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DRAFT

RECOMMENDED METHODS FOR BLOOD GROUPING REAGENTS EVALUATION

March 1992

For further information about this draft, contact:

Center for Biologics Evaluation and Research (HFB-940)
Food and Drug Administration
8800 Rockville Pike
Bethesda, MD 20892
301-227-6487.

Submit written comments on this draft to:

Dockets Management Branch (HFA-305)
Food and Drug Administration
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Rockville, MD 20857.

Submit requests* for single copies of this draft to:

Congressional, Consumer, and International Affairs Branch (HFB-142)
Food and Drug Administration
5600 Fishers Lane
Rockville, MD 20857
301-295-8228
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Congressional, Consumer, and International Affairs Staff (HFB-142)
Food and Drug Administration
Suite 109, Metro Park North 3
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Rockville, MD 20855.

Comments and requests should be identified with the docket number found in brackets in the heading of this document.

DRAFT

PROPOSED REVISION

RECOMMENDED METHODS FOR

BLOOD GROUPING REAGENTS EVALUATION

MARCH 1992

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ABO BLOOD GROUPING REAGENTS

GENERAL INFORMATION

These recommended methods are provided to help assist manufacturers in pursuing new product license applications and amendments to existing product license applications. The methods described herein do not bind the agency, and manufacturers may consider use of other methods. In cases where manufacturers wish to use methods other than those described herein, FDA recommends that the matter be discussed with FDA in advance. The methods, potency titer values, specificity results, and other matters referred to in this document are intended to assist manufacturers in preparing submissions to FDA. The information is based on current knowledge and is not meant to be all inclusive and should not be viewed as ensuring approval or the only means of achieving FDA approval. Following the methods provided in this document will assist in the approval process, but does not guarantee approval. FDA will review applications on an individual basis and approvals will be granted when supported by scientific evidence.

I. REFERENCE PREPARATIONS

A. The Reference Blood Grouping Reagents listed below can be obtained from:

Center for Biologics Evaluation and Research
Food and Drug Administration
8800 Rockville Pike
Bethesda, MD. 20892
USA

Anti-A

Anti-B

NOTE: FDA Reference Blood Grouping Reagents are not routinely available to anyone except U.S. licensed manufacturers and amounts issued will be proportional to lots released in the previous year.

B. Reference sera are to be used according to the accompanying package insert only for determining the potency of Blood Grouping Reagents as part of their final lot release testing.

In-house reference materials should be developed for all stability testing, in process testing or product development purposes.

II. POTENCY TESTING

A. REAGENT DILUTIONS

1. Beginning with the undiluted reagent, prepare separate master two-fold serial dilutions (1 in 2, 1 in 4, etc.) of the test reagent using isotonic saline containing 1-2% bovine albumin or another diluent approved by the Director, Center for Biologics Evaluation and Research. Test tubes should be of a size that facilitates adequate mixing of the contents (12 X 75 mm or larger).

   If the endpoint is expected to exceed 1024, accuracy will be improved if direct intermediate dilutions are done to keep the number of serial transfers to less than 10. (e.g., If the expected endpoint is 4096, prepare an initial 1:10 dilution with the same diluent as used above.)

   NOTE: All titrations should be carried to a negative endpoint. (See E.4)

2. Prepare master dilutions of the Reference Blood Grouping Reagent(s) as in paragraph 1 of this section. For Anti-A,B and Anti-A and B prepare dilutions of each Reference Blood Grouping Reagent separately.

3. A separate, clean pipet or pipet tip should be used for each dilution (including any intermediate dilutions) to avoid carryover of higher reagent concentrations.

4. The last tube should contain diluent only and serve as a diluent control.

B. RED BLOOD CELL PREPARATIONS

Fresh or frozen red blood cells may be used for preparing cell suspensions for the potency testing of all Blood Grouping Reagents under the following conditions:

1. Red blood cells of any age may be used, provided the titer values of the reference reagents are within an acceptable range.

2. Red blood cells may be frozen and thawed for use in the preparation of cell suspensions for reagent evaluation. To ensure that the correct cell has been thawed, appropriate controls should be used to demonstrate the desired reactivity and identity of the thawed red blood cells on the day of use.

The method of freezing, storing, and thawing red blood cells, including a description of the cryoprotective medium should be described in detail and should be approved by the Director, Center for Biologics Evaluation and Research before use in control testing of licensed reagents.

3. Red blood cells should be washed at least twice in isotonic saline or until a clear supernate is obtained and then resuspended to a 2% suspension in isotonic saline containing 1-2% bovine albumin or another diluent approved by the Director, Center for Biologics Evaluation and Research.

C. MINIMUM TEST CELLS FOR POTENCY

REAGENT	RED BLOOD CELLS
Anti-A	A_1 and 3 DIFFERENT A_2B *
Anti-B	B and A_1B
Anti-A,B	A_1, A_2 **, and B
Anti-A and B	A_1, A_2 **, and B

* AB cells which do not react with anti-A_1 and do react with anti-H.

** A cells which do not react with anti-A_1 and do react with anti-H.

D. THE TEST (BY TUBE METHOD)

1. Place 0.1 milliliter of each reagent dilution in a separate, clean test tube (approximately 10 X 75 mm or 12 X 75 mm).

2. Place 0.1 milliliter of each Reference Blood Grouping Reagent dilution in a separate, clean test tube (approximately 10 X 75 mm or 12 X 75 mm).

3. Add 0.1 milliliter of the appropriate 2% cell suspension to each test tube.

4. Mix the contents of each tube thoroughly. Incubate for 5 minutes at room temperature (RT; 20-30 C) and centrifuge for 1 minute at approximately 1000 rpm (100-125 rcf) or 15 seconds at approximately 3400 rpm (900-1000 rcf) or at a time and speed appropriate for the centrifuge being used.

E. INTERPRETATION OF THE TEST

1. The cell buttons of each test tube should be gently dislodged and examined macroscopically.

2. The reactions should be graded as follows:

   4+ Cell button remains in one clump.

   3+ Cell button dislodges into several clumps.

   2+ Cell button dislodges into many small clumps of equal size.

   1+ Cell button dislodges into finely granular, but definite, small clumps.

   D Cell button dislodges into fine granules, but not definite small clumps. Results should be recorded as doubtful. For purposes of this paragraph, doubtful reactions are deemed to be negative.

   0 Negative reaction - cell button dislodges into no visible clumps.

3. The potency titer value is the reciprocal of the greatest reagent dilution for which the reaction is graded at 1+.

   The dilution caused by the addition of the red blood cells should not be considered as contributing to the dilution of the reagent.

4. Test results should include at least one tube with no agglutination after the endpoint. The diluent control tube should be negative.

F. POTENCY TITER VALUES

1. ABO Reagents should have an average potency titer value at least equal to that of the reference reagent.

2. Products recommended for use in automated or microplate systems without user dilution (as supplied) should be sufficiently potent that a two-fold dilution prepared with an approved diluent will produce the same qualitative test result as the undiluted product when tested in accordance with the manufacturer's package insert.

III. SPECIFICITY TESTING

A. REAGENT DILUTIONS

1. No dilution of the reagent under test is permitted.

B. RED BLOOD CELL PREPARATIONS

Fresh or frozen red blood cells may be used for preparing cell suspensions for the specificity testing of all Blood Grouping Reagents under the following conditions:

1. Any cells of any age may be used in the "Test to Confirm Reactivity with Antigen Positive Cells" (III.C.1). In the "Test to Confirm Absence of Contaminating Antibodies" (III.C.2) licensed reagent red blood cells may be used any time before their expiration date. All other red blood cell samples should be used within 7 days of collection from the donor.

   Manufacturers that wish to use cells more than 7 days after collection from the donor are to obtain approval from the Director, Center for Biologics Evaluation and Research, and are to provide sufficient data to support the request.

2. Red blood cells meeting the criteria of paragraph 1 of this section may be frozen and thawed for use in the preparation of cell suspensions for reagent evaluation. To ensure that the correct cell has been thawed, appropriate controls should be used to demonstrate the desired reactivity and identity of the thawed red blood cells on the day of use. In the case of cells expressing low frequency antigens, testing for several common antigens may serve to adequately identify the cell.

   The method of freezing, storing, and thawing red blood cells, including a description of the cryoprotective medium should be described in detail and should be approved by the Director, Center for Biologics Evaluation and Research before use in control testing of licensed reagents.

3. Licensed reagent red blood cells may be used as provided.

   All other red blood cell samples should be washed at least twice in isotonic saline or until a clear supernate is obtained and then resuspended with an approved diluent to the cell concentration listed in the manufacturer's package insert.

C. MINIMUM TESTING FOR SPECIFICITY

1. TEST TO CONFIRM REACTIVITY WITH ANTIGEN POSITIVE CELLS

a. At least 4 different donors whose red blood cells exhibit expression of the antigen should be tested.

b. When testing Anti-A,B and Anti-A and B reagents, the reactivity with both group A and group B red blood cells should be confirmed separately, i.e. at least four group A donors should be used to confirm the reactivity of the Anti-A component and at least four group B donors should be used to confirm the reactivity of the Anti-B component.

c. Minimum test red blood cells recommended:

REAGENT	RED BLOOD CELLS
Anti-A	A_1 (1) and A_1B (3) *
Anti-B	A_1B (3) and B (1)
Anti-A,B	A_1 (2), A_2 ** (2), B (4), at least 3 different A_x ***
Anti-A and B	A_1 (2), A_2 ** (2), B (4), at least 3 different A_x ***

* AB cells which do not react with anti-A_1 and do react with anti-H.

** A cells which do not react with anti-A_1 and do react with anti-H.

*** A_x cells are recommended; labeling should indicate detection of group A variants. Include examples of "strong A_x cells" and "moderate A_x cells". "Weak A_x cells" are optional.

d. Include at least one red blood cell which does not exhibit expression of the antigen as a negative control.

2. TEST TO CONFIRM ABSENCE OF CONTAMINATING ANTIBODIES

Test the reagent for the presence of antibodies corresponding to the following antigens by one of the methods listed below.

A, B, H, Le^a, Le^b, I, K, k, Kp^a, Kp^b, Js^b, P_1, D, C, E, c, e, C^w, M, N, S, s, U, Lu^a, Lu^b, Jk^a, Jk^b, Fy^a, Fy^b, Xg^a, Do^a, Do^b, Yt^a, Yt^b, Lan, Co^a, Co^b, M^g, Wr^a, Sd^a, and Vw.

If the source material is a monoclonal antibody from a previously characterized and licensed clone, this list may be shortened as follows:

A, B, H, Le^a, Le^b, I, K, k, Kp^b, Js^b, P_1, D, C, E, c, e, M, N, S, s, U, Lu^b, Jk^a, Jk^b, Fy^a, and Fy^b.

Approval for the use of fewer antigens than included on this list may be requested from the Director, Center for Biologics Evaluation and Research by a manufacturer at the time of submission of the first protocol.

a. Perform a direct test for the presence of contaminating antibodies by using red blood cells from at least 4 different donors whose cells lack the antigen corresponding to the reagent antibody.

b. When red blood cells lacking the antigen corresponding to the reagent antibody under test are not available, the reagent antibody may be adsorbed to exhaustion with cells of a known phenotype.

   The adsorbed serum may then be tested against red blood cells which exhibit any antigens which were not present on the cells used for adsorption. The methods for adsorption and subsequent testing should be approved by the Director, Center for Biologics Evaluation and Research.

c. When direct tests are impractical, the Director, Center for Biologics Evaluation and Research may approve procedures whereby antibodies may be presumptively excluded by testing an appropriate number of non-reactive red blood cell samples to provide statistical assurance of the absence of contaminating antibodies.

d. Red blood cell samples from four different donors may be used to confirm presumptively the absence of contaminating antibodies to antigens having an incidence of greater than 99% in the general population of the United States or the country in which it is sold.

D. THE TESTS

1. To confirm reactivity with antigen positive cells, each lot of Blood Grouping Reagent should be tested and results interpreted by all test methods described in the manufacturer's package insert. Minimum parameters (drops of reagent, incubation time, centrifugation, etc.) should be followed.

2. To confirm absence of contaminating antibodies, each lot of Blood Grouping Reagent should be tested and results interpreted by the most sensitive test method(s) described in the manufacturer's package insert. Maximum parameters (drops of reagent, incubation time, centrifugation, etc.) should be followed.

E. SPECIFICITY RESULTS

1. No hemolysis or rouleaux formation should be detected by any of the methods in the manufacturer's package insert.

2. Red blood cells which exhibit the antigen corresponding to the reagent antibody should yield at least a 2+ reaction. If any of the four samples tested yields less than a 2+ reaction, red blood cells from four additional donors who exhibit the antigen should be tested. The test is considered satisfactory if no more than one of eight red blood cell samples yields less than a 2+ reaction with the test reagent.

   When testing unusual phenotypes, other criteria for reactivity may apply. For example, a larger percentage of A_x red blood cells may not yield a 2+ reaction with Anti-A,B and Anti-A and B but should yield a clearly positive macroscopic result.

3. The negative control cell(s) in step III.C.1 should yield a negative reaction by each test method described in the manufacturer's package insert.

4. Tests with red blood cells which lack the antigen corresponding to the reagent antibody and tests with adsorbed reagent should be negative, thus confirming the absence of significant contaminating

4. Tests with red blood cells which lack the antigen corresponding to the reagent antibody and tests with adsorbed reagent should be negative, thus confirming the absence of significant contaminating antibodies directed at the antigens listed in III.C.2

5. The manufacturer should list on the lot release protocol and in the "Specific Performance Characteristics" section of the package insert those red blood cell antigens listed in III.C.2 for which no specificity tests have been performed.

   If desired, the red blood cell phenotype of the antibody donor(s) may also be listed as presumptive evidence that antibodies to those factors are not present.

6. Confirmation by the manufacturer of nonspecific reactions after a lot of Blood Grouping Reagent has been released should be reported promptly by the manufacturer to the Director, Center for Biologics Evaluation and Research.

IV. AVIDITY TEST FOR SLIDE REAGENTS

A. REAGENT DILUTIONS

1. Prepare a 1 in 2 dilution of the reagent under test by mixing equal parts of the reagent and AB serum which is free of antibodies or a diluent approved by the Director, Center for Biologics Evaluation and Research.

B. RED BLOOD CELL PREPARATIONS

1. Red blood cells should be prepared according to the manufacturer's package insert.

C. MINIMUM TEST CELLS FOR AVIDITY

REAGENT	RED BLOOD CELLS
Anti-A	A_1, and A_2B *
Anti-B	B and A_1B
Anti-A,B	A_1, A_2 **, B, and A_x ***
Anti-A and B	A_1, A_2 **, B, and A_x ***

* AB cells which do not react with anti-A_1 and do react with anti-H.

** A cells which do not react with anti-A_1 and do react with anti-H.

*** A_x red blood cells are recommended only if the reagent is recommended for detection of weak subgroups of A by slide technique.

D. THE TEST (BY SLIDE METHOD)

1. The test is to be performed with both undiluted reagent and the diluted reagent prepared in step IV.A by the method recommended in the manufacturer's package insert.

E. INTERPRETATION OF THE TEST

1. Test results are observed and recorded at one half of the manufacturer's recommended observation time and at the end of the full recommended observation time.

F. AVIDITY TESTING RESULTS

1. Signs of agglutination should be observed with both the undiluted and diluted reagent at the end of the first half of the observation time.

2. Clear macroscopic agglutination should be observed with both the undiluted and diluted reagent at the end of the observation time and should be reported as greater than or less than 1 mm in diameter.

V. TEST FOR SPONTANEOUS AGGLUTINATION

A. REAGENT DILUTIONS

1. No dilution of the reagent under test is permitted.

B. RED BLOOD CELL PREPARATION

1. Obtain c positive group O red blood cells from one donor.

2. Coat the red blood cells heavily with an IgG anti-c such that a 3-4+ direct antiglobulin test (DAT) is achieved and positive reactions are obtained with a high protein Rh control reagent, but negative reactions are obtained with a saline control.

   The exact procedure for coating the red blood cells will depend on the specific antibody chosen for coating and its strength.

C. THE TEST

1. Test the coated cell sample according to the manufacturer's package insert.

D. INTERPRETATION

1. Blood Grouping Reagents for use by a low protein tube test method should not spontaneously agglutinate immunoglobulin coated red blood cells.

2. In the event that the reagent under test does agglutinate the coated red blood cells, an effective control test or a control reagent adequate to prevent misinterpretation of blood group results should be recommended or supplied.

3. If a control test or reagent is recommended by the manufacturer, cells for use in control testing in sections II, II, and IV should give a negative direct antiglobulin test (DAT).

GENERAL INFORMATION

These recommended methods are provided to help assist manufacturers in pursuing new product license applications and amendments to existing product license applications. The methods described herein do not bind the agency, and manufacturers may consider use of other methods. In cases where manufacturers wish to use methods other than those described herein, FDA recommends that the matter be discussed with FDA in advance. The methods, potency titer values, specificity results, and other matters referred to in this document are intended to assist manufacturers in preparing submissions to FDA. The information is based on current knowledge and is not meant to be all inclusive and should not be viewed as ensuring approval or the only means of achieving FDA approval. Following the methods provided in this document will assist in the approval process, but does not guarantee approval. FDA will review applications on an individual basis and approvals will be granted when supported by scientific evidence.

I. REFERENCE PREPARATIONS

A. The Reference Blood Grouping Reagents listed below can be obtained from:

Center for Biologics Evaluation and Research
Food and Drug Administration
8800 Rockville Pike
Bethesda, MD. 20892
USA

Anti-D for evaluation of IgG products

Anti-C for rapid tube test

Anti-E for rapid tube test

Anti-c for rapid tube test

Anti-e for rapid tube test

NOTE: FDA Reference Blood Grouping Reagents are not routinely available to anyone except U.S. licensed manufacturers and amounts issued will be proportional to lots released in the previous year.

B. All reference sera are to be used according to the accompanying package insert only for determining the potency of Blood Grouping Reagents as part of their final lot release testing.

In-house reference materials should be developed for all stability testing, in process testing or product development purposes.

II. POTENCY TESTING

A. REAGENT DILUTIONS

1. Beginning with the undiluted reagent, prepare separate master two-fold dilutions (1 in 2, 1 in 4, etc.) of the test reagent using 20-22% bovine albumin or another diluent approved by the Director, Center for Biologics Evaluation and Research. Test tubes should be of a size that facilitates adequate mixing of the contents (12 X 75 mm or larger).

   If the endpoint is expected to exceed 1024, accuracy will be improved if direct intermediate dilutions are done to keep the number of serial transfers to less than 10. (e.g., If the expected endpoint is 4096, prepare an initial 1:10 dilution with the same diluent as used above.)

   NOTE: All titrations should be carried to a negative endpoint. (See E.4)

2. Prepare master dilutions of the Reference Blood Grouping Reagent(s) as in paragraph 1 of this section. For test reagents containing multiple antibodies (ex. Anti-CDE) dilutions of each of the corresponding Reference Blood Grouping Reagents should be made separately.

3. A separate, clean pipet or pipet tip should be used for each dilution (including any intermediate dilutions) to avoid carryover of higher reagent concentrations.

4. The last tube should contain diluent only and serve as a diluent control.

B. RED BLOOD CELL PREPARATIONS

Fresh or frozen red blood cells may be used for preparing cell suspensions for the potency testing of all Blood Grouping Reagents under the following conditions:

1. Red blood cells of any age may be used, provided the titer values of the Reference Blood Grouping Reagent(s) are within an acceptable range.

2. Red blood cells may be frozen and thawed for use in the preparation of cell suspensions for reagent evaluation. To ensure that the correct cell has been thawed, appropriate controls should be used to demonstrate the desired reactivity and identity of the thawed red blood cells on the day of use.

   The method of freezing, storing, and thawing red blood cells, including a description of the cryoprotective medium should be described in detail and should be approved by the Director, Center for Biologics Evaluation and Research before use in control testing of licensed reagents.

3. Each batch of red blood cells for use in control testing of reagents requiring indirect antiglobulin technique should be tested for absence of complement or immunoglobulin molecules (DAT negative) on the day of use.

4. Red blood cells should be washed at least twice in isotonic saline or until a clear supernate is obtained and then resuspended to a 2% suspension in isotonic saline containing 1-2% bovine albumin or another diluent approved by the Director, Center for Biologics Evaluation and Research.

C. MINIMUM TEST CELLS FOR POTENCY

REAGENT	RED BLOOD CELLS
Anti-D	Dce (R_0r)
Anti-C	dCce (r'r)
Anti-E	dcEe (r"r)
Anti-c	DCcEe (R_1R_2)
Anti-e	dcEe (r"r)
Anti-CD	dCce and Dce (r'r and R_0r)
Anti-DE	Dce and dcEe (R_0r and r"r)
Anti-CDE	dCce and Dce and dcEe (r'r and R_0r and r"r)

D. THE TEST (BY TUBE METHOD)

1. Place 0.1 milliliter of each reagent dilution in a separate, clean test tube (approximately 10 X 75 mm or 12 X 75 mm).

2. Place 0.1 milliliter of each Reference Blood Grouping Reagent dilution in a separate, clean test tube (approximately 10 X 75 mm or 12 X 75 mm).

3. Add 0.1 milliliter of the appropriate 2% cell suspension to each test tube.

4. Mix the contents of each tube thoroughly and incubate the test tubes for 15 minutes at 37 C.

5. Centrifuge for 2 minutes at approximately 1000 rpm (100-125 rcf) or 45 seconds at approximately 3400 rpm (900-1000 rcf) or at a time and speed appropriate for the centrifuge being used.

E. INTERPRETATION OF THE TEST

1. The cell buttons of each test tube should be gently dislodged and examined macroscopically.

2. The reactions should be graded as follows:

   4+ Cell button remains in one clump.

   3+ Cell button dislodges into several clumps.

   2+ Cell button dislodges into many small clumps of equal size.

   1+ Cell button dislodges into finely granular, but definite, small clumps.

   D Cell button dislodges into fine granules, but not definite small clumps. Results should be recorded as doubtful. For purposes of this paragraph, doubtful reactions are deemed to be negative.

   0 Negative reaction- cell button dislodges into no visible clumps.

3. The potency titer value is the reciprocal of the greatest reagent dilution for which the reaction is graded at 1+.

The dilution caused by the addition of the red blood cells should not be considered as contributing to the dilution of the reagent.

4. Test results should show at least one tube with no agglutination after the endpoint. The diluent control tube should be negative.

F. POTENCY TITER VALUES

1. Slide and Modified Tube Rh Blood Grouping Reagents should have an average potency titer value at least equal to that of the reference reagent.

2. Products recommended for use in automated or microplate systems without user dilution (as supplied) should be sufficiently potent that a two-fold dilution prepared with an approved diluent will produce the same qualitative test result as the undiluted product when tested in accordance with the manufacturer's package insert.

III. SPECIFICITY TESTING

A. REAGENT DILUTIONS

1. No dilution of the reagent under test is permitted.

B. RED BLOOD CELL PREPARATIONS

Fresh or frozen red blood cells may be used for preparing cell suspensions for the specificity testing of all Blood Grouping Reagents under the following conditions:

1. Any cells of any age may be used in the "Test to Confirm Reactivity with Antigen Positive Cells" (III.C.1). In the "Test to Confirm Absence of Contaminating Antibodies" (III.C.2) Licensed reagent red blood cells may be used any time before their expiration date. All other red blood cell samples should be used within 7 days of collection from the donor.

Manufacturers that wish to use cells more than 7 days after collection from the donor are to obtain approval from the Director, Center for Biologics Evaluation and Research, and are to provide sufficient data to support the request.

2. Red blood cells meeting the criteria of paragraph 1 of this section may be frozen and thawed for use in the preparation of cell suspensions for reagent evaluation. To ensure that the correct cell has been thawed, appropriate controls should be used to

demonstrate the desired reactivity and identity of the thawed red blood cells on the day of use. In the case of cells expressing low frequency antigens, testing for several common antigens may serve to adequately identify the cell.

The method of freezing, storing, and thawing red blood cells, including a description of the cryoprotective medium should be described in detail and should be approved by the Director, Center for Biologics Evaluation and Research before use in control testing of licensed reagents.

3. Each batch of red blood cells for use in control testing of reagents requiring indirect antiglobulin technique should be tested for absence of complement or immunoglobulin molecules (DAT negative) on the day of use.

4. Licensed reagent red blood cells may be used as provided.

   All other red blood cell samples should be washed at least twice in isotonic saline or until a clear supernatant is obtained and then resuspended with an approved diluent to the cell concentration listed in the manufacturer's package insert.

C. MINIMUM TESTING FOR SPECIFICITY

1. TEST TO CONFIRM REACTIVITY WITH ANTIGEN POSITIVE CELLS

   a. At least 4 different donors whose red blood cells exhibit weak or heterozygous expression of the antigen should be tested.

   b. When testing reagents containing multiple antibodies, the reactivity of each specificity should be confirmed separately by using 4 different red blood cells possessing only one of the antigens for each different specificity.

      ex. For Anti-CDE reagents, at least four donors should be used to confirm the reactivity of the Anti-C component, at least four donors should be used to confirm the reactivity of the Anti-D component, and at least four donors should be used to confirm the reactivity of the Anti-E component.

c. Include at least one red blood cell which does not exhibit the expression of the antigen as a negative control.

2. TEST TO CONFIRM ABSENCE OF CONTAMINATING ANTIBODIES

Test the reagent for the presence of antibodies corresponding to the following antigens by one of the methods listed below.

A, B, H, Le^a, Le^b, I, K, k, Kp^a, Kp^b, Js^b, P_1, D, C, E, c, e, C^w, M, N, S, s, U, Lu^a, Lu^b, Jk^a, Jk^b, Fy^a, Fy^b, Xg^a, Do^a, Do^b, Yt^a, Yt^b, Lan, Co^a, Co^b, M^g, Wr^a, and Sd^a.

If the source material is a monoclonal antibody from a previously characterized and licensed clone, this list may be shortened as follows:

A, B, H, Le^a, Le^b, I, K, k, Kp^b, Js^b, P_1, D, C, E, c, e, M, N, S, s, U, Lu^b, Jk^a, Jk^b, Fy^a, and Fy^b.

Approval for the use of fewer antigens than included on this list may be requested from the Director, Center for Biologics Evaluation and Research by a manufacturer at the time of submission of the first protocol.

a. Perform a direct test for the presence of contaminating antibodies by using red blood cells from at least 4 different donors whose cells lack the antigen corresponding to the reagent antibody.

b. When red blood cells lacking the antigen corresponding to the reagent antibody under test are not available, the reagent antibody may be adsorbed to exhaustion with cells of a known phenotype.

The adsorbed serum may then be tested against red blood cells which exhibit any antigens which were not present on the cells used for adsorption. The methods for adsorption and subsequent testing should be approved by the Director, Center for Biologics Evaluation and Research.

c. When direct tests are impractical, the Director, Center for Biologics Evaluation and Research may approve procedures whereby antibodies may be presumptively excluded by testing an appropriate number of non-reactive red blood cell samples to provide statistical

assurance of the absence of contaminating antibodies.

d. Red blood cell samples from four different donors may be used to confirm presumptively the absence of contaminating antibodies to antigens having an incidence of greater than 99% in the general population of the United States.

3. TEST TO CONFIRM ABSENCE OF ANTI-A AND ANTI-B

a. Group A_1 and B red blood cells lacking the antigen corresponding to the reagent antibody should be tested. Group A_1B red blood cells may be substituted for A_1 and/or B red blood cells if either are unavailable.

Adsorbed serum may be used as in III.C.2.b above.

4. PHENOTYPES RECOMMENDED FOR TESTING

As a minimum, red blood cells exhibiting the following phenotypes should be used in the specificity testing outlined in steps 1, 2, and 3 above.

MINIMUM	RED BLOOD CELLS
Anti-D	DCce, Dce, dCce, and dcEe (R_1r, Ror, r'r, and r"r) A_1 dce, B dce, and O dce (rr) Vw positive
	3 different dce (rr) Bg(a+) *
	6 D^u samples representing different Rh phenotypes and reactive by the Indirect Antiglobulin Test only *
Anti-D (monoclonal)	Category IV, V, and VI cells
Anti-C	Dce, dCce, and dcEe or dcE (R_0r, r'r, and r"r or r"r") C+ Ce- (e.g. R_2R_2 or R_2r) ** C^w positive (e.g. R_1^wr) A_1 dce, B dce, and O dce (rr)
Anti-E	Dce, dCce or dCe, and dcEe (R_0r, r'r or r'r', and r"r) E+ cE- (e.g. R_2R_1 or R_2r) A_1 dce, B dce, and O dce (rr)

Anti-c	dCce and DCEe or DCE or dCE (r'r and R_zR_1 or R_zR_z or r^yr^y) A_1 DCe, B DCe, and O DCe (R_1R_1) DCcEe f neg (R_1R_z)
Anti-e	dcEe and DCcE or DCE or dCE (r"r and R_zR_z or R_zR_z or r^yr^y) A_1 DcE, B DcE, and O DcE (R_zR_z) DCcEe f neg (R_1R_z)
Anti-CD	Dce, dCce, and dcE or dcEe (R_or, r'r, and r"r" or r"r) A_1 dce, B dce, and O dce (rr) r^Gr or $r"^Gr$ ***
Anti-DE	Dce, dCce or dCe, and dcEe (R_or, r'r or r'r',and r"r) A_1 dce, B dce, and O dce (rr)
Anti-CDE	Dce, dCce, and dcEe (R_or, r'r, and r"r) A_1 dce, B dce, and O dce (rr) r^Gr ***

* For Anti-D reagents recommended for D^u testing

** r'^{s}r cells may be used in addition to but not as a substitute for C+ Ce- cells

*** Recommended if labeling indicates detection of G antigen

D. THE TESTS

1. To confirm reactivity with antigen positive cells, each lot of Blood Grouping Reagent should be tested and results interpreted by all test methods described in the manufacturer's package insert. Minimum parameters (drops of reagent, incubation time, centrifugation, etc.) should be followed.

2. To confirm absence of contaminating antibodies, each lot of Blood Grouping Reagent should be tested and results interpreted by the most sensitive test method(s) described in the manufacturer's package insert. Maximum parameters (drops of reagent, incubation time, centrifugation, etc.) should be followed.

E. SPECIFICITY RESULTS

1. No hemolysis or rouleaux formation should be detected by any of the methods in the manufacturer's package insert.

2. Red blood cells which exhibit the antigen corresponding to the reagent antibody should yield at least a 2+ reaction. If any of the four samples tested yields less than a 2+ reaction, red blood cells from four additional donors who exhibit the antigen should be tested. The test is considered satisfactory if no more than one of eight red blood cell samples yields less than a 2+ reaction with the test reagent.

   When testing unusual phenotypes, other criteria for reactivity may apply. For example, a larger percentage of C+ Ce- red blood cells may not yield a 2+ reaction with Anti-C but should yield a clearly positive macroscopic result.

3. The negative control cell(s) in step III.C.1 should yield a negative reaction by each test method described in the manufacturer's package insert.

4. Tests with red blood cells which lack the antigen corresponding to the reagent antibody and tests with adsorbed reagent should be negative, thus confirming the absence of significant contaminating antibodies directed at the antigens listed in III.C.2

5. The manufacturer should list on the lot release protocol and in the "Specific Performance Characteristics" section of the package insert those red blood cell antigens listed in III.C.2 for which no specificity tests have been performed.

   If desired, the red blood cell phenotype of the antibody donor(s) may also be listed as presumptive evidence that antibodies to those factors are not present.

6. Tests with group A_1 and B red blood cells should be negative, thus confirming the absence of anti-A and anti-B.

7. Confirmation by the manufacturer of nonspecific reactions after a lot of Blood Grouping Reagent has been released should be reported promptly by the manufacturer to the Director, Center for Biologics Evaluation and Research.

IV. AVIDITY TEST FOR SLIDE REAGENTS

A. REAGENT DILUTIONS

1. Prepare a 1 in 2 dilution of the reagent under test by mixing equal parts of the reagent and AB serum, group compatible serum, or a diluent approved by the Director, Center for Biologics Evaluation and Research.

B. RED BLOOD CELL PREPARATIONS

1. Red blood cells should be prepared according to the manufacturer's package insert.

C. MINIMUM TEST CELLS FOR AVIDITY

REAGENT	RED BLOOD CELLS
Anti-D	DCe (R_1r)
Anti-C	dCce (r'r) C+ Ce- (R_2R_2 or R_2r) C^w positive (R_1^wr)
Anti-E	dcEe (r"r) E+ cE- (R_2R_1 or R_2r)
Anti-c	dCce (r'r)
Anti-e	dcEe (r"r)
Anti-CD	Dce and dCce (R_0r and r'r) r^Gr or r''^Gr *
Anti-DE	Dce and dcEe (R_0r and r"r)
Anti-CDE	Dce, dCce, and dcEe (R_0r, r'r, and r"r) r^Gr *

* Only if the reagent is recommended for detection of the G antigen by slide technique.

D. THE TEST (BY SLIDE METHOD)

1. The test is to be performed with both undiluted reagent and the diluted reagent prepared in step IV,A by the method recommended in the manufacturer's package insert.

E. INTERPRETATION OF THE TEST

1. Test results are observed and recorded at one half of the manufacturer's recommended observation time and at the end of the full recommended observation time.

F. AVIDITY TESTING RESULTS

1. Signs of agglutination should be observed with both the undiluted and diluted reagent at one half of the recommended observation time.

2. Clear macroscopic agglutination should be observed with both the undiluted and diluted reagent at the end of the recommended observation time and should be reported as greater than or less than 1 mm.

V. TEST FOR PROZONE

A. REAGENT DILUTIONS

1. No dilution of the reagent under test is permitted.

B. RED BLOOD CELL PREPARATIONS

1. Obtain at least three red blood cell samples representing three different Rh phenotypes which exhibit heterozygous or weak expression of the antigen corresponding with the reagent antibody.

2. Fresh or frozen red blood cells may be used under the following conditions:

   a. Licensed reagent red blood cells may be used any time before their expiration date.

   b. Frozen red blood cells should have been frozen within 7 days of collection from the donor and should be used on the day of thawing.

   c. All other red blood cell samples should be used within 7 days of collection from the donor.

3. Red blood cells should not be coated with complement or immunoglobulin (should be direct antiglobulin test negative).

4. Licensed reagent red blood cells may be used as provided.

   All other red blood cell samples should be washed at least twice in isotonic saline or until a clear

supernatant is obtained and then resuspended with an approved diluent to the cell concentration listed in the manufacturer's package insert.

C. CELLS SUGGESTED FOR USE IN THE TEST FOR PROZONE

REAGENT	RED BLOOD CELLS
Anti-D	DCce (R_1r)
Anti-C	DCcEe (R_1R_2)
Anti-E	DCcEe (R_1R_2)
Anti-c	DCce and DCcEe (R_1r and R_1R_2)
Anti-e	DcEe and DCcEe (R_2r and R_1R_2)

D. THE TEST

1. For each cell sample to be tested label three tubes, "15 minutes", "30 minutes", and "60 minutes" respectively.

2. Add the appropriate amount of the reagent under test to all tubes.

   If the manufacturer's package insert recommends the use of 1 drop of reagent, use 1 drop for this test.

   If the manufacturer's package insert recommends the use of 2 drops of reagent or 1 or 2 drops of reagent, use 2 drops for this test.

3. Add 1 drop of each cell sample to its respective tubes.

4. Mix and incubate for the time indicated on the tube label according to the manufacturer's package insert, i.e. at the temperature recommended for those tests giving a negative result.

5. Centrifuge according to the package insert and examine for agglutination. Grade the reactions as in II.E.

E. INTERPRETATION OF THE TEST

1. If the reaction grades are the same or increase as the incubation time increases, no prozone is present.

2. If the reaction grades decrease as the incubation time increases, a prozone is present.

F. RESULTS

1. At least a 2+ reaction should be obtained with ALL samples at ALL incubation times.

VI. TEST TO DETECT PROZONES - METHOD 2

A. REAGENT DILUTIONS

1. Prepare a 1+5 dilution of the reagent under test in inert human serum (group AB or compatible with the cells to be tested).

B. RED BLOOD CELL PREPARATIONS

1. See V.B.

C. CELLS SUGGESTED FOR USE IN THE TEST FOR PROZONE

1. See V.C.

D. THE TEST

The 1+5 dilution and undiluted reagent will be tested in parallel.

1. For each cell to be tested, label two sets of tubes, "I.S.", "1 minute", "3 minutes", "5 minutes", and "10 minutes".

2. Add the appropriate amount of the reagent under test to all tubes.

   If the manufacturer's package insert recommends the use of 1 drop of reagent, use 1 drop for this test.

   If the manufacturer's package insert recommends the use of 2 drops of reagent or 1 or 2 drops of reagent, use 2 drops for this test.

3. Add 1 drop of each cell sample to its respective tubes.

4. Mix and incubate at room temperature for the time indicated on the tube label.

5. Centrifuge according to the package insert and examine for agglutination. Grade the reactions as in II.E.

E. INTERPRETATION OF THE TEST

1. If the reaction grades with the diluted reagent are stronger than the reaction grades with the undiluted reagent, a prozone is present.

2. If the reaction grades with the diluted reagent are equal to or weaker than the reaction grades with the undiluted reagent, no prozone is present.

F. RESULTS

1. The reagent should not exhibit any prozone.

LOW PROTEIN RH BLOOD GROUPING REAGENTS

GENERAL INFORMATION

These recommended methods are provided to help assist manufacturers in pursuing new product license applications and amendments to existing product license applications. The methods described herein do not bind the agency, and manufacturers may consider use of other methods. In cases where manufacturers wish to use methods other than those described herein, FDA recommends that the matter be discussed with FDA in advance. The methods, potency titer values, specificity results, and other matters referred to in this document are intended to assist manufacturers in preparing submissions to FDA. The information is based on current knowledge and is not meant to be all inclusive and should not be viewed as ensuring approval or the only means of achieving FDA approval. Following the methods provided in this document will assist in the approval process, but does not guarantee approval. FDA will review applications on an individual basis and approvals will be granted when supported by scientific evidence.

I. REFERENCE PREPARATIONS

A. The Reference Blood Grouping Reagents listed below can be obtained from:

Center for Biologics Evaluation and Research
Food and Drug Administration
8800 Rockville Pike
Bethesda, MD. 20892
USA

Anti-CD for evaluation of IgM, Anti-D products

Anti-C for saline tube test

Anti-E for saline tube test

NOTE: FDA Reference Blood Grouping Reagents are not routinely available to anyone except U.S. licensed manufacturers and amounts issued will be proportional to lots released in the previous year.

For Blood Grouping Reagents for which there is no Reference Blood Grouping Reagent, it is strongly recommended that a previously approved lot of reagent be used as an in-house control reagent.

B. All reference sera are to be used according to the accompanying package insert only for determining the potency of Blood Grouping Reagents as part of their final lot release testing.

In-house reference materials should be developed for all stability testing, in process testing or product development purposes.

II. POTENCY TESTING

A. REAGENT DILUTIONS

1. Beginning with the undiluted reagent, prepare separate master two-fold serial dilutions (1 in 2, 1 in 4, etc.) of the test reagent using isotonic saline containing 1-2% bovine albumin or another diluent approved by the Director, Center for Biologics Evaluation and Research. Test tubes should be of a size that facilitates adequate mixing of the contents (12 X 75 mm or larger).

If the endpoint is expected to exceed 1024, accuracy will be improved if direct intermediate dilutions are done to keep the number of serial transfers to less than 10. (e.g., If the expected endpoint is 4096, prepare an initial 1:10 dilution with the same diluent as used above.)

NOTE: All titrations should be carried to a negative endpoint. (See E.4)

2. Prepare master dilutions of the Reference Blood Grouping Reagent(s) or in-house control reagent as in paragraph 1 of this section. For test reagents containing multiple antibodies (ex. Anti-CDE), dilutions of each of the corresponding Reference Blood Grouping Reagents should be made separately.

3. A separate, clean pipet or pipet tip should be used for each dilution (including any intermediate dilutions) to avoid carryover of higher reagent concentrations.

4. The last tube should contain diluent only and serve as a diluent control.

B. RED BLOOD CELL PREPARATIONS

Fresh or frozen red blood cells may be used for preparing cell suspensions for the potency testing of all Blood Grouping Reagents under the following conditions:

1. Red blood cells of any age may be used, provided the titer values of the Reference Blood Grouping Reagent(s) or the in-house control reagent are within an acceptable range.

2. Red blood cells may be frozen and thawed for use in the preparation of cell suspensions for reagent evaluation. To ensure that the correct cell has been thawed, appropriate controls should be used to demonstrate the desired reactivity and identity of the thawed red blood cells on the day of use.

   The method of freezing, storing, and thawing red blood cells, including a description of the cryoprotective medium should be described in detail and should be approved by the Director, Center for Biologics Evaluation and Research as a license amendment before use in control testing.

3. Each batch of red blood cells for use in control testing of reagents requiring indirect antiglobulin technique should be tested for absence of complement or immunoglobulin molecules (DAT negative) on the day of use.

4. Red blood cells should be washed at least twice in isotonic saline or until a clear supernate is obtained and then resuspended to a 2% suspension in isotonic saline containing 1-2% bovine albumin or another diluent approved by the Director, Center for Biologics Evaluation and Research.

C. MINIMUM TEST CELLS FOR POTENCY

REAGENT	RED BLOOD CELLS
Anti-D	Dce (R_0r)
Anti-C	dCce (r'r)
Anti-E	dcEe (r"r)
Anti-c	DCcEe (R_1R_2)
Anti-e	dcEe (r"r)
Anti-CD	dCce and Dce (r'r and R_0r)
Anti-DE	Dce and dcEe (R_0r and r"r)
Anti-CDE	dCce and Dce and dcEe (r'r and R_0r and r"r)

D. THE TEST (BY TUBE METHOD)

1. Place 0.1 milliliter of each reagent dilution in a separate, clean test tube (approximately 10 X 75 mm or 12 X 75 mm).

2. If a Reference Blood Grouping Reagent is available, place 0.1 milliliter of each Reference Blood Grouping Reagent dilution in a separate, clean test tube (approximately 10 X 75 mm or 12 X 75 mm).

3. Add 0.1 milliliter of the appropriate 2% cell suspension to each test tube.

4. Mix the contents of each tube thoroughly and incubate the test tubes at 37 C for 15 minutes.

   If no Reference Blood Grouping Reagent is available, incubate at 37 C for the shortest period of time recommended in the manufacturer's package insert.

5. Centrifuge for 1 minute at approximately 1000 rpm (100-125 rcf) or 15 seconds at approximately 3400

rpm (900-1000 rcf) or at a time and speed appropriate for the centrifuge being used.

In the case of reagents for which no Reference Blood Grouping Reagent is available, centrifuge for the shortest period of time at the lowest speed recommended in the manufacturer's package insert.

E. INTERPRETATION OF THE TEST

1. The cell buttons of each test tube should be gently dislodged and examined macroscopically.

2. The reactions should be graded as follows:

4+ Cell button remains in one clump.

3+ Cell button dislodges into several clumps.

2+ Cell button dislodges into many small clumps of equal size.

1+ Cell button dislodges into finely granular, but definite, small clumps.

D Cell button dislodges into fine granules, but not definite small clumps. Results should be recorded as doubtful. For purposes of this paragraph, doubtful reactions are deemed to be negative.

0 Negative reaction- cell button dislodges into no visible clumps.

3. The potency titer value is the reciprocal of the greatest reagent dilution for which the reaction is graded at 1+.

The dilution caused by the addition of the red blood cells should not be considered as contributing to the dilution of the reagent.

4. Test results should show at least one tube with no agglutination after the endpoint. The diluent control tube should be negative.

F. POTENCY TITER VALUES

1. Products for which Reference Blood Grouping Reagents are available should have an average potency titer value at least equal to that of the reference reagent.

2. Products of polyclonal origin which are recommended for tube test methods for which there are no Reference Blood Grouping Reagents available should have a potency titer value of at least a 1+ reaction with a 1:4 dilution of reagent:

   eg. Anti-c (saline)
   Anti-e (saline)

3. Products of monoclonal origin which are recommended for tube test methods for which there are no Reference Blood Grouping Reagents available should have a potency titer value of at least a 1+ reaction with a 1:8 dilution of reagent:

   eg. Anti-c (saline)
   Anti-e (saline)

   Manufacturers that wish to establish potency titer values other than these are to obtain approval from the Director, Center for Biologics Evaluation and Research at the time of license application.

4. Products recommended for use in automated or microplate systems without user dilution (as supplied) should be sufficiently potent that a two-fold dilution prepared with an approved diluent will produce the same qualitative test result as the undiluted product when tested in accordance with the manufacturer's package insert.

III. SPECIFICITY TESTING

A. REAGENT DILUTIONS

1. No dilution of the reagent under test is permitted.

B. RED BLOOD CELL PREPARATIONS

Fresh or frozen red blood cells may be used for preparing cell suspensions for the specificity testing of all Blood Grouping Reagents under the following conditions:

1. Any cells of any age may be used in the "Test to Confirm Reactivity with Antigen Positive Cells" (III.C.1). In the "Test to Confirm Absence of Contaminating Antibodies" (III.C.2) licensed reagent red blood cells may be used any time before their expiration date. All other red blood cell samples should be used within 7 days of collection from the donor.

Manufacturers that wish to use cells more than 7 days after collection from the donor are to obtain approval from the Director, Center for Biologics Evaluation and Research, and are to provide sufficient data to support the request.

2. Red blood cells meeting the criteria of paragraph 1 of this section may be frozen and thawed for use in the preparation of cell suspensions for reagent evaluation. To ensure that the correct cell has been thawed, appropriate controls should be used to demonstrate the desired reactivity and identity of the thawed red blood cells on the day of use. In the case of cells expressing low frequency antigens, testing for several common antigens may serve to adequately identify the cell.

   The method of freezing, storing, and thawing red blood cells, including a description of the cryoprotective medium should be described in detail and should be approved by the Director, Center for Biologics Evaluation and Research before use in control testing of licensed reagents.

3. Each batch of red blood cells for use in control testing of reagents requiring indirect antiglobulin technique should be tested for absence of complement or immunoglobulin molecules (DAT negative) on the day of use.

4. Licensed reagent red blood cells may be used as provided.

   All other red blood cell samples should be washed at least twice in isotonic saline or until a clear supernatant is obtained and then resuspended with an approved diluent to the cell concentration listed in the manufacturer's package insert.

C. MINIMUM TESTING FOR SPECIFICITY

1. TEST TO CONFIRM REACTIVITY WITH ANTIGEN POSITIVE CELLS

   a. At least 4 different donors whose red blood cells exhibit weak or heterozygous expression of the antigen should be tested.

b. When testing reagents containing multiple antibodies, the reactivity of each specificity should be confirmed separately by using 4 different red blood cells possessing only one of the antigens for each different specificity.

ex. For Anti-CDE reagents, at least four donors should be used to confirm the reactivity of the Anti-C component, at least four donors should be used to confirm the reactivity of the Anti-D component, and at least four donors should be used to confirm the reactivity of the Anti-E component.

c. Include at least one red blood cell which does not exhibit the expression of the antigen as a negative control.

2. TEST TO CONFIRM ABSENCE OF CONTAMINATING ANTIBODIES

Test the reagent for the presence of antibodies corresponding to the following antigens by one of the methods listed below.

A, B, H, Le^a, Le^b, I, K, k, Kp^a, Kp^b, Js^b, P_1, D, C, E, c, e, C^w, M, N, S, s, U, Lu^a, Lu^b, Jk^a, Jk^b, Fy^a, Fy^b, Xg^a, Do^a, Do^b, Yt^a, Yt^b, Lan, Co^a, Co^b, M^g, Wr^a, and Sd^a.

If the source material is a monoclonal antibody from a previously characterized and licensed clone, this list may be shortened as follows:

A, B, H, Le^a, Le^b, I, K, k, Kp^b, Js^b, P_1, D, C, E, c, e, M, N, S, s, U, Lu^b, Jk^a, Jk^b, Fy^a, and Fy^b.

Approval for the use of fewer antigens than included on this list may be requested from the Director, Center for Biologics Evaluation and Research by a manufacturer at the time of submission of the first protocol.

a. Perform a direct test for the presence of contaminating antibodies by using red blood cells from at least 4 different donors whose cells lack the antigen corresponding to the reagent antibody.

b. When red blood cells lacking the antigen corresponding to the reagent antibody under test are not available, the reagent antibody may be adsorbed to exhaustion with cells of a known phenotype.

The adsorbed serum may then be tested against red blood cells which exhibit any antigens which were not present on the cells used for adsorption. The methods for adsorption and subsequent testing should be approved by the Director, Center for Biologics Evaluation and Research.

c. When direct tests are impractical, the Director, Center for Biologics Evaluation and Research may approve procedures whereby antibodies may be presumptively excluded by testing an appropriate number of non-reactive red blood cell samples to provide statistical assurance of the absence of contaminating antibodies.

d. Red blood cell samples from four different donors may be used to confirm presumptively the absence of contaminating antibodies to antigens having an incidence of greater than 99% in the general population of the United States.

3. TEST TO CONFIRM ABSENCE OF ANTI-A AND ANTI-B

a. Group A_1 and B red blood cells lacking the antigen corresponding to the reagent antibody should be tested. Group A_1B red blood cells may be substituted for A_1 and/or B red blood cells if either are unavailable.

Adsorbed serum may be used as in III.C.2.b above.

4. PHENOTYPES RECOMMENDED FOR TESTING

As a minimum, red blood cells exhibiting the following phenotypes should be used in the specificity testing outlined in steps 1, 2, and 3 above.

REAGENT	RED BLOOD CELLS
Anti-D	DCce, Dce, dCce, and dcEe (R_1r, Ror, r'r, and r"r) A_1 dce, B dce, and O dce (rr) Vw positive
	3 different dce (rr) Bg(a+) cells *
	6 D^u samples representing different Rh phenotypes and reactive by Indirect Antiglobulin Test only *
Anti-D (monoclonal)	Category IV, V, and VI cells
Anti-C	Dce, dCce, and dcEe or dcE (R_0r, r'r, and r"r or r"r") C+ Ce- (ex. R_zR_2 or R_zr) ** C^w positive (ex. R_1^wr) A_1 dce, B dce, and O dce (rr)
Anti-E	Dce, dCce or dCe, and dcEe (R_0r, r'r or r'r', and r"r) E+ cE- (ex.R_zR_1 or R_zr) A_1 dce, B dce, and O dce (rr)
Anti-c	dCce and DCEe or DCE or dCE (r'r and R_zR_1 or R_zR_z or r^yr^y) A_1 DCe, B DCe, and O DCe (R_1R_1) DCcEe f neg (R_1R_2)
Anti-e	dcEe and DCcE or DCE or dCE (r"r and R_zR_2 or R_zR_z or r^yr^y) A_1 DcE, B DcE, and O DcE (R_2R_2) DCcEe f neg (R_1R_2)
Anti-CD	Dce, dCce, and dcE or dcEe (R_0r, r'r, and r"r" or r"r) A_1 dce, B dce, and O dce (rr) r^Gr or $r"^Gr$ ***
Anti-DE	Dce, dCce or dCe, and dcEe (R_0r, r'r or r'r',and r"r) A_1 dce, B dce, and O dce (rr)
Anti-CDE	Dce, dCce, and dcEe (R_0r, r'r, and r"r) A_1 dce, B dce, and O dce (rr) r^Gr ***

* For Anti-D reagents recommended for D^u testing.

** r'^{s}r cells may be used in addition to but not as a substitute for C+ Ce- cells

*** Recommended if labeling indicates detection of G antigen

D. THE TESTS

1. To confirm reactivity with antigen positive cells, each lot of Blood Grouping Reagent should be tested and results interpreted by all test methods described in the manufacturer's package insert. Minimum parameters (drops of reagent, incubation time, centrifugation, etc.) should be followed.

2. To confirm absence of contaminating antibodies, each lot of Blood Grouping Reagent should be tested and results interpreted by the most sensitive test method(s) described in the manufacturer's package insert. Maximum parameters (drops of reagent, incubation time, centrifugation, etc.) should be followed.

E. SPECIFICITY RESULTS

1. No hemolysis or rouleaux formation should be detected by any of the methods in the manufacturer's package insert.

2. Red blood cells which exhibit the antigen corresponding to the reagent antibody should yield at least a 2+ reaction. If any of the four samples tested yields less than a 2+ reaction, red blood cells from four additional donors who exhibit the antigen should be tested. The test is considered satisfactory if no more than one of eight red blood cell samples yields less than a 2+ reaction with the test reagent.

   When testing unusual phenotypes, other criteria for reactivity may apply. For example, a larger percentage of C+ Ce- red blood cells may not yield a 2+ reaction with Anti-C but should yield a clearly positive macroscopic result.

3. The negative control cell(s) in step III.C.1 should yield a negative reaction by each test method described in the manufacturer's package insert.

4. Tests with red blood cells which lack the antigen corresponding to the reagent antibody and tests with adsorbed reagent should be negative, thus confirming the absence of significant contaminating antibodies directed at the antigens listed in III.C.2

5. The manufacturer should list on the lot release protocol and in the "Specific Performance Characteristics" section of the package insert those red blood cell antigens listed in III.C.2 for which no specificity tests have been performed.

   If desired, the red blood cell phenotype of the antibody donor(s) may also be listed as presumptive evidence that antibodies to those factors are not present.

6. Tests with group A_1 and B red blood cells should be negative, thus confirming the absence of anti-A and anti-B.

7. Confirmation by the manufacturer of nonspecific reactions after a lot of Blood Grouping Reagent has been released should be reported promptly by the manufacturer to the Director, Center for Biologics Evaluation and Research.

IV. AVIDITY TEST FOR SLIDE REAGENTS

A. REAGENT DILUTIONS

1. Prepare a 1 in 2 dilution of the reagent under test by mixing equal parts of the reagent and AB serum, group compatible serum, or a diluent approved by the Director, Center for Biologics Evaluation and Research.

B. RED BLOOD CELL PREPARATIONS

1. Red blood cells should be prepared according to the manufacturer's package insert.

C. MINIMUM TEST CELLS FOR AVIDITY

REAGENT	RED BLOOD CELLS
Anti-D	DCe (R_1r)
Anti-C	dCce (r'r) C+ Ce- (R_2R_2 or R_r) C^w positive (R_1^wr)
Anti-E	dcEe (r"r) E+ cE- (R_2R_2 or R_zr)
Anti-c	dCce (r'r)
Anti-e	dcEe (r"r)
Anti-CD	Dce and dCce (R_or and r'r) r^Gr or $r"^Gr$ *
Anti-DE	Dce and dcEe (R_or and r"r)
Anti-CDE	Dce, dCce, and dcEe (R_or, r'r, and r"r) r^Gr *

* Only if the reagent is recommended for detection of the G antigen by slide technique.

D. THE TEST (BY SLIDE METHOD)

1. The test is to be performed with both undiluted reagent and the diluted reagent prepared in step IV,A by the method recommended in the manufacturer's package insert.

E. INTERPRETATION OF THE TEST

1. Test results are observed and recorded at one half of the manufacturer's recommended observation time and at the end of the full recommended observation time.

F. AVIDITY TESTING RESULTS

1. Signs of agglutination should be observed with both the undiluted and diluted reagent at one half of the manufacturer's recommended observation time.

2. Clear macroscopic agglutination should be observed with both the undiluted and diluted reagent at the

end of the manufacturer's recommended observation time and should be reported as greater than or less than 1 mm.

V. TEST FOR SPONTANEOUS AGGLUTINATION

A. REAGENT DILUTIONS

1. No dilution of the reagent under test is permitted.

B. RED BLOOD CELL PREPARATION

1. Obtain c positive group O red blood cells from one donor. (When testing an Anti-c reagent, use Rh(D) positive group O cells.)

2. Coat the red blood cells heavily with an IgG anti-c (anti-D when testing an Anti-c reagent) such that a 3-4+ direct antiglobulin test (DAT) is achieved and positive reactions are obtained with a high protein Rh control reagent, but negative reactions are obtained with a saline control.

   The exact procedure for coating the red blood cells will depend on the specific antibody chosen for coating and its strength.

C. THE TEST

1. Test the coated cell sample according to the manufacturer's package insert.

D. INTERPRETATION

1. Blood Grouping Reagents for use by the saline tube test method should not spontaneously agglutinate immunoglobulin coated red blood cells.

2. In the event that the reagent under test does agglutinate the coated red blood cells, an effective control test or a control reagent adequate to prevent misinterpretation of blood group results should be recommended or supplied.

VI. TEST FOR PROZONE

A. REAGENT DILUTIONS

1. No dilution of the reagent under test is permitted.

B. RED BLOOD CELL PREPARATIONS

1. Obtain at least three red blood cell samples representing three different Rh phenotypes which exhibit heterozygous or weak expression of the antigen corresponding with the reagent antibody.

2. Fresh or frozen red blood cells may be used under the following conditions:

   a. Licensed reagent red blood cells may be used any time before their expiration date.

   b. Frozen red blood cells should have been frozen within 7 days of collection from the donor and should be used on the day of thawing.

   c. All other red blood cell samples should be used within 7 days of collection from the donor.

3. Red blood cells should not be coated with complement or immunoglobulin (should be direct antiglobulin test negative).

4. Licensed reagent red blood cells may be used as provided.

   All other red blood cell samples should be washed at least twice in isotonic saline or until a clear supernatant is obtained and then resuspended with an approved diluent to the cell concentration listed in the manufacturer's package insert.

C. CELLS SUGGESTED FOR USE IN THE TEST FOR PROZONE

REAGENT	RED BLOOD CELLS
Anti-D	DCce (R_1r)
Anti-C	DCcEe (R_1R_2)
Anti-E	DCcEe (R_1R_2)
Anti-c	DCce and DCcEe (R_1r and R_1R_2)
Anti-e	DcEe and DCcEe (R_2r and R_1R_2)

D. THE TEST

1. For each cell sample to be tested label three tubes, "15 minutes", "30 minutes", and "60 minutes" respectively.

2. Add the appropriate amount of the reagent under test to all tubes.

   If the manufacturer's package insert recommends the use of 1 drop of reagent, use 1 drop for this test.

   If the manufacturer's package insert recommends the use of 2 drops of reagent or 1 or 2 drops of reagent, use 2 drops for this test.

3. Add 1 drop of each cell sample to its respective tubes.

4. Mix and incubate for the time indicated on the tube label according to the manufacturer's package insert, i.e. at the temperature recommended for those tests giving a negative result.

5. Centrifuge according to the package insert and examine for agglutination. Grade the reactions as in II.E.

E. INTERPRETATION OF THE TEST

1. If the reaction grades are the same or increase as the incubation time increases, no prozone is present.

2. If the reaction grades decrease as the incubation time increases, a prozone is present.

F. RESULTS

1. At least a 2+ reaction should be obtained with ALL samples at ALL incubation times.

VII. TEST TO DETECT PROZONES - METHOD 2

A. REAGENT DILUTIONS

1. Prepare a 1+5 dilution of the reagent under test in inert human serum (group AB or compatible with the cells to be tested.)

B. RED BLOOD CELL PREPARATIONS

1. See VI.B.

C. CELLS SUGGESTED FOR USE IN THE TEST FOR PROZONE

1. See VI.C.

D. THE TEST

The 1+5 dilution and undiluted reagent will be tested in parallel.

1. For each cell to be tested, label two sets of tubes, "I.S.", "1 minute", "3 minutes", "5 minutes", and "10 minutes".

2. Add the appropriate amount of the reagent under test to all tubes.

   If the manufacturer's package insert recommends the use of 1 drop of reagent, use 1 drop for this test.

   If the manufacturer's package insert recommends the use of 2 drops of reagent or 1 or 2 drops of reagent, use 2 drops for this test.

3. Add 1 drop of each cell sample to its respective tubes.

4. Mix and incubate at room temperature for the time indicated on the tube label.

5. Centrifuge according to the package insert and examine for agglutination. Grade the reactions as in II.E.

E. INTERPRETATION OF THE TEST

1. If the reaction grades with the diluted reagent are stronger than the reaction grades with the undiluted reagent, a prozone is present.

2. If the reaction grades with the diluted reagent are equal to or weaker than the reaction grades with the undiluted reagent, no prozone is present.

F. RESULTS

1. The reagent should not exhibit any prozone.

RARE BLOOD GROUPING REAGENTS

GENERAL INFORMATION

These recommended methods are provided to help assist manufacturers in pursuing new product license applications and amendments to existing product license applications. The methods described herein do not bind the agency, and manufacturers may consider use of other methods. In cases where manufacturers wish to use methods other than those described herein, FDA recommends that the matter be discussed with FDA in advance. The methods, potency titer values, specificity results, and other matters referred to in this document are intended to assist manufacturers in preparing submissions to FDA. The information is based on current knowledge and is not meant to be all inclusive and should not be viewed as ensuring approval or the only means of achieving FDA approval. Following the methods provided in this document will assist in the approval process, but does not guarantee approval. FDA will review applications on an individual basis and approvals will be granted when supported by scientific evidence.

I. REFERENCE PREPARATIONS

A. There are no Reference Blood Grouping Reagents available for the reagents covered in this document. It is strongly recommended that a previously approved lot of reagent be used as an in-house control reagent.

II. POTENCY TESTING

A. REAGENT DILUTIONS

1. Beginning with the undiluted reagent, prepare separate master two-fold serial dilutions (1 in 2, 1 in 4, etc.) of the test reagent using isotonic saline containing 1-2% bovine albumin or another diluent approved by the Director, Center for Biologics Evaluation and Research. Test tubes should be of a size that facilitates adequate mixing of the contents (12 X 75 mm or larger).

   If the endpoint is expected to exceed 1024, accuracy will be improved if direct intermediate dilutions are done to keep the number of serial transfers to less than 10. (e.g., If the expected endpoint is 4096, prepare an initial 1:10 dilution with the same diluent as used above.)

   NOTE: All titrations should be carried to a negative endpoint. (See E.4)

2. Prepare master dilutions of the in-house control reagent as in paragraph 1 of this section.

3. A separate, clean pipet or pipet tip should be used for each dilution (including any intermediate dilutions) to avoid carryover of higher reagent concentrations.

4. The last tube should contain diluent only and serve as a diluent control.

B. RED BLOOD CELL PREPARATIONS

Fresh or frozen red blood cells may be used for preparing cell suspensions for the potency testing of all Blood Grouping Reagents under the following conditions:

1. Red blood cells of any age may be used, provided the titer values of the in-house control reagent are within an acceptable range.

2. Red blood cells may be frozen and thawed for use in the preparation of cell suspensions for reagent evaluation. To ensure that the correct cell has been thawed, appropriate controls should be used to demonstrate the desired reactivity and identity of the thawed red blood cells on the day of use. In the case of cells expressing low frequency antigens, testing for several common antigens may serve to adequately identify the cell.

   The method of freezing, storing, and thawing red blood cells, including a description of the cryoprotective medium should be described in detail and should be approved by the Director, Center for Biologics Evaluation and Research as a license amendment before use in control testing of reagent.

3. Each batch of red blood cells for use in control testing of reagents requiring indirect antiglobulin technique should be tested for absence of complement or immunoglobulin molecules (DAT negative) on the day of use.

4. Red blood cells should be washed at least twice in isotonic saline or until a clear supernate is obtained and then resuspended to a 2% suspension in isotonic saline containing 1-2% bovine albumin or another diluent approved by the Director, Center for Biologics Evaluation and Research.

C. MINIMUM TEST CELLS FOR POTENCY

1. At least 2 different donors with phenotypes exhibiting weak and/or heterozygous expression of the antigen, where applicable.

   For example, Anti-Le^a and Anti-Le^b are excluded.

D. THE TEST (BY TUBE METHOD)

1. Place 0.1 milliliter of each reagent dilution in a separate, clean test tube (approximately 10 X 75 mm or 12 X 75 mm).

2. Add 0.1 milliliter of the appropriate 2% cell suspension to each test tube.

3. Mix the contents of each tube thoroughly and incubate the test tubes for the shortest incubation time at the temperature recommended in the manufacturer's package insert for the product. For rapid tube reagents incubate at room temperature (RT; 20-30 C) for 5 minutes.

4. Centrifuge at the lowest speed and for the shortest period of time recommended in the manufacturer's package insert.

5. Perform the indirect antiglobulin test according to the manufacturer's package insert, if recommended.

E. INTERPRETATION OF THE TEST

1. The cell buttons of each test tube should be gently dislodged and examined macroscopically.

2. The reactions should be graded as follows:

   4+ Cell button remains in one clump.

   3+ Cell button dislodges into several clumps.

   2+ Cell button dislodges into many small clumps of equal size.

   1+ Cell button dislodges into finely granular, but definite, small clumps.

   D Cell button dislodges into fine granules, but not definite small clumps. Results should be recorded as doubtful. For purposes of this paragraph, doubtful reactions are deemed to be negative.

   0 Negative reaction- cell button dislodges into no visible clumps.

3. The potency titer value is the reciprocal of the greatest reagent dilution for which the reaction is graded at 1+.

   The dilution caused by the addition of the red blood cells should not be considered as contributing to the dilution of the reagent.

4. Test results should show at least one tube with no agglutination after the endpoint. The diluent control tube should be negative.

F. POTENCY TITER VALUES

1. Products of polyclonal origin which are recommended for tube test methods should have an average potency titer value as follows:

   a. At least a 1+ reaction with a 1:8 dilution of reagent:

      Anti-K
      Anti-k
      Anti-Jk^a
      Anti-Fy^a
      Anti-C^w

   b. At least a 1+ reaction with a 1:4 dilution of reagent:

      Anti-S
      Anti-s
      Anti-P_1
      Anti-M
      Anti-I
      Anti-A_1

   c. At least a 2+ reaction with undiluted reagent:

      Anti-U
      Anti-Kp^a
      Anti-Kp^b
      Anti-Js^a
      Anti-Js^b
      Anti-Fy^b
      Anti-N
      Anti-Le^a
      Anti-Le^b
      Anti-Di^a
      Anti-M^g
      Anti-Jk^b
      Anti-Xg^a
      Anti-Co^b
      Anti-Wr^a

2. Products of monoclonal origin which are recommended for tube test methods should have an average potency titer value of at least 1+ with a 1:8 dilution of reagent.

   Manufacturers that wish to establish potency titer values other than these are to obtain approval from the Director, Center for Biologics Evaluation and Research at the time of license application.

3. Products recommended for use in automated or microplate systems without user dilution (as supplied) should be sufficiently potent that a two-fold dilution prepared with an approved diluent will produce the same qualitative test result as the undiluted product when tested in accordance with the manufacturer's package insert.

III. SPECIFICITY TESTING

A. REAGENT DILUTIONS

1. No dilution of the reagent under test is permitted.

B. RED BLOOD CELL PREPARATIONS

Fresh or frozen red blood cells may be used for preparing cell suspensions for the specificity testing of all Blood Grouping Reagents under the following conditions:

1. Any cells of any age may be used in the "Test to Confirm Reactivity with Antigen Positive Cells" (III.C.1). In the "Test to Confirm Absence of Contaminating Antibodies" (III.C.2) licensed reagent red blood cells may be used any time before their expiration date. All other red blood cell samples should be used within 7 days of collection from the donor.

   Manufacturers that wish to use cells more than 7 days after collection from the donor are to obtain approval from the Director, Center for Biologics Evaluation and Research, and are to provide sufficient data to support the request.

2. Red blood cells meeting the criteria of paragraph 1 of this section may be frozen and thawed for use in the preparation of cell suspensions for reagent evaluation. To ensure that the correct cell has been thawed, appropriate controls should be used to demonstrate the desired reactivity and identity of the thawed red blood cells on the day of use. In the case of cells expressing low frequency antigens, testing for several common antigens may serve to adequately identify the cell.

The method of freezing, storing, and thawing red blood cells, including a description of the cryoprotective medium should be described in detail and should be approved by the Director, Center for Biologics Evaluation and Research before use in control testing of licensed reagents.

3. Each batch of red blood cells for use in control testing of reagents requiring indirect antiglobulin technique should be tested for absence of complement or immunoglobulin molecules (DAT negative) on the day of use.

4. Licensed reagent red blood cells may be used as provided.

   All other red blood cell samples should be washed at least twice in isotonic saline or until a clear supernatant is obtained and then resuspended with an approved diluent to the cell concentration listed in the manufacturer's package insert.

C. MINIMUM TESTING FOR SPECIFICITY

1. TEST TO CONFIRM REACTIVITY WITH ANTIGEN POSITIVE CELLS

   a. At least 4 different donors whose red blood cells exhibit weak or heterozygous expression of the antigen should be tested.

   b. When testing reagents containing multiple antibodies, the reactivity of each specificity should be confirmed separately by using 4 different cells possessing only one of the antigens for each different specificity.

   c. Include at least one red blood cell which does not exhibit the expression of the antigen as a negative control.

2. TEST TO CONFIRM ABSENCE OF CONTAMINATING ANTIBODIES

   Test the reagent for the presence of antibodies corresponding to the following antigens by one of the methods listed below.

   A, B, H, Le^a, Le^b, I, K, k, Kp^a, Kp^b, Js^b, P_1, D, C, E, c, e, C^w, M, N, S, s, U, Lu^a, Lu^b, Jk^a, Jk^b, Fy^a, Fy^b, Xg^a, Do^a, Do^b, Yt^a, Yt^b, Lan, Co^a, Co^b, M^g, Wr^a, and Sd^a.

If the source material is a monoclonal antibody from a previously characterized and licensed clone, this list may be shortened as follows:

A, B, H, Le^a, Le^b, I, K, k, $\overline{Kp}^b$, Js^b, P_1, D, C, E, c, e, M, N, S, s, U, Lu^b, Jk^a, Jk^b, Fy^a, and Fy^b

Approval for the use of fewer antigens than included on this list may be requested from the Director, Center for Biologics Evaluation and Research by a manufacturer at the time of submission of the first protocol.

a. Perform a direct test for the presence of contaminating antibodies by using red blood cells from at least 4 different donors whose cells lack the antigen corresponding to the reagent antibody.

b. When red blood cells lacking the antigen corresponding to the reagent antibody under test are not available, the reagent antibody may be adsorbed to exhaustion with cells of a known phenotype.

   The adsorbed serum may then be tested against red blood cells which exhibit any antigens which were not present on the cells used for adsorption. The methods for adsorption and subsequent testing should be approved by the Director, Center for Biologics Evaluation and Research.

c. When direct tests are impractical, the Director, Center for Biologics Evaluation and Research may approve procedures whereby antibodies may be presumptively excluded by testing an appropriate number of non-reactive red blood cell samples to provide statistical assurance of the absence of contaminating antibodies.

d. Red blood cell samples from four different donors may be used to confirm presumptively the absence of contaminating antibodies to antigens having an incidence of greater than 99% in the general population of the United States.

3. TEST TO CONFIRM ABSENCE OF ANTI-A AND ANTI-B

a. Group A_1 and B red blood cells lacking the antigen corresponding to the reagent antibody should be tested. Group A_1B red blood cells may be substituted for A_1 and/or B red blood cells if either are unavailable.

Adsorbed serum may be used as in III.C.2.b above.

4. ADDITIONAL PHENOTYPES RECOMMENDED FOR TESTING

REAGENT	RED BLOOD CELLS
Anti-A_1	A_1, A_2, A_1B, A_2B, B, and O
Anti-k	K+k+ Kp(a+b+)
Anti-Le^b	6 different A_1 and/or A_1B Le(b+) *
Anti-P_1	At least 2 P_1 weak (as determined by titration studies)
Anti-U	S-, s-, U+
Anti-Fy^b	Fy^x
Anti-Jk^a	Jk(a-b-)
Anti-Jk^b	Jk(a-b-)

* Group A and AB cells which do react with anti-A_1 and do not react with anti-H.

D. THE TESTS

1. To confirm reactivity with antigen positive cells, each lot of Blood Grouping Reagent should be tested and results interpreted by all test methods described in the manufacturer's package insert. Minimum parameters (drops of reagent, incubation time, centrifugation, etc.) should be followed.

2. To confirm absence of contaminating antibodies, each lot of Blood Grouping Reagent should be tested and results interpreted by the most sensitive test method(s) described in the manufacturer's package insert. Maximum parameters (drops of reagent, incubation time, centrifugation, etc.) should be followed.

E. SPECIFICITY RESULTS

1. No hemolysis or rouleaux formation should be detected by any of the methods in the manufacturer's package insert.

2. Red blood cells which exhibit the antigen corresponding to the reagent antibody should yield at least a 2+ reaction. If any of the four samples tested yields less than a 2+ reaction, red blood cells from four additional donors who exhibit the antigen should be tested. The test is considered satisfactory if no more than one of eight red blood cell samples yields less than a 2+ reaction with the test reagent.

   When testing unusual phenotypes, other criteria for reactivity may apply. For example, Fy^x red blood cells may not yield a 2+ reaction with Anti-Fy^b but should yield a clearly positive macroscopic result.

3. The negative control cell(s) in step III.C.1 should yield a negative reaction by each test method described in the manufacturer's package insert.

4. Tests with red blood cells which lack the antigen corresponding to the reagent antibody and tests with adsorbed reagent should be negative, thus confirming the absence of significant contaminating antibodies directed at the antigens listed in III.C.2

5. The manufacturer should list on the lot release protocol and in the "Specific Performance Characteristics" section of the package insert those red blood cell antigens listed in III.C.2 for which no specificity tests have been performed.

   If desired, the red blood cell phenotype of the antibody donor(s) may also be listed as presumptive evidence that antibodies to those factors are not present.

6. Tests with group A_1 and B red blood cells should be negative, thus confirming the absence of anti-A and anti-B.

7. Confirmation by the manufacturer of nonspecific reactions after a lot of Blood Grouping Reagent has been released should be reported promptly by the manufacturer to the Director, Center for Biologics Evaluation and Research.

IV. AVIDITY TEST FOR SLIDE REAGENTS

A. REAGENT DILUTIONS

1. Prepare a 1 in 2 dilution of the reagent under test by mixing equal parts of the reagent and AB serum, group compatible serum, or a diluent approved by the Director, Center for Biologics Evaluation and Research.

B. RED BLOOD CELL PREPARATIONS

1. Red blood cells should be prepared according to the manufacturer's package insert.

C. MINIMUM TEST CELLS FOR AVIDITY

1. Red blood cells from at least two different donors exhibiting weak and/or heterozygous expression of the antigen corresponding to the reagent antibody should be used.

D. THE TEST (BY SLIDE METHOD)

1. The test is to be performed with both undiluted reagent and the diluted reagent prepared in step IV,A by the method recommended in the manufacturer's package insert.

E. INTERPRETATION OF THE TEST

1. Test results are observed and recorded at one half of the manufacturer's recommended observation time and at the end of the full recommended observation time.

F. AVIDITY TESTING RESULTS

1. Signs of agglutination should be observed with both the undiluted and diluted reagent at one half of the manufacturer's recommended observation time.

2. Clear macroscopic agglutination should be observed with both the undiluted and diluted reagent at the end of the manufacturer's recommended observation time and should be reported as greater than or less than 1 mm.