

Department Laboratory
 Laboratory Blood Bank
 Section Technical Procedures
 Sites(s) UMMC/UMMCH
 Document# D-5988 BB v1
Subject A₁ ANTIGEN TYPING

Method Manual tube testing using Anti-A₁ lectin.

Purpose This procedure describes the method for antigen typing red blood cells (RBCs) for the ABO Blood Group antigen, A₁. Testing for the A₁ antigen is required for some solid organ transplant evaluations.

This procedure meets CAP Requirement TRM.31400, “There are records of acceptable reactivity and specificity of typing sera and reagent cells on each day of use, including a check against known positive and negative controls or antisera, or manufacturer’s directions for daily quality control are followed.”

Policy Standard procedures and protocols are used to produce quality laboratory results.

Specimen Recipient or donor whole blood collected in EDTA. Specimen can be tested up to 10 days after day of draw. Recipients will arrive ordered with normal MRNs, but donors will must be ordered in Blood Bank as shown below with UNOS IDs:

LifeSource Donor Orders:

1. Create a Patient ID and order test.
 - a. In Sunquest function Order Entry, at Patient ID prompt, enter Blood Bank LIFESOURCE UAKD location number followed by a hyphen. This will bill LifeSource. Enter the code is “Z544-,” then select the “Search” option.
 - b. The next screen will require creation of a patient record. Choose HID “F” and select “Create.”
 - c. At Patient Name, enter “UNOS” as the last name and use UNOS ID number as the first name (UNOS,xxx123).
 - d. At DOB, enter the correct birthdate or 01/01/1900 if unknown.
 - e. At sex, choose Unknown.
 - f. Select “New Episode” option and then “Save” option.
 - g. Ordering physician, if required, enter “0” to indicate Unknown.
 - h. Client ID will be highlighted, enter “c” for client or Z544.
 - i. The Order Entry screen will appear. Order test code A1SUB and choose “Save.”

Equipment/ 12 x 75 mm tubes
 Supplies Blood Bank Saline
 Calibrated Centrifuge
 Pipettes

Reagents Current Lot of Immucor Anti-A₁ Lectin
 Current Lot of A₁ Reagent Cells
 Current Lot of A₂ Reagent Cells

- Procedure
1. Check patient blood type. If patient is ABO type A or AB, perform A₁ antigen typing. If patient is ABO type O or B, do not proceed with test, and refer to Test Cancellation section of this procedure.
 2. Check patient transfusion history. If patient has been transfused in the last 3 months, antigen typing results will be invalid; therefore, we cannot perform the test. Refer to Test Cancellation section of this procedure.
 3. Select current in use lot of A₁ cells as the positive control cell.
 4. Select current in use lot of A₂ cells as the negative control cell.
 5. In a tube labeled with the patient's initials, wash patient cells one time per standard procedure and suspend in saline to a 3-5% red cell suspension.
 6. Label 3 - 12 x 75mm plastic tubes with cell type and name of antigen as shown below:
 - a. Positive Control: A₁C and A₁
 - b. Negative Control: A₂C and A₁
 - c. Patient's initials and A₁
 7. Add 1 drop of Anti-A₁ antisera to each appropriately labeled tube.
 8. Add 1 drop of 3-5% suspension of the appropriate red cells to each corresponding tube.
 9. Mix contents of tubes thoroughly before placing tubes in centrifuge.
 10. Centrifuge the tubes for the saline time listed on the centrifuge.
 11. Immediately following centrifugation, gently agitate the tube to suspend the red blood cell button and examine the reactions macroscopically.
 12. Record patient results in Blood Order Processing A1SUB accession number. Refer to Computer Entry section of this procedure.
 13. Record control results in SmartTerm function QCE using Grid Result Entry Battery Code ANTIA1.

Interpretation of
Results

Positive Test:

- Positive Control and Recipient: Agglutination of control and patient red cells with reactions of at least $\geq 2+$.
- Donor (UNOS specimen): Any agglutination of any grade observed shall be resulted as A₁ positive.

Negative Test:

- Negative Control and Recipient: Agglutination of control and recipient red blood cells with reactions of $\leq 1+$, mixed field, or negative (0 grade).
 - Negative or 0 grade reactions may be reported without further investigation.
 - Reactions of $\leq 1+$ or mixed field with recipient red cells should be investigated before it is assumed the red blood cells carry a weakened expression of A.
 - If reactions of $\leq 1+$ or mixed field are observed, there may be an indication that the environmental temperature, centrifugation speed or time, or the volume of reagent of red blood cell suspension used is not optimum or that the reagent is deteriorating.
 - Refer to Limitations and Troubleshooting section of this procedure for next steps.
- Donor (UNOS specimen): Only negative (0 grade) reactions observed shall be resulted as A₁ negative.

Expected Typing Results per Immucor Anti-A₁ Lectin Package Insert

Blood Group	Reaction with Anti-A ₁	Percent
A ₁	+	80% of all group A
A ₁ B	+	80% of all group AB
A ₂	0	20% of all group A
A ₂ B	0	20% of all group AB
A _{int}	+w*	Uncommon
A ₃ , A _m , A _x , etc.	0	Uncommon

*Weak agglutination may occur with some red blood cells that are most properly classified as A₂ or A₂B.

Limitations and Troubleshooting Falsely positive or falsely negative results can occur from bacterial or chemical contamination of test materials, inadequate incubation time and temperature, improper centrifugation, improper storage of materials, or omission of test reagents.

Mixed field reactions may occur if patient has recently been transfused. If no history at UMMC, it may be necessary to check Epic or call LifeSource to investigate if any outside transfusions have occurred. If patient has been transfused within last 3 months, do not perform testing. Refer to Test Cancellation section of the procedure.

Mixed field reactions may also occur with weak A subgroups. If mixed field is observed without history of recent transfusion, follow the steps below before resulting patient as A₁ negative.

Follow the following steps before resulting patient as A₁ negative:

1. Ensure Anti-A₁ is in-dated. If expiration date has passed, discard bottle and repeat testing with an in-dated bottle.
2. Visually inspect Anti-A₁ lectin for turbidity, cloudiness, or floating particulates. If antiserum appears contaminated, discard bottle and repeat testing with a bottle that appears clear and free of contamination.
3. Ensure all test reagents have been added. Repeat testing if necessary.
4. Ensure testing is performed per times specified in testing procedure. Ensure proper centrifugation speed and times have been performed. If not, correct test time and centrifugation to test requirements. Alert Technical Supervisor/Specialist or Lead if help with resolution needed.
5. Ensure red cell suspension is 3-5% and isn't too heavy or too light.
6. If steps 1-4 have been ruled out as issues, repeat testing with a new bottle of the same lot of Anti-A₁. If same results are acquired, interpret patient A1 Test (A1 Ag Type) as A₁ negative. If discrepant results are acquired, repeat testing with a new lot of Anti-A₁. Alert Technical Supervisor/Specialist of discrepancy for help with troubleshooting before test results released.

- Computer Entry
1. Result A1 Test (A1 Ag Type) reaction grid with reaction grade (e.g. 4+). If MF is resulted, but patient is interpreted as A₁ negative, you will need to override a QA failure. Override the QA failure with Reason Code TE and free text "MF observed, pt determined to be A₁ neg."
 2. Interpret A1 Test (A1 Ag Type) Interp grid with following ETC codes.
 - a. PA1 if patient is A₁ positive.
 - b. NA1 if patient is A₁ negative.

Test	Description	Result
A1	A1 Ag Type	
AGI	Antigen Type	

3. Result AGI (Antigen Type)
 - a. PA1 if patient is A₁ positive.
 - b. NA1 if patient is A₁ negative.

Test	Description	Result
A1	A1 Ag Type	
AGI	Antigen Type	

4. If patient is A₁ negative, add a BBC and result it with ETC code A1NEG. This code expands out to “Serologic tests for A1 subtype were negative, indicating the patient is a subgroup of A, such as A2. For clinical relevance of this finding, please page the Transfusion MD on call.”

BBC	Blood Bank Comment	A1NEG
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BBC	Blood Bank Comment	Serologic tests for A1 subtype were negative, indicating the patient is a subgroup of A, such as A2.
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5. If patient is A₁ positive, no BBC is needed.

Critical Values **None**

Test Evaluate Cancellation Eligibility

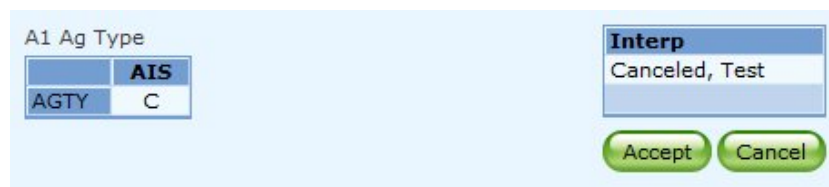
- Cancellation
- Cancel an A1SUB test if patient is not type A or AB. Cancel using BBC Example 1.
 - If we receive two A1SUBs on an A or AB patient, one is not a duplicate test unless they both have the same collection time. A difference of a few minutes almost always means the same draw and one is considered a duplicate test. Try getting in touch with the phlebotomist if you're unsure. If the A1SUBs were drawn at the same time, cancel one

test using BBC Example 2.

- Certain A or AB transplant patients require A1SUBs on two separate draws, just like they all require two ABOs on separate draws. Perform the testing if two A1SUBs are received on two separate draws. Do not cancel.
- If patient has been transfused in the last 3 months, testing results will be invalid. Cancel using BBC Example 3.

Performing Cancellation

1. Fill in the A1 grid with a C and, for the Interp, enter ;DEL (will expand to Canceled, Test Credited). You will get a QA failure. To acknowledge the QA failure, check the box in the Acknowledge column and click Okay.



2. Result AGI with ;DEL (will expand to Canceled, Test Credited). This interpretation goes to the BAD File. Remove this from BAD File once resulted.

AGI	Antigen Type	Canceled, Test credited
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3. Add a BBC and free text your reason for canceling. Include the date and your initials at the end.
 - a. Example 1: Patient is not ABO type A or AB. A subtyping not applicable. 10/27/16 KH
 - b. Example 2: Duplicate test received. Two A1SUB tests drawn at same time. 10/27/16 KH
 - c. Example 3: Patient transfused in last 3 months. Transfusions invalidate test. Genotyping may be needed. Contact Blood Bank with questions and for genotyping info. 10/27/16 KH

BBC	Blood Bank Comment	;Patient is not ABO type A or AB. A subtyping not applicable. 10.27.16 KH
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4. There is no code for crediting the test. In order to credit for the test, make a screenshot of the A1SUB accession in Sunquest function BOP and give to Primary Specimen Receiving Tech (PSRT) for crediting.
5. Click Save button.
6. Override the QA failure with Reason Code TE and free text "Canceling A1SUB."



References “Anti-A₁ Lectin (*Dolichos biflorus*) Blood Grouping Lectin For Slide and Tube Tests”
 Immucor manufacturer’s insert. Insert code 313-7, Rev 02/13.

Summary of New Procedure
 Changes

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