


TS - Rho(D) Typing	SFO-WI.0084	Page 1 of 9
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PURPOSE

The presence of the Rho(D) antigen is determined by testing the red cells with Anti-D reagent using specific test methods. A positive reaction indicates the presence of the antigen on the red cells being tested, while a negative reaction signifies its absence. Cells possessing the weaker form of the D antigen and/or fewer antigen sites (weak D) may give a negative or perceptibly weaker than normal reaction in the direct agglutination phase of the test but may produce a readily detectable positive reaction in the antiglobulin phase.

REAGENTS

- A. Anti-D (human monoclonal polyclonal blend) contains human cell-line IgM anti-D blended with a prescribed amount of polyclonal IgG anti-D. The IgM portion of this reagent causes direct agglutination of D positive red cell while the IgG portion reacts in the antiglobulin phase of the test for weak D (formerly Du).
- B. Anti-D (monoclonal blend) is manufactured by blending the secretions of two human/murine heterohybridomas grown in fluid culture. The IgM (saline agglutinating) component is contributed by the cell line GAMA401 and the IgG component by the cell line F8D8. There is no human serum component.
- C. Rh Control depends on the anti-D reagent used. Refer to the manufacturer's insert for each specific anti-D reagent. (Gamma-clone Control, Monoclonal Control, patient's own serum, or 7% bovine albumin).
- D. Antihuman globulin (polyspecific or anti-IgG).
- E. IgG coated Coombs control cells.

EQUIPMENT

- A. 12x75 mm test tubes
- B. Serologic centrifuge
- C. Agglutination viewer
- D. Transfer pipets
- E. Cell washer
- F. Microscope

SPECIMEN

- A. EDTA specimen
- B. Clotted specimen

CONTROLS

- A. In cases where the test samples show definite or doubtful agglutination with anti-D, a control test should be performed to investigate the reliability of the reactions observed in the tests. Refer to the manufacturer's insert for each specific anti-D reagent.

Note: Gamma-clone Control should be used as Rh Control when using Gamma-clone anti-D reagent.

- B. In cases where the red blood cells being tested are previously known to have a positive direct antiglobulin test (DAT), the Gamma-clone Control or Monoclonal control is

recommended as being the most suitable control.

- C. Either a direct antiglobulin test or an indirect antiglobulin test (after incubating the red blood cells with a serologically inert control reagent) is required before interpreting the result of the test for weak D as valid.

PROCEDURE

- A. If test sample shows visible hemolysis, lipemia or icterus, the cells should be washed at least one time in physiologic saline and suspended in saline for testing.
- B. **Vision D testing**
1. Refer to **Vision** SOP for operating instructions.

Vision Results Interpretation Table:

If	Then
Anti-D is 3+ or greater	Rh positive unless discrepant from historical.
Anti-D is 3+ or greater (historically Rh Neg, no ID error)	Perform tube test. Refer to Flowchart.
Anti-D is less than 3+	Perform tube test. Refer to Flowchart.
Anti-D is dp (dual population or mixed field)	Perform tube test. Check recent tx history of different Rh RBC. Check BMT/ Stem Cells Tx / IUT / Exchange transfusion history. Refer to Cord Blood Testing SOP for neonates. MF in cord blood may indicate contamination with mother's blood.
Anti-D is '-'	Rh negative unless discrepant from historical.
Rh Control is negative	Rh result is valid.
Rh Control is positive	Rh result is invalid. Wash cells and perform tube test.

- C. **Tube Method: Immucor Gamma clone anti-D is the primary reagent for manual tube test.**

Note: Refer to Flow Chart for patients whose current Rh typing is discrepant from historical.

1. Add one drop of anti-D to a properly labeled test tube.
2. Add one drop of 3-5 % cell suspension to each tube.
3. Mix the contents of the tubes thoroughly and centrifuge for 15 seconds at 3400 rpm or a time appropriate to the calibration of the centrifuge.
4. Resuspend the cells by gentle agitation and examine **macroscopically** for agglutination using the agglutination viewer.
5. Record the test result immediately in the computer (Refer to Computer SOP).
6. When a patient types as AB positive, a D control according to manufacturer's instruction must be run in parallel to rule out spontaneous agglutination. **Note:**

Gamma-clone Control should be used as the Rh Control when using Gamma-clone anti-D reagent.

7. If no agglutination is observed, the patient is Rh negative.
8. If agglutination is present (**2+ or greater**), interpret as **Rh positive** for patients **without historical Rh typing discrepancy**.
9. If the test result is **less than 2+**, interpret as **Rh negative** for patients **4 months or older without historical Rh typing discrepancy**.
10. For **neonates** or if test result is **doubtful**, proceed to **weak D testing**.
11. See section for Rh Typing Discrepancy.

D. When to perform Weak D testing:

Note: Cells with positive Direct Coombs Test due to IgG cannot be tested for weak D by the indirect antiglobulin method. See Neonate Rho(D) Typing Flowchart for interpretation.

1. A test for weak D is not required for women who are pregnant or who have been pregnant recently when the woman's test for D antigen is negative.
2. Weak D test will be performed in the following situations:
 - a. Neonates who tested negative or less than 2+ with anti-D at immediate spin.
 - b. Doubtful Rh(D) results.
 - c. Rh discrepancy investigation when needed.

E. Weak D testing procedure:

1. Add one drop of Anti-D to a properly labeled test tube.
2. Add one drop of Rh control to a properly labeled test tube.
3. Add one drop of 3-5 % cell suspension to each tube.
4. Mix thoroughly and incubate at 37 C for 15-30 minutes.
5. Centrifuge after incubation and read **macroscopically** for agglutination.
6. If agglutination is present (**2+ or greater**) and the **control is negative**, it is unnecessary to continue. Interpret the patient as **Rh positive unless patient is historically Rh negative (no patient ID error), then work it up as Rh discrepancy and report Rh Negative Weak D Positive**.
7. If reaction is **less than 2+**, proceed to the Coombs phase.
8. Wash the patient and control tubes 3-4 times in saline.
9. Decant the saline completely following the last wash.
10. Add two drops of antihuman globulin (anti-IgG) to each tube.
11. Mix well and centrifuge.
12. Immediately, read macroscopically and record results.
13. **Macroscopic agglutination with anti-D at AHG phase, and no agglutination in the control tube indicate weak or partial D antigen. See Table for interpretation.**
14. If **no** agglutination occurs, add a drop of Coombs control cells to each tube, then centrifuge.
 - a. If Coombs control is positive, the test may be reported as Rh negative.
 - b. If Coombs control is negative, repeat testing.

F. Tube Method Results Interpretation Table:

If	Then	Result Comments (use specified template when applicable)	Blood Bank Comments
Immediate Spin with anti-D is negative or less than 2+	Rh negative	None	None
Immediate Spin with anti-D is 2+ or greater	Rh positive	None	None
37°C with anti-D is 2+ or greater	Rh positive Or If patient is historically Rh negative (no patient ID error), then work it up as Rh discrepancy and report Rh Negative with Result Comments (Weak D positive).	None Or SF_WKD	None
AHG phase with anti-D is Positive (macroscopic agglutination)	Adults: Rh negative (Weak D positive) Neonates: Rh Indeterminate (technically Rh negative Weak D positive)	Adults: SF_WKD. Neonates: SF_DU	Adults: None Neonates: Weak D positive. Give Rh negative blood products.

Anti-D is mixed field	Check recent tx history of different Rh RBC. Check BMT/ Stem cells/ IUT/ Exchange transfusion history. MF in a recently delivered mother may indicate mixture of maternal and cord red cells. Refer to Cord Blood Testing SOP for neonates. MF in cord blood may indicate contamination with mother's blood.	Result Note: SF_MF or free text comments for transplant history.	Free text comments for transplant history.
Rh Control is negative	Rh result is valid.	None	None
Rh Control is positive	Wash cells and repeat tests. Warm saline may be used if cold autoagglutinin is suspected. Give Rh negative blood products if the Rh control is still positive and interpret as Rh 'Indeterminate'	Unable to interpret Rh due to positive Rh control.	Unable to interpret Rh due to positive Rh control. Give Rh negative blood products.

Notes: SF_DU - Infant is Weak D Positive. Note: RHIG should be given to Rh Neg mother.

SF_WKD - Weak D Positive. Due to new clinical standards, this patient is considered Rh neg and women of child bearing age are candidates for RhIg.

G. Computer Results Entry for Rh Negative Weak D Positive

NOTE: Refer to RILIS Millennium Quick Reference for detailed instructions.

1. ADULTS

NOTE: RILIS Millennium automatically interprets as Rh positive when the reaction with anti-D is positive (W+, 1+, 2+, 3+, or 4+).

- To interpret as **Rh negative when anti-D reaction is positive**, remove the positive reaction and enter '**Not Valid**' with **Result Note: anti-D = ____**.
- Interpret as **Rh Neg** and add template SF_WKD as Result Comments at ABORh interpretation field. Refer to the Table above for details.

2. NEONATES

STEP I:

- Enter results for Anti-A, Anti-B, Anti-D, D Con. Leave blanks the

following: Weak D, Con AHG, Wk D CC, Con CC.

- b. Interpret as **Rh Indeterminate** and add template SF_DU as Result Comments at ABORh interpretation field. Refer to the Table above for details.
- c. Verify results.

STEP II:

- a. Return to Result Entry and enter the accession number for the ABORh again. Enter results for Weak D testing.
- b. Verify results.

H. Factors that may cause false test results include the following:

1. Contamination of blood specimens, reagent and/or supplementary materials.
2. Aged blood specimens, which may yield weaker reactions than those obtained with fresh cells.
3. Too light or too heavy cell suspension.
4. Improper incubation time or temperature.
5. Excessive centrifugation may lead to difficulty in resuspending the cell button. Conversely, inadequate centrifugation may yield unclear cell button patterns and agglutinates that are too readily dispersed.
6. Improper examination of agglutination (usually, excessively vigorous shaking). The resuspension of the cell button in the tube must be carried out by gentle rocking motion. Shaking too vigorously may cause agglutinates to be dispersed.
7. Deviation from the recommended test procedure.
8. Proteolytic enzymes may have a deleterious effect on components of anti-D. Accordingly, its use by one-stage enzyme test procedure (manual or automated) is not recommended.
9. Failure to add reagent.
10. The wrong reagent being used.
11. Warm or cold autoagglutinins causing immunoglobulin coating of the cells.
12. High plasma proteins causing rouleaux and spontaneous red cell aggregation.

I. Rh Typing Discrepancy

If there is a **discrepancy with the historical Rh type**, one or more of the following steps should be performed:

1. False positive Rh typing can result from warm or cold autoagglutinins, and high plasma proteins. Wash patient's cells (with warm saline if appropriate) and repeat Rh typing.
2. Check identification of all samples. Request for a second sample and repeat test if sample identification is suspect.
3. Investigate where, when and what methodology or reagent clone was used for the historical typing.
4. Check patient's clinical information to see if the patient received bone marrow/stem cells transplant, IUT, Exchange or Massive blood transfusion of a different Rh type from their own.

5. See table below, refer to flowcharts, and refer to section for computer resulting of Rh Negative Weak D Postive.

If Rh typing	then
Current and second specimens are Rh positive, no patient ID error	i) Repeat testing with different manufacturers' anti-D reagents. Unnecessary to carry to Weak D testing. ii) If patient tests negative or less than 2+ with one or more of the reagents, report Rh negative Weak D positive. iii) Give Rh negative blood products.
Current and second specimens are Rh negative, no patient ID error	Perform weak D testing.
Current and second specimens are Weak D positive, no patient ID error	i) Interpret as Rh negative Weak D positive. ii) Give Rh negative blood products.
Current and second specimens still differ from historical after further testing	i) Initial and second specimens are Rh negative after Weak D testing, report Rh negative. Historical sample may have been misidentified. ii) Initial and second specimens are Rh positive (2+ or greater at IS with all anti-D reagents), report Rh positive. Historical sample may have been misidentified.
Unresolved Rh Discrepancy	If discrepancy is still unresolved after testing, notify the supervisor (unless not on site) and give Rh negative blood products. Interpret as Rh 'Indeterminate' . Supervisor will review and decide if the Rh interpretation should be changed.

NOTE: For L&D patients identified to be Weak D positive, request additional sample for reference laboratory Weak D Analysis.

J. Management of Weak D Analysis

- Weak D Analysis is a test offered by Blood Center of Wisconsin and it includes serological typing with four different anti-D reagents and Rh(D) Genotyping. It is a useful tool to determine a patient's correct Rh(D) assignment in cases of historical typing discrepancy and RhIg candidacy.
- When the sample is received, order ABORh and BB Ref Lab Wkup.
- Perform tube ABORh testing. Enter 'Not Valid' for the D typing reaction with **Result Note: anti-D = ___** in LIS. Do not interpret or verify.
- Fill out the appropriate reference laboratory requisition and send sample to BCW. Make a copy and affix the RILIS labels on the retained copy.
- When the Weak D Analysis result is received, verify the ABORh according to the reference laboratory interpretation. Add the appropriate Result Comments and BB Comments.

Result Comments and Blood Bank Comment in PPI:

- a. SFO; D Genotype: Weak D Type ___ (**Rh Negative**); IS a candidate for

antenatal/postpartum RhIg and SHOULD receive Rh Negative blood.

- b. SFO; D Genotype: Weak D Type __ (**Rh Positive**); NOT a candidate for antenatal/postpartum RhIg and CAN receive Rh Positive blood.
6. Then result the BB Ref Wkup with freetext comments based on the reference laboratory report.
 7. Send a copy of the Reference Lab report to Medical Records to be scanned into the patient's chart.

PROCEDURE NOTE(S)

A. Repeat of Weak D Test

1. It is not necessary to perform weak D testing on each sample collected from a weak D positive patient. Interpret and enter as Rh negative but add 'Weak D positive' or appropriate template as Result Comments at ABORh Interpretation.
2. However, there must be two concurrent ABORh to qualify patient for computer crossmatch.

B. Rho(D) typing discrepancy in persons who do not have the entire complement of the D antigen, may be observed when different clones of anti-D and/or different methodologies are used.

C. RhIg Candidacy and Dosage

1. **Candidates for Rh immune globulin** are Rh negative (including Rh Negative Weak D Positive) or indeterminate and not immunized to Rho(D).
 - a. Pregnant female at prenatal 28 to 32 weeks gestation or abortion, miscarriage, ectopic pregnancy, antepartum hemorrhage, fetal death or amniocentesis.
 - b. Mother at post-partum, baby is Rh positive, weak D positive or Rh indeterminate.
 - c. Mother at post-partum, baby is Rh positive or weak D positive and mother has anti-D due to Rh immune globulin..
 - d. Females <50 years received Rh positive red cells contaminated platelets or granulocytes. Refer to SFOWI-0078 Platelet Transfusion and SFOWI-0115 Granulocyte Transfusion.

NOTE: Be cognizant when a D-negative mother who has apparent anti-D along with anti-C, that it may be anti-G and RhIg administration is recommended as precaution.

2. **Non-candidates for Rh immune globulin** are as follows:
 - a. Rh positive women.
 - b. Rh negative women who have Rh negative babies.
 - c. Rh negative women known to be immunized to Rho(D) antigen.
3. Rh immune globulin (RhIG) should be administered within 72 hours post Rh

Positive red cells exposure.

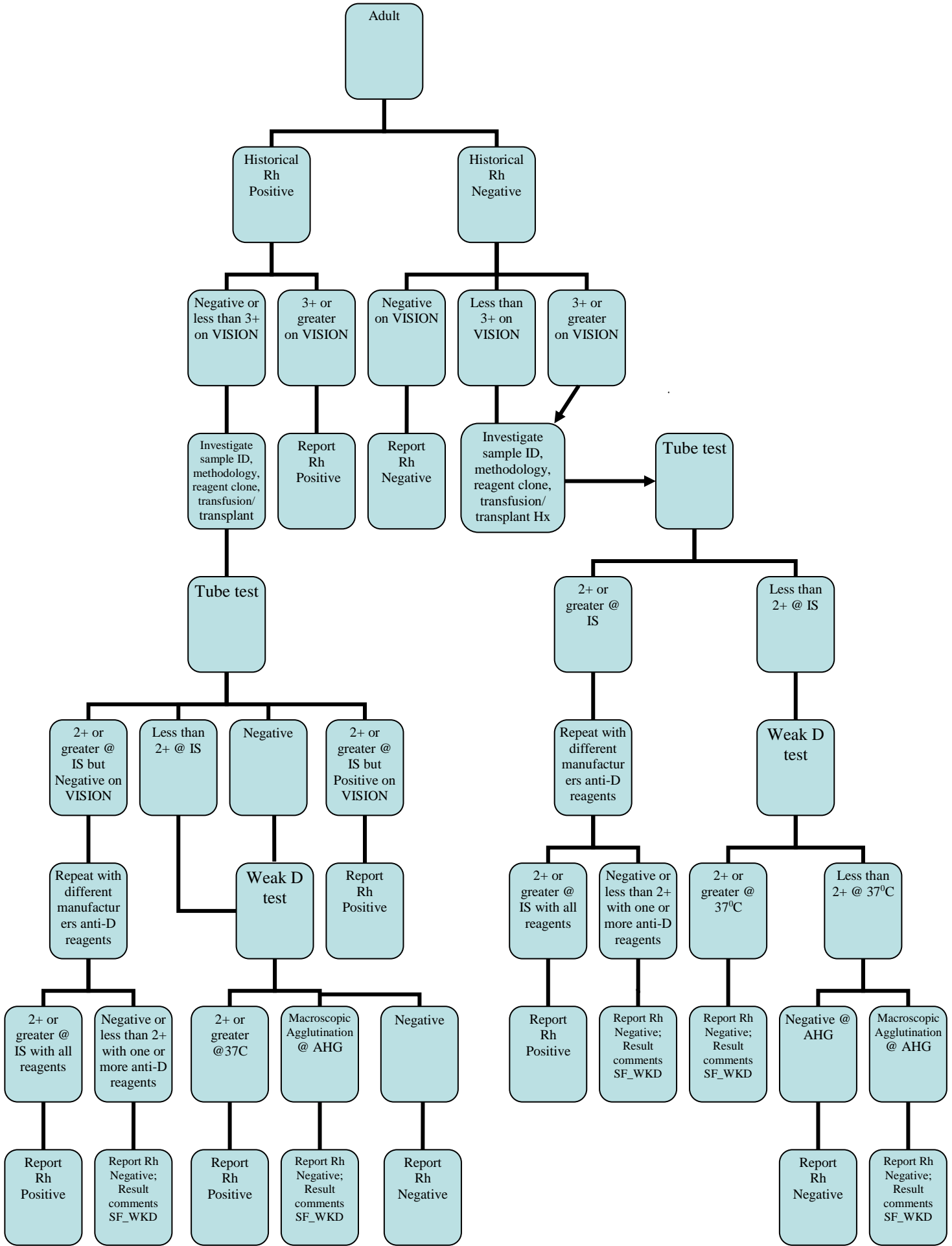
4. **Dosage**
 - a. A full dose (300ug) of RhIg or IV RhIg (both are obtained from pharmacy) would provide protection against 15 mL of red cells or 30 mL of whole blood.
 - b. A full dose (300ug) of RhIg or IV RhIg (both are obtained from pharmacy) would provide protection against red cells in 30 units of Rh positive platelet concentrates or 5 units of platelet pheresis.

5. **Selection of Rh Negative cellular blood products for Weak D Positive patients:**
 - a. These patients are reported as Rh Negative with 'Weak D Positive' as comments.
 - b. Rh Negative cellular blood products will be selected for routine transfusion.
 - c. Selection of Rh Positive cellular blood products will generate a LIS warning at the time of crossmatch or dispense.

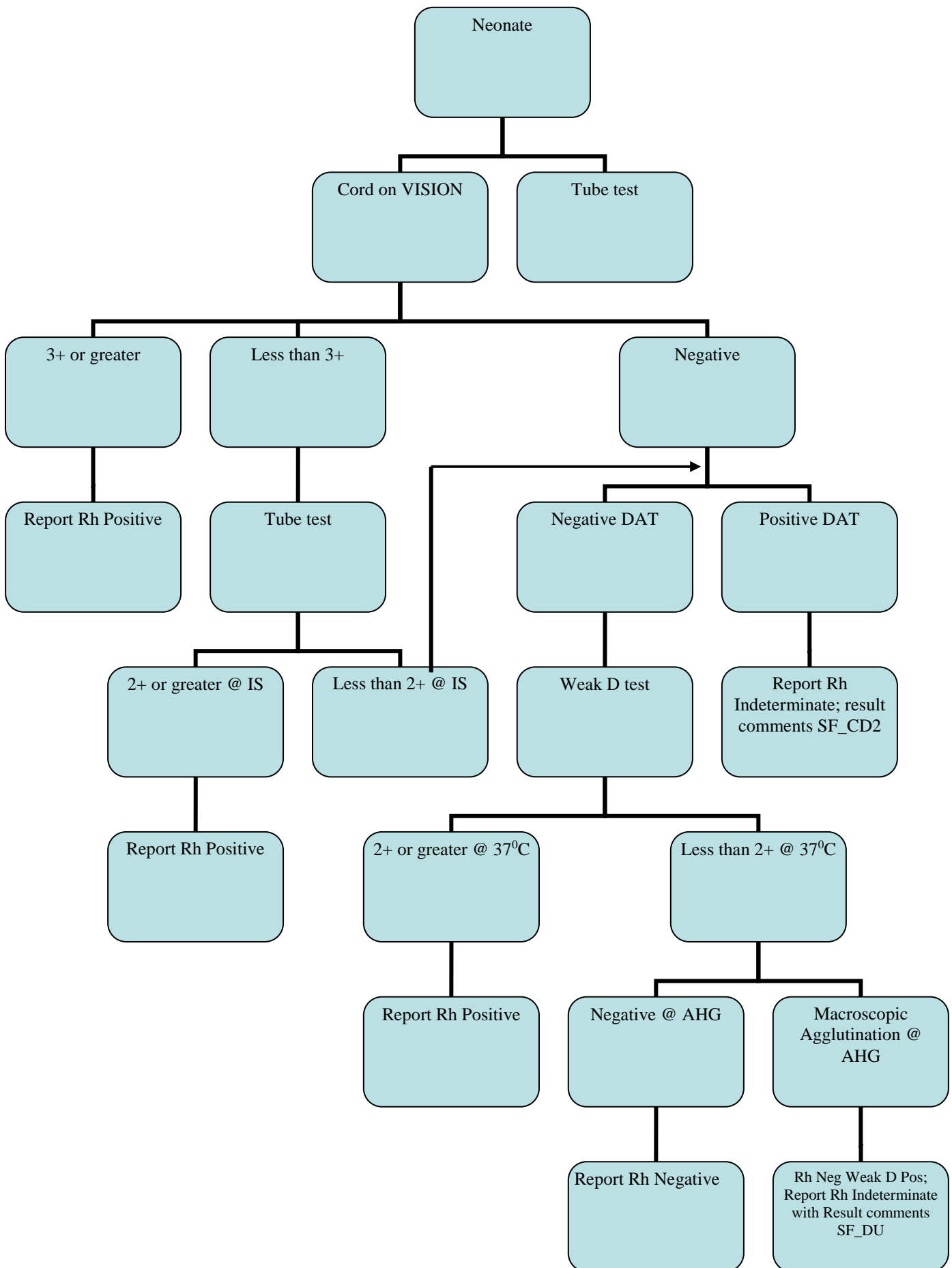
REFERENCE:

- A. AABB, Standards for Blood Banks and Transfusion Services, current edition, Bethesda, MD.
- B. AABB, Technical Manual, current edition, Bethesda, MD.

Adult Rho(D) Typing Flowchart for Patients with History



Neonate Rho(D) Typing Flowchart



Adult Rho(D) Typing Flowchart for Patients with NO History

