

# Antibodies to Co-trimoxazole interfering with routine antibody screening: Case

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# Background

- ▶ **In-vitro reactions not due to blood group antibodies** are encountered when typing red cells /performing compatibility testing /antibody screen
- ▶ Patient has an antibody that reacts with a chemical present in –
  - ▶ The commercial RBC suspension
  - ▶ Commercial Anti-sera
  - ▶ Commercial Antibody Potentiators (Albumin or LISS)
- ▶ These reactions can lead to **serious errors or incompatible crossmatches or delay** in supplying blood for transfusion

## Table : Reported antibodies to chemicals added to reagents

Antibiotics	Bacteriostatic /antifungal reagents	Sugars	Dyes	Miscellaneous
neomycin	Paraben®	glucose	acriflavine	EDTA
chloramphenicol	thimerosal		yellow #5 tartrazine	inosine citrate
gentamycin	sodium azide			sodium caprylate

# Chemical Present in Commercial RBC suspending Media

## Many companies

adenine  
chloramphenicol  
glucose  
inosine  
neomycin sulfate  
sodium chloride  
sodium citrate

## A few companies\*

adenosine  
citric acid  
gentamicin  
guanosine  
hydrocortisone  
magnesium sulfate  
potassium chloride  
potassium phosphate  
sodium acetate  
sodium bicarbonate  
sodium gluconate  
sodium phosphate  
sodium pyruvate  
sucrose

# Case

- ▶ 36 year old female,
- ▶ Admitted with complaints of Generalized weakness and headache for 3 days
- ▶ a known case of hydrocephalus, Aseptic meningitis
- ▶ On regular antiepileptic, antidepressant, anti-osteoporotic medications
- ▶ VP shunt was done in 2009 and admitted for surgery for revision of V-P shunt

# Request for Type and screen

- ▶ Hemoglobin -11.5
- ▶ **Blood group – ABO Discrepancy**
- ▶ Forward Grouping – A positive
- ▶ Reverse grouping – Mixed field reactions with A cells and O cells
- ▶ ? Cold Agglutinins
- ▶ Tube method confirmed BGP– A Positive



# Antibody screen

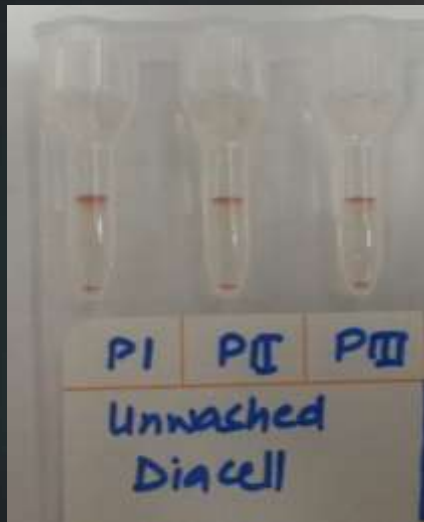
- ▶ Using Gel method (Biorad ID-Diacell I-II-III),
- ▶ 4+ Panreactive antibody detected in AHG phase,
- ▶ Direct Coombs' Test – Negative
- ▶ Auto control - Negative

## 1. Autoimmune etiology was ruled out

Negative DCT (*anti-IgG and anti-C3d*) and autocontrol



# Antibody Identification



## 2. Antibody against High frequency antigen

- ▶ (anti-k, anti-kpb, anti-Lub) was suspected.

## 3. Panreactive antibody in saline phase

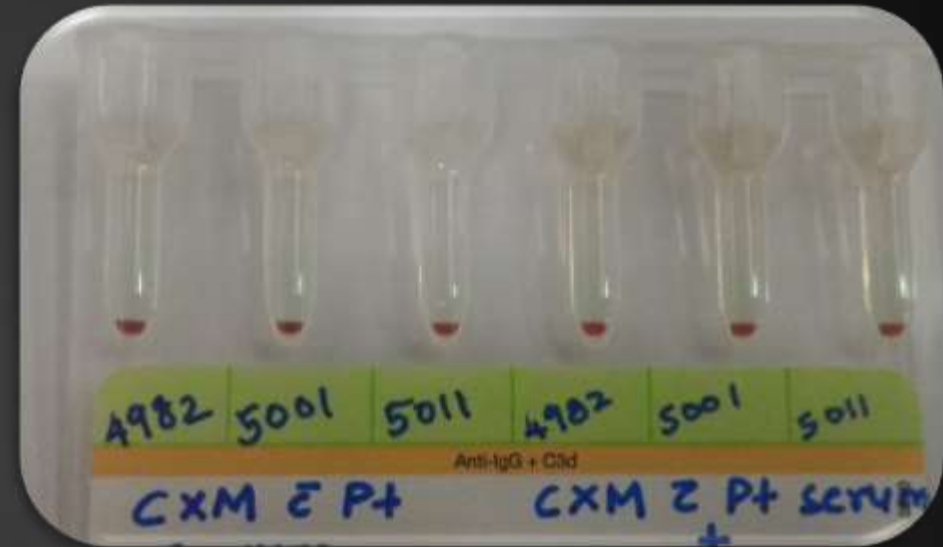
- ▶ Anti-IH antibody was also kept as a possibility.

The Neurosurgery Team was informed for Positive antibody screen and possibility of Anti-HFA



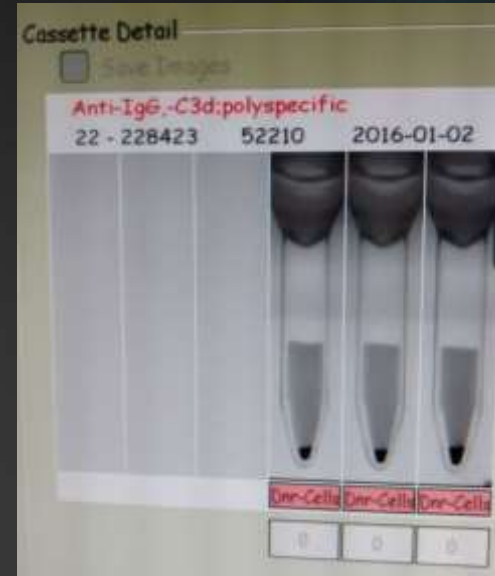
# Conflicting Crossmatch results

- ▶ To rule out Anti-IH, crossmatching was performed with both A and O positive units, It showed compatible results with both A/O units.
- ▶ **Indirect Coombs Test by Tube method- Negative**

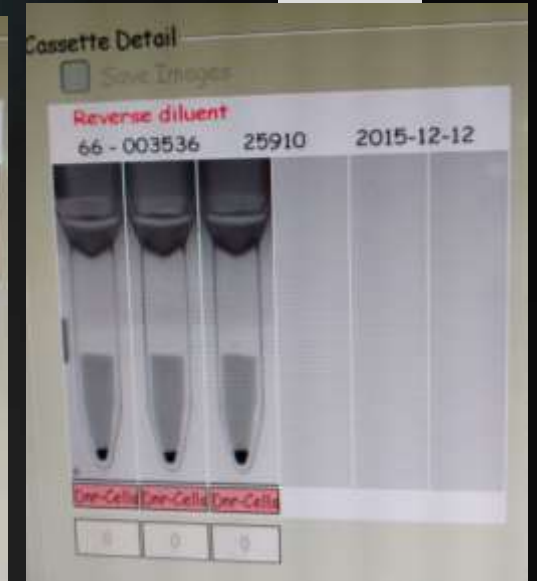


- ▶ Sample were tested with commercial screen cells of different manufacturers
- ▶ a. **Surgiscreen cells** using Glass bead method, Orthoclinical diagnostics
- ▶ B. **Matrix ERYGEN AS reagent red cells** ; Tulip diagnostics
- ▶ **The results with other commercial red cells were nonreactive.**

**? Antibody to reagent red cell preservative**



**Nonreactive with Surgiscreen reagent red cells**



**Nonreactive with Matrix Erygen AS cells**

# Manufacturing Inserts

All test cell reagents are of human origin, in a buffered suspension medium at 0.1% ( $\pm 0.01\%$ ).  
Preservatives: the antibiotics trimethoprim and sulfamethoxazole.

For antibody screening, single donors, blood group O:

ID-DiaCell I-II	$R_1^w R_1 + R_2 R_2$ for IAT and NaCl test
ID-DiaCell I-II-III	$R_1^w R_1 + R_2 R_2 + rr$ for IAT and NaCl test
ID-DiaCell IP-IIP-IIIP	papainized, for enzyme technique
ID-DiaCell Pool	$R_1 R_1 + R_2 R_2$ (2 pooled cells for donor screening)
ID-DiaCell I-II-III Asia	$R_1 R_1 + R_2 R_2 +$ cell of the GP.MUR phenotype, for IAT and NaCl test

Ortho Clinical Diagnostics

**Surgiscreen Reagent Red cells**,  
Phosphate citrate buffered diluent, Steroid,  
Chloramphenicol, neomycin sulphate, Gentamycin

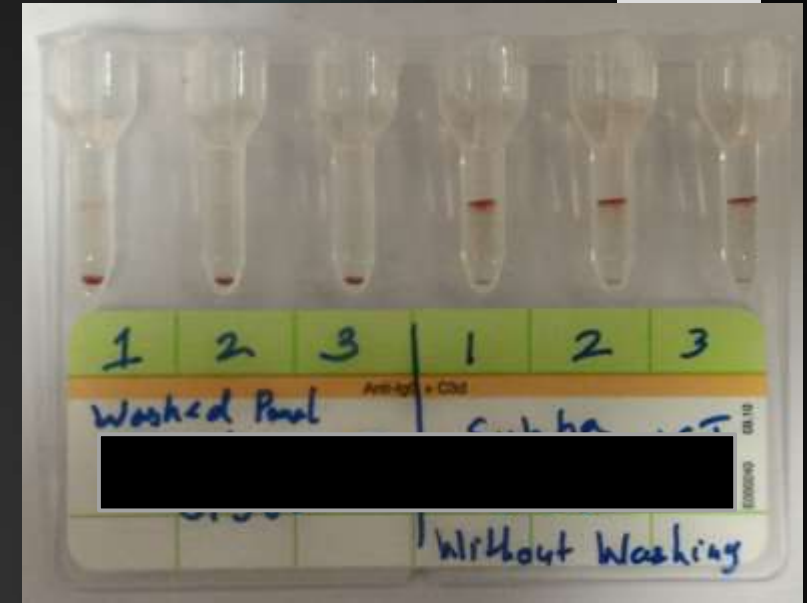
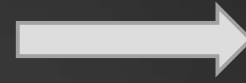


Special preservative media

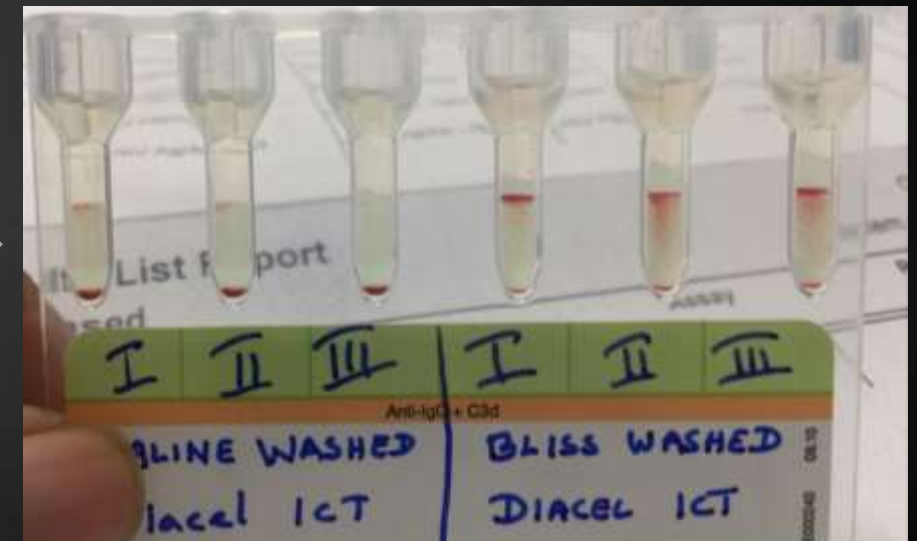
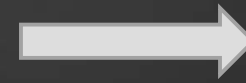
Table 1: Patient red cell antibody screen results with commercial red cell reagents

<b>Manufacturer</b>	<b>Bio-Rad Laboratories ID-Diacell I-II-III</b>	<b>Ortho clinical Diagnostics Surgiscreen cells</b>	<b>Tulip diagnostics Matrix ERYGEN AS reagent red cells</b>	<b>Tube method Using in-house Pooled O cells</b>
<b>Preservative</b>	Trimethoprim and sulphamethoxazole	Chloramphenicol, neomycin sulphate, Gentamycin, Steroid	Contents not disclosed	<b>None</b>
<b>Antibody screen results</b>	<b>Panreactive 4+ in AHG as well as phase</b>	<b>Negative</b>	<b>Negative</b>	<b>Negative</b>

▶ To confirm the nature of drug induced antibodies, reagent red cells were washed and ICT was found negative with washed reagent red cells.

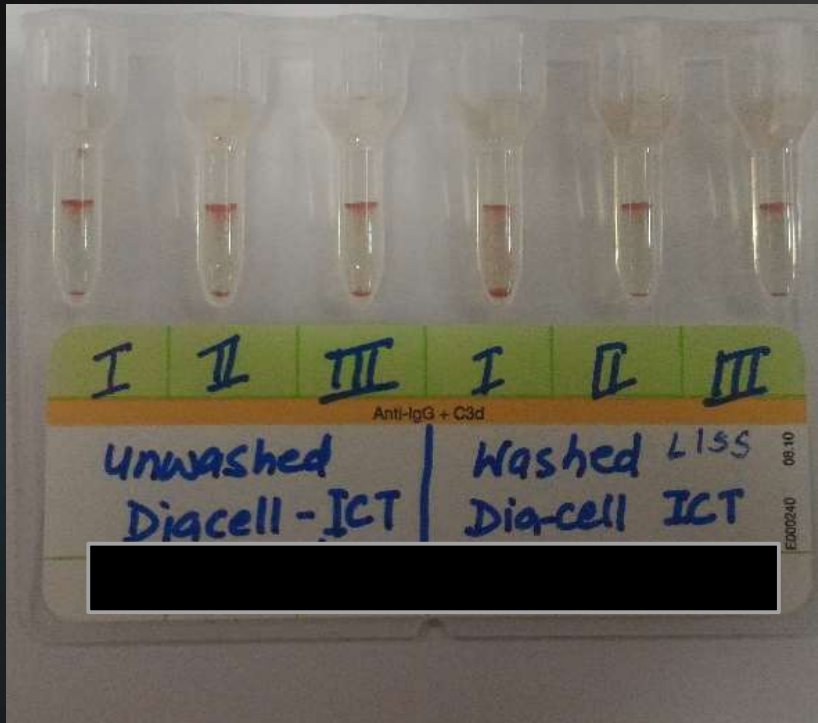


- ▶ On incubating the reagent red cells in BLISS, ICT was reactive
- ▶ Also, on incubating the donor red cells with BLISS, the crossmatch was showing reactive due to presence of some preservative drug in BLISS as Co-trimoxazole



# Red cell diluents

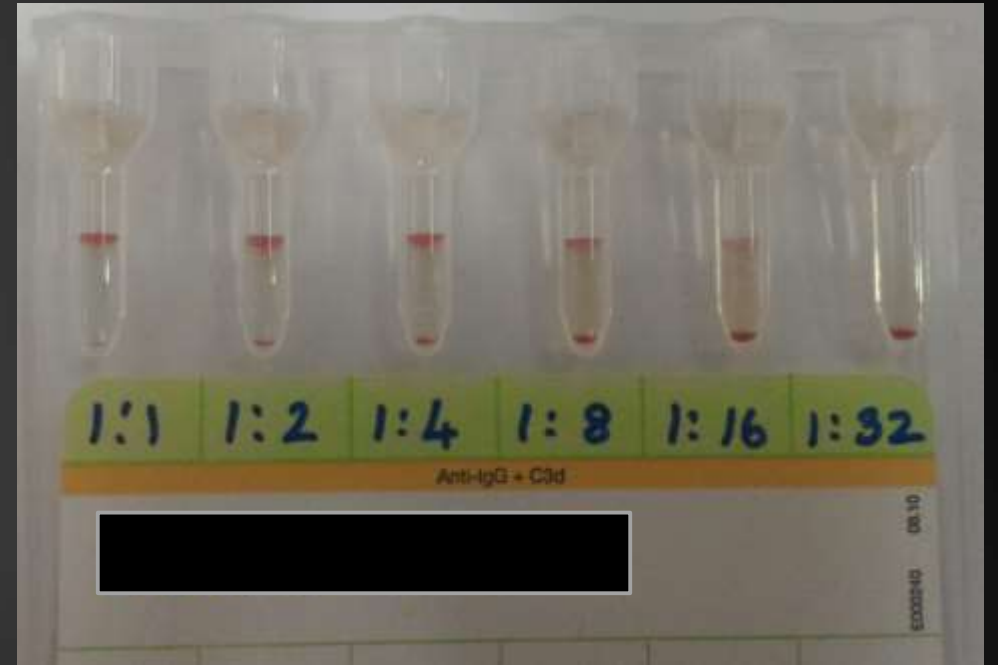
- ▶ **ID –Diluent 2** : Modified LISS for red cell Suspensions:
  - ▶ Preservative : the antibiotics trimethoprim and sulphamethoxazole
- ▶ **Ortho BLISS**
  - ▶ Preservative: Chloramphenicol, Trimethoprim and sulphamethoxazole



Washed reagent red cells incubated with LISS for More than 1 hour RT



Donor cells + LISS 1 hr Incubation+ Pt serum



Titration 1:16

# Final Diagnosis

- ▶ On review of past history, Tab Bactrim was administered for UTI two month before
- ▶ It was confirmed to be an anti- Co-trimoxazole drug induced antibody against preservative media in reagent red cells.
- ▶ Co-trimoxazole is an antibacterial drug, a combination of two drugs: Trimethoprim (TMP) and Sulphamethoxazole (SMX)



# Patient evaluation

- ▶ There were no clinical features of hemolysis.
- ▶ Blood transfusion was not required during surgery.
- ▶ The patient and the physician were explained regarding potential risk of hemolysis if the same drug is taken.
- ▶ Patient was discharged with stable status with proper advice.
- ▶ The possibility of **Co-trimoxazole Drug induced aseptic meningitis** was also considered.

**Garratty G Am J Med Technol 1976**

**Problems in pre-transfusion tests related to drugs and chemicals.**

Howard PL et al. *Transfusion* 1976 Mar-Apr;16(2):166-9.

**Blood bank reagents: some problems related to preservatives and dyes.**

Beattie KM et al. Transfusion. 1976 Mar-Apr;16(2):174-7.

**Chloramphenicol** antibody causing interference in antibody detection and identification tests.

Shulman IA et al *Transfusion* 1984 Jul-Aug;24(4):365-7.

**Thimerosal**-dependent agglutination complicating the serologic evaluation for unexpected antibodies.

Umlas J et al *Transfusion* 1993 .

**Antibodies to hydrocortisone** in reagent red cells causing positive antibody screening tests.

Pham BN et al. Antibodies to **co-trimoxazole (trimethoprim and/or sulfamethoxazole)** related to the presence of the drug in a commercial low-ionic-strength solution. *Transfusion*. 2012 Apr;52 (4):844-8.

# Discussion

- ▶ These in-vitro reaction due to preservative antibiotic drugs are similar to reactions of DIHA
- ▶ Follow “Immune complex mechanism”
- ▶ Reaction is seen only when patient serum containing antibody is added to RBCs in the presence of antibiotic (i.e. the commercial RBC suspension media)
- ▶ Certain drug antibodies reported has shown Antibody specificity
  - ▶ Paraben – Anti-Jka

# Discussion

- ▶ Co-trimoxazole is an antibacterial drug, a combination of two drugs: Trimethoprim (TMP) and Sulphamethoxazole (SMX).
- ▶ These antibodies have shown to be associated with Hemolytic anemia, renal failure, aseptic meningitis
- ▶ Antibodies against Co-trimoxazole shows three Patterns
  - ▶ Anti-TMP + Anti-SMX
  - ▶ Anti-TMP alone (anti-Ku specificity)
  - ▶ Anti – SMX (Anti-H specificity)
- ▶ These anti-preservative antibodies should be assessed carefully, and despite absence of clinical events, the avoidance of drug should be recommended

# To summarise ..

- ▶ These anti preservative antibodies should be suspected and given attention whenever
  - ▶ Conflicting results are noted in antibody screening with different techniques of similar sensitivities,
  - ▶ Panreactive results and absence of clinical and biochemical data matching with results.



**Thank  
you**

#### Acknowledgements

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