1. **Principle**

Determination of the titre of an antibody in a patient's blood sample is performed by straightforward serial dilutions of the plasma. This provides a semi-quantitative estimate of the concentration of the antibody being tested and is accurate enough for most purposes. It does not measure the total strength of the antibody in the plasma; the relatively short incubation period and serum/cell ratio does not permit equilibrium to be reached between antigen/antibody. Despite these limitations a titer performed using the same technique and the same cells, in parallel, can identify whether there has been an increase/decrease in antibody. This may help the physician to determine if further tests are required to monitor the wellbeing of the fetus *in utero*.

In this gel test, the reagent red blood cells in a hypotonic saline solution are combined with patient plasma to allow antigen/antibody interaction in the upper chamber of the microtube. The cards are then incubated to enhance antigen/antibody interaction, and if the cells become sensitized the sensitized cells will react with the Anti-IgG incorporated in the gel of the microtube during centrifugation. Agglutination indicates the presence of an antigen/antibody reaction while lack of agglutination indicates no antigen/antibody reaction.

1. **Scope and Related Policies**
   1. Clinically significant IgG antibodies will be titred using a cell that is homozygous (if possible) for the antigen corresponding antibody under investiga­tion.

There are those that believe red cells selected for testing should be obtained from heterozygous donors to mimic the red cells of the fetus. Others prefer to use red cells from homozygous donors for increased sensitivity. Whichever method is chosen by an institution should be carefully validated and used consistently. 9.1

* 1. All titres will be tested in parallel with the plasma frozen from the previous titre (if available) 9.1. Any discrepancies must be followed according to institution policy (i.e. reported to the Chief Technologist or designate). Any significant increase must be telephoned to the physician and the call documented on the requisition. If the titre is +/- one tube difference, it will be reported as no change.
  2. Titres are not required to be done at the time of delivery unless the patient is a new encounter or the antibody has not been previously detected.
  3. Therapeutic Abortions: Titres are not applicable.
  4. Only isotonic saline can be used to prepare the dilutions. See Procedural Notes 8.5.

1. **Specimen**

EDTA anticoagulated whole blood within 14 days of collection.

Hemolyzed and grossly icteric specimens may cause difficulty in interpretation. See Procedure Notes 8.13

Grossly lipemic specimens containing particles that clog the gel, as indicated by diffuse blotches of red cells, may be clarified by centrifugation or filtration and re-tested.

1. **Material**

**Equipment:** ID – Micro Typing System™:

Centrifuge

Incubator

Pipettor

Dispenser

Set-up workstation, optional

Serologic centrifuge

**Supplies:** Pipette tips

Test tubes – 12 x 75 mm

Serologic pipettes

Package insert

**Reagents:** MTS Anti-IgG Card, Anti-IgG (Rabbit) suspended in gel

Indicator cell for antibody being titred 3%, to be prepared in-house for use in MTS Anti-IgG testing

MTS Diluent 2, a hypotonic buffered saline solution (for in-house preparation only)

Do not use beyond expiration date. Store cards at 2 to 25°C. Store diluent and red cells at 2 to 8°C. Bring reagents to room temperature (18 to 25°C) prior to use.

1. **Quality Control**
   1. To recognize reagent deterioration, the reagents must be tested on day of use with appropriate controls.
   2. MTS Diluent 2™ must be visually checked to ensure that the liquid is not discolored, turbid or showing any signs of bacterial contamination.
   3. To confirm the specificity and reactivity of the MTS Anti-IgG Card, it is recommended that each lot be tested on each day of use with known positive and negative antibody samples with the appropriate red cells. Reactivity must be present with the positive sample only.
   4. Do not freeze or expose cards to excessive heat. Store upright at 2 to 25°C. If the cards have not been stored in an upright position, centrifuge the cards before use.
   5. Do not use cards that show signs of drying. A liquid layer should appear on top of the gel in each microtube.
   6. Do not use cards in which the microtubes show discoloration, bubbles or crystals.
   7. Do not use the microtube cards where the seal to the microtube appears to be damaged or opened.
   8. Do not remove the foil seal to the microtubes until ready to use.
   9. MTS Diluent 2 must be visually checked to ensure that the liquid is not discolored, turbid or showing any signs of bacterial contamination. Red blood cells must be suspended in MTS™ Diluent 2 or be a commercial 0.8% red blood cell in low ionic strength diluent specifically approved for use with the ID-Micro Typing System™.
   10. The manufacturer recommends that, following centrifugation, results should be read immediately. Results may be affected by drying of the gel, hemolysis of the red cells and slanting of the reaction patterns due to storage in a non-upright position.
2. **Procedure**

|  |  |
| --- | --- |
| * 1. Plasma Master Dilutions | * + 1. Centrifuge the specimen for 5 minutes at 3500 rpm or equivalent. |
| * + 1. After centrifugation, transfer all plasma into a clean test tube labeled with patient’s full name. Transcribe the information from the patient specimen label(not from the request form). Patient specimen labels may be used (ensure the information coincides exactly to the specimen label). |
| * + 1. Label 13 – 12 x 75 mm tubes numerically and with the first three letters of the patient's last name. |
| * + 1. Dispense 0.2 mL Saline in tubes 2 -13. |
| * + 1. Dispense 0.2 mL test serum in tubes 1 and 2. |
| * + 1. Mix the contents of tube 2 ten times avoiding air bubbles. Using a clean pipette tip transfer 0.2 mL to tube 3. |
| * + 1. Repeat step 6.1.6 for each adjacent tube moving down the row until the last 0.2 mL from tube 12 has been dispensed into tube 13. |
| * + 1. Set this last tube aside in case endpoint of titration extends past the dilution in tube 12. |
| * 1. Cell Preparation (This step is not required if 0.8% commercially prepared screen/panel cells are used.) | * + 1. Label test tube with the identification of the indicator cell. |
| * + 1. Prepare a volume of saline washed indicator cells sufficient to provide 10μL of packed red blood cells. |
| * + 1. In a separate labeled tube, dispense 1.0 mL of MTS Diluent 2. Add 10μL of the packed indicator cell to the labeled tube. |
| * + 1. Mix gently. Final cell suspension should be approximately 0.8% and stable for 24 hours. For best results, the suspension should not be less than 0.6% or exceed 1.0%. |
| * 1. Bring specimens and reagents to room temperature (18-25°C). | |
| * 1. Antibody Titre Test Procedure | * + 1. Label the MTS Anti-IgG Card with the appropriate identification and test information. Label the individual microtubes 1 - 12 for each plasma or serum dilution. |
| * + 1. Remove the foil seal from the microtubes to be used.   **Note**: Foil should be removed immediately before testing or within one hour of testing. Once opened, the gel may begin to dry out which could affect test results. Ensure that residual foil does not block the opening of any microtube after removal of the foil. |
| * + 1. Using an appropriate pipette, add 50μL of 0.8% indicator cell suspension to each microtube. Do not touch pipette to gel card. |
| * + 1. Using an appropriate pipette, add 25μL of plasma from each of the master dilution tubes to the correct microtubes. |
| * + 1. Incubate at 37± 2ºC for 15 minutes. Refer to the instructions for use for comment on extending incubation times. See Procedure Note 8.16 |
| * + 1. Centrifuge the gel cards at the preset conditions of 895±25 rpm for 10 minutes. |
| * + 1. After centrifugation, remove the card(s) from the centrifuge and observe each card for the following signs: * Unagglutinated red cells observed in the gel are usually caused by an interrupted centrifuge cycle. These red cells will appear dark pink and hazy. * A line of red cells streaming down one side and forming a “J” Appearance is caused by improperly seated card in the card holder. * If the card(s) show a sign of improper centrifugation, repeat the test. Do not recentrifuge the card(s) |
| * + 1. Read the front and the back of each microtube. |
| * + 1. Record reactions from the chart below.  |  |  | | --- | --- | | **Grade** | **Description of Reaction\*** | | Neg | Unagglutinated red blood cells form a well-defined button at the bottom of the microtube. See Procedural Notes 8.1 if a few unagglutinated cells are trapped at the top or sides of the gel. | |  | No W in updated guide | | 1 | Agglutinates predominantly observed in the lower half of the microtube. Unagglutinated red cells form a button in the bottom of the microtube. | | 2 | Agglutinates dispersed throughout the length of the gel column. A few unagglutinated cells may be observed in the bottom of the microtube. | | 3 | Majority of agglutinates trapped in the upper half of the microtube. | | 4 | A solid band of red cell agglutinates on top of the gel. A few agglutinates may filter into the gel, but remain near the predominant band. | | H | Hemolysis with few or no red cells in the gel. Report if hemolysis is present in the microtube but not in the specimen. | | mf | A band of red cell agglutinates at the top of the gel or dispersed throughout the gel accompanied by unagglutinated cells in the bottom of the microtube. | | NT or ND | Not tested or not done |   Do not use half grade, superscript or “plus signs”  See the discussion section of each grading in the MTS Interpretation Guide for more information on grading. |

1. **Reporting** 
   1. No agglutination of the red cells is a negative test result and indicates the absence of an antigen/antibody reaction
   2. Hemolysis in the absence of a hemolyzed sample or agglutination of any of the red cells in a microtube of the gel card indicates the presence of an antibody directed against the corresponding antigen that is present on the cells.
   3. The endpoint of the titration is considered to be the most dilute tube to have a grade 1+ reaction.
   4. The reciprocal will be reported for all titres (e.g. 256 not 1/256).
   5. An increase of two tubes in the titre compared to the previous sample is considered a significant rise in titre. Where a difference of more than one tube occurs, the results should not be reported until the result of the previous sample has been reviewed and confirmed. See Procedural Notes 8.3.
   6. Report Results
2. **Procedural Notes**
   1. The indicator cells are selected to be homozygous for the antigen corresponding to the maternal antibody. It is important that the phenotype of the indicator cell remains consistent (e.g. when testing for anti-D, if an R1 R1 is used for the first titration, do not use an R1R2 cell for subsequent titration). See Scope homo/hetero/NB same type all titres.
   2. Failure to use separate pipettes for each master dilution will result in falsely high tires due to carryover.
   3. When comparing results of a previous titration ensure that the same test procedure was used (e.g. Gel, not SIDAT or PEG). If the procedure was not the same the comparison may not be valid.
   4. In general antibody titres may be higher in the gel test because of increased sensitivity with this method.9.2
   5. Inert serum/plasma should not be used as a diluent in preparing dilutions for the gel test. 6% BSA should also be avoided because false positive results may occur. Only isotonic saline should be used for preparing dilutions.
   6. Antibodies specific for low-incidence antigens not represented on the test cells will not be detected.
   7. Antibodies below the threshold level may not be detected by this test.
   8. Anti-IgG may occasionally fail to detect antibodies that are demonstrable by the use of antiglobulin reagents that contain anti-C3.
   9. False-positive results may occur if antibodies to components of the preservative solution are present in the serum tested.
   10. Addition of cells and plasma.
       1. Red cell suspension should be added before the plasma because the volume of red cell suspension is greater than the volume of plasma. Insufficient mixing may occur if the smaller volume of plasma is added before the red cell suspension.
       2. Plasma should be added within 10-15 minutes of adding the red cell suspension to the reaction chambers. Any red cells that come in contact with the gel column prior to centrifugation may not have the opportunity to come in contact with the plasma and may begin to migrate through the gel potentially giving a weaker reaction after centrifugation.
   11. Interpretation of mixed-field reactions must be done with caution. The presence of fibrin, clots or particulates may result in some cells layering at the top of the gel. Mixed-field reactions are generally only observed in tests containing a dual population of red cells, such as a transfused patient, bone marrow recipient or when a pooled cell sample is used for testing. However, not all mixed cell situations have a sufficient minor population to be detected.
   12. Rouleaux is a property of test plasma resulting in a characteristic pattern of red cell aggregation. It can occur if sufficient quantities of abnormal proteins are present in the test sample and may infrequently cause difficulties in gel test interpretation. Rouleaux must be confirmed using tube hemagglutination methods and saline replacement performed when necessary.
   13. Red cells present in the gel and hemolysis in the liquid portion is usually due to a hemolyzed specimen. In this case, hemolysis should not be reported as a positive test result. If hemolysis occurs during centrifugation, the liquid portion above the gel will appear pink or red but there will be few or no cells in the gel.
   14. False positive or false negative test results can occur from bacterial contamination of test materials, inadequate incubation time or temperature, improper centrifugation, improper storage of materials or omission of test samples.
   15. False-positive results may occur in gel cards showing signs of drying
   16. Incubation times in low ionic strength solutions between 5 – 40 minutes have been recommended in the literature. No single incubation time will be optimal for all antibodies. If the incubation time is changed from the manufacturer’s recommendation, validation studies are required.
   17. Anomalous results may be caused by fresh serum, fibrin or particulate matter in serum or plasma, or red cells that stick to the sides of the microtube. Use of EDTA plasma will minimize this problem.
   18. Adherence to the manufacturer’s instructions for use is critical to test performance.
3. **References**
   1. Roback JD, ed. American Association of Blood Banks Technical Manual, 17th ed. Bethesda, MD: American Association of Blood Banks, 2011:633, 935-937,907-908
   2. INSTRUCTIONS FOR USE Anti-Human Globulin Anti-IgG (Rabbit) MTS™ Anti-IgG Card, Version 2.0

Append current manufacturer’s insert here

1. **Revision History**

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| --- | --- |
| **Revision Date** | **Summary of Revision** |
| April 30, 2014 | * Revised name of manual * Changed document number from GM.010 to GM.011 * Revised wording in section 1.0 * Revised and renumbered sections 2.0, 5.0, 6.0, 7.0 and 8.0 * Added reference to 9.1 in section 2.2 * Specified that the preferred sample is EDTA anticoagulated whole blood within 14 days of collection in section 3.0 * Revised wording in section 3.0 to include “See Procedure Notes 8.13” * Revised list of references |