# **Principle**

To remove unbound human plasma proteins before the addition of antiglobulin reagents or to resolve test discrepancies.

Incomplete washing may leave residual proteins which may neutralize antihuman globulin used in indirect antiglobulin testing. This neutralization will be detected by the failure of IgG coated red cells to agglutinate resulting in an invalid test result.

1. **Scope and Related Policies**
   1. Red cells are washed when performing a direct antiglobulin test(s) (DAT) or indirect antiglobulin test(s) (IAT).
   2. Washing patient and/or donor cells before preparing a 3% cell suspension may be done when resolving problems detected in compatibility testing.
2. **Specimens – N/A**
3. **Materials**

**Equipment:** Automated cell washer

## Serologic centrifuge

Pipette vacuum device, if applicable

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

Biohazard container

Saline dispenser

Absorbent material

Serological pipettes

Personal protective attire, if applicable

**Reagents:** 3% cell suspension

Normal saline

1. **Quality Control**

See QCA.007 – Functional Calibration of Serologic Centrifuges

1. **Procedure**

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| **STEP** | **ACTION** |
| 1. Automatic Washing of IAT or DAT tubes | 1. Automated cell washing of IAT or DAT must be done following the cell washer manufacturer’s instructions. |
| 1. Manual cell washing of IAT or DAT tubes | 1. Add normal saline using a plastic bottle with a spout, squeeze the bottle (or alternative dispensing device) and dispense 5 to 6 cm of normal saline in each tube (leave 1 cm from the top of the tube empty). See Procedural Notes 8.1. 2. Place the tube(s) in a serologic centrifuge and centrifuge for 45 to 60 seconds at 3500 rpm or  equivalent. See Procedural Notes 8.2. 3. Decant the normal saline from the tubes as follows:  |  |  | | --- | --- | | By | then | | Holding the tube(s) between the thumb and four fingers | * Quickly invert the tube(s) upside down to allow all the normal saline to drain out of the tube(s)   *If manually decanting into a biohazard container (see Procedural Notes 8.3), follow established safety procedures (i.e., use protective equipment such as goggles, face shield or safety shield).*   * + - * Firmly shake your arm down to expel the normal saline remaining.       * Blot any normal saline left at the top of the tube(s) on absorbent material.       * Turn the tube(s) up and mix them gently to dislodge the cell button. |  1. Repeat the above action three more times. 2. After the last wash, thoroughly blot the normal  saline left at the top of the tube(s) on absorbent material. 3. Check that the size of the cell button is approximately 3-4 mm in diameter. |
| 1. Manual cell washing to prepare a 3% suspension. | 1. Dispense one drop of packed cells (or two drops of whole blood) in a labeled tube. 2. Add normal saline using a plastic bottle with a spout, squeeze the bottle (or alternative dispensing device) and dispense 5 to 6 cm of normal saline in each tube (leave 1 cm from the top of the tube empty). See Procedural Notes 8.1. 3. Place the tubes in a serological centrifuge and centrifuge for 45 to 60 seconds. See Procedural Notes 8.2. 4. Remove the normal saline using a pipette or  vacuum aspiration equipment, leaving the cell  button intact.   *If manually decanting into a biohazard container, follow established safety procedures (i.e., use protective equipment such as goggles, face shield or safety shield). See Procedural Notes 8.3. Ensure decant of saline is gentle to avoid loss of cells.*   1. Mix the tube(s) gently to dislodge the cell button. 2. Repeat steps 6.3.2 through 6.3.5. if more than one wash is required 3. Add 0.5 to 1.0 mL of normal saline resuspend to 3% after the last wash; mix well and compare with a commercial 3% suspension. If the cell  suspension is too heavy add additional normal  saline. If the cell suspension is too light repeat steps 6.3.3 – 6.3.5 adding less normal saline. |

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1. **Reporting – N/A**
2. **Procedural Notes**
   1. Direct the normal saline into the bottom of the tube gently so that the cells are mixed homogeneously with the normal saline but no splashing (and loss of cells) occurs. Do not allow the spout of the bottle to touch the inside of the tube. This is important to avoid contamination from one tube to the next while dispensing normal saline into several tubes.
   2. After centrifugation, the cells should be packed at the bottom of the tube. There should not be a line of red cells formed along the side of the tube. If a line of red cells is seen along the side of the tube, the centrifugation time should be extended until all the red cells are packed at the bottom of the tube.
   3. Biohazard Container: A container approved by the Laboratory Safety Committee that can be used to prevent potential exposure to biohazardous material.
3. **References**
   1. Refer to manufacturer’s operating manual for automated cell washers in use.
4. **Revision History**

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| **Revision Date** | **Summary of Revision** |
| January 31, 2014 | * Revised name of manual * Changed document number from PA 005 to RT 002 * Removed section 6.1.1 to 6.1.3 regarding steps to take if the instructions are not available * Renumbered sections 6.2 and 8.0 |