ANTISERA EVALUATION
AND
OTHER CONSULTATION SERVICES

Brochure

The
Blood Bank Center Reference Laboratory*

US ARMY MEDICAL RESEARCH LABORATORY
Fort Knox, Kentucky 40121
June 1972

*Accredited by the American Association of Blood Banks, October 1971.
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ANTISERA EVALUATION AND OTHER SERVICES AVAILABLE IN THE BLOOD BANK CENTER REFERENCE LABORATORY

A brochure has been prepared describing the various quality control tests of blood group reagents and consultation services available at The Blood Bank Center Reference Laboratory. The role of The Blood Bank Center Reference Laboratory in evaluating blood group reagents for the Armed Services is described as well as the interrelationship of this quality control testing with the Defense Medical Material Board, the Defense Personnel Support Center, and the Division of Biologics Standards of the National Institutes of Health. Other consultation and testing services include immunohematological studies, forensic studies, Gm testing, and pyrogen testing.

A listing of available scientific literature includes 121 laboratory reports, five monographs, and a translation series in blood group immunology.
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ANTISERA EVALUATION
AND
OTHER CONSULTATION SERVICES

BROCHURE

by

MAJ Virgil R. Coley, MSC
Mary J. Levan
COL Frank R. Camp, Jr., MSC
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and
Ima G. Shirley

Blood Bank Center
US ARMY MEDICAL RESEARCH LABORATORY
Fort Knox, Kentucky 40121

June 1972

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MISSION STATEMENTS

Defense Medical Materiel Board: Established by the Secretary of Defense to provide coordination, advice, and assistance on the professional/technical aspects of medical materiel and in the field of medical supply.

Captain R. F. C. MacPherson,  
MC, USN, Director, Defense Medical Materiel Board

Mrs. Elise N. Hayes,  
Staff Member, Defense Medical Materiel Board

Defense Personnel Support Center Medical Mission: Procures, stores, stocks, and issues items of medical materiel standardized by the Defense Medical Materiel Board, based on the logistic requirements of the individual medical services.
SUPPORT AGREEMENT

1. The US Army Medical Research Laboratory (USAMRL) agrees to provide services upon written request from the Directorate of Medical Materiel, Defense Personnel Support Center (DPSC), on the following types of items supplied by the receiving activity:

   b. Blood grouping sera.
   c. Bromelin, ficin, papain, and trypsin enzyme solutions.
   d. Serum, antihuman, Coombs test.
   e. Dextran.
   f. Albumin normal human serum.
   g. Albumin serum reagent, bovine.
   h. Globulin, tetanus immune.
   i. Globulin, immune serum.
   j. Globulin, Rho immune.
   k. Other blood derivatives and related products.
   l. Pyrogen testing.
   m. Blood bags.

2. USAMRL will test other blood related equipment and supplies not described above upon mutual agreement with DPSC.

3. USAMRL will conduct workshop courses (duration: 5 days) for medical materiel inspectors of DPSC.

4. Upon completion of any examination, USAMRL will notify DPSC of any evidence of noncompliance with specifications and/or nonsuitability for issue and use.

ACKNOWLEDGMENTS

The authors wish to express their thanks to Rachel G. Bertram, Cynthia S. Carter, and Dorothy P. Stiglitz for the format, typing, and editing of this monograph.

We also acknowledge the photographic support and technical advice of Richard A. Wheeler, George W. Weeks, James E. Smith, Mary Jo Wyatt, and Philip E. Corbit.
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Alfred Chenevix
William O. Conolly
Daniel J. Collins
Allen D. Clark

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INTRODUCTION

The Blood Bank Center (BBC), US Army Medical Research Laboratory (USAMRL), Fort Knox, Kentucky, operates a reference laboratory. One important function is to evaluate each lot of blood bank antisera purchased on government contract.* The criteria for evaluation are developed from the performance requirements (essential characteristics) which are established by the Defense Medical Materiel Board (DMMB) and incorporated in the purchase description by the Defense Personnel Support Center (DPSC).

Before any laboratory evaluation, a blood bank reagent must conform to the existing minimum requirements established by the Division of Biologics Standards (DBS), National Institutes of Health (NIH). A copy of the NIH release form must accompany the material submitted. Reference standard reagents from the Division of Biologics Standards, National Institutes of Health, are tested in parallel with all blood group reagents submitted to the Blood Bank Center Reference Laboratory for evaluation.

A contract for a particular antiserum is awarded by the Defense Personnel Support Center (DPSC) after the reference laboratory certifies that the antiserum conforms to DPSC specifications. During bottling of any lot, a quality assurance representative from DPSC is present; at this time 12 bottles are selected at random and shipped directly to the BBC Reference Laboratory by him. Six of these samples are tested before shipment is released; the remaining six are stored as reference samples at the laboratory.

The following products currently under contract are tested:

1. Anti-A, liquid 5 ml, 6505-159-8475.
2. Anti-A, dried, equivalent to 5 ml, 6505-975-0614.
3. Anti-B, liquid, 5 ml, 6505-159-8500.
4. Anti-B, dried, equivalent to 5 ml, 6505-975-0615.
5. Anti-A,B, dried, equivalent to 5 ml, 6505-935-3998.
6. Anti-A,B, liquid, 5 ml, 6505-584-3038.
7. Anti-Rh, liquid, 5 ml, 6505-159-8575.
8. Anti-Rh, dried, equivalent to 5 ml, 6505-975-0613.
10. Antihuman, 2 ml, 6505-071-0611.
11. Antihuman, 10 ml, 6505-065-0024.
The procedures used in testing antiserum are detailed in Annexes A-G.

The DMMB performance requirements for titer, avidity, and specificity for ABO and Rh sera follow:

<table>
<thead>
<tr>
<th>TABLE 1</th>
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<tr>
<td>ABO Grouping Sera</td>
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<th>Cells</th>
<th>Anti-A (Avidity)</th>
<th>Anti-B (Avidity)</th>
<th>Anti-A,B (Avidity)</th>
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<td></td>
<td>Titer</td>
<td>Beginning</td>
<td>Complete</td>
</tr>
<tr>
<td>A1</td>
<td>512*</td>
<td>5&quot;</td>
<td>30&quot;</td>
</tr>
<tr>
<td>A2</td>
<td>128</td>
<td>5&quot;</td>
<td>30&quot;</td>
</tr>
<tr>
<td>A1B</td>
<td>256</td>
<td>5&quot;</td>
<td>30&quot;</td>
</tr>
<tr>
<td>A2B</td>
<td>64</td>
<td>45&quot;</td>
<td>3'</td>
</tr>
<tr>
<td>B</td>
<td>512*</td>
<td>5&quot;</td>
<td>30&quot;</td>
</tr>
</tbody>
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*1:256, June 1972.

Antibodies must react with the corresponding antigens only. This is tested by using both serum and saline suspensions of group A, B, O, and AB bloods. The tube and slide methods are used in testing at least ten A's, ten B's, ten O's, and five AB's. A 4+ reaction after centrifugation is required with the tube method.

Tests are performed to insure that no hemolysins and/or nonspecific immune antibodies are present.

<table>
<thead>
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<th>TABLE 2</th>
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<tr>
<td>Rh Typing Sera</td>
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<table>
<thead>
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<th>Cells</th>
<th>Anti-Rh (Avidity)</th>
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<td>Titer</td>
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<td>R1R1</td>
<td>64</td>
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<td>R2R2</td>
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<td>R1R2</td>
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<tr>
<td>R1r</td>
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<tr>
<td>r'r</td>
<td>4</td>
<td>30&quot;</td>
</tr>
<tr>
<td>r''r</td>
<td>4</td>
<td>30&quot;</td>
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</table>
Antibodies must react with the corresponding antigens only and are tested with serum suspended cells by the tube and slide methods. In the test tube method at room temperature, the degree of positive agglutination reaction with Rh0 cells must be ++++ when used according to the directions of the manufacturer. No incubation is permitted. Reading of reaction must be made immediately after spin. At least ten random positive bloods and five negative bloods are used in testing. The anti-Rh0 must be suitable for testing for the Rh0 variant Rh0 by the indirect Coombs method.

Some of the more important DMMB performance requirements for anti-human sera and bovine albumin follow:

**Antihuman Serum**

Antihuman serum is tested using the block Coombs titration. The antihuman serum, when diluted 1:16 in saline, must cause agglutination of sensitized Rh0 positive erythrocytes producing a 1+ reaction, one serial dilution higher than the basic titer of the anti-Rh0 serum. It must be capable of detecting antibodies in the Rh, Duffy, Kidd, Lewis, and Kell systems and of detecting immune anti-A and immune anti-B in serum by the indirect antiglobulin method.

Using the direct antiglobulin method, the antiserum must detect coated cells from an acquired hemolytic anemia, as well as coated cord cells in cases of mother-child ABO incompatibility. It must detect both gamma and nongamma immunoglobulins.

Specificity of reagent must permit microscopic examination in blood transfusion compatibility testing.

**Albumin, Bovine 22%**

Albumin, bovine, must be a concentrated 22% (±2%) solution suitable for use in Rh testing, Rh antibody titrations, and compatibility testing. The pH must be between 7.0 and 8.0; the sodium chloride content between 700 and 1,000 mg/100 ml. The albumin solution must not cause hemolysis, crenation, or rouleaux formation of red blood cells.

In addition to testing blood bank reagents, the BBC Reference Laboratory offers the following consultation services to any military installation.
CONSULTATION SERVICES AVAILABLE AT USAMRL

1. Immunohematological studies.
   a. Antibody detection and identification.
   b. Crossmatch problem assistance.
   c. Transfusion reaction studies and assistance.
   d. Screening for rare donors.

2. Forensic studies.
   a. ABO determinations.
      (1) Blood crusts.
      (2) Blood stains.
      (3) Seminal stains.
      (4) Saliva stains.
      (5) Bone.
      (6) Hair.
      (7) Fingernails.
   b. Precipitin testing.
   c. Paternity studies.
   d. Hemoglobin studies.

3. Miscellaneous studies.
   a. Pyrogen studies.
   b. Gm testing (referral).
   c. Special studies upon request.
   d. Hepatitis (Australia antigen) screening.
   e. Analysis of blood bank reagents, purchased through DPSC involved in complaints.
   f. Coagulation studies.
Consultation forms are available upon request. See Annex H for a sample form.

The BBC Reference Laboratory may be reached by telephone:

Day (502) 624-6656/7051
Night (CQ) (502) 624-1647
Autovon 464-6656/7051/1647

BLOOD BANK FELLOWS
Blood Bank Fellows (cont)

DPSC WORKSHOP

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ANNEX A

PROCEDURE FOR TESTING ANTI-A

1. **Titer.**

   a. Five rows of test tubes (12 x 75) are set up--ten tubes to each row (1:2, 1:4, etc.).

   b. In the first row place 0.7 ml of saline in each of the ten tubes, using a 1 ml pipette.

   c. With a 1 ml pipette, place 0.7 ml of anti-A in the first tube. Discard pipette.

   d. Using a clean 1 ml pipette, mix and transfer 0.1 ml to each of the four tubes in the back and 0.7 ml in the tube on the right (1:4). Discard pipette.

   e. Using a clean pipette, mix and repeat as in d above through the tenth tube.

   f. Place 0.1 ml of a 2% suspension of A1 cells in the second row, 0.1 ml of a 2% suspension of A2 cells in the third row, 0.1 ml of a 2% suspension of A1B cells in the fourth row, and 0.1 ml of a 2% suspension of A2B cells in the fifth row. Mix. Spin in a serofuge for 30 seconds. Read for agglutination. Let tubes sit at room temperature for 15 minutes and reread.

2. **Avidity.**

   a. Use a 10% suspension of the same cells used in the titer (A1, A2, A1B, and A2B).

   b. Place one drop of the cell suspension on a slide and one drop of anti-A. Mix. Observe for beginning agglutination and complete agglutination. **(Complete agglutination is the point at which 1 square mm of agglutinated RBC is obtained.)**

3. **Specificity.**

   a. Usually ten random group A bloods are tested by the slide and stick-tube method. (The amount of cells and antiserum used is equivalent to a 2% suspension.)

   b. Using blood of A1, A2, A1B, A2B, B, and O, an approximate 2% suspension in saline is made and equal volumes of cell suspension and anti-A are used. Spin immediately. Read for agglutination.
c. These same six blood groups are tested by the stick-tube method giving a serum suspended cell of a 2% suspension in whole blood. Spin immediately. Read for agglutination.

4. Test for hemolysins and nonspecific immune antibodies. A 2% suspension of group O, Rh negative blood is made. Equal volumes of the cell suspension and anti-A are placed in three tubes. Place one tube at room temperature, one tube at 37 C, and one tube at 4 C for 1 hour. Observe for hemolysis and/or agglutination. Then the three tubes are kept at room temperature for 2 hours. Observe for hemolysis and/or agglutination.

5. Clarity.
   a. Liquid antiserum. Material should be clear and free of particulate matter.
   b. Dried antiserum. Material should have a minimum of turbidity and particulate matter.
ANNEX B

PROCEDURE FOR TESTING ANTI-B

1. Titer.
   a. Four rows of test tubes (12 x 75) are set up—ten tubes to each row (1:2, 1:4, etc.).
   b. In the first row, place 0.7 ml of saline in each of the tubes, using a 1 ml pipette.
   c. With a 1 ml pipette, place 0.7 ml of anti-B in the first tube. Discard pipette.
   d. Using a clean 1 ml pipette, mix and transfer 0.1 ml to each of the three tubes in the back and 0.7 ml in the tube on the right (1:4). Discard pipette.
   e. Using a clean pipette, mix and repeat as in d above through the tenth tube.
   f. Place 0.1 ml of a 2% suspension of B₁ cells in the second row, 0.1 ml of a 2% suspension of A₁B cells in the third row, 0.1 ml of a 2% suspension of A₂B cells in the fourth row. Mix. Spin in a serofuge for 30 seconds. Read for agglutination. Set tubes aside for 15 minutes at room temperature and reread.

2. Avidity.
   a. Use a 10% suspension of the same cells used in the titer (B, A₁B, and A₂B).
   b. Place one drop of the cell suspension on a slide and one drop of anti-B. Mix. Observe for beginning and complete agglutination. (Complete agglutination is the point at which 1 square mm of agglutinated rbc is obtained.)

   a. Usually ten random group B bloods are tested by the slide and stick-tube method. (The amount of cells and antiserum used is equivalent to a 2% suspension.)
   b. Using blood from A₁, A₂, A₁B, A₂B, B, and O, an approximate 2% suspension in saline is made and equal volumes of cell suspension and anti-B are used. Spin immediately. Read for agglutination.
c. These same six blood groups are tested by the stick-tube method, giving a serum suspended cell of a 2% suspension in whole blood. Spin. Read for agglutination.

4. Test for hemolysins and nonspecific immune antibodies. A 2% suspension of group O, Rh negative blood is made. Equal volumes of the cell suspension and anti-B are placed in three tubes. Place one tube at room temperature, one at 37 C, and one at 4 C for 1 hour. Observe for hemolysis and/or agglutination. Keep the three tubes at room temperature for 2 hours. Observe for hemolysis and/or agglutination.

5. Clarity.

a. Liquid antiserum. Material should be clear and free of particulate matter.

b. Dried antiserum. Material should have a minimum of turbidity and particulate matter.
ANNEX C

PROCEDURE FOR TESTING ANTI-A,B

Cells needed: A₁, A₂, B, A₁B, and A₂B - 2% suspensions in saline.

1. Titer.
   a. Six rows of test tubes (12 x 75) are set up--ten tubes to each row (1:2, 1:4, etc.).
   b. In the first row place 0.8 ml of saline in each of the ten tubes, using a 1 ml pipette.
   c. With a 1 ml pipette, place 0.8 ml of anti-A,B in the first tube. Discard pipette.
   d. Using a clean 1 ml pipette, mix and transfer 0.1 ml to each of the five tubes in the back and 0.8 ml in the tube on the right (1:4). Discard pipette.
   e. Using a clean pipette, mix and repeat as in d above through the tenth tube.
   f. Place 0.1 ml of a 2% suspension of A₁ cells in the second row, 0.1 ml of a 2% suspension of A₂ cells in the third row, 0.1 ml of a 2% suspension of B cells in the fourth row, 0.1 ml of a 2% suspension of A₁B cells in the fifth row, and 0.1 ml of a 2% suspension of A₂B cells in the sixth row. Mix. Spin in serofuge for 30 seconds. Read for agglutination. Let tubes sit at room temperature for 15 minutes and reread without spinning.

2. Avidity.
   a. Use a 10% saline suspension of same cells used in the titer (A₁, A₂, B, A₁B, and A₂B).
   b. Place one drop of the cell suspension on a slide and one drop of anti-A,B. Mix. Observe for beginning agglutination and complete agglutination. (Complete agglutination is the point at which 1 square mm of agglutinated rbc is obtained.)

   a. Usually 10-15 random group A, B, and AB bloods are tested by the slide and stick-tube method. (The amount of cells and antiserum used is equivalent to a 2% suspension.)
b. Using blood of A₁, A₂, B, A₁B, A₂B, and O, an approximate 2% suspension in saline is made and equal volumes of cell suspension and anti-A,B are used. Spin immediately. Read for agglutination.

c. The same six blood groups are tested by the stick-tube method giving a serum suspended cell of a 2% suspension in whole blood. Spin immediately. Read for agglutination.

4. Test for hemolysins and nonspecific immune antibodies. A 2% suspension of group O, Rh negative blood is made. Equal volumes of the cell suspension and anti-A,B are placed in three tubes. Place one tube at room temperature, one tube at 37°C, and one tube at 4°C for 1 hour. Observe for hemolysis and/or agglutination. Then set aside the three tubes for 2 hours at room temperature. Observe for hemolysis and/or agglutination.

5. Clarity.

a. Liquid antiserum. Material should be clear and free of particulate matter.

b. Dried antiserum. Material should have a minimum of turbidity and particulate matter.
ANNEX D

PROCEDURE FOR TESTING ANTI-Rh0

Cells needed: Group O, R1r, R1R1, R1R2, R2R2 - 2% suspensions in 22% albumin.

1. Titer.
   a. Five rows of test tubes (12 x 75) are set up--ten tubes to each row (1:2, 1:4, etc.).
   b. In the first row place 0.7 ml of group AB serum in each of the ten tubes, using a 1 ml pipette.
   c. With a 1 ml pipette, place 0.7 ml of anti-Rh0 in the first tube. Discard pipette.
   d. Using a clean 1 ml pipette, mix and transfer 0.1 ml to each of the four tubes in the back and 0.7 ml in the tube on the right (1:4). Discard pipette.
   e. Using a clean pipette, mix and repeat as in d above through the tenth tube.
   f. Place 0.1 ml of a 2% suspension of R1r cells in the second row, 0.1 ml of a 2% suspension of R1R1 cells in the third row, 0.1 ml of a 2% suspension of R1R2 cells in the fourth row, and 0.1 ml of a 2% suspension of R2R2 cells in the fifth row. Mix. Incubate at 37 C for 1 hour. Mix. Spin in a serofuge for 45 seconds. Read for agglutination.

2. Avidity.
   a. Use as whole blood the same cells used in the titer (R1r, R1R1, R1R2, and R2R2).
   b. Place two drops of the cell suspension on a slide (heated to 37 C), add one drop of anti-Rh0. Mix. Observe for beginning agglutination and complete agglutination. (Complete agglutination is the point at which 1 square mm of agglutinated rbc is obtained.)

   a. Usually ten random Rh positive and five Rh negative bloods are tested by the slide and stick-tube methods.
   b. Test, by slide and stick-tube methods, using cells of R1r, R1R1, R1R2, and R2R2 in the appropriate suspensions.
c. Using known Rh positive and negative cells, test for this Rh variant. Place two drops of anti-Rh in a tube, two drops of 22% albumin in a second tube (negative control). Add one drop of a 2% suspension of cells to each tube. Incubate at 37° C for 30 minutes. Wash three times, add two drops of Coombs, spin and read.

4. Clarity. Material should be clear and free of particulate matter.
ANNEX E
PROCEDURE FOR TESTING ANTI-Rh0rh'rh'

Cells needed: Group O, R0r, r'r, r"r - 2% suspension in 22% albumin.

1. Titer.
   a. Four rows of test tubes (12 x 75) are set up--ten tubes to each row (1:2, 1:4, etc.).
   b. In the first row place 0.6 ml of group AB serum in each of the ten tubes, using a 1 ml pipette.
   c. With a 1 ml pipette, place 0.6 ml of anti-Rh0rh'rh" in the first tube. Discard pipette.
   d. Using a clean 1 ml pipette, mix and transfer 0.1 ml to each of the three tubes in the back and 0.6 ml in the tube on the right (1:4). Discard pipette.
   e. Using a clean pipette, mix and repeat as in d above through the tenth tube.
   f. Place 0.1 ml of a 2% suspension of R0r cells in the second row, 0.1 ml of a 2% suspension of r'r cells in the third row, and 0.1 ml of a 2% suspension of r"r cells in the fourth row. Mix. Incubate at 37° C for 1 hour. Mix. Spin in a serofuge for 45 seconds. Read for agglutination.

2. Avidity.
   a. Use as whole blood the same cells used in the titer (R0r, r'r, and r"r).
   b. Place two drops of the cell suspension on a slide (heated to 37° C), add one drop of anti-Rh0rh'rh". Mix. Observe for beginning agglutination and complete agglutination. (Complete agglutination is the point at which 1 square mm of agglutinated rbc is obtained.)

   a. Usually ten random Rh positive and five Rh negative bloods are tested by the slide and stick-tube methods.
   b. Test by slide and stick-tube methods, using cells of R0r, r'r, and r"r in the appropriate suspensions.

4. Clarity. Material should be clear and free of particulate matter.
ANNEX F

PROCEDURE FOR TESTING ANTIHUMAN SERUM

1. Materials.

   a. Red blood cells. For more reactive tests, use homozygous Rh\(_{0}\) positive cells preferably of genotype \(R_2R_2\). If these are not available use genotype \(R_1R_1\) or \(R_1R_2\). Reasonably fresh cells should be used.

   b. Anti-Rh\(_{0}\) antiserum. Use anti-Rh\(_{0}\) antiserum which has a minimum titer of 1:32.

2. Quantitative test procedure.

   Sensitization of cells with dilutions of anti-Rh\(_{0}\).

   a. Wash cells in normal saline once. After washing, there should be a minimum packed cell volume of 0.8 ml.

   b. Make a 2% cell suspension.

   c. Place 5.0 ml of the 2% cell suspension into each of six test tubes (use graduated centrifuge tubes, if possible). Label the tubes 1:16, 1:32, 1:64, 1:128, 1:256, 1:512.

   d. Add more normal saline to each tube and centrifuge. After centrifugation, each tube should have a 0.1 ml packed cell volume. Aspirate all of the saline from each tube.

   e. While the cells are being centrifuged, the dilutions of the anti-Rh\(_{0}\) antiserum can be made. Label six 13 x 100 mm test tubes 1:16, 1:32, 1:64, 1:128, 1:256, 1:512.

   f. Place 12.0 ml normal saline in tube labeled 1:16, place 6.0 ml normal saline in the remaining tubes.

   g. Add 0.8 ml anti-Rh\(_{0}\) to tube 1:16. Rinse the pipette in the tube several times. This gives a dilution of 1:16 anti-Rh\(_{0}\) in a total volume of 12.8 ml.

   h. Make twofold dilutions in the remaining tubes by removing 6.0 ml from tube 1:16 into tube 1:32, and so on. Discard the remaining 6.0 ml of the 1:512 dilution.

   i. Place 4.9 ml of each dilution of anti-Rh\(_{0}\) into the appropriately labeled tube containing 0.1 ml packed cells. Mix well to resuspend cells.
j. Incubate at 37 C for 1 hour.

k. Wash four times with normal saline. This is important because insufficient washing may cause a false negative reaction.

l. Add 4.9 ml normal saline and resuspend packed cells.

m. Place 0.1 ml of sensitized cells in six tubes from each dilution of anti-Rh0, a total of 36 tubes. Add 0.1 ml of antihuman (Coombs) serum, using undiluted 1:2, 1:4, 1:8, 1:16, and 1:32 dilutions. (See Diagram #3. Titration of Antihuman Serum, Appendix A, Minimum Requirements: Antihuman Serum for the Antiglobulin Test, NIH.)

n. Centrifuge and read. (See 3.5 potency requirements, Minimum Requirements: Antihuman Serum for the Antiglobulin Test, NIH.)

3. Qualitative test procedure.

a. Place two drops of Rh0 positive cells (2% cell suspension) in a 12 x 75 mm test tube.

b. Add two drops of 1:16 dilution of anti-Rh0.

c. Spin and read. (Test should be negative; if positive, make a higher dilution of anti-Rh0.)

d. Incubate at 37 C for 1 hour.

e. Spin and read. (Test should still be negative.)

f. Wash four times with saline and decant completely after last wash.

g. Add two drops of antihuman serum. Centrifuge and read. (This should be positive.)

4. Potency testing (using a known positive antigen-antibody system):

a. Depending on the titer of the antisera used, make either a 1:10 or a 1:20 dilution of anti-rh', anti-rh", anti-hr', and anti-hr".

b. Place two drops of each dilution into a test tube.

c. Add two drops of a 2% cell suspension of the corresponding Rh antigen.

d. Incubate at 37 C for 30 minutes.

e. Wash four times with normal saline and decant completely after last wash.
f. Add antihuman serum, spin, and read. (Tests should be positive.)

g. Repeat, using undiluted anti-K, anti-Jka, anti-Lea, and anti-Fya antisera with two drops of a 2% cell suspension of their corresponding antigens. (Tests should be positive.)

h. Test for immune anti-A and anti-B, using group O serum (previously known to have immune A and B) according to the AABB screening method. (See pages 59 and 60 of AABB Manual, 5th edition.)

### BLOCK COOMBS TITRATION

<table>
<thead>
<tr>
<th>Coombs Dilution</th>
<th>Dilution of Anti-Rh(\text{O}) Sensitized Cells (Group 0)</th>
<th>Amount of Saline</th>
<th>Amount of Coombs</th>
</tr>
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<tr>
<td>Undiluted</td>
<td>1:16, 1:32, 1:64, 1:128, 1:256, 1:512</td>
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<tr>
<td>1:2</td>
<td></td>
<td>0.4 ml</td>
<td>0.4 ml</td>
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<tr>
<td>1:4</td>
<td></td>
<td>0.6 ml</td>
<td>0.2 ml</td>
</tr>
<tr>
<td>1:8</td>
<td></td>
<td>0.7 ml</td>
<td>0.1 ml</td>
</tr>
<tr>
<td>1:16</td>
<td></td>
<td>0.75 ml</td>
<td>0.05 ml</td>
</tr>
<tr>
<td>1:32</td>
<td></td>
<td>1.55 ml</td>
<td>0.05 ml</td>
</tr>
</tbody>
</table>

...
5. Anticomplement activity (C'4 and C'3).
   
   (1) Five percent dextrose in water.
   (2) Isotonic saline.
   (3) Fresh clotted blood (less than 24 hours old), clot and serum separated.
   (4) Liquid $\frac{1}{2}$ EDTA, 5 mg per drop.
   (5) Parafilm.
   (5) Five Pasteur pipettes.
   
b. Procedure.
   (1) Mark three 13 x 100 mm tubes at the 2 ml level and number them 1, 2, and 3.
   (2) Transfer 5% dextrose in water to tubes 1 and 2 and fill to the 2 ml level.
   (3) Transfer isotonic saline to tube 3 and fill to 2 ml level.
   (4) Add three drops of liquid EDTA to tube 2.
   (5) Add five drops of fresh serum to each tube.
   (6) Cover tubes with parafilm and mix several times.
   (7) Add three drops of fresh whole clotted blood (from the same donor as the serum) to each tube.
   (8) Cover tubes with parafilm and mix well.
   (9) Incubate all three tubes for 10 minutes at 37 C.
   (10) Label three 10 x 75 mm tubes 1, 2, and 3.
   (11) Place two drops of the mixed cell suspensions from the larger tubes into the appropriately numbered small tubes.
   (12) Wash the cells three times in saline.
   (13) Add one drop antiglobulin reagent, serofuge, and read.
**RECORD RESULTS**

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<th>Cell</th>
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<th>Normal Ionic Strength</th>
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<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Complement Coated</td>
<td>Complement Blocked</td>
</tr>
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</table>

Antiglobulin Test

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ANNEX G

PROCEDURE FOR TESTING BOVINE ALBUMIN

Cells needed: Group 0, R_l, R_lR_l, R_lR_2, R_2R_2.

1. Test for hemolysis, crenation, and rouleaux formation of red blood cells.
   a. Place two drops of albumin into several tubes.
   b. Add two drops of a 2% suspension of group 0 cells to each tube.
   c. Observe macroscopically and microscopically for crenation of cells, hemolysis, and rouleaux formation. None should be present.

2. Test for clot formation in crossmatching procedure.
   a. Place two drops of plasma in tube.
   b. Add one drop of a 2% suspension of cells (obtained from segment on bag).
   c. Add two drops of albumin.
   d. Incubate at 37°C for 30 minutes, spin, and read. Observe closely for clot formation. Test should be negative.

3. Test for observing hemolysis.
   a. Place 0.1 ml of serum (known to have a hemolysin) in a tube.
   b. Add 0.1 ml of a 2% suspension of A_l or B cells.
   c. Add 0.1 ml of albumin.
   d. Incubate at 37°C for 1 hour.
   e. Spin and read. Hemolysin should be present.

4. Quantitative testing.
   a. Make serial dilutions of a previously tested anti-Rh_o in group AB serum.
   b. Make 2% cell suspensions of group 0, R_l, R_lR_l, R_lR_2, and R_2R_2 cells in the albumin.
c. Add 0.1 ml of the cell suspension to 0.1 ml of the anti-Rh₀ dilution.

d. Incubate at 37°C for 1 hour.

e. Mix. Spin for 45 seconds and read. Titer should be the same as when previously tested.

5. Sodium chloride content.
   a. Determine the chloride content.
   b. Sodium chloride content may then be determined by using this formula:

      \[
      \text{mEq Cl}/\text{l} \times 5.85 = \text{mg NaCl}/100 \ \text{ml}
      \]

c. Should be between 700-1000 mg/100 ml.

6. pH determination. pH of the albumin should be between 7.0 and 8.0.

7. Percent of albumin. Albumin content should be 22% ± 2%.
ANNEX H

Blood Bank Center
US ARMY MEDICAL RESEARCH LABORATORY
Fort Knox, Kentucky 40121

REQUEST FOR CONSULTATION

Send Report To: ___________________________ Date: ________________

Name: ____________________________________________________________________________

Hospital: __________________________________________________________________________

Street: _____________________________________________________________________________

City & State: ___________________________ Zip Code: ____________________________

Telephone No: ___________________________ Area Code: _______________________

Send specimen to: Reference Laboratory
Blood Bank Center
US Army Medical Research Laboratory
Fort Knox, Kentucky 40121

Procedure for submitting samples:

1. Send freshly drawn samples, clearly labeled with full name and date.

2. Send 15 to 20 ml clotted blood and 5 ml anticoagulated blood. SEPARATE MOST OF SERUM FROM CLOT

3. Send specimens AIR MAIL, SPECIAL DELIVERY, and label "Blood specimen - refrigerate as soon as possible." Mail container to arrive at Reference Laboratory between Monday and Friday, if possible.

4. Notify the Reference Laboratory by telephone of the shipment.
   Autojun: 464-6656
   Commercial: 624-6656, Area Code 502

INFORMATION CONCERNING CASE

1. Patient's name ___________________________ Serial No. ____________________
   Sex ___________________________ Age ___________________________ Race ___________________________

   Diagnosis ________________________________________________________________________________

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2. ALL FEMALE PATIENTS:
   Number of pregnancies _____ Any difficulty? ____________________________
   Number of exchanges, if necessary ________________________________

3. ALL PATIENTS:
   Number of transfusions and dates____________________________________
   Number of group 0 (universal donor) units received____________________
   Type of reaction and number of units received________________________
   Estimate number of units needed____________________________________

4. DIFFICULTY ENCOUNTERED:
   A. Crossmatch problem_______________________________________________
      1. Saline______________ 2. Albumin______________________________
      3. Coombs______________ 4. Enzyme______________________________
      No. of donors compatible______ No. of donors incompatible______
      Is patient receiving any drugs?____ List drugs______________________

   B. Antibody identification___________________________________________
      1. Saline______________ 2. Albumin______________________________
      3. Coombs______________ 4. Enzyme______________________________

   C. Hemolytic Disease of the Newborn______________________________

   D. Other (explain in detail)__________________________________________

   ________________________________________________________________

   ________________________________________________________________

   ________________________________________________________________

   ________________________________________________________________

   ________________________________________________________________
## ANNEX I

### AVAILABLE SCIENTIFIC LITERATURE

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<td>The Detection of Sickle Cell Disease in Large Human Populations by an Automated Technique</td>
</tr>
<tr>
<td>937</td>
<td>The Forensic Testing Laboratory, 1971--Problems, Progress, and People</td>
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<td>938</td>
<td>Physicochemical Changes in Erythrocyte Membranes During Cold Storage in the Presence of Progesterone</td>
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<td>939</td>
<td>Effect of Varying Concentrations of Adenine, Inosine, and Methylene Blue on the Useful Storage Life of Blood</td>
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<td>Title</td>
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<td>942</td>
<td>Dithionite Tube Test - A Rapid, Inexpensive Technique for the Detection of Hemoglobin S and Non-S Sickling Hemoglobin</td>
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<td>943</td>
<td>Automated Dithionite Test for the Rapid, Inexpensive Detection of Hemoglobin S and Non-S Sickling Hemoglobinopathies</td>
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<td>944</td>
<td>The Murayama Test for Hemoglobin S (A Simplification in Technique)</td>
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<td>945</td>
<td>Sickledex Test for S Hemoglobin: A Critique</td>
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<td>955</td>
<td>Automated Quantitation of A and B Blood Group Substances</td>
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<td>958</td>
<td>Blood Component Logistics</td>
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<td>960</td>
<td>Hemolytic, Coagulant, and Renal Effects of Transfused IgG and IgM Derived from Plasma of Isoimmunized Monkeys</td>
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<td>961</td>
<td>Ability of Rabbit IgG Fab' Fragment Specific for a Human Species Antigen to Block Reactivity of HL-A Antisera</td>
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<td>Specificity of a Rabbit Antihuman Lymphocyte Serum</td>
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<td>Management in Military Blood Banking for Conservation of Blood Resources: New Aspects Concerning the Blood Donor Base</td>
</tr>
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<td>Mass Screening of Military Populations for Hemoglobin S by the Automated Dithionite Test</td>
</tr>
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<td>A Collected Bibliography of Clinical Advances in Sickle Cell Disease Based on the Murayama Molecular Hypothesis</td>
</tr>
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<td>969</td>
<td>Pulmonary Hemorrhage Syndrome as a Manifestation of Disseminated Intravascular Coagulation: Analysis of 10 Cases</td>
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<td>972</td>
<td>Thermal Destruction of Anti-A1 and Anti-A(A2) from Group O and Group B Serum</td>
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<td>973</td>
<td>Electrocardiographic and Respiratory Changes Observed in Blood Donors During Phlebotomy</td>
</tr>
<tr>
<td>974</td>
<td>Hemoglobin Function in Stored Blood: XII. Effects of Varying Phosphate Concentrations on Red Cell ATP and 2,3-DPG with Adenine and Inosine</td>
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Report Number | Title
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975 | Blood Preservation Solutions. XI: Raising the pH to Improve Hemoglobin Function
978 | Urea, Urease, Cyanate, and the Sickling of Hemoglobin S
979 | The Effects of Platelets on the Storage Properties of Human Erythrocytes
980 | Sickle Cell Disease: Clinical Advances by the Murayama Molecular Hypothesis

Monograph | Pitfalls of Blood Grouping and Pretransfusion Tests (Library of Congress Catalog Card Number 75-606639)
Monograph | Genetics for the Reference and Forensic Testing Laboratory (Library of Congress Catalog Card Number 77-175026)
Monograph | Military Blood Banking 1941-1971. Lessons Learned Applicable to Civil Disasters and Other Considerations (Library of Congress Catalog Card Number 78-184862)
Monograph | Immunohematology (Library of Congress Catalog Card Number 77-175027)
Monograph | Blood Group Immunology: Translation and Reproduction of Early Scientific Treatises (Library of Congress Catalog Card Number 76-188448)
Brochure | Antisera Evaluation and Other Consultation Services Available at The Blood Bank Center Reference Laboratory

Translation Series

Gammelgaard, Arne. On Rare, Weak A Antigens (A3, A4, A5, and Ax) in Man (Library of Congress Catalog Card Number 64-65449)
Hartman, Crethe. Group Antigens in Human Organs (Library of Congress Catalog Card Number 71-606638)
Selected Contributions to the Literature of Blood Groups and Immunology: Volume I. The ABO System (Dunsford Memorial)
Volume II. Secretion of Blood Group Substances and Lewis System

Volume III. Part I. Constitutional Serology and Blood Group Research
Part II. M, N, and P Systems

Volume IV. Part I. Anthropologic Data
Part II. Blood Groups and Their Areas of Application

Volume V. Landsteiner Centennial
ANNEX J

MISCELLANEOUS PHOTOGRAPHS

ACTIVATION AND GROWTH OF BLOOD PROGRAMS
US ARMY MEDICAL RESEARCH LABORATORY
Fort Knox, Kentucky 40121

*1964 Staff Study
1965 Blood Transfusion Research Division
1965 Blood Group Reference Laboratory
**1965 Quality Control Monitoring (DPSC)
1965 Blood Bank Fellowship Program (3 Fellows) Army
1966 Medical Corps Officer Training Program
1966 Reference and Forensic Testing Laboratory
1966 Blood Transfusion Division
1967 Institutional Membership, AABB
***1967 Approved Institution of Training AABB-ASCP
1969 Blood Coagulation Laboratory
1969 Transfusion Reaction Model
1969 Blood Components Center
1969 Blood Bank Fellowship (4 Fellows) 3 Army, 1 Navy
1970 Histocompatibility (Lymphocyte Typing) Laboratory
1970 Field Testing Laboratory
1970 311-f1 Blood Bank Training for Enlisted Personnel
1971 Blood Bank Center
1971 Blood Research Division
1971 AABB Reference Laboratory
1971 Blood Bank Fellowship (5 Fellows) 3 Army, 1 Navy, 1 Air Force

FUTURE GOALS

Frozen Red Blood Cell Bank
Rare Donor Registry

*Crosby & Camp
**Defense Personnel Support Center
***American Association of Blood Banks
****American Society of Clinical Pathology
US ARMY
BLOOD TRANSFUSION RESEARCH STAFF 1965-1971
FORT KNOX, KENTUCKY

CONTE, N. F.
1969-71
CAMP, E. R., JR.
1965-71
SHIELDS, C. E.
1965-70
DAUBER, L. G.
1966-68
REED, L. J.
1955-68
BUNN, H. F.
1966-68
KAPLAN, H. S.
1967-69
LITWIN, S. D.
1967-69
DAWSON, R. B., JR.
1968-71
LOPAS, H.
1968-71
BIRNDORF, N. I.
1958-71
BELL, C. E., JR.
1968-71
US ARMY BLOOD BANK FELLOWSHIP PROGRAM
WALTER REED ARMY INSTITUTE OF RESEARCH
WASHINGTON, D.C.
1958 - 1965

US ARMY MEDICAL RESEARCH LABORATORY
FORT KNOX, KENTUCKY
Left to right: J. A. Maples, A. G. Cumuze, Jr., R. G. DeBonville, R. F. C. MacPherson, L. R. McKinley, Jr., J. H. Young, Margaret E. McPeak, and Elise N. Hayes.
Left to right: Margaret E. McPeak, Elise N. Hayes, and R. F. C. MacPherson.