DOCKET NO. 84S-0181

RECOMMENDED METHODS

FOR

BLOOD GROUPING REAGENTS EVALUATION

Revised: March 1988

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Center for Biologics Evaluation and Research
Food and Drug Administration

84B-0181

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CENTER FOR BIOLOGICS EVALUATION AND RESEARCH

RECOMMENDED METHODS FOR

BLOOD GROUPING REAGENTS EVALUATION

I. Reference preparations

The following reference blood grouping reagents are obtained from the Center for Biologics Evaluation and Research, Food and Drug Administration, 8800 Rockville Pike, Bethesda, MD 20892, and should be used as described in the accompanying package insert for determining the potency of blood grouping reagents.

Anti-A

Anti-B

Anti-D for evaluation of IgG products

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

Anti-C for rapid tube test

Anti-C for saline tube test

Anti-E for rapid tube test

Anti-E for saline tube test

Anti-c for rapid tube test

Anti-e for rapid tube test

Red blood cell preparations. Fresh or frozen red blood cells may be used for preparing cell suspensions for the testing of all blood grouping reagents under the following conditions:

(a) Unlicensed red blood cells used for specificity testing should be used within 7 days of collection from the donor. Unlicensed red blood cells of any age may be used for potency testing. Licensed reagent red blood cells may be used for potency and specificity testing any time before their expiration date.

(b) Red blood cells meeting the criteria of paragraph (a) of this section may be frozen and thawed for use in the preparation of all cell suspensions for testing antisera. Appropriate positive and negative controls should be used to demonstrate the desired reactivity and specificity 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 must be approved by the Director, Center for Biologics Evaluation and Research as a license amendment before use in control testing of antisera.

(c) Red blood cells for use in control testing of reagents requiring indirect antiglobulin technique should be tested for absence of complement or immunoglobulin molecules on the day of use or each time frozen blood cells are thawed, whichever is applicable.

II. Potency test with reference preparations. Products for which reference blood grouping reagents are available should be tested as follows:

(a) Test procedures for ABO Blood Grouping Reagents. (1) Cell suspension. (i) A 2-percent suspension of red blood cells, in isotonic saline containing 1 to 2 percent bovine albumin should be prepared on the day of use. The red blood cells used should have been washed with isotonic saline at least twice and should have a clear supernate.

(ii) As a minimum, the following cells should be used:

Blood Grouping Reagent	Cells
Anti-A	A_1, A_2B (A_2B cells from 3 different donors shall be used)
Anti-A,B	A_1, A_2, B.
Anti-B	B

(2) Reagent dilutions. (i) Beginning with the undiluted reagent, separate two-fold dilutions (1 in 2, 1 in 4, etc.) of the test reagent and the reference reagent should be prepared using isotonic saline containing 1 to 2 percent bovine albumin.

(ii) A separate clean pipette should be used for each dilution to avoid carryover of higher reagent concentrations.

(iii) For an anti-A,B reagent, dilutions of Reference Blood Grouping Reagents Anti-A and Anti-B should be made separately.

(3) The test. Using cells listed in paragraph (a)(1)(ii) of this section, Reference Blood Grouping Reagents Anti-A and Anti-B should be tested in parallel with the test reagent.

(i) Place 0.1 milliliter of each reagent dilution in a separate clean test tube approximately 10 x 75 millimeters or 12x75 millimeters. Add 0.1 milliliter of the appropriate 2 percent cell suspension to each test tube.

(ii) Mix contents of each test tube thoroughly and centrifuge immediately for 1 minute at approximately 150 relative centrifugal force (rcf) or 20 seconds at approximately 1,000 rcf.

(4) Interpretation of the test. (i) The cell buttons of each test tube should be gently dislodged and examined macroscopically. 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.

(ii) 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 cells should not be considered as contributing to the dilution of the reagent.

(b) Test procedures for anti-D, anti-CD, anti-DE, anti-CDE, anti-C, anti-E, anti-c and anti-e for slide test or rapid tube test-(1) Cell suspensions. (i) A 2 percent suspension of red blood cells in 11 to 15 percent bovine albumin should be prepared on the day of use. The red blood cells used should have been washed with isotonic saline at least twice and should have a clear supernate.

(ii) As a minimum, the following cells should be used:

Blood Grouping Reagent	Cells (A, B, A B, or O)
Anti-D	cDe
Anti-C	Ccde
Anti-E	cdEe
Anti-c	CcDEe
Anti-e	cdEe
Anti-CD	Ccde,cDe
Anti-DE	cDe, cdEe
Anti-CDE	Ccde,cDe,cdEe,

(2) Reagent dilutions. (i) Beginning with the undiluted reagent, separate twofold dilutions (1 in 2, 1 in 4, etc.) of the test reagent and the reference reagent should be prepared using 20 to 22 percent bovine albumin.

(ii) A separate clean pipette should be used for each dilution to avoid carryover of higher reagent concentrations.

(iii) For test reagents containing multiple antibodies (e.g., anti-CDE) dilutions of the corresponding reference blood grouping reagents should be made separately.

(3) The test. Using cells listed in paragraph (b)(1)(ii) of this section, reference blood grouping reagents should be tested in parallel with the test reagents.

(i) Place 0.1 milliliter of each reagent dilution in a separate clean test tube approximately 10 x 75 millimeters. Add 0.1 milliliter of the appropriate 2 percent cell suspension to each test tube.

(ii) Mix contents of each test tube thoroughly and incubate test tubes at 37 C for 1 hour.

(iii) Centrifuge test tubes for 2 minutes at approximately 150 relative centrifugal force (rcf) or for 45 seconds at approximately 1,000 rcf.

(4) Interpretation of the test. The interpretation of the test should be the same as that described in paragraph (a)(4) of this section.

(c) Test procedure for anti-C, anti-D, and anti-E for saline tube test. The test procedure should be the same as that described in paragraphs (b)(1) through (4) of this section except for the following:

(1) The 2-percent suspensions of red blood cells and the two-fold reagent dilutions should be made in isotonic saline containing 1 to 2 percent bovine albumin.

(2) Test tubes should be centrifuged for 1 minute at approximately 150 relative centrifugal force (rcf) or 20 seconds at approximately 1,000 rcf.

III. Potency test without reference preparations. Products for which reference blood grouping reagents are not available should be tested as follows:

(a) Products recommended for tube tests - (1) Cell suspensions. (i) A 2 percent suspension of red blood cells in isotonic saline containing 1-2 percent bovine albumin or in a diluent approved by the Director, Center for Biologics Evaluation and Research, should be prepared on the day of use. The red blood cells used should have been washed with isotonic saline at least twice and should have a clear supernate.

(ii) As a minimum, red blood cells from two donors should be used. Phenotypes exhibiting heterozygous expression for the antigen should be selected if reagents defining antigens corresponding to products of all alleles of that blood group system exist. When necessary, other cells may be used and reported on the protocol for that lot.

(2) Reagent dilutions. (i) Beginning with the undiluted reagent, separate two fold dilutions (1 in 2, 1 in 4, etc.) of the test reagent should be prepared using isotonic saline, or other approved diluent.

(ii) A separate clean pipette should be used for each dilution to avoid carryover of higher reagent concentrations.

(3) The test. (i) Place 0.1 milliliter of each reagent dilution in a separate clean test tube approximately 10 x 75 millimeters. Add 0.1 milliliter of the appropriate cell suspension to each test tube.

(ii) Mix contents of each test tube thoroughly and incubate test tubes at the temperature and for the shortest length of time recommended in the manufacturer's package insert for the product.

(iii) Perform antiglobulin test, if required in the manufacturer's package insert for the product.

(iv) Centrifuge test tubes according to the instructions that result in the lowest rcf and the least time recommended in the manufacturer's package insert.

(4) Interpretation of the test. The interpretation of the test should be the same as described in II(a)(4)(i).

IV. Specificity tests.

(a) Test procedures. (1) Each lot of blood grouping reagent should be tested by all test methods described in the manufacturer's package insert.

(2) Red blood cell samples from at least four different donors whose cells have the antigen and red blood cell samples from at least four different donors whose cells lack the antigen corresponding to the specificity of the antibody should be tested.

When testing reagents containing multiple antibodies, the reactivity of each specificity should be confirmed separately by using an appropriate number of cells positive only for that antigen. For example, when testing anti-A,B reagents, at least four donors should be used to confirm the reactivity of the anti-A component and at least four different donors should be used to confirm the anti-B component.

(3) Approval for exceptions to paragraph (a)(2) of this section may be requested from the Director, Center for Biologics Evaluation and Research, by a manufacturer at the time of submission of the first protocol if the blood grouping reagent is specific for a high-incidence antigen.

(b) Red blood cells listed below are to be used as a minimum in specificity tests of the following reagents.

(1) ABO and Rh blood grouping reagents:

Blood Grouping Reagent	Cells
Anti-A	A_1, A_2B, B, O
Anti-B	A_1, B, O
Anti-A,B	A_1, A_2, B, O, Ax cells from 3 different donors*
Anti-A_1	A_1, A_2, A_1B, A_2B, B, O
Anti-D	CcDe, cDe, Ccde, cdEe, A_1 cde B cde, O cde,

*Only if the labeling recommends anti-A,B blood grouping reagent for detection of group A variants.

Additional testing for Anti-D recommended for Du testing	cde Bg(a+) cells from 3 different donors and 6 Du samples including different Rh phenotypes and possessing the weakest forms of D expression, as represented by those showing distinct reaction only by indirect antiglobulin test.
Anti-C	cDe, Ccde, cdEe or cdE, C+rhi negative cells, A_1 cde, B cde, O cde
Anti-E	cDe, Ccde or Cde, cdEe, A_1 cde, B cde, O cde
Anti-CD	cDe, Ccde or cdE, cdEe, A_1 cde, B cde, O cde, r^Gr**
Anti-DE	cDe, Ccde or Cde, cdEe, A_1 cde, B cde, O cde

**Only if the reagent is recommended for detection of G antigen

Anti-CDE.....cDe, Ccde, cdEe, A_1 cde, B cde, O cde, $r^G r$**

Anti-c.....Ccde, A_1 CDe, B CDe, O CDe and CDEe or CDE, or CdE

Anti-e.....cdEe, A_1 cDE, B cDE, O cDE and CcDE or CDE, or CdE

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(2) Low protein Rh reagents should not react with cells heavily coated with IgG antibodies prepared in vitro. (Heavily coated is defined as a positive direct antiglobulin test of 3+ or stronger). These reagents should be tested with three different donor cells sensitized with IgG antibody. Low protein anti-D should give acceptable positive results with cord bloods representing phenotypes CcDe, cDE, cDe, and CcDEe.

(3) The manufacturer of a blood grouping reagent that is recommended for use in an automated system may do manual tests on the base material (before dilution) to demonstrate the absence of contaminating antibodies. The manual tests should be performed by test procedures approved by the Director, Center for Biologics Evaluation and Research.

(c) Specificity criteria for all blood grouping reagents:

**Only if the reagent is recommended for detection of G antigen

(1) Red blood cells of appropriate ABO blood group may be used. Phenotypes exhibiting heterozygous expression of the antigen should be selected if reagents exist to enable recognition of diminished antigen expression.

(2) In addition, group O, A_1 and B red blood cells lacking the corresponding antigen should be tested. Group A_1B cells may be substituted for A_1 and B cells when either or both are unavailable.

(3) Specificity tests should include red blood cells having the following antigens:

Antigens: 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. For Anti-A, Anti-B, Anti A,B and Anti-D, this list should be expanded to include the V^w antigen.

(4) Approval for exceptions to paragraph (3) of this section may be requested from the Director, Center for Biologics Evaluation and Research, by a manufacturer at the time of submission of the first protocol if the blood grouping reagent is specific for a high incidence antigen.

(5) 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 antibody.

(d) Specificity requirements. (1) Specificity tests should demonstrate the absence of hemolysis and rouleaux formation in all tests performed on the finished lot.

(2) If one of four red blood cell samples with the antigen corresponding to the specific antibody gives less than a 2+ reaction, additional red blood cell samples from four donors whose cells have the antigen should be tested. The test is considered satisfactory if no more than one of eight red blood cell samples gives less than a 2+ reaction with the test reagent.

(3) Exceptions to (d)(2) may be granted when testing unusual phenotypes. For example, a larger percentage of Ax cells may not give a 2+ reaction with anti-A,B but should give a clearly positive macroscopic result.

(4) The manufacturer should list on the protocol and in the "Specific Performance Characteristics" section of the package insert red blood cell antigens listed in (c)(3) 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.

(5) Specificity tests should confirm the absence of significant contaminating antibodies reactive with red blood cell antigens listed in (c)(3) by the most sensitive test technique recommended by the manufacturer for use of the blood grouping reagent.

(6) Confirmation by manufacturers 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.

(a) Blood grouping reagents recommended for use by a slide method should be diluted with an equal part of human serum or with a diluent approved by the Director, Center for Biologics Evaluation and Research, before being tested for avidity by the slide method recommended in the manufacturer's package insert.

(1) Cells exhibiting heterozygous expression of the corresponding antigen should be used.

As a minimum, the following cells should be used:

Blood Grouping Reagent	Cells
Anti-A	A_1, A_2B
Anti-B	B
Anti-A,B	A_1, A_2, B, Ax*
Anti-D	cDe
Anti-C	Ccde
Anti-E	ccEe
Anti-c	Ccde
Anti-e	ccEe

*Only if the labeling recommends anti-A,B blood grouping reagent for detection of weak subgroups of A by slide technique.

Anti-CDE.....cDe, Ccde, cdEe $r^{G}r$**

Anti-CD.....cDe, Ccde, $r^{G}r$**

Anti-DE.....cDe, cdEe

(2) At the end of the first half of the recommended observation period, clear macroscopic agglutination should be observed.

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

Document I.D. 0070j; prepared by JWilczek 9/26/85:lp; revised by JWilczek 1/21/86:lp/dcq:2/10/88