

# Discrepancies in Immunohaematological Testing-Importance of Use of Multiple Modalities of Testing

SOUMEE BANERJEE<sup>1</sup>, PARIMALA PUTTAIAH<sup>2</sup>, ANUPA JACOB<sup>3</sup>, SITALAKSHMI SUBRAMANIAN<sup>4</sup>

## ABSTRACT

Two cases are being reported here which shows that in case of immuno-hematological discrepancies, the standard mode of testing should always be supplemented by additional methods to gain an insight into further work-up. Case 1: A 28-year-old multigravida without history of co-morbidities, transfusion or drug intake, showed pan-agglutination with all commercial cells- three reverse grouping cells, Indirect Antiglobulin Test (IAT) and antibody screening on automated and manual gel-card and tube methods. Auto-control and Direct Antiglobulin Test (DAT) were negative on both platforms. Forward grouping was B positive on both platforms. Using in-house cells, automated and manual gel-card and tube methods, IAT was negative and reverse grouping was B positive. Suspecting reagent dependant reactivity and washing the commercial cells resolved all discrepancies on all platforms. Case 2: A 20-year-old lady without any history of any major illness or drug intake came for a routine check-up. Forward grouping showed 4+ reaction with Anti-A and Anti-D on automated and manual gel-card methods, but Anti- B showed double population with multiple commercial and donor-derived anti-B antisera. By tube method, it showed mixed field reaction. Reverse grouping was consistent with AB positive and Coomb's minor cross-matches with AB positive units were compatible, with B and O units cross-matching was incompatible and with A positive units, it showed double population. DAT was negative and saliva testing confirmed secretor status for A and B antigens. Suspecting AB and A mosaic, cells were agglutinated with anti-B, the agglutinates allowed to settle and free cells re-suspended. This was done repeatedly to get a population of completely free cells that showed A positive on forward grouping. Both patients lacked any significant relevant history and did not want further testing. Counselling was done for them and their physicians about the discrepancies and safety of future transfusions. Hence in discrepant cases, repeat testing with atleast one additional modality is highly recommended as a first step to sculpting out a patient-specific line of management.

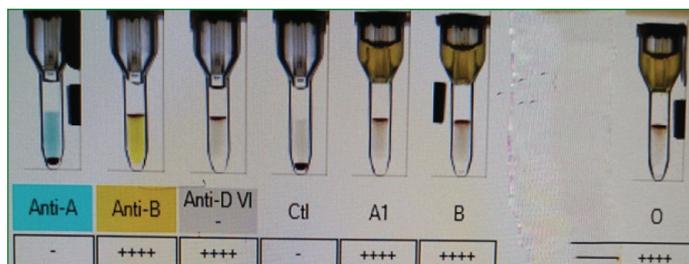
**Keywords:** Antiglobulin test, Automation, Mosaic

## INTRODUCTION

Automation has advantages like high through-put, facilities for data storage and retrieval, better standardisation, accuracy and reproducibility [1]. However, ancillary methods are required to resolve discrepancies identified by automated testing platforms. In non-automated settings, Column Agglutination Technology (CAT) and Conventional Tube Test (CTT) should be used to supplement discrepant findings in immuno-hematology. Two cases are presented here. Although examples of both cases have been reported previously in the literature, the novelty of these case reports presented lies in the interpretation of the significance of discrepant and similar findings, respectively, on different modalities of immuno-haematological testing. These reports also illustrate a step-wise and patient-specific approach to discrepancies and the importance of prioritisation, that can be particularly relevant in resource limited settings.

## CASE 1

A 28-year-old multigravida (G3P3L1A1) presented at 36+3 weeks of gestation with pain in the abdomen. There was no associated co-morbidity, drug or transfusion history. One unit of Packed Red Blood Cells (PRBC) was requested in anticipation of a caesarean section.



**[Table/Fig-1]:** ABO Grouping and RhD typing on IH-500 with commercial cells- While forward grouping shows reaction consistent with "B Positive", there is pan-agglutination with all cells in reverse grouping.

## Investigations

### Step 1: ABO-Grouping and RhD Typing

The forward grouping on our automated platform (IH-500, Biorad, Cressier, Switzerland) with commercial cells (ID-Diacell ABO, Biorad, Cressier, Switzerland) and grouping gelcards (ID-card, DiaClon ABO/D+reverse grouping, Biorad, Cressier, Switzerland) was 'B Positive'. In reverse grouping, there was pan-agglutination (4<sup>+</sup>) with A, B and O cells as seen in [Table/Fig-1]. By CTT with inhouse cells, the grouping was 'B Positive' as seen in [Table/Fig-2].

	Anti-A	Anti-B	Anti-D	Control	Result	A-cell	B-cell	O-cell	Result
IH500 (with Biorad ID cells)	0	4 <sup>+</sup>	4 <sup>+</sup>	0	B Positive	4 <sup>+</sup>	4 <sup>+</sup>	4 <sup>+</sup>	?
*CTT (inhouse cells)	0	4 <sup>+</sup>	4 <sup>+</sup>		B Positive	4 <sup>+</sup>	0	0	B

**[Table/Fig-2]:** ABO Grouping and RhD typing on IH-500 with commercial cells and by conventional tube method with inhouse cells showed a discrepancy in the reverse grouping.

\*CTT: Conventional tube testing;

† with commercial cells, there is pan-agglutination on the automated platform, leaving reverse grouping discrepant; with inhouse cells by CTT, grouping is B Positive

### Step 2: Evaluation of Panagglutination (Indirect Antiglobulin Test and Antibody Screen)

Indirect Antiglobulin Test (IAT) with commercial cells (ID-Diacell Pool, Biorad, Cressier, Switzerland) on IH500-positive (4+). With inhouse cells, IAT by manual gelcard (ID card- LISS/Coombs, Biorad, Cressier, Switzerland)-negative. Antibody screen with commercial 3-cell panel (ID-Diacell I-II-III, Biorad, Cressier, Switzerland) by manual gelcard method was panreactive (4+) with negative autocontrol.

### Step 3: Reaffirming Discrepancy- Interchanging Platforms

With commercial cells the reverse grouping and IAT were positive by CTT and manual gelcard methods. With inhouse cells, the reverse grouping showed "B" and the IAT with pooled O cells was negative on IH500. This is highlighted in [Table/Fig-3,4]. On IH500, The grouping control and DAT were both negative.

	Anti-A	Anti-B	Anti-D	Control	Result	A-cell	B-cell	O-cell	Result
*CTT (with commercial grouping cells)	0	4+	4+	0	B Positive	4+	4+	4+	?
IH500 (with inhouse pooled cells)	0	4+	4+		B Positive	4+	0	0	B

**[Table/Fig-3]:** ABO Grouping and RhD typing on interchanging platforms- by conventional tube method with commercial cells and on IH500 with inhouse cells, showing that reverse grouping showed panagglutination with commercial cells irrespective of platform.  
\*CTT: Conventional tube testing;  
† with commercial cells, there is pan-agglutination by CTT, leaving reverse grouping discrepant but with inhouse cells on automated platform, grouping is B Positive

*IAT (commercial O-pooled cells by manual CAT)	4+
IAT (inhouse O-pooled cells on IH500)	0

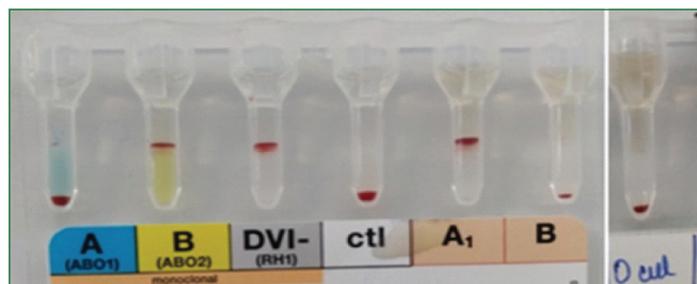
**[Table/Fig-4]:** IAT on interchanging platforms- manually by CAT with commercial cells and on IH500 with inhouse cells showing a positive reaction with commercial cells only.  
\*IAT: indirect antiglobulin test

### Step 4: Identifying the Discrepancy

Since positive reactions were seen only with commercial cells and not donor cells at the same phase of testing, reagent-dependent reactivity was suspected [2,3]. Antibodies have been described against various components of the reagents [4] e.g., bacteriostatic agents.

### Step 5: Resolving Discrepancy and Establishing Safety of Transfusion

The grouping was done on IH500 and ID Card- NaCl, enzyme and cold agglutinins (Biorad, Cressier, Switzerland) and IAT on gelcard with saline washed commercial reagent cells. There was resolution of both issues. The grouping was 'B Positive' and IAT was negative. This is shown in [Table/Fig-5].



**[Table/Fig-5]:** ABO Grouping and RhD typing with commercial cells washed thrice with 0.9% normal saline showed reaction consistent with "B Positive" by both forward and reverse grouping.

Coomb's Major cross-matches with 2 B Positive units were compatible both by automated and manual gelcard methods.

On repeating the complete work-up with a fresh sample, same results were obtained.

### Management

The patient did not require any transfusion during the current admission. Her treating physicians were informed of this serological issue. Due to her financial constraints, it was decided to limit the work-up to resolving her serological issue and assessing safety of future transfusions rather than further characterising the implicated antibody. She was grouped as "B Positive".

### CASE 2

A 20-year-old female had asked for a blood grouping as part of a routine check-up.

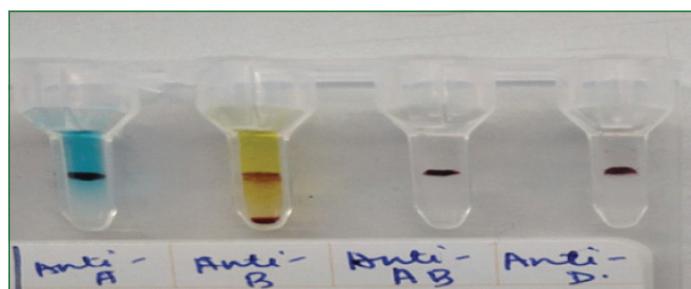
### Investigations

#### Step 1: ABO-Grouping and RhD Typing

ABO Grouping and RhD typing showed double population with anti-B on DiaClon ABO/D+ reverse grouping (Biorad, Cressier, Switzerland) gelcards in both IH-500 and manual methods. When done by manual tube method, there was mixed field reaction with anti-B antisera (Monoclonal, Eryclone, Tulip Diagnostics, Goa). There was 4+ agglutination with anti-A and anti-D. Reverse grouping was consistent with AB. This is shown in [Table/Fig-6,7].

	Anti-A	Anti-B	Anti-D	Ctl	Result	A cell	B cell	O cell	Result
On IH-500	4+	dp	4+	0	?	0	0	0	AB
CTT	4+	Mf	4+		?	0	0	0	AB

**[Table/Fig-6]:** ABO Grouping and RhD typing on IH500 and by conventional tube testing- indeterminate as sample shows discrepant reaction with anti-B antisera, on both platforms of testing.  
\*\*dp- double population; Mf-mixed field



**[Table/Fig-7]:** ABO Grouping and RhD typing by manual CAT showing double population with anti-B antisera. It was done manually by CAT after a similar pattern was seen in automated CAT. The pattern was same with multiple anti-B commercial and donor derived antisera on CAT.

#### Step 2: Confirming the Discrepancy

The forward grouping showed the same pattern with antisera from 2 different manufacturers (Combined ABO Monoclonal Antibodies, J.Mitra, New Delhi and Span Clone ABD agglutinating antisera, Span Akrey Healthcare, Surat) and with sera obtained from donors.

#### Step 3: Assessing RBC antigenic and antibody expression patterns

Her DAT by manual gelcard was negative. With Anti-AB and Anti-H lectin as highlighted in [Table/Fig-8].

Anti-AB	Anti-H
4+	2+

**[Table/Fig-8]:** Reaction patterns with Anti-AB and Anti-H Lectin.

#### Step 4: Documenting Secretor Status

Her secretor status by a saliva test was as illustrated in [Table/Fig-9].

	Anti-A (1:256)	Anti-B (1:512)
Saline control	2 <sup>+</sup>	2 <sup>+</sup>
Known Secretor (AB)	0	0
Known non-secretor (O)	2 <sup>+</sup>	2 <sup>+</sup>
Test	0	0
Interpretation	Secretor of A	Secretor of B

**[Table/Fig-9]:** Secretor status by saliva testing showing secretor status for both A and B antigens.

### Step 5: Attempting to Resolve the Discrepancy

After a thorough literature search, suspecting a AB Positive and A Positive mosaic [5], agglutination of cells with anti-B antisera was done, followed by separation of the agglutinated cells by repeated sedimentation and resuspension [6]. With the free cells, forward grouping by CTT was A Positive.

### Step 6: Assessing Safety of Transfusion

Since this seemed to be a RBC-related issue, to assess the safety of any future transfusions, Coomb's minor cross-matching with sera from A, B, O and AB donors was tried.

There was compatibility with all 3 AB Positive donors and 1 out of 3 A positive donors. With the other 2 A positive donors, there was double population. The findings are summarised in [Table/Fig-10].

	A positive donor	B positive donor	O positive donor	AB positive donor
1	Compatible	Incompatible (4 <sup>+</sup> )	Incompatible (4 <sup>+</sup> )	Compatible
2	Incompatible (dp)	Incompatible (4 <sup>+</sup> )	Incompatible (4 <sup>+</sup> )	Compatible
3	Incompatible (dp)	Incompatible (4 <sup>+</sup> )	Incompatible (4 <sup>+</sup> )	Compatible

**[Table/Fig-10]:** Minor Coomb's Compatibility testing of patient with A, B, O and AB donors to assess safety of transfusion.

\*dp: double population;

† There was clear incompatibility with B Positive and O positive donors and clear compatibility with AB positive donors, but with A positive donors, the incompatibility pattern mirrors that with commercial and other donor derived anti-B antisera

### Management

After discussing the findings with the patient it was elicited that she had no significant past history of illness, transfusion or hematopoietic stem cell transplantation. She has two elder siblings and she was unaware of their blood groups. She did not want any further testing on herself or her family. She was grouped as AB Positive and in view of compatibility with "AB Positive" donors she was advised that for future transfusions, AB PRBCs would be safe for her.

### DISCUSSION

An immuno-haematological work-up ideally should begin with ABO grouping and RhD typing. Many discrepancies can be detected at this step itself. The nature of discrepancy and the results of any attempts made to resolve it can help in deciding the next steps of the work-up. These steps often include- DAT (to detect in-vivo sensitisation of RBCs by antibodies or complement) and IAT (to detect in-vitro sensitisation of reagent RBCs by antibodies in patient's serum) [1]. Any implicating antibodies thus detected can be identified by antibody screening and identification by multiple cell panels. Two common methods to perform these tests are by CTT with appropriate antisera and pooled cells, where presence/absence of agglutination determines a positive/negative reaction, or gel-based CAT. Here, after a centrifugation step, the gel traps agglutinated RBCs at the top of the column and free RBCs form a cell button at the bottom. Positive and negative reactions are read accordingly. CAT is also available on automated platforms. Antigens of the ABO system are secreted in other body fluids as well, and non-serological tests to detect them, like saliva test can provide helpful supplemental information in resolving immuno-haematological discrepancies.

In the first case, the difference in reaction patterns on the two platforms in the same phase prompted consideration of reagent-dependant reactivity as a possible cause. This helped rule out other differential diagnoses like antibodies against High Frequency Antigens (HFA) [7]. Discrepancies in reactions seen only with commercial cells and not donor cells at the same phase of testing may be due to reagent-dependent reactivity.

For the second case, confirming the pattern of agglutination with three modes- automated gelcard, manual gelcard and conventional tube method with different commercial and donor-derived antisera helped ascertain the uniformly aberrant pattern of reactivity with anti-B antisera and come-up with possible explanations for the same. Relying on any one modality may have caused attribution of this phenomenon to specific antisera-related issue or a weak reaction. Performing a simple test like saliva secretor status helped reaffirm the serological findings. Even in resource limited settings, this can prove to be a useful supplement to routine serological tests. Persistent double population with antisera of different manufacturers or donor-derived antisera in patients with no history of transfusion or transplant, may indicate a mosaic.

All work-up was done as part of our routine protocol of investigating a discrepancy with the ultimate goal of providing safe blood for the patients and were requested by the treating physicians. The patients have given written consent permitting use of their investigation details for publication under the condition of anonymity. However, the antibody in the first case and the suspected mosaic phenotype in the second case required further serological and gene-based testing, respectively, for confirmation. Both patients were, however, unable to afford and unwilling to do any further testing. So the focus was on establishing the safety of future transfusions and counselling them and their physicians accordingly. The biggest challenge for most such discrepancies is that their clinical significance is often ill-defined. They also prolong the time required to provide safe blood products to these patients. So the extent and course of investigation must be tailored to the needs of each specific patient. These logistical issues highlight the need for a high degree of suspicion and a proper investigative protocol in cases of discrepancies encountered in our daily practise so that we know how and when to further evaluate such cases to best suit specific patient requirements.

### CONCLUSION

Most institutions supplement discrepant findings on one modality with at least one other mode of testing. The cases discussed above highlight the importance of that practice. So, discrepant cases should be approached in a systematic manner and repeating the tests on at least one other modality should be the first step in that approach. Assessing secretor status can also substantiate the findings of routine serological testing.

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**PARTICULARS OF CONTRIBUTORS:**

1. 2<sup>nd</sup> Year Postgraduate Trainee, Department of Transfusion Medicine and Immunohematology, St John's Medical College, Bangalore, Karnataka, India.
2. Lecturer, Department of Transfusion Medicine and Immunohematology, St John's Medical College, Bangalore, Karnataka, India.
3. 3<sup>rd</sup> Year Postgraduate Trainee, Department of Transfusion Medicine and Immunohematology, St John's Medical College, Bangalore, Karnataka, India.
4. Professor and Head, Department of Transfusion Medicine and Immunohematology, St John's Medical College, Bangalore, Karnataka, India.

**NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:**

Dr. Sitalakshmi Subramanian

Department of Transfusion Medicine and Immunohematology, St John's Medical College, Bangalore, Karnataka, India.

E-mail: slvbs@yahoo.co.in

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