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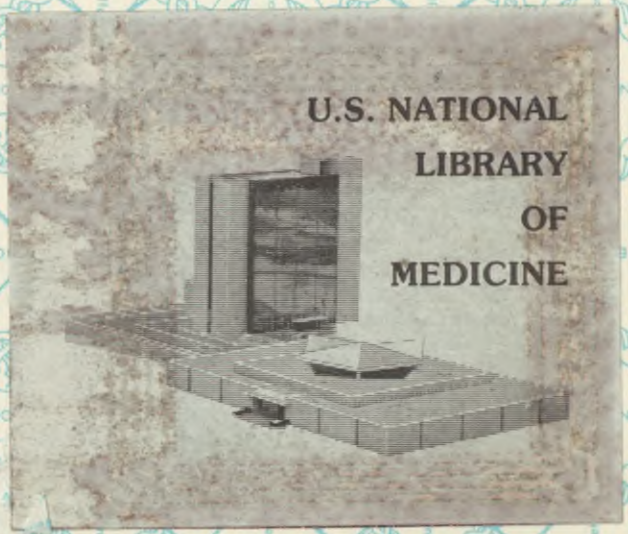

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STANDARD RUBELLA HEMAGGLUTINATION-INHIBITION TEST

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE

OCTOBER 1970

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We have received a number of reports from persons using this protocol, indicating problems with indistinct patterns of red blood cells in the 1:8 serum dilution. We are currently investigating this problem. Should you encounter this problem, we would like to suggest that the concentration of $MnCl_2$ be reduced to 0.75 M. This has eliminated the agglutination problem in many instances without adversely affecting the removal of non-specific inhibitor. If you do not experience this agglutination problem, we recommend that you continue to use a 1.0 M concentration of $MnCl_2$.

12-75

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PREFACE

A number of methods for the serological diagnosis of rubella virus infections have been introduced in recent years. Of these methods the hemagglutination-inhibition technique has been the one most widely applied because of its relative simplicity and generally accepted reliability. Since the medical handling of patients is based on the results of these tests, it is of obvious importance that the laboratory procedures used be of proven reproducibility and reliability.

Dr. U. Pentti Kokko, Director, Laboratory Division, Center for Disease Control, appointed the following committee and charged it with establishing a standard protocol for the performance of the rubella hemagglutination-inhibition test based on sound scientific principles and proven by comparative study:

Chairman

Dr. Robert E. Kissling
Virology Section
Center for Disease Control
Atlanta, Georgia

Dr. J. V. Baublis
Dept. of Pediatrics and Communicable Diseases
University of Michigan Medical Center
Ann Arbor, Michigan

Dr. Louis Z. Cooper
New York University Medical Center
New York, New York

Dr. Kenneth L. Herrman
Viral Exanthems Unit
Center for Disease Control
Atlanta, Georgia

Dr. Dorothy Horstmann
Dept. of Epidemiology and Public Health
Yale University School of Medicine
New Haven, Connecticut

Dr. Harvey Liebhaber
Dept. of Epidemiology and Public Health
Yale University School of Medicine
New Haven, Connecticut

Dr. Nathalie J. Schmidt
Dept. of Public Health
Berkeley, California

During the final stages of the development of this standard protocol 10 additional laboratories were asked to participate in the evaluations. The following individuals generously contributed time and effort to the development of the protocol set down in this booklet.

Dr. Charles Alford
Dept. of Pediatrics
University of Alabama Medical School
Birmingham, Alabama

Dr. Henry Bauer, Director
Division of Medical Laboratories
Minnesota Dept. of Health
Minneapolis, Minnesota

Dr. Rudolph Deibel, Director
Virus Laboratories
State Dept. of Health
Albany, New York

Dr. Paul D. Ellner
College of Physicians & Surgeons
Columbia University
New York, New York

Mr. Maurice R. Miot
Virology Section
Department of Public Health
Atlanta, Georgia

Mr. Paul Bonin
Director of Laboratories
Seattle-King County Dept. of Health
Seattle, Washington

Miss Elsie E. Buff
Virologist in Charge
Dept. of Health and Rehabilitative Services
Jacksonville, Florida

Dr. Paul D. Parkman
Laboratory of Viral Immunology
National Institutes of Health
Bethesda, Maryland

Dr. Jay E. Satz
Clinical Immunology
Pennsylvania Dept. of Health
Philadelphia, Pennsylvania

Dr. Yau Wai Wong, Assistant Director
State Hygienic Laboratory
University of Iowa
Iowa City, Iowa

The Committee realizes that future developments may render this protocol obsolete. However, the trials leading to the selected protocol indicate that reproducibility of results within a laboratory can and should be in excess of 93% if the protocol is faithfully followed.

A detailed account of the many trials leading to the development of this protocol will be published in an appropriate scientific journal.

NOTE: A training manual giving detailed instructions for performance of the test is available from the Center for Disease Control, Laboratory Division, Atlanta, Georgia 30333.

CDC STANDARD RUBELLA HEMAGGLUTINATION-INHIBITION TEST

INTRODUCTION

The following protocols describe the materials and methods to be used in the CDC standard rubella hemagglutination-inhibition (HAI) test. The information presented in these protocols is necessarily limited to the actual performance of the HAI test. The detailed studies of the variables affecting rubella hemagglutination and the removal of nonspecific serum inhibitors upon which the present protocols were largely based have been published. It would be advisable to consult these papers if one wishes to gain an understanding of this test system beyond the procedural details described herein.

Included in this booklet is an approved alternate method for removing nonspecific inhibitors from serum using dextran sulfate-$CaCl_2$ and instructions for preparing goose erythrocytes which may be used if chick cells are not available.

THESE POINTS ARE IMPORTANT AND MUST BE STRICTLY OBSERVED:

1. **Do not heat the test serums.**
2. **Reagents must be kept sterile.**
3. **Reagents must not be used when stored beyond the specified time.**
4. **The pH of the reagents must be within the specified limits.**
5. **The working fowl erythrocyte suspensions must be prepared fresh daily for each test run.**
6. **The antigen must titer 1:64 or greater.**
7. **The antigen must be titrated daily before each test run.**
8. **Reagents must be added exactly in the order given.**
9. **All specified controls must be included in each test.**
10. **Serial samples from an individual patient should always be assayed for HAI antibody in the same test.**
11. **Screening of serums at one or two low dilutions only is not advised.**

In addition to the usual laboratory equipment which includes a refrigerator, refrigerated centrifuge, spectrophotometer and miscellaneous glassware, the following equipment is required:

Microtitration "V" bottom plates (either rigid or flexible plastic).
Diluting loops, 0.025 ml capacity.
Dropping pipettes, 0.025 ml drops.
Reading mirror.
Vibrating platform shaker.

The following reagents are needed for the performance of the standard rubella HAI test:

Rubella HA antigen with titer of 1:64 or greater.
Chick erythrocytes.
Alsever's solution.
Dextrose-Gelatin-Veronal (DGV) buffer.
HEPES-saline-albumin-gelatin (HSAG) diluent.
High titer rubella antibody positive control human serum.
Low titer rubella antibody positive control human serum.
Negative rubella antibody control human serum.
Cyanmethemoglobin reagent.

REFERENCES

Liebhaber, H. Measurement of rubella antibody by hemagglutination-inhibition. I. Variables affecting rubella hemagglutination. J. Immunol. *104*:818-825 (1970).

Liebhaber, H. Measurement of rubella antibody by hemagglutination-inhibition. II. Characteristics of an improved HAI test employing a new method for the removal of non-immunoglobulin HA inhibitors from serum. J. Immunol. *104*:826-834 (1970).

Hierholzer, J. C., and Suggs, M. T. Standardized viral hemagglutination and hemagglutination-inhibition tests. I. Standardization of erythrocyte suspensions. Applied Microbiology *18*:816-823 (1969).

Cooper, L. Z., Matters, B., Rosenblum, J. K., and Krugman, S. Experience with a modified rubella hemagglutination-inhibition antibody test. J.A.M.A. *207*:89-93 (1969).

FORMULAE

ALSEVER'S SOLUTION (Modified)

Dextrose	20.5 gms
Sodium Citrate, $Na_3C_6H_5O_7 \cdot 2H_2O$	8.0 gms
Citric Acid, $C_6H_8O_7$	0.55 gms
Sodium Chloride	4.2 gms
Distilled Water q.s.ad	1.0 liter

Sterilize by filtration through Millipore membrane 0.22 μ pore size; pH should be 6.0-6.2. Store at 4°C in convenient aliquots.

DEXTROSE-GELATIN-VERONAL (DGV)

Barbituric Acid	0.58 gm
Gelatin	0.60 gm
Sodium Barbital	0.38 gm
$CaCl_2$ (anhydrous)	0.02 gm
$MgSO_4 \cdot 7\ H_2O$	0.12 gm
NaCl	8.5 gms
Dextrose	10.0 gms
Distilled H_2O q.s.ad	1.0 liter

Dissolve barbituric acid and gelatin in 250 ml of water by heating. Combine this solution with the remaining reagents.

Sterilize by filtration through Millipore membrane 0.22 μ pore size; pH should be 7.2. Store at 4°C.

HSAG Diluent (HEPES-saline-albumin-gelatin)

I. HEPES saline 5X stock solution

HEPES (N-2-Hydroxyethylpiperazine-N-2-ethanesulfonic acid)	29.8 gms
NaCl	40.95 gms
$CaCl_2 \cdot 2H_2O$	0.74 gms
Distilled H_2O q.s.ad	1.0 liter

Dissolve in 900 ml distilled water. Adjust pH of this solution to 6.5 by adding 1 N NaOH (approximately 12.0 ml). Add distilled water to bring volume of solution to 1,000 ml.

Sterilize by filtration through Millipore membrane 0.22 μ pore size. Store at 4°C.

II. Bovine serum albumin 2X stock solution.

Bovine albumin powder	20 gms
Distilled H_2O q.s.ad	1.0 liter

Dissolve bovine albumin in 900 ml water. Adjust volume to 1,000 ml by adding distilled water.

Sterilize by filtration through Millipore membrane 0.22 μ pore size. Store at 4°C.

III. Gelatin 10X stock solution

Gelatin	25 milligrams
Distilled H_2O	1.0 liter

The gelatin is dissolved and the solution sterilized by autoclaving for 15 minutes at 120°C. Store at 4°C.

IV. To Make HSAG Diluent

Combine:

HEPES saline 5X stock solution	200 ml
Bovine serum albumin 2X stock solution	500 ml
Gelatin 10X stock solution	100 ml
Sterile distilled H_2O	200 ml

At 25°C the pH of HSAG should be 6.2 $\pm$ 0.05. If pH is below 6.2, adjust with 1.0 N NaOH. If pH is above 6.2, adjust with 1.0 N HCl. This solution should be stored at 4°C and may be used for 2 months if kept sterile.

HEPARIN-$MnCl_2$ REAGENT

I. Heparin Stock Solution

Sodium heparin (USP) ..... 5,000 units/ml

This concentration may be obtained from commercial sources. If heparin is obtained as a more concentrated preparation (10,000 units/ml or more), dilute to 5,000 units/ml with distilled H_2O.

II. 1 Molar Manganous Chloride Solution

$MnCl_2 \cdot 4H_2O$ ..... 39.6 gms
Distilled H_2O q.s.ad ..... 200 ml

Sterilize by filtration through Millipore membrane 0.22 μ pore size. Store in dark at 4°C. Discard if brown precipitate appears.

III. Heparin-$MnCl_2$ 1:1 Working Solution

Combine equal parts heparin (5,000 units/ml) and 1.0 M $MnCl_2$.
Store at 4°C no longer than 2 weeks.
Keep sterile.

COLLECTION OF CHICK ERYTHROCYTES

One-to three-day-old chicks are bled from the heart using a 19-gauge needle on a 5.0 ml syringe. Before bleeding each chick, take up 3.0 ml of Alsever's solution into the syringe. From 0.5 to 1.5 ml of blood may be collected from each chick. Pool the blood from six chicks in a bottle containing 20 ml of Alsever's solution. The volume of Alsever's should always be at least four times that of the blood. Cells may be used for no longer than two weeks from the date of bleeding.

STANDARDIZATION OF CHICK ERYTHROCYTES

I. Calculation of Target O.D.

A. Construct standard cyanmethemoglobin curve.

Prepare dilutions of cyanmethemoglobin (cmg) in cmg reagent (Hycel) containing 80, 60, 40, 20, and 0 mg% cmg. Read each dilution in a spectrophotometer at 540 nm wavelength. These readings should fall on a straight line when plotted on regular graph paper against the mg% cmg of the standards.

B. Calculate the factor which will be used for determining the target O.D.

Factor = sum of concentrations of standards (80 + 60 + 40 + 20 + 0) divided by the sum of the optical density readings of the standards.

C. Calculate target O.D.

Target O.D. = target mg% cmg* divided by the factor.

*Target mg% cmg for 0.25% baby chick erythrocytes = 2.746.

The factor and target $O.D._{540}$ can be used for all subsequent cell standardizations made with your spectrophotometer, provided the instrument is not moved or unduly jarred.

II. Preparation of Chick Erythrocyte Suspension

A. The volume of erythrocytes needed over a period of a few days is prepared as a 4% suspension of washed cells.

Wash erythrocytes three times in dextrose-gelatin-veronal buffer (DGV) centrifuging at 900 xg for 10 min. in a graduated conical

centrifuge tube for each wash. Carefully remove the buffy coat after each wash. After the third wash read the packed cell volume and dilute in DGV to a 4% cell suspension. Store at 4°C for no longer than 3 days.

B. Prepare a 0.25% erythrocyte suspension daily according to the following directions:

1. Transfer 1.0 ml of the 4% suspension to a 25 ml volumetric flask.
2. Fill to the mark with cyanmethemoglobin reagent, add 30 mg of saponin and mix well.
3. Let stand for 15 to 45 minutes at room temperature until cells are lysed.
4. Centrifuge a sample of the cell solution at 900 xg/10 min. and transfer the supernate to a spectrophotometer cuvette.
5. Read the adsorbance (O.D.) at 540 nm against a reagent blank set at zero.
6. Check a reading of the adsorbance of a 1:2 dilution of standard cmg (40 mg% cmg) against the standard curve.
7. Calculate dilution necessary to obtain the desired 0.25% eyrthrocyte suspension.

$$\frac{(\text{O.D. of test suspension}) \times (\text{volume of 4\% cell suspension})}{\text{Target O.D.}} = \text{final volume of 0.25\% cell suspension}$$

8. Dilute to 0.25% cell suspension in HSAG.

PREPARATION OF 50% CHICKEN ERYTHROCYTE SUSPENSION

Chick erythrocytes too old for use in the HAI test proper may be used for absorbing natural agglutinins, provided the cells do not show significant hemolysis. Fresh erythrocytes from adult or baby chickens may also be used for this purpose.

1. Collect erythrocytes in Alsever's solution as described previously.
2. Wash erythrocytes three times in dextrose-gelatin-veronal buffer (DGV), centrifuging at 900 xg/10 min. in a graduated conical centrifuge tube for each wash. Carefully remove the buffy coat after each wash. After the third wash read the packed cell volume and dilute in HSAG to a 50% cell suspension. Prepare fresh daily.

ANTIGEN TITRATION

Perform in triplicate

1. Since the expected titer of the antigen is 1:64 or greater, prepare a preliminary 1:4 dilution of antigen by combining 0.1 ml of antigen and 0.3 ml of cold HSAG diluent. Allow to stand at 4°C for 15 minutes.
2. Add 0.025 ml cold HSAG diluent to wells 2 through 9 in each of three rows of wells in a "V"-type microtiter plate.
3. Add 0.05 ml of the 1:4 dilution of rubella antigen to well 1 in each of the three rows.
4. Using 0.025 ml diluting loops prepare 2-fold dilutions of the antigen in each row of wells (final dilution 1:1024).
5. Add 0.025 ml HSAG to each well of diluted antigen.
6. Add 0.05 ml HSAG to three additional wells (for cell control).
7. Place the plate in the refrigerator for 15 minutes.
8. Place the plate on a shaking machine (vibrator) and add 0.05 ml cold 0.25 per cent chick erythrocytes to each well of diluted antigen and to the three wells containing only HSAG.
9. Seal the plate with tape and incubate at 4°C for 1½ hours.
10. Remove the plate to room temperature. After 15 minutes, read and record the agglutination pattern.

The antigen titer in each row is the highest dilution of antigen causing *complete* agglutination of the cells. (See illustration.) The titers of antigen in the three rows must differ by no more than one 2-fold dilution. If the differences are greater than this, repeat the titration.

Since the titer obtained in at least two of the rows must agree, this is considered the final antigen titer. The final antigen titer must be at least 1:64.

To obtain the proper antigen dilution to use in test (4 units of antigen), multiply the final antigen titer by 4. Dilute the antigen accordingly with cold HSAG.

Example: (final titer = 1:64)

$$\frac{1}{64} \times 4 = \frac{1}{16}$$ or 1:16 dilution of antigen contains 4 hemagglutinating units

Dilute 1.0 ml of antigen with
15.0 ml of HSAG.

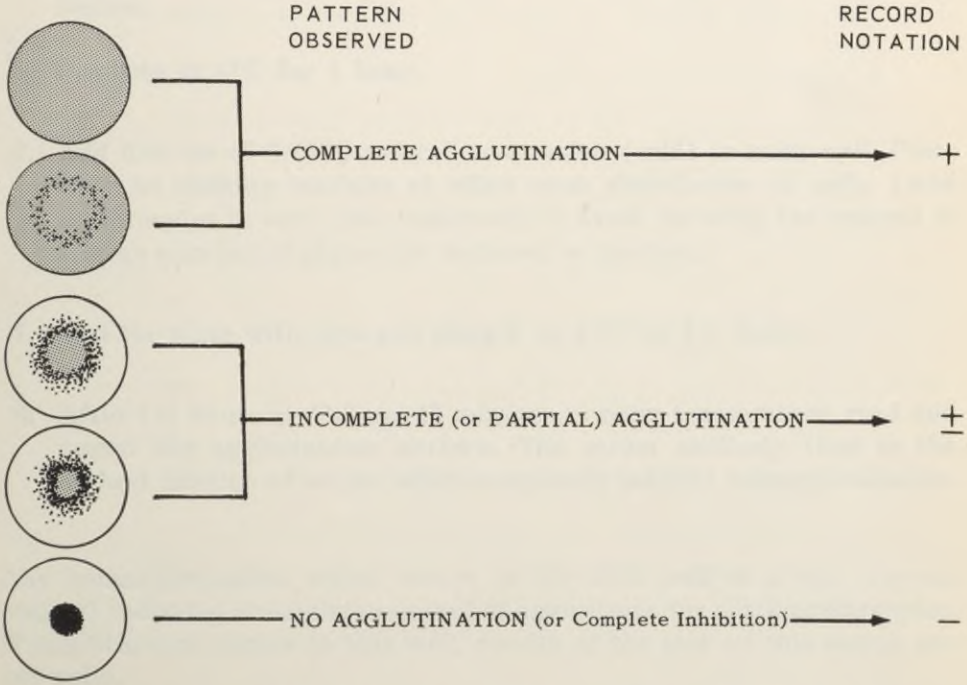
READING HEMAGGLUTINATION PATTERNS

Complete agglutination of erythrocytes has taken place when the cells are distributed in an even shield over the bottom of the well. The diluting loops, on occasion, may etch a ring on the bottom of the well. The agglutinated cells may settle, forming a roughened appearance on this etched portion. This pattern should not be confused with incomplete agglutination where a moderately heavy ring of nonagglutinated cells settles over a thin shield of agglutinated cells.

When no agglutination or complete inhibition of agglutination has occurred, the cells settle into a smooth compact button in the bottom of the well. This is not to be confused with the occasional slippage of a shield of agglutinated cells which may result in a dense ragged button in the bottom of the well. If doubt occurs as to whether the pattern consists of a button of nonagglutinated cells or a "slipped" agglutination pattern, the plate may be tipped at an angle. Nonagglutinated cells will flow along the side of the well.

Illustrations of complete, partial, and no agglutination are shown in the accompanying figure.

GUIDE FOR READING PATTERNS IN THE RUBELLA HA-HAI TEST



Note: Complete negatives (no agglutination) will "run" when microtitration plate is tilted on its side for 15-30 seconds before reading.

TREATMENT OF SERUM SPECIMEN TO REMOVE NONSPECIFIC INHIBITORS AND AGGLUTININS

Do not heat serum.

1. Mix 0.2 ml serum with 0.3 ml HSAG.
2. Add 0.2 ml of the 1:1 Heparin-$MnCl_2$ working solution and mix gently.
3. Incubate at 4°C for 15 minutes.
4. Add 0.2 ml of 50% chicken erythrocytes and suspend evenly by gentle mixing.
5. Incubate at 4°C for 1 hour.
6. Add 0.8 ml HSAG.
7. Centrifuge 900 xg for 15 minutes at 4°C.
8. Pipette supernate into an appropriately labeled, stoppered tube, taking care not to disturb the precipitate and cell pack.

This is considered a 1:8 dilution of serum and may be stored at 4°C for up to 2 weeks before testing.

CONDUCT OF THE HAI TEST

All reagents must be cooled to 4°C before use in the hemagglutination-inhibition test.

A row of 10 wells should be set up for each serum specimen in order to use nine wells for the serum dilutions of 1:8 to 1:2048 and the 10th well for the serum control.

1. **Add 0.025 ml of diluent (HSAG) to all wells in each row except the first well.**

2. **Pipette 0.05 ml of each 1:8 serum dilution into the first well containing no diluent and 0.025 ml into the last well (serum control).**

3. **Serial 2-fold dilutions of serum are made with 0.025 ml diluter (loop) beginning from the first well through the ninth well.**

4. **When serum dilutions are completed, add 0.025 ml of antigen dilution containing 4 hemagglutinating units to each well except the serum control.**

5. **Incubate at 4°C for 1 hour.**

6. **Add 0.05 ml of 0.25% red cell suspension (cold) to each well. Place plate on shaking machine to effect even distribution of cells. (Add erythrocytes to each plate separately to avoid warming the reagent if a large number of plates are included in the test.)**

7. **Seal the plate with tape and place it at 4°C for 1½ hours.**

8. **After 1½ hours at 4°C and 15 minutes at room temperature, read and record the agglutination pattern. The serum antibody titer is the highest dilution of serum which completely inhibits hemagglutination.**

Any hemagglutination which occurs in the 10th well of a row (serum control) indicates incomplete removal of agglutinins for chick erythrocytes. If agglutination occurs in this well, results of the test on this serum are not valid.

CONTROLS: In addition to the serum controls, each test must include the following controls:

A. Antigen Back Titration (perform in triplicate).

1. Add 0.025 ml of cold HSAG to wells 2 through 5 in each of three rows. Leave the first well of each row empty.
2. Add 0.025 ml of the antigen dilution used in the test (4 units) to the first and second well of each row.
3. Incubate with test at 4°C for 1 hour.
4. Using a 0.025 diluting loop, dilute the antigen from the second through the fifth well. The five wells should now contain 4, 2, 1, ½, and ¼ units of antigen.
5. Add 0.025 ml HSAG to each well.
6. Add 0.05 ml cold 0.25% chick erythrocytes. Place plate on a vibrating shaker to effect even distribution of cells, seal and incubate with test for 1½ hours at 4°C. Remove to room temperature and after 15 minutes read the hemagglutination pattern.

   Complete agglutination should occur in wells 1, 2 and 3 indicating that 4 units of antigen were used in the test.

   If less than two full units or more than eight full units were actually used, the entire test must be considered invalid.

B. Positive Serum Controls.

Two serums known to contain rubella HAI antibody are to be included in each test. They are treated for removal of nonspecific inhibitor and are tested identically as the test serums.

One serum should have a high antibody titer (1:256 or greater). The second serum should have a low antibody titer (1:16-1:32).

The titers obtained in each test on these positive serum controls must vary no more than ± one 2-fold dilution from the average titer obtained in previous tests with these serums; otherwise the entire test is considered invalid.

C. Negative Serum Control.

A serum known to contain no rubella HAI antibody is to be included in each test. It is treated and tested in a manner identical to that used for the test serums. This serum must show no inhibition of agglutination in any of the dilutions; otherwise the entire test is considered invalid.

D. Cell Controls.

1. Add 0.05 ml of HSAG to two wells.
2. When erythrocytes are added to test, add 0.05 ml 0.25% chick erythrocytes to these two wells. Incubate with test at 4°C for 1½ hours.
3. Read hemagglutination pattern. No hemagglutination must occur in these wells.

ALTERNATIVE PROCEDURES

In the study which led to the adoption of a standard protocol for the rubella HAI test, a dextran sulfate-$CaCl_2$ technique for removing nonspecific inhibitors from serum was found to give results which were as reproducible as those obtained following treatment of serum with heparin-$MnCl_2$. This technique is given here as an acceptable alternative to the heparin-$MnCl_2$ method.

Goose erythrocytes have also been found to give results equal to those obtained with chick erythrocytes. The method for collecting goose erythrocytes is given.

DEXTRAN SULFATE 5% SOLUTION

Dextran sulfate	5 gms
Distilled H_2O q.s.ad	100 ml

Sterilize by filtration through Millipore filter 0.22 μ pore size. Store at 4°C.

CALCIUM CHLORIDE—1 Molar Solution

$CaCl_2 \cdot 2H_2O$	29.4 gms
Distilled H_2O q.s.ad	200 ml

Sterilize by filtration through Millipore membrane 0.22 μ pore size. Store at 4°C.

COLLECTION AND PREPARTION OF GOOSE ERYTHROCYTES

I. Collection of adult goose erythrocytes:

Adult geese are bled aseptically from the jugular vein into a 50-ml syringe fitted with an 18-gauge needle. Before bleeding each animal of up to 25 ml of blood, take up 25 ml of Alsever's solution into the syringe. Use a fresh syringe and needle for each goose. Pool the blood from at least three geese in a bottle or flask containing 100 ml of Alsever's solution. It is most important to avoid the formation of clots (even small ones) in the syringe while the animal is being bled. Cells from partially clotted bleedings form clumped agglutination patterns which are unstable. Goose erythrocytes collected in this manner are available commercially.

II. Preparation of erythrocyte suspension:

Goose erythrocytes are used at an 0.08% concentration in the test proper and at a 50% concentration for absorbing agglutinins from serum. These suspensions are prepared in a manner similar to that described for chick erythrocytes. The target mg% cyanmethemoglobin for 0.08% goose cells is 0.9629.

ALTERNATE METHOD FOR TREATMENT OF SERUM SPECIMEN TO REMOVE NONSPECIFIC INHIBITORS

Do not heat serum.

1. Mix 0.2 ml serum with 0.35 ml HSAG.
2. Add 0.05 ml 5% dextran sulfate solution and mix gently.
3. Add 0.1 ml 1 M $CaCl_2$ solution; shake to mix.
4. Incubate at 4°C for 2 hours.
5. Centrifuge 900 xg for 15 min. at 4°C.
6. Without removing the packed precipitate, add 0.2 ml of 50% fowl erythrocytes; agitate gently to evenly suspend the cells without disturbing the precipitate.
7. Incubate at 4°C for 30 min.
8. Add 0.8 ml HSAG.
9. Centrifuge 900 xg for 15 min. at 4°C.
10. Pipette supernate into an appropriately labeled, stoppered tube, taking care not to disturb the precipitate and cell pack.

This is considered a 1:8 dilution of serum and may be stored at 4°C for one week before testing. If a longer holding period is anticipated, storage at —20°C is recommended.

SOURCES OF SUPPLIES

The following supplies are available from the indicated sources. There is no intention to imply that these are the only satisfactory sources.

HEPES (N-2-Hydroxyethylpiperazine-N^1-2-ethanesulfonic acid)—
Calbiochem No. 391338

Bovine Serum Albumin (Fraction V from Bovine Plasma)—
Armour Pharmaceutical Co.—No. 2293

Pooled goose cells—Flow Laboratories, Rockville, Md.

Sodium Dextran Sulfate. Preparations of this compound with molecular weights of 8,000 to 20,000, containing 12% to 18% sulfur, are suitable for use.—

Nutritional Biochemicals, Cleveland, Ohio (Catalogue listing: Dextran sulfate reagent grade for serum lipoprotein determinations.)

Mann Research Laboratories, New York City, New York No. 10300—9006, Dextran sulate, sodium salt [low MW] MW 18,000, S:14-38%)

Sodium Heparin 5,000 units/ml (sterile aqueous solution)—Upjohn Co.
Abbott Laboratories

Gelatin (Bacteriological Grade)—Difco Laboratories

Cyanmethemoglobin standard and diluent reagent—

Hycel, Inc.
P. O. Box 56329
Houston, Texas 77036

Clinical & Diagnostics Div.
Armour Pharmaceutical Co.
530 E. 31st St.
Chicago, Ill. 60616

Ortho Diagnostics
Raritan, New Jersey 08869

Unitek
7901 San Fernando Rd.
Sun Valley, Calif. 91350

Microplates

Disposable Autotrays Vee Bottom, clear or opaque —

Canalco
5635 Fisher Lane
Rockville, Md. 20852

No. 220-25—Disposable "V" plates (flexible vinyl)
No. 220-25A—Disposable "V" plates (rigid styrene)—

Cooke Engineering Co.
900 Slaters Lane
Alexandria, Va. 22314

Model 1S-MVC-96 Micro Disposo-Tray—

Linbro Chemical Co.
681 Dixwell Avenue
New Haven, Conn. 06511

Miscellaneous Micro Equipment

Pipette droppers (0.025 ml and 0.05 ml)
Diluting loops (0.025 ml and 0.05 ml)
Miscellaneous equipment (delivery testers, reading mirrors, etc.)—

Cooke Engineering Co.
900 Slaters Lane
Alexandria, Va. 22314

Linbro Chemical Co.
681 Dixwell Avenue
New Haven, Conn. 06511

Miscellaneous salts etc. should be reagent grade and are available from many sources.

Use of trade names is for identification only and does not constitute endorsement by the Public Health Service or by the U. S. Department of Health, Education, and Welfare.

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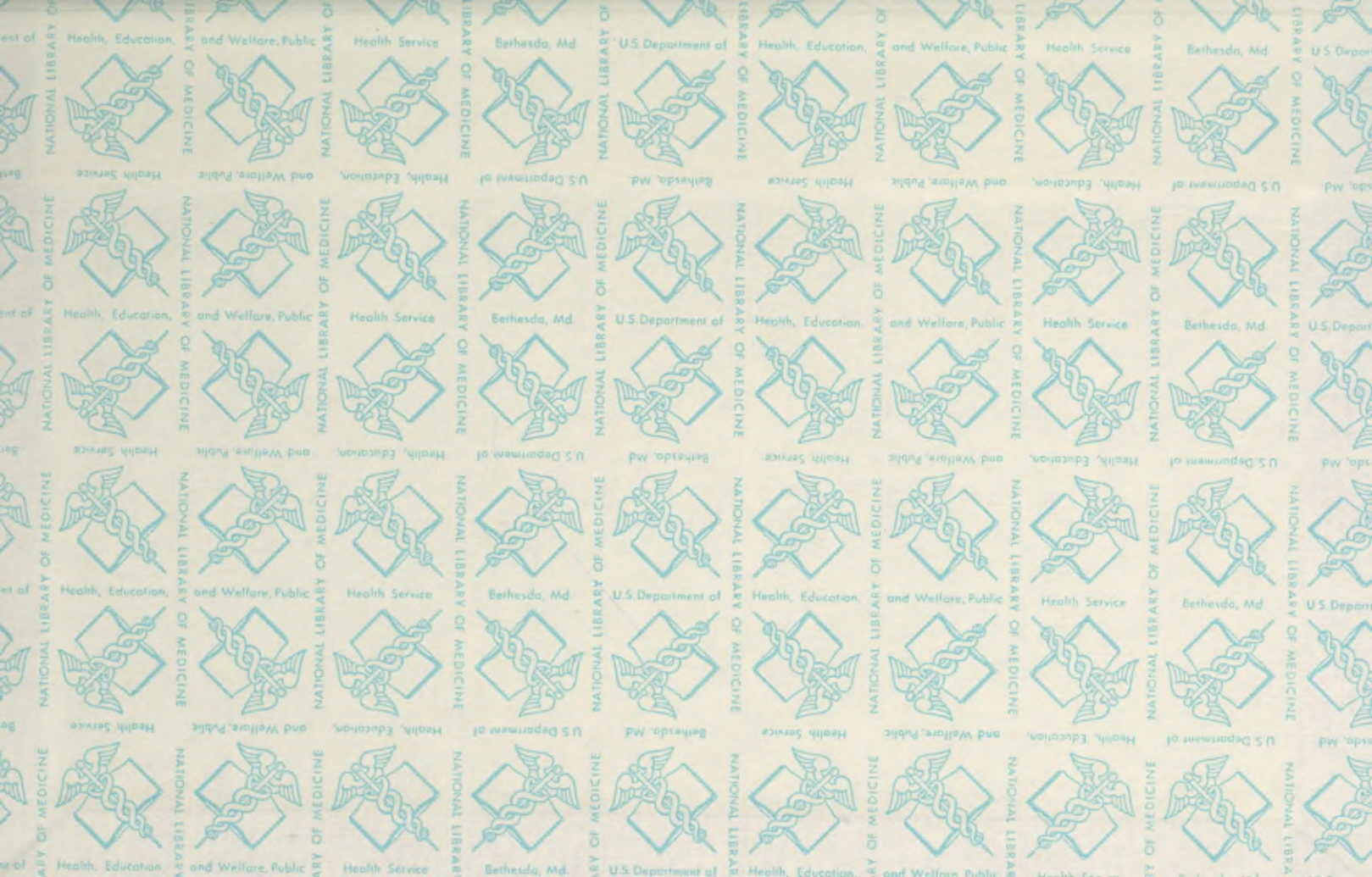
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