**Purpose**

To provide instructions for how to detect in vivo sensitization of patient red cells with immunoglobulin, and/or complement by performing a direct antiglobulin test using the tube method.

**Background**

Broad Spectrum Antiglobulin reagent is used for the **initial** Direct Antiglobulin testing, unless the specimen is Cord Blood, in which case only Anti-IgG is used. If a Broad Spectrum AHG test is resulted as “positive”, both mono-specific AHG antisera (Anti-IgG and Anti-C3) will be used to perform further testing to distinguish between complement-coated cells, and IgG coated cells.

**Sample Precautions**

Testing should be performed on fresh specimens. If unable to test immediately; clotted or EDTA specimens may be stored at 2 – 8 degrees C and citrated specimens (donor segments) may be stored at 1 – 6 degrees C. Polyspecific and Anti-IgG testing must be performed within 10 days of sample collection, and Anti-C3 testing must be performed within 48 hrs of collection. It is preferable to test all specimens within the first 48 hrs of collection. If a clotted sample is used, positive test results must be confirmed using an EDTA sample.

**Testing Limitations**

* False negatives: May be caused by insufficiently washed RBCs, patient RBCs left in suspension too long, or centrifuged tubes read too long after spin.
* False positives: May be caused by over-centrifuged tubes or contaminated reagents.
* Samples with a positive DAT may show false positive results when testing with monoclonal antibodies (i.e. antigen typing). It is recommended that an appropriate control be tested in parallel.

**Procedure:**

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| **Step** | **Action** | **Related Documents**  |
| *Test Broad Spectrum Antiglobulin reagent for the* ***initial*** *adult Direct Antiglobulin testing.**If a Broad Spectrum AHG test is resulted as “positive”, test both mono-specific AHG antisera (Anti-IgG and Anti-C3) to distinguish between complement-coated cells and IgG coated cells.****QC for anti-C3 reagent and Complement Coated Check Cells may need to be performed.*** |
| 1 | Label **each** tube with the patient’s identification and **reagent** used:* Polyspecific, Anti-IgG, and/or Anti-C3 reagent
* Saline control—Negative control (perform once with first antisera test.)
 | Labeling tubes for manual bench testing |
| 2 | Prepare a 3% to 5% suspension of patient test red cells per established procedure. | Preparation of 3-5% Suspension of Red Cells |
| **Step** | **Action** | **Related Documents**  |
| 3 | Add 1 drop of the patient red cell suspension into each labelled tube.  |  |
| 4 | Perform saline wash on each tube:* Wash 4 X with saline either manually or in the cell washer.
* Decant completely
 | Washing Red Cell Samples (Manual or Automated) |
|  | **Phase** | **Test QC** | **Poly AHG Test** | **IgG Test** | **C3 Test** |
|  |  | **Patient Control Tube** | **Patient Test Tubes** |
| 5 | **Anti-human****globulin** | Add 2 drops of Saline | Add 2 drops of Poly | Add 2 drops of Anti-IgG | Add 2 drops of Anti-C3 |
| 6 | Centrifuge, read immediately, and record per SOP. |
| If result is positive, test is invalid. | If Result is positive, compare to Patient Control Tube:* If control is NEG, test is complete.
	+ Interpret any macroscopic agglutination as positive. Note mixed field findings in BBCS comments.
* If control is POS, test is invalid.
	+ Do not interpret DAT until investigation and resolution have been completed. Send out to Red Cell Reference may be required.
 |
| If Result is negative, go to step 7. | If result is negative, incubate for 5 min at room temp, spin, read and record. If still negative, go to step 7. |
| 7 | **Coombs control cells*****Note:*** *In order for a valid test result, the check cell reactions must be ≥ 2+ for Poly AHG and IgG, ≥ 1+ for C3.* | N/A(Do not add to saline control tube.) | Add 1 drop of ***IgG coated*** cells to patient tube. | Add 1 drop of ***IgG coated*** cells to patient tube. | Add 1 drop of ***C3 coated*** cells to patient tube. |
| 8 | Centrifuge, read immediately, and record. | Incubate 5-10 minutes at room temp., centrifuge, read immediately, and record. |
| If positive reaction ≥ 2+, test is valid. | If positive reaction ≥ 1+, test is valid. |
| If reaction is negative, repeat test. Negative results indicate an invalid test. |
| 9 | Interpret test results:* + POSITIVE: any grade of reaction in the antihuman globulin or anti-complement tube and a negative patient control tube
	+ NEGATIVE: no reaction in the antihuman globulin or anti-complement tube with valid coombs control cell results and a negative patient control tube.
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| 10 | Further investigation indicated:* + POSITIVE reactions require investigation including but not limited to elution and antibody identification when correlated with the antibody detection test results.
	+ MIXED FIELD reactions require investigation as described above. In addition, transfusion reaction with destruction of donor cells by patient antibody must be considered. The Medical Director must be notified immediately if the findings indicate any possibility of transfusion reaction.
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**References**

AABB Technical Manual, Current Edition.

Manufacturer’s package insert