

International Journal of Biomedical and Advance Research

ISSN: 2229-3809 (Online); 2455-0558 (Print)

Journal DOI: <https://doi.org/10.7439/ijbar>

CODEN: IJBABN

Case Report

A case report on weak subgroup of A end Subgroup**Saurabh Lahare*** and Sankalp Sharma*Department of Transfusion Medicine and Blood Bank, AIIMS, Raipur, C.G., India*

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Article History:*Received:** 06/06/2017**Revised:** 26/10/2017**Accepted:** 29/10/2017**DOI:** <https://doi.org/10.7439/ijbar.v8i10.4218>**Abstract**

A end is a weak subgroup of Blood group “A”, found rarely in general population, not detected by routine forward and reverse blood grouping, detected by Adsorption/Elution technique along with saliva testing for A, B and H antigens. Although it is subgroup of “A” but it lacks “A” antigen in saliva and contains only “H” antigen.

Keywords: A end, subgroup, A blood group, saliva testing.

1. Introduction

A end is a weak subgroup of Blood group “A”, not detected by routine forward and reverse blood grouping, detected by Adsorption/Elution technique along with saliva testing for A, B and H antigens[3]. Although it is subgroup of “A” but it lacks “A” antigen in saliva and contains only “H” antigen [4].

It is a weak “subgroup of A” and seems as “O blood group” in routine forward and reverse blood grouping, on transfusion of O whole blood, there is a slight risk of hemolytic transfusion reaction [5]. On routine forward grouping it may give 0 to weak reaction and mixed field agglutination on microscope with anti-A and anti-AB antisera’s on reverse grouping it gives 3+/4+ agglutination with B cells and cold reacting anti-A1 antibody may be demonstrated [3].

In our case study the subject was admitted in the Dept. of orthopedics, AIIMS Raipur, C.G., no history of previous blood transfusion or any alloantibody identification.

2. Materials required

Test tubes, glass slides, microscope, polyvalent human B serum, 37⁰C incubator, 2-6⁰C refrigerator, table top centrifuge, Saliva, Anti-A,B,H,AB, O cells of R1R1, R2R2, and RR phenotype.

Method: -Adsorption and Elution procedure for confirming weak subgroup of A or B[1]

- 1) 1 mL of red cells to be tested is washed at least three times with saline. Supernatant saline is removed and discarded after the last wash.
- 2) 1 mL of reagent anti-A (if weak A variant suspected) or 1 mL reagent anti-B (if weak B variant suspected) is added to washed red cells.
- 3) Red cells are mixed with reagent antisera, and incubated at 4⁰C for 1 hour, mixing occasionally.
- 4) Red cells are centrifuged to pack the red cells. All supernatant reagents are removed.
- 5) Red cells are transferred to a clean test tube.
- 6) Red cells are washed at least eight times with large volumes (10 mL or more) of cold (4⁰C) saline. An aliquot of the final wash supernatant fluid is saved, and tested in parallel with the eluate.
- 7) An elution method suitable for recovery of ABO antibodies is used, Heat elution method was used here, where equal volumes of Bovine serum albumin was added first, then incubated at 56⁰C for 10 min, then centrifuged at 1000 x g for 3 minutes and supernatant separated as eluate.
- 8) Eluate and the final wash solution (from step 6) is tested, in parallel, against three examples of group O

red cells and three examples of red cells of the appropriate ABO type (A1 or B cells). 2 drops of eluate or wash is added to 1 drop of red cells, and examined for agglutination after immediate centrifugation.

- 9) If no agglutination is observed after centrifugation then incubated for 15 to 30 minutes at room temperature and centrifuged.
- 10) If no agglutination is observed after room-temperature incubation then incubated at 37°C (15 minutes) an indirect antiglobulin test is performed.

Interpretation

- 1) The presence of anti-A or anti-B in the eluate indicates the presence of A or B antigen on test red cells. The results of the eluate are valid only if the following occur:
 - a. The eluate is reactive with all three antigen-positive cells, at any phase.
 - b. The eluate is nonreactive with all three group O red cells.
 - c. The final wash solution is nonreactive with all six cells tested.
- 2) Nonreaction of the eluate with antigen-positive red cells may indicate that the test red cells did not express A or B antigen. Alternatively, the lack of reaction could reflect failure to perform the eluate correctly.
- 3) Reactivity in the eluate with some or all of both antigen-positive and group O red cells indicates recovery of some other or additional antibody in the process.
- 4) If the wash solution is reactive with antigen-positive red cells, the eluate results are invalid. The reaction can occur if unbound reagent antibody was not adequately removed by washing before the elution step or by dissociation of bound antibody during the wash process.
- 5) Adsorption and elution of A1, B, or O red cells, or all three, can be performed and tested in parallel as positive and negative controls.

Testing Saliva FOR A, B, H, Antigens [2]

- 1) 5 to 10 mL of saliva is collected in a small beaker or wide-mouth test tube. Most individuals can accumulate this amount in several minutes. To encourage salivation, the person may chew wax, paraffin, or a clean rubber band, but not gum or anything else that contains sugar or protein.
- 2) Saliva is centrifuged at 900 to 1000 × g for 8 to 10 minutes.
- 3) Supernatant is transferred to a clean test tube, and placed in a boiling water bath for 8 to 10 minutes to inactivate salivary enzymes.

- 4) Then again centrifuged at 900 to 1000 × g for 8 to 10 minutes then clear or slightly opalescent supernatant fluid is removed and opaque or semisolid material is discarded. Supernatant fluid is mixed with an equal volume of saline.
- 5) Sample is refrigerate, if testing is to be done within several hours. If testing will not be done on the day of collection, sample is frozen and stored at -20°C. Frozen samples retain activity for several years.

Selection of Blood Grouping Reagent Dilution

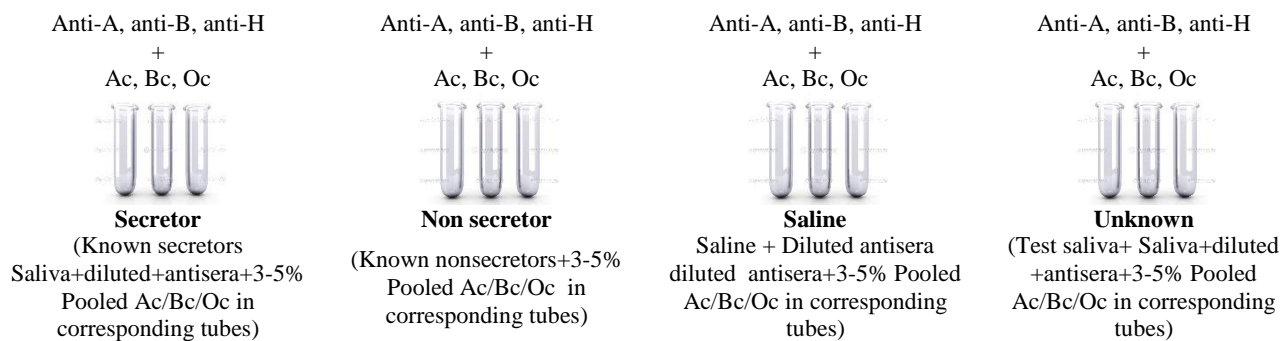
- 1) Doubling dilutions of the appropriate blood typing reagent is prepared: anti-A, anti-B, anti-H to determine ABH secretor status
- 2) To 1 drop of each reagent dilution, 1 drop of 2% to 5% saline suspension of red cells [A, B, O, or Le (a+) as appropriate] is added.
- 3) Each tube and is centrifuged and examined macroscopically for agglutination.
- 4) Highest reagent dilution is selected that gives 2+ agglutination.

Inhibition test for saliva

- 1) 1 drop of appropriately diluted blood grouping reagent is added to each of four tubes. For ABH studies, the tubes should be labeled "Secretor," "Nonsecretor," "Saline," and "Unknown".
- 2) 1 drop of the appropriate saliva is added to each of the "Secretor," "Nonsecretor," and "Unknown" tubes, and 1 drop of saline to the tube marked "Saline."
- 3) Contents of the tubes are mixed. The tubes are incubated for 8 to 10 minutes at room temperature.
- 4) 3-5% cell suspension of pooled Ac, Bc and Oc are added in the corresponding test tubes of all "Secretor," "Nonsecretor," "Saline," and "Unknown" test tubes. Contents of the tubes are mixed. Tubes are incubated for 30 to 60 minutes at room temperature.
- 5) Each tube is centrifuged and red cell button is inspected macroscopically for agglutination

Interpretation

- 1) Agglutination of indicator red cells by antibody in tubes containing saliva indicates that the saliva does not contain the corresponding antigen.
- 2) The failure of known antibody to agglutinate indicator red cells after incubation with saliva indicates that the saliva contains the corresponding antigen.
- 3) The failure of antibody in the saline control tube to agglutinate indicator red cells invalidates the results of saliva tests; the failure usually reflects use of reagents that are too dilute.
- 4) Redetermine the appropriate reagent dilution, as described earlier, and repeat the testing.



3. Testing Results

Blood Grouping

Forward grouping: - Anti-A:-w/mf, Anti-B:-0, Anti-D1:-4+, Anti-D2:-4+, Anti-H:-4+, Anti-AB:-w/mf

Reverse grouping: - A cell:-2+ on 4°C incubation for 30 minute, B cell:-4+, O cell:-0, Auto control:-0

Adsorption/Elution Procedure results

Reactivity for eluate with known A1 cells: - 2+

Reactivity for eluate with known B cells: - 0

Reactivity for eluate with known R1R1, R2R2 & rr type O cells: - 0

Saliva testing results

Reactivity with secretor test tubes: - No reactivity with A and H test tubes, 1+ reactivity present with B test tubes (A positive was the blood group of secretor sample)

Reactivity with non-secretor test tubes: - 1+ in all A, B and H test tubes (B positive was the blood group of non-secretor sample)

Reactivity with saline test tubes: - 1+ Reactivity present in all three A, B and H test tubes

Reactivity with unknown test tubes (Test sample saliva): - 1+ reactivity present with A and B test tubes, 0 reactivity present with H test tube.

4. Conclusion

Only H substance present in our test sample, all the reagents were had undergone quality control procedure so our test was valid, Adsorption/Elution confirmed it to be a weak subgroup of A, and Saliva testing confirmed it to be the weak subgroup of A having only H substance in saliva, mixed field reaction was also present on forward grouping with anti-A anti-AB, so best possible subgroup showing these reaction pattern could be A end.

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