an isotonic citrate phosphate buffered solution containing glucose and amino acids.
- Thiomersal.

Why is this happening?

- Many reasons however the main cause is the antibiotics.
- Majority of manufacturers use Trimethoprim (TMP) and Sulfamethoxazole (SMX) in their 0.8% cells and some in their 3% cells.
- When antibiotics are administered patients can develop antibodies.
- These antibodies are present in the patients plasma and react with the antibiotics in the reagent red cells.
  - They are not reacting with patients antigens.
  - They are interfering with pre-transfusion testing.

What do we do about it?

Once identified it is easy:

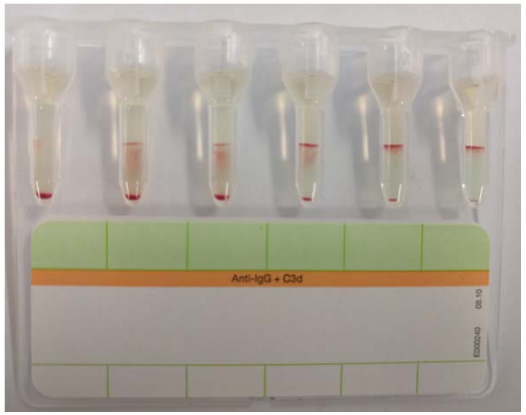
- Repeat testing with a different product.
  - Eg. bioCSL 3% cells have different antibiotics to their 0.8% cells.
    - Repeat testing by tube with 3% cells.
    - Dilute 3% cells to a 0.8% cell suspension and repeat testing by Column Agglutination Technique (CAT).
- Wash cells and resuspend in a different diluent.
  - Eg. BioRad ID-Diluent 2 contains SMX and TMP whereas bioCSL Celpresol contains Chloramphenicol and Neomycin Sulphate.

How do we identify the cause in the first place?

- This is the problem, it can be time consuming.
- Common features are:
  - All cells react with a similar pattern and strength suggesting an antibody to a high incidence antigen.
  - Direct Coombs Test (DCT) typically negative, excluding an auto-antibody.
  - Reactions with CAT testing but none with tube testing.
- May have a Positive auto control but Negative DCT.

What can I do?

- I reviewed 9 cases to see if I could identify any patterns to assist.
  - Initial testing performed using:
    - bioCSL 0.8% Abtectcells which contain the antibiotics SMX and TMP.
    - BioRad Diamed LISS/Coombs cards.

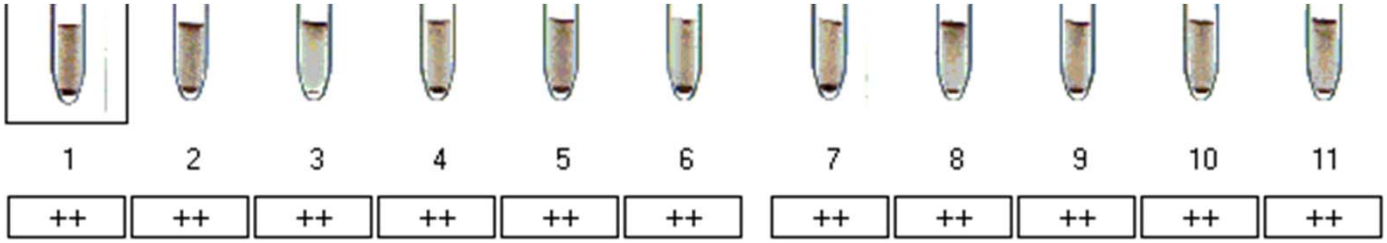


- 3 patterns were identified.

Footn

Pattern 1 – Strange DP like reactions

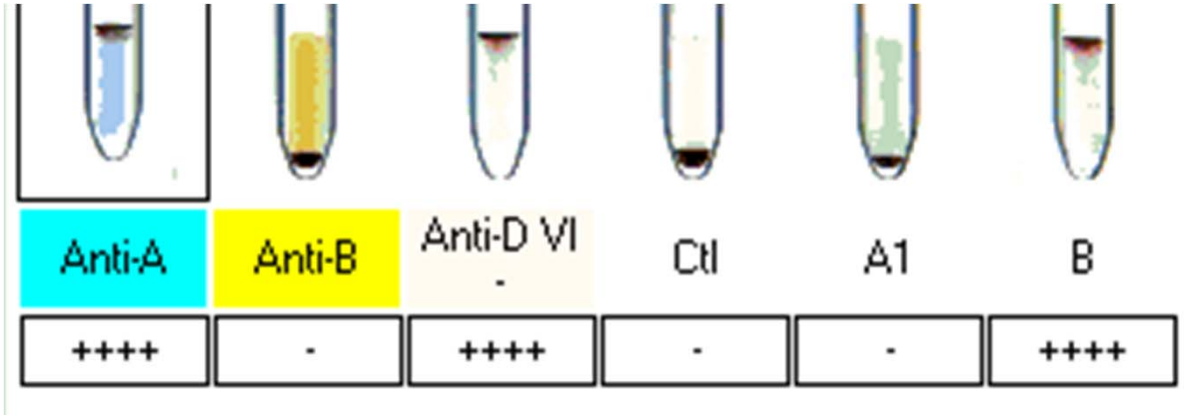
- Easiest to identify.
- Look strange and unlike most reactions caused by allo-antibodies.
- Dual population-2+ reactions in all cells
- 5 patients resembled this picture.



Pattern 1 – Blood Group

- No interference with Blood Group using BioCSL 0.8% Revercells.

- BioCSL 0.8% and 3% Revercells contain antibiotics Chloramphenicol and Neomycin Sulphate.



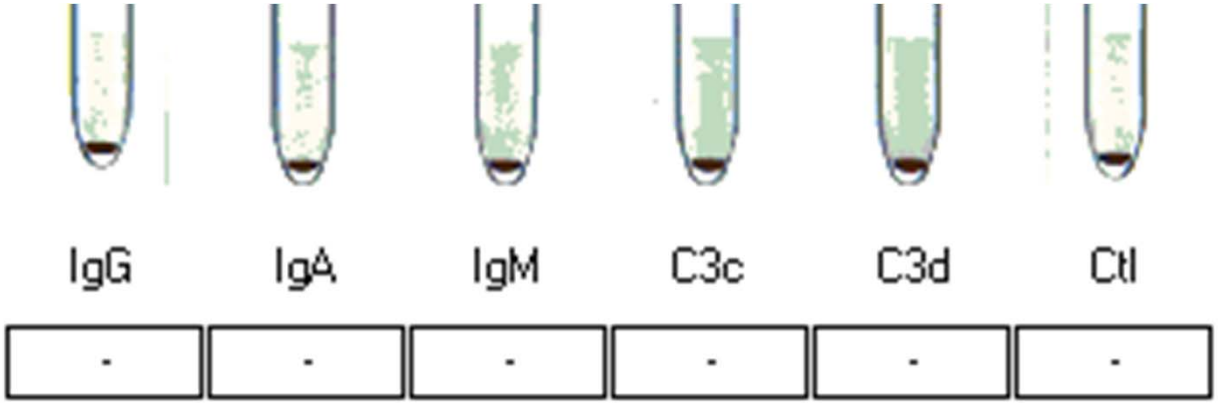
Pattern 1 – Other manufactures

- BioRad ID-DiaCell 0.8% cells screening cells:
  - Similar reactions however appeared weaker.
  - Also contains antibiotics TMP and SMX.
  - ? Do these cells have a lower concentration of the antibiotic.

- Grifols Tube Perfect 11 cell 0.8% panel.
  - Identical reactions to bioCSL 0.8% cells.
  - Also contains antibiotics TMP and SMX.

Pattern 1 – Further testing

- Direct Coombs Test Negative



- All tube testing with bioCSL 3% cells at Room Temp (RT), 37C and IAT are Negative.

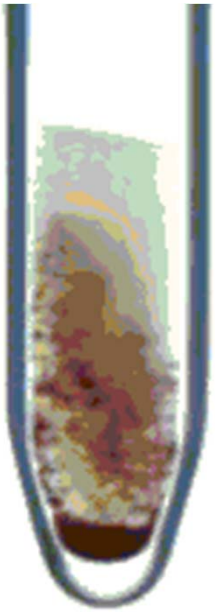
Pattern 1 – Further testing

- BioRad Pre-Papainised Panel tested on Neutral/Saline Card:
  - 1 of 5 patients was tested.
  - Negative reactions.
  - However, panel contains SMX and TMP.

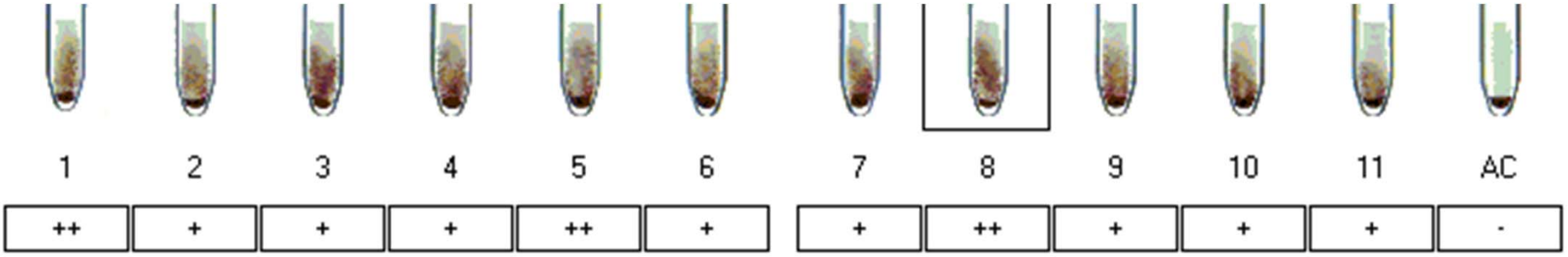
Pattern 1 – Anti-TMP

- Similar findings reported in literature suggests these reactions may be due to trimethoprim (TMP).
- Both TMP and SMX are recommended first line treatment for uncomplicated Urinary Tract Infections (UTIs).
- Women are more susceptible to UTIs.
- Occasionally prescribed for chest infections.
- All 5 of patients female aged 33-53.
- Clinical notes “history of UTIs in childhood”, “COPD”and “THR”.

Pattern 2



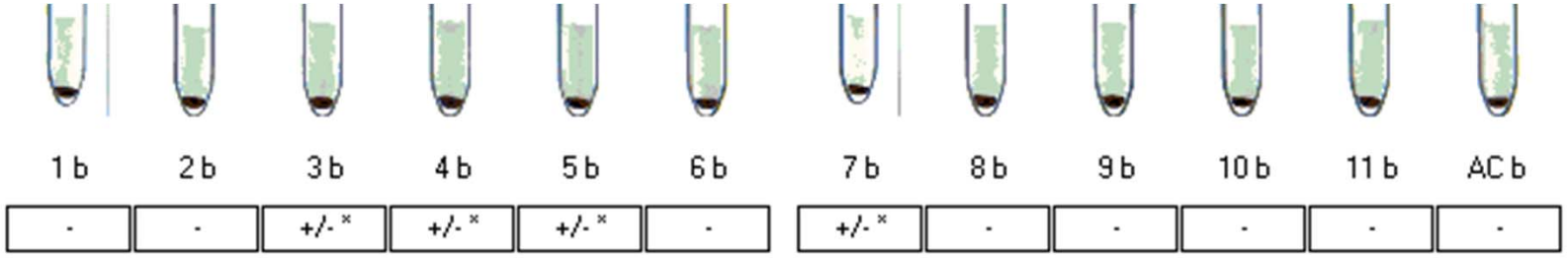
- 3 patients.
- “Real allo-antibody-like reactions.
- Reactions 1-2+



Pattern 2 – Further testing

- Results all same as pattern 1.

- Additional testing performed on 1 patient sample
  - Different enzyme: Bromelin (Diluent 1) with bioCSL panel tested on saline/neutral Card.
  - Results also Negative to very weak.



Pattern 2

- Patients all female aged 29-59.
- Clinical notes: “Pregnant, UTI” , “?UTI” and “recurrent bladder infections”.
- Results suggest these reactions may also be due to trimethoprim.
- Anti-TMP has an apparent anti-Ku specificity due to negative reactions with K_o RBCs.
- Hypothesised that TMP may bind to the Kell glycoprotein and that IgG antibodies against TMP may react with RBC-bound TMP.

Pattern 1 and 2 both TMP

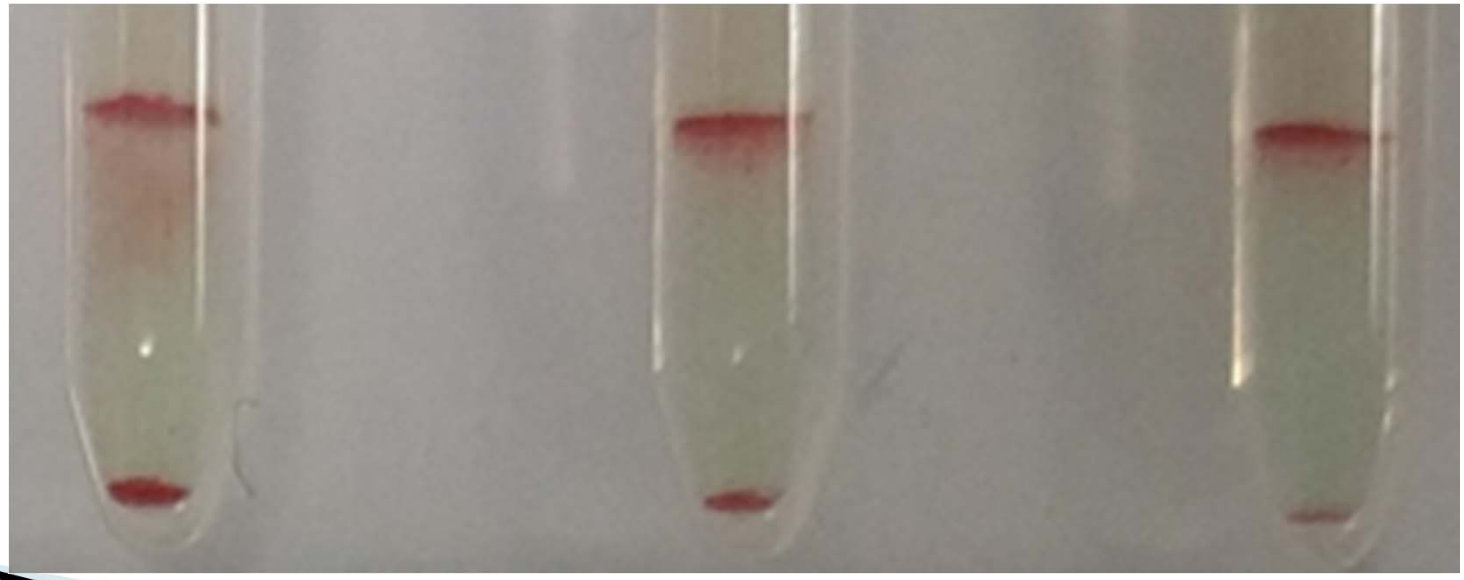
- Quoted from journal article by Pham et al:

The different patterns of antibody reactivity support the evidence that the immune response elicited by a given drug is an individual response to antigenic challenge governed by many factors.

Pattern 3 – The difficult one

- 2 patients, both female with multiple co-morbidities, aged 68 and 82.

- Stronger “DP like reactions”.



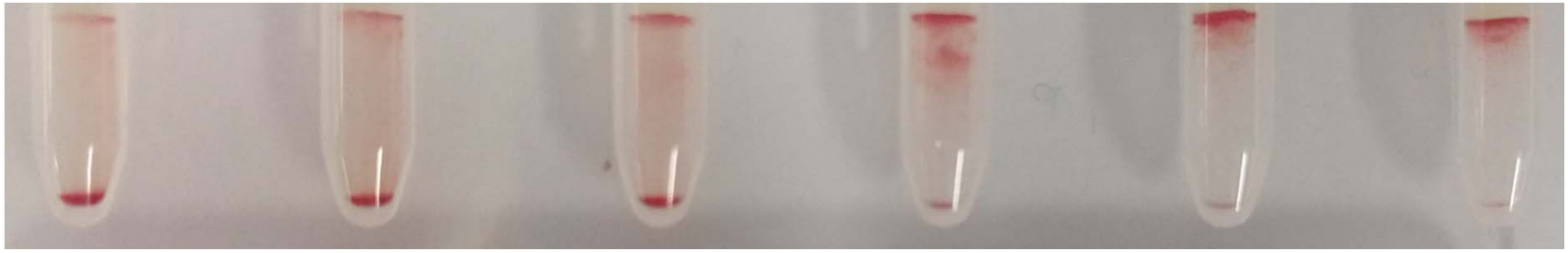
Pattern 3 – Investigations

- Diluent confirmed as:
  - 3% cells diluted to 0.8%, tested by CAT: Negative.
  - All 37C IAT tube testing: Negative.
  - Grifols 0.8% cells similar reactions to CSL 0.8%.
  - BioRad 0.8% cells had typical weaker reactions.

- However, appears as a cold auto antibody and antibody to diluent.
  - DCT: C_3d (1+).
  - Papain: 4+ reactions, Bromelin 3+.
  - Tube RT 1-2+ reactions.

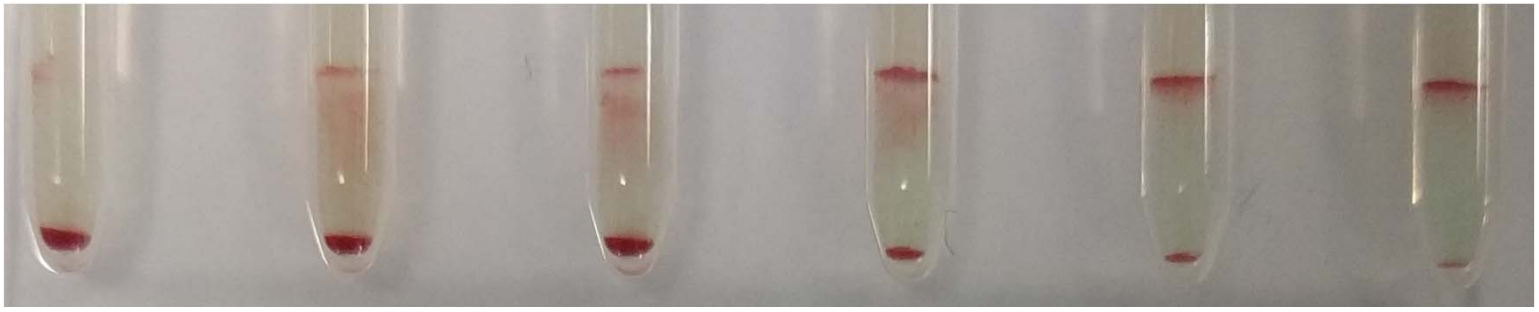
Comparing 0.8% cells containing TMP and SMX

- Saline/neutral card incubated at RT for 15mins:



Biorad screening cells CSL screening cells

- LISS/Coombs card:



Biorad screening cells CSL screening cells

Pattern 3

- Literature suggests reactions may be due anti-SMX or both anti-SMX and anti-TMP.
- Reported that antibodies to SMX have an apparent anti-H specificity.
- May bind to a component of H substance.
- Patients were both A Pos.

In conclusion

- Reactions due to diluents/antibiotics appear as an antibody to a high incidence antigen.
- Results of testing often don't make sense.

- Understand your reagents.
- Understand different antibiotics used by manufacturers.

- Although my aim was to identify some patterns and reactions I realised it can really vary according to an individuals immune response.
- This topic needs more investigating to obtain more helpful conclusions.

References

- Arndt PA, Garratty G, Wolf CF, Riviera M. Haemolytic anaemia and renal failure associated with antibodies to trimethoprim and sulfamethoxazole. Transfusion Medicine 2011; 21:194-8.

- Le Pennec PY, Babinet J, Noizat-Pirenne F, Dubeaux I, Rouger P. Sulfamethoxazole and trimethoprim dependent antibodies with respective anti-H (H1) and anti-Ku (KEL5) specificity (abstract). Transfusion 1999;39 Suppl:81S.

- Pham BN, Gien D, Bensaad F, Babinet J. Antibodies to co-trimoxazole (trimethoprim and/or sulfamethoxazole) related to the presence of the drug in commercial low-ionic-strength-solution. Transfusion 2012;52:844-848.