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| **Isohemagglutination Titer** |
| **Purpose** | This procedure provides instructions for determining the amount of IgM Anti-A and/or Anti-B present in a patient's serum. [Isohemagglutination Titer](http://www.childrensmn.org/Manuals/Lab/TransfusionSvc/012725.asp) |
| **Policy Statements** | * The test is inappropriate for patients less than 4 months of age, and may be inconclusive for patients less than 12 months old. Normal individuals may not reach full antibody titer until 5 years of age.
* Isohemagglutination titers will be performed on patient’s that are going to receive an ABO incompatible heart transplant regardless of age.
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| **Test Codes** | **TIA (key F) = Titer of Anti-A****TITB (key D) = Titer of Anti-B****TA15 (key u) =Titer of Anti-A 15 minute incubation****TB15 (key v) = Titer of Anti-B 15 minute incubation**CTIA=Credit of Anti-A titerCTIB=Credit of Anti-B titer |
| **Related****Documents** | [TS 4.8 Grading and interpretation of tube reactions](http://khan.childrensmn.org/Manuals/Lab/SOP/TS/PatTest/202225.pdf) |
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| **Materials** | **Equipment** | **Reagents** | **Supplies** |
| * + Centrifuge
	+ Pipettes (50μl and 100μl)

Optical aid | * Biotestcell A1 and B
 | * 10 x 75 test tube

Normal saline |
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| **Sample** | No special preparation of the patient is required prior to specimen collection. Blood should be collected and labeled according to approved policies and procedures.  [Collection of Patient Specimens](http://www.childrensmn.org/Manuals/Lab/TransfusionSvc/012709.asp)EDTA or clotted specimen should be tested within 10 days and stored at 2-8°C. |
| **Quality Control** | Reagents must be evaluated each day of use with appropriate controls. Refer to [TS 18.2 Daily Reagent Quality Control](http://khan.childrensmn.org/Manuals/Lab/SOP/TS/OperCon/202407.pdf) |
| **Before** **You Begin** | 1. Perform an ABO grouping and Rh typing on the patient specimen.  *Testing is not required on group AB patients or Group O, A, or B patient that do not demonstrate a* *backtype reaction at immediate spin. See* [*Interpretation*](#Interpretation) *section.* |
| **Procedure** |  |
|  | **Step** | Action |
| Test ABTI | 1 | Determine titer(s) to be done:

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| **If** | **Then** |
| Patient's ABO is O |  titer Anti-A and Anti-B. |
| Patient's ABO is A | titer Anti-B. |
| Patient's ABO is B | titer Anti-A. |
| Patient's ABO is AB | No titers to be done. |

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|  | 2 | Label ten 10x75 test tubes for each titer to be done. PI PI PI PI PI PI PI PI PI PI 1 2 4 8 16 32 64 128 256 512**U U U U U U U U U U**Replace PI with the patient's initials. Indicate A1 or B cells. |
|  | 3 | Deliver 0.1ml of saline into all tubes except for the first tube (1:1). |
|  | 4 | Deliver 0.1ml of patient’s serum/plasma into tubes labeled 1:1 and 1:2 using a **clean** pipette tip. |
|  | 5 | Using a **clean** pipette tip, mix the contents in the 1:2 tube 5 times. |
|  | 6 | Transfer 0.1ml of the dilution from the 1:2 tube to the 1:4 tube. |
|  | 7 | Using a **clean** pipette tip, mix the contents in the 1:4 tube 5 times. |
|  | 8 | Transfer 0.1ml of the dilution from the 1:4 tube to the 1:8 tube. |
|  | 9 | Continue this process for each remaining tube using a clean tip each time. |
|  | 10 | Remove 0.1ml of diluted plasma/serum from the final tube (1:512) and save in another labeled tube in case additional dilutions are required. |
|  | 11 | Add 50μl of A1 or B cells to each tube appropriate for the titer dependent on the patient’s ABO grouping. |
|  | 12 | Centrifuge for the appropriate time for saline reactions as indicated on the centrifuge. |
|  | 13 | Gently dislodge the red cell button from the 1:512 tube and observe macroscopically for agglutination. |
|  | 14 | Continue reading each tube from 1:512 to 1:1.Note: If >1+ reactions is noted in the 1:512 tube, additional dilutions are required. |
|  | 15 | Record reactions and interpretation. |
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|  | 1 | Determine titer(s) to be done:

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| **If** | **Then** |
| Patient's ABO is O |  titer Anti-A and Anti-B both initial and 15 minute incubation |
| Patient's ABO is A | titer Anti-B both initial and 15 minute incubation |
| Patient's ABO is B | titer Anti-A both initial and 15 minute incubation |
| Patient's ABO is AB | No titers to be done. |

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| Test ABTIH | 2 | Label ten 10x75 test tubes for each titer to be done. PI PI PI PI PI PI PI PI PI PI IN or  15 1 2 4 8 16 32 64 128 256 512**U U U U U U U U U U**Replace PI with the patient's initials. Indicate A1 or B cells and IN(initial spin) or 15 (15 Incubation). |
|  | 3 | Deliver 0.1ml of saline into all tubes except for the first tube (1:1). |
|  | 4 | Deliver 0.1ml of patient’s serum/plasma into tubes labeled 1:1 and 1:2 using a **clean** pipette tip. |
|  | 5 | Using a **clean** pipette tip, mix the contents in the 1:2 tube 5 times. |
|  | 6 | Transfer 0.1ml of the dilution from the 1:2 tube to the 1:4 tube. |
|  | 7 | Using a **clean** pipette tip, mix the contents in the 1:4 tube 5 times. |
|  | 8 | Transfer 0.1ml of the dilution from the 1:4 tube to the 1:8 tube. |
|  | 9 | Continue this process for each remaining tube using a clean tip each time. |
|  | 10 | Remove 0.1ml of diluted plasma/serum from the final tube (1:512) and save in another labeled tube in case additional dilutions are required. |
|  | 11 | Add 50μl of A1 or B cells to each tube appropriate for the titer dependent on the patient’s ABO grouping. |
|  | 12 | Incubate room temp titers for 15 minutes and centrifuge after incubation and centrifuge initial spin titers right away. |
|  | 12 | Centrifuge titers for the appropriate time for saline reactions as indicated on the centrifuge. |
|  | 13 | Gently dislodge the red cell button from the 1:512 tube and observe macroscopically for agglutination. |
|  | 14 | Continue reading each tube from 1:512 to 1:1.Note: If >1+ reactions is noted in the 1:512 tube, additional dilutions are required. |
|  | 15 | Record reactions and interpretation. |
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| **Interpretation** | Result the titer as the dilution value of the first tube that produces a 1+ macroscopic agglutination reaction. E.g. Result titer as DI32 for the reaction pattern illustrated below. 512 256 128 64 32 16 8 4 2 1**U U U U U U U U U U**0 0 0 w+ 1+ 1+ 2+ 3+ 3+ 4+ tube reaction Group O, A, or B patient that do not demonstrate a backtype reaction at immediate spin:* Enter a reaction result of NT for "Not Tested" for all tubes
* Enter interpretation as DI0
* Credit the resulted titers. CTIA for Anti-A and CTIB for Anti-B

Group AB patients:* Enter a reaction result of NT for "Not Tested" for all tubes
* Enter interpretation as DI0
* Credit the resulted titers. CTIA for Anti-A and CTIB for Anti-B
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| **Limitations** | Individuals produce different titers of isohemagglutinin antibodies based on genetics and state of health. The typical range observed for anti-A is a titer of 32, while anti-B is 8. However, no reference range can be established since the clinical significance of the titer results are a function of the individual patient’s titer increasing or decreasing and should not be used in abstract. |
| **Result Reporting** |  |
| **Step** | Action |
| 1 | Enter Blood Order Processing by the patient sample accession number.  |
| 2 | Click on the Patient Specimen tab. |
| 3 | Add the appropriate test for the titer(s) performed into the **Add spec. test** box, by entering a semi-colon and test code (TIA, TITB, TA15, or TB15) or by selecting the test from the keyboard. |
| 4 | Press the “Home” key or click in the D512 cell in the result field. |
| 5 | Enter the graded agglutination for each dilution tube. |
| 6 | Click in the Interp. Cell and enter the interpretation of the titer.* Enter a semi-colon
* Enter DI then the titer value of first tube showing 1+ agglutination. Example: **;DI32**

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| 7 | Press Tab twice to file results. |
| 8 | Repeat result entry procedure for the second titer if applicable.  |
| 9 | Save results.Note: Sunquest maintenance for test TIA, TITB, TA15, and TB15 assumes the first tube with any positive reaction to be the dilution interpretation value. If the first positive tube shows only a w+ reaction a QA failure will result as the first tube with a 1+ reaction must be reported as the final interpretation. Respond to QA failure resulting from w+ reactions not corresponding to final end point by entering Reason code **WRX-Weak reaction not used to determine titer.** |
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| **References** | *Product Insert, Biotestcell A1 and B, Bio*-Rad Medical Diagnostics, current editionAABB Technical Manual, current editionCambridge Biomedical Research Group. [www.**cambridgebiomedical**.com/](http://www.cambridgebiomedical.com/) |
| **Approval****Workflow** | Transfusion Service/Laboratory Director |
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| **Historical Record** | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
| 1 | K. Hartley  | 1978 | Initial Version |
| 2 | J. Wenzel | 8/1990 |  |
| 3 | J. Wenzel | 5/1994 | Dec’d testing in 1995 |
| 4 | J. Wenzel | 5/1997 | Reinstated in SOP |
| 5 | J. Wenzel | 9/1999 |  |
| 6 | J. Wenzel | 7/24/2001 | New Format |
| 7 | S. Cassidy | 10/27/08 | New Format |
|  | 8 | J. Wenzel | 8/22/2011 | BioRad reagentsAdded Result Reporting steps previously in TS 5.19QA Reason code WRX |
|  | 9 | S. Cassidy | 3/15/2019 | Added steps for test ABTIH |