M E M O R A N D U M

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service
Food and Drug Administration
Center for Drugs and Biologics

TO : Dockets Management Branch (HFA-305)

DATE: MAR 04 1985

FROM : Consumer Safety Officer (HFN-368)

SUBJECT: Material for Public Display under Docket Number 83N-0169

At the time of publication of the proposed rule amending the additional standards for Blood Grouping Serum, please place the following on public display:

Recommended Methods for Blood Grouping Sera Evaluation Docket No. 84S-0181.

An additional ten copies of the recommended methods are attached for your convenience.

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Consumer Safety Officer (HFN-368)

84B-0181

RMI

DOCKET NO. 84S-0181

RECOMMENDED METHODS

FOR

BLOOD GROUPING SERA EVALUATION

Prepared: March 1985

Prepared by: Division of Blood and Blood Products
Office of Biologics Research and Review
Center for Drugs and Biologics
Food and Drug Administration

CENTER FOR DRUGS AND BIOLOGICS'

RECOMMENDED METHODS FOR

BLOOD GROUPING SERA EVALUATION

I. Reference Preparations

The following reference Blood Grouping Sera are obtained from the Office of Biologics Research and Review, Center for Drugs and Biologics, Food and Drug Administration, 8800 Rockville Pike, Bethesda, MD 20205, and should be used as described in the accompanying package insert for determining the potency of Blood Grouping Sera.

Anti-A

Anti-B

Anti-D for evaluation of IgG products

Anti-CD 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 Sera under the following conditions:

(a) Unlicensed fresh red blood cells used for specificity testing should be used within 7 days of withdrawal 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 controls should be used to demonstrate the desired reactivity of the thawed red blood cells on the day of use. The method of freezing, storing, and thawing red blood cells, including description of the cryoprotective medium, should be described in detail and must be approved by the Director, Office of Biologics Research and Review, as a license amendment before use in control testing of antisera.

(c) Red blood cells for use in control testing of antisera requiring indirect antiglobulin technique should first be tested and found negative by direct antiglobulin technique 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 Sera are available should be tested as follows:

(a) Test procedures for ABO Blood Grouping Serum. (1) Cell suspension. (i) A 2-percent suspension of red blood cells, in isotonic saline containing 1 to 2 percent bovine serum 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 serum	Cells
Anti-A	A_1, A_2B cells from 3 different donors.
Anti-A,B	A_1, A_2, B.
Anti-B	B

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

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

(iii) For anti-A,B serum, dilutions of Reference Blood Grouping Sera 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 Sera Anti-A and anti-B should be tested in parallel with the test serum.

(i) Place 0.1 milliliter of each serum 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 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 serum 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 antiserum.

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

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

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

(iii) For test sera containing multiple antibodies (e.g., Anti-CDE) dilutions of the corresponding Reference Blood Grouping Sera should be made separately.

(3) The test. Using cells listed in paragraph (b)(1)(ii) of this section, Reference Blood Grouping Sera should be tested in parallel with the test sera.

(i) Place 0.1 milliliter of each serum 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 serum dilutions should be made in isotonic saline containing 1 to 2 percent bovine serum 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 Sera 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 serum albumin or in a diluent approved by the Director, Office of Biologics Research and Review, 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 representing heterozygous expression of the antigen should be selected if antisera exist to enable recognition of heterozygous antigen expression. When necessary, other cells may be used and reported on the protocol for that lot.

(2) Serum dilutions. (i) Beginning with undiluted serum, separate two-fold dilutions (1:2, 1:4, etc.) of the test serum 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 serum concentrations.

(3) The test. (i) Place 0.1 milliliter of each serum 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 Serum 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, Office of Biologics Research and Review, by a manufacturer at the time of submission of the first protocol if the Blood Grouping Serum is specific for high-incidence antigens.

(b) Red blood cells to be used as a minimum in specificity tests of the following antisera:

(1) ABO and Rh Blood Grouping Sera:

Blood grouping serum	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, O
Anti-D	CcDe, cDe, Ccde, cdEe, A_1 cde, B cde, O cde,
Additional testing for Anti-D recommended for Du testing	cde Bg(a+) cells from 3 different donors and Du positive samples of at least 3 different Rh phenotypes.
Anti-C	cDe, Ccde, cdEe or cdE, C+rh_i negative cells, A_1 cde, B cde, O cde
Anti-E	cDe, Ccde or Cde, cdEe, A_1 cde, B cde, Ocde
Anti-CD	cDe, Ccde or cdE, cdEe, A_1 cde, Bcde, Ocde, r^Gr**

Anti-DE.....cDe, Ccde or Cde, cdEe, A_1 cde, B cde, O cde

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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*Only if the labeling recommends Anti-A,B Blood Grouping Serum for detection of Group A variants.

**Only if antiserum is recommended for detection of G antigen

(2) Low protein Rh antisera should not react with cells heavily coated with IgG antibodies. (Heavily coated is defined as a positive direct antiglobulin test of 3+ or stronger). Low protein Anti-D should give acceptable positive results with cord bloods representing phenotypes CcDe, cDE, cDe, and CcDEe.

(3) The manufacturer of Blood Grouping Serum 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, Office of Biologics Research and Review.

(c) All Blood Grouping Sera:

(1) Red blood cells of appropriate ABO blood group may be used. Phenotypes representing heterozygous expression of the antigen should be selected if antersera exist to enable recognition of heterozygous 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, Kp^a, P_1, D, C, E, c, e, C^w, M, N, S, s, Lu^a, Lu^b, Jk^a, Jk^b, Fy^a, Fy^b, Xg^a, Do^a, Do^b, Yt^a, Yt^b, Co^a, Co^b, and Sd^a. For Anti-A, Anti-B, and Anti-D, this list should be expanded to include M^g, Wr^a and V^w.

(4) Approval for exceptions to paragraph (3) of this section may be requested from the Director, Office of Biologics Research and Review, by a manufacturer at the time of submission of the first protocol if Blood Grouping Serum is specific for high incidence antigens.

(5) When direct tests are impractical, the Director, Office of Biologics Research and Review, 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 no hemolysis or 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 serum.

(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 have been excluded.

(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 Serum.

(6) Confirmation of nonspecific reactions by manufacturers after a lot of Blood Grouping Serum has been released should be reported promptly by the manufacturer to the Director, Office of Biologics Research and Review.

IV. Avidity test.

(a) Blood Grouping Sera recommended for use by a slide method should be diluted with an equal part of human serum before being tested for avidity by the slide method recommended in the manufacturer's package insert.

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

As a minimum, the following cells should be used:

Blood grouping serum	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	cdEe
Anti-c	Ccde
Anti-e	cdEe
Anti-CDE	cDe, Ccde, cdEe r^Gr**
Anti-CD	cDe, Ccde, r^{Gr}**
Anti-DE	cDe, cdEe

*Only if the labeling recommends Anti-A,B Blood Grouping Serum for detection of weak subgroups of A by slide technique.

**Only if antiserum is recommended for detection of G antigen by slide techniques.

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