1. **Principle**

Anti-Ch and Anti-Rg are antibodies directed against the C4 component of human complement. Neutralisation of these antibodies can be achieved by incubation with plasma from antigen positive individuals.

1. **Scope and Related Policies**
	1. Chido and Rodgers antigens are epitopes on the C4 component of human complement.
	2. Plasma that contains C4 from Ch/Rg postivie individuals can be used to inhibit the reaction of Ch/Rg antibodies.
	3. Plasma that has undergone inhibition can be used to identify the presence of other alloantibodies that may have been previously masked by the Ch/Rg antibody.
2. **Specimen**

EDTA anticoagulated whole blood preferably less than 72 hours old.

1. **Material**

**Equipment:** Cell Washer

 Serological centrifuge

 Block for test tubes

Microscope

Waterbath/Heating block at 37°C

**Supplies:** Test tubes – 10 x 75 mm

 Serological pipettes

**Reagents:** 0.9% saline

 Anti-IgG

 IgG-coated cells

Inert Plasma – Group compatible (Pool of at leastsix normal plasma samples known to be non-reactive with screening cells).

Test cells (antibody screen cells or a panel of cells for antibody identifi­cation).

6% Bovine serum albumin (BSA).

1. **Quality Control**
	1. A control consisting of the indicator red cell and the pooled neutralising plasma must always be included.
	2. The absence of activity in the 6% albumin control indicates dilution of a weakly reactive antibody and an invalid test.
2. **Procedure**

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| * 1. Prepare serial twofold dilutions of patient’s plasma in saline. The dilution range should be from 1 in 2 to 1 in 512, or to one tube beyond the known titer. Refer to SP.002 – Titrations. The volume prepared should be not less than 0.3 mL for each red cell sample to be tested.
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| * 1. For each red cell sample to be tested, place 2 drops of each plasma dilution into each of two sets of appropriately labeled 10 x 75 mm test tubes.
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| * 1. To one set, add 2 drops of inert pooled plasma to each tube.
 |
| * 1. To the other set, add 2 drops of 6% albumin to each tube.
 |
| * 1. Gently agitate the contents of each tube and incubate the tubes at room temperature for at least 30 minutes.
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| * 1. Add 1 drop of a 3% suspension of red cells to each tube.
 |
| * 1. Gently agitate the contents of each tube and incubate the tubes at 37°C for 1 hour.
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| * 1. Wash the cells four times in saline, add anti-IgG, and centrifuge for 10-15 seconds at 3400 rpm.
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| * 1. Resuspend the cell buttons and examine for agglutination; confirm all non-reactive tests microscopically. Grade and record the results.
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| * 1. Confirm the validity of negative results by adding IgG-coated red cells.
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1. **Reporting**
	1. Inhibition of antibody activity in the tubes to which plasma has been added suggests anti-Ch or anti-Rg specificity; this inhibition is often complete.
	2. The presence of partial inhibition suggests the possibility of additional alloantibodies. These antibodies should be identified before reporting the final results, see table below:

Interpretation of Results:

|  |  |  |
| --- | --- | --- |
|  TEST PLASMA | CONTROL PLASMA |  INTERPRETATION |
| No reac­tivity withall cells tested | Reactivity withall cells tested | Antibody neutralised specificityChido or Rodgers |
| No reac­tivity | No reactivity | Dilution of antibody has occurredNo conclusion can be made |
| Reac­tivity withsome cells tested | Reactivity withall cells tested | Anti-Ch or Anti-Rg plus an additionalantibody maybe present |

* 1. To confirm the presence of additional antibody(ies) prepare a large volume of inhibited plasma and test it against a reagent red cell panel to determine whether the non-neutralized activity displays specificity towards a particular antigen.
1. **Procedural Notes**
	1. Some red cell related HLA antibodies (e.g. Bg) may be neutralised with human plasma.
	2. Plasma may also inhibit anti-Cromer.

1. **References**
	1. Judd, WJ ed. Judd’s Methods in Immunohematology, 3rd ed, Bethesda, MD: pg. 345-347.
	2. Roback, JD. ed. AABB Technical Manual, 17th ed. Bethesda, MD: American Association of Blood Banks, 2011: pg 911-912.
2. **Revision History**

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| --- | --- |
| **Revision Date** | **Summary of Revision** |
| September 1, 2014 | * Revised name of manual
* Revised section 2.0
* Replaced “red cells” with “whole blood” in section 3.0
* Replaced “Normal Saline” with “0.9% Saline” in section 4.0- Reagents
* Revised wording of section 6.1; added section 6.10
* Updated list of references to include most recent editions
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