1. **Principle**

The red cells from a patient with a positive direct antiglobulin test (DAT) and free plasma autoantibody are treated with W.A.R.M. This treatment removes autoantibody, thereby reducing the strength of the positive DAT and freeing antigen sites for adsorption. Concomitantly, the enzyme premodifies the red cells. The red cells, thus prepared, are combined with autologous plasma and the mixture is incubated at 37°C. The warm-reactive autoantibody binds to the antigen present on the patient’s red cells. This antigen-antibody interaction is enhanced by the enzyme treatment of the cells. Following incubation, the mixture is centrifuged and adsorbed plasma is harvested and tested. By comparing the results of W.A.R.M.-adsorbed plasma with those obtained with the unadsorbed plasma, it is usually possible to confirm the presence of the warm-reactive autoantibody, and detect or identify any additional alloantibody(ies) that may be present.

1. **Scope and Related Policies**
	1. Warm-reactive autoantibodies create complex serological problems that may be extremely time-consuming to resolve, particularly in those patients requiring pretransfusion compatibility testing. Such autoantibodies are produced by patients with warm autoimmune hemolytic anemia (WAIHA), drug-induced immune hemolytic anemia (DIHA) and in a certain population of normal individuals with no overt clinical symptoms.
	2. Some of the autoantibodies have a single specificity (e.g. anti-c, -E, -e, Jka). However, the majority of autoantibodies encountered have specificities that cannot be classified without the use of rare cells such as Rh deletion of Ko cells. Samples from patients with autoantibodies and requiring transfusion can present with 1) a positive DAT due to in vivo coating of autologous red cells and 2) a positive indirect antiglobulin test (IAT) or antibody screen due to free plasma autoantibody. The autoantibody often reacts with virtually all reagent and donor red cells. Consequently, it is difficult to ascertain whether underlying alloantibodies of potential clinical significance are present.
	3. Allogeneic adsorption procedures can also be useful in the recognition of underlying alloantibody(ies) in patients where pre-transfusion autologous cells are not available. Adsorption of the patient’s plasma with donor R1R1 R2R2 and rr red cells has been successfully employed to determine the presence of alloantibodies in patients with free plasma autoantibody. Although the procedure is helpful, it is time-consuming and laborious. The required red cell phenotypes are not readily available in sufficient quantity in most laboratories. Additionally, there is an inherent risk that an undetected alloantibody may be simultaneously adsorbed by the donor red cells.
	4. An autologous adsorption procedure to remove warm-reactive autoantibody is the most suitable technique to aid in the resolution of these serological complexities particularly in patients who have not received recent transfusion. Red cells needed to perform the procedures are available in the patient specimen. Since the procedure is an autoadsorption, there is little concern that foreign antigens will remove a hidden alloantibody unless the patient was recently transfused.
2. **Specimen**

EDTA anticoagulated whole blood.

1. **Materials**

**Equipment:** Serological centrifuge

 Block for test tubes

 Water bath/Heating block at 37°C

 Timer

**Supplies:** Test tubes - 10 x 75 mm

Serological pipettes

Transfer pipettes

**Reagents:** 0.9% saline

Deionized Water

 W.A.R.M. reagent

1. **Quality Control**
	1. The activity of W.A.R.M. may be demonstrated upon reconstitution to ensure the effectiveness of the solution. W.A.R.M. may be deemed sufficiently active if it can be shown that treated cells show enhancement of Rh antigen and denaturation of a Kell system antigen. It is recommended that a reagent red cell of known phenotype be treated with W.A.R.M to demonstrate enhancement and denaturation with appropriate antibodies.
	2. Store lyophilized W.A.R.M. and reconstituted W.A.R.M. at 1-10°C. Do not freeze. Do not use beyond expiration date. Do not use beyond five (5) days after reconstitution.
	3. Some particulate matter and slight turbidity may be noted upon reconstitution. Gross turbidity may indicate possible reagent deterioration that may be confirmed by serological tests as outlined in section 5.1.
2. **Procedure**

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| * 1. Label the tubes to be used in the adsorption procedure.
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| * 1. Reconstitute a vial of W.A.R.M. by adding 5mL of deionized water. Mix thoroughly.
 |
| * 1. Add 1 volume of packed patient red cells to a labeled tube. It is not necessary to wash patient red cells prior to W.A.R.M. treatment.
 |
| * 1. Add 2 volumes of reconstituted W.A.R.M. to the tube (for example 1mL red cells to 2 mL W.A.R.M.)
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| * 1. Mix well and incubate at 37°C for approximately 30 minutes.
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| * 1. Wash the W.A.R.M.-treated cells with saline a minimum of three times. Remove as much saline as possible after each wash.
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| * 1. Add an equal volume of patient plasma to the W.A.R.M.-treated, packed red cells.
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| * 1. Mix well and incubate for 30-60 minutes at 37°C.

**Note:** Due to the fragility of certain red cells, some hemolysis may be observed upon treatment. The treatment of such red cells with W.A.R.M. should be limited to thirty minutes. |
| * 1. Centrifuge for approximately two minutes or until red cells are well packed.
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| * 1. Harvest the adsorbed plasma using a transfer pipette.
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| * 1. Test an aliquot of the adsorbed specimen at 37°C and at the antiglobulin phase to determine if all warm-reactive autoantibody has been removed. See 7.0 Reporting. If results indicate that adsorption was insufficient, repeat steps 6.7-6.8 using a new aliquot of treated patient red cells (see steps 6.1-6.6) and incubate for 60 minutes. If adsorption is complete, the adsorbed specimen is ready for use in antibody detection and identification.
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1. **Reporting**
	1. Complete Adsorption:
		1. Complete adsorption of warm-reactive autoantibody has been achieved when the adsorbed plasma no longer reacts where previously detected with both:
2. the patient’s own W.A.R.M.-treated red cells; and
3. reagent red cells that lack antigens toward which additional alloantibodies are directed.
	1. Incomplete Adsorption:
		1. If warm-reactive autoantibody has not been sufficiently removed, or the autoantibody cannot be adsorbed by W.A.R.M.-treated cells, agglutination will be detected when the plasma is tested as in a or b above.

**Note:** Complete adsorption of warm-reactive autoantibodies may not always be possible. However, in some instances, only partial adsorption of the autoantibody may be sufficient to reveal underlying alloautoantibody (ies) that might also be present.

1. **Procedural Notes**
	1. Limitations:
		1. Some autoantibodies are directed toward Kell system antigens or enzyme sensitive antigens, such as M or Xga. Since W.A.R.M. treatment destroys these antigens, autoadsorption using W.A.R.M.-treated cells will not remove the corresponding autoantibody.
		2. The use of W.A.R.M.-treated red cells for antigen typing is not recommended since commercial antisera may or may not be specific when tested in this manner.
		3. On occasion, it is possible that a warm-reactive autoantibody will not be completely adsorbed by W.A.R.M.-treated red cells. As a result, it may not be possible to assign a definitive specificity to those reactions obtained following adsorption. Further complete adsorption of warm-reactive autoantibodies does not assure that those antibodies remaining in the adsorbed plasma can be readily identified.
		4. Crossmatches performed on a W.A.R.M. autoadsorbed plasma cannot be considered “compatible”, since the antibodies removed by W.A.R.M. autoadsorption procedures are active at 37°C. Any warm autoadsorption procedure serves only as an aid in the detection and identification of underlying alloantibodies potentially masked by a warm-reactive autoantibody.
		5. Because the potential exists for adsorption of clinically significant alloantibodies onto transfused red cells, the results of autoadsorption procedures performed on samples from recently transfused patients should be interpreted with caution.
2. **References**
	1. Roback JD, ed.AABB Technical manual, 17th ed. Bethesda, MD: American Association of Blood Banks: 2011:506-508.
	2. Issitt PD, Anstee DJ. Applied blood group serology. 4th ed. Durham, NC: Montgomery Scientific Publications, 1998.
	3. Refer to current manufacturer’s insert for W.A.R.M.
3. **Revision History**

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| **Revision Date** | **Summary of Revision** |
| September 1, 2014 | * Revised name of manual
* Specified “allogeneic adsorption” in section 2.3
* Revised wording to include “in patients where pre-transfusion autologous cells are not available” in section 2.3
* Revised wording of section 3.0
* Changed “normal saline” to “0.9% saline” in section 4.0- *Reagents*
* Changed incubation temperature to 37ºC in sections 6.5, 6.8 & 6.11
* Revised and renumbered section 8.0
* Updated list of references to include most recent editions
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