

**PURPOSE**

The presence of the RhD 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 Variant) 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. RhD 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. Cells that are Partial D Variant may or may not give a positive reaction depending on the missing epitope and the anti-D clone in the reagent used.

This procedure provides instructions for anti-D testing and details the workflow for managing RhD interpretations for different groups of patients based on age and gender.

**SCOPE**

Transfusion Service CLS trained on this procedure.

**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 hetero hybridomas 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. Vision Anti-D Testing****1. Female Patient 50 years and younger**

- a. All ABORh test should be tested on the Vision.
  - b. Tube testing may be used on patients who have:
    - i. a recent Vision 4+ anti-D result OR
    - ii. a pending D Genotype result OR
    - iii. a historical D Genotype result (Versiti's D Genotype result older than 2017 is unacceptable).
2. Refer to **Vision** SOP for operating instructions.
3. **Vision Results Interpretation**
- a. Refer to the table below and flowcharts for RhD interpretation.

**NOTE:** Any female patients 50 years and younger (except neonates) without a D Genotyping results (Versiti's D Genotype result older than 2017 is unacceptable) who has a RhD reaction that is less than 4+ (i.e. 1+, 2+, 3+) on the Vision will be reported as RhD Negative with Result Comment **SF\_WKD** until a final 'true type' can be determined through D genotyping.

**SF\_WKD:** Current serological testing indicates that the patient **may** be **Weak D Variant or Partial D Variant. Women of childbearing age should be considered Rh Negative and given RhIg** until the Weak D or Partial D status can be confirmed by molecular genotyping.

<b>Female 50 years and younger on Vision</b>	<b>Then</b>
Patient has a <b>historical D Genotype</b> (Versiti's D Genotype result older than 2017 is unacceptable)	Report RhD type that is consistent with the RhD Genotype result.
History of ABORh	Refer to <i>'Vision RhD Typing Flowchart for All Patients Except Neonates'</i> .
No History of D Genotype or ABORh Anti-D is <b>0</b>	Report RhD Negative.
No History of D Genotype or ABORh Anti-D is <b>1+, 2+ or 3+</b>	Report RhD Negative with Result Comment <b>SF_WKD</b> . Send out to Regional Lab for <b>RhD Geno</b> . Refer to <i>'Vision RhD Typing Flowchart for All Patients Except Neonates'</i> .
<b>Female Neonates</b>	Refer to <i>'Female Neonate Rho(D) Typing Flowchart for Vision'</i> .
Anti-D is <b>dp</b> (dual population or mixed field)	Perform tube test. Check recent tx history of <b>different</b> Rh RBC. Check BMT/ Stem Cells Tx / IUT / Exchange transfusion history. Refer to <i>SFO-WI.0104 Cord Blood Testing</i> for neonates. MF in cord blood may indicate contamination with mother's blood.
Rh Control is negative	Rh result is valid.
Rh Control is positive	Rh result is invalid. Wash cells and perform tube test.

<b>Female over 50 years and Male</b>	<b>Then</b>
Patient has a <b>historical D Genotype</b> (Versiti's D Genotype result older than 2017 is unacceptable)	Report RhD type that is consistent with the D Genotype result.
<b>Male Neonates</b>	Refer to <i>'Male Neonate Rho(D) Typing Flowchart'</i> .
Anti-D is <b>3+ or 4+</b>	RhD positive <b>unless</b> discrepant from historical. Investigate discrepancy. Refer to <i>'Vision RhD Typing Flowchart for All Patients Except Neonates'</i> .
Anti-D is <b>0, 1+ or 2+</b>	RhD negative <b>unless</b> discrepant from historical. Investigate discrepancy. Perform tube test. Refer to <i>'Vision RhD Typing Flowchart for All Patients Except Neonates'</i> .
Anti-D is <b>dp</b> (dual population or mixed field)	Perform tube test. Check recent tx history of <b>different</b> RhD RBC. Check BMT/ Stem Cells Tx / IUT / Exchange transfusion history. Refer to <i>SFO-WI.0104 Cord Blood Testing</i> for neonates. MF in cord blood may indicate contamination with mother's blood.
Rh Control is negative	Rh result is valid.
Rh Control is positive	Rh result is invalid. Wash cells and perform tube test.

**B. Tube Method:**

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

1. 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.
2. Add one drop of anti-D to a properly labeled test tube.
3. Add one drop of 3-5 % cell suspension to each tube.
4. 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.
5. Resuspend the cells by gentle agitation and examine **macroscopically** for agglutination using the agglutination viewer.
6. Record the test result immediately in the computer (Refer to Computer SOP).
7. 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.**
8. If no agglutination is observed, anti-D is nonreactive.
9. If agglutination is present, anti-D is reactive.
10. Refer to the appropriate flowcharts for interpreting the anti-D results.
11. For **neonates** or if test result is **doubtful**, proceed to **Weak D testing**.
12. See section for **Rh Typing Discrepancy** for resolution of discrepant results.

**C. 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. Refer to the appropriate **Rho(D) Typing Flowcharts** 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 with anti-D at immediate spin.
  - b. Doubtful RhD results.
  - c. RhD discrepancy investigation when needed.
3. **Repeat of Weak D Test**
  - a. It is not necessary to perform Weak D testing on each sample collected from a Weak D positive patient. Attach an appropriate template as Result Comments at ABORh Interpretation or free text.

**NOTE:** There must be two concurrent ABORh to qualify patient for computer crossmatch.

**D. 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 **Rh control is negative**, it is unnecessary to continue. **Refer to the appropriate Rho(D) Typing Flowcharts for interpretation.**
7. If reaction is **less than 2+**, proceed to the AHG 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 Rh control tube indicate a weak or partial D antigen. Refer to the appropriate *Rho(D) Typing Flowcharts* for interpretation.**
14. If **no** agglutination occurs, add **a drop of AHG Coombs control cells (Checkcell ®)** to each tube, then centrifuge.
  - a. If AHG Coombs control is positive, the test may be reported as RhD negative.
  - b. If AHG Coombs control is negative, repeat testing (increase wash cycles to 4 or perform manual wash).

## E. Flowcharts and Templates for RhD Interpretation

### 1. Flowcharts

Refer to the appropriate Rho(D) Typing Flowchart (see attachments) for guidance on interpreting anti-D results.

- a. **Female Neonate** Rho(D) Typing Flowchart for **Vision**
- b. **Female Neonate** Rho(D) Typing Flowchart for **Tube Test**
- c. **Male Neonate** Rho(D) Typing Flowchart
- d. **Vision RhD Typing** Flowchart for **All Patients Except Neonates**
- e. **Tube and Weak D Testing** Flowchart for **All Patients Except Neonates**

### 2. Templates to be attached as Result Comment and/or BB Comments in Millennium when appropriate:

- a. **SF\_DU:** Infant is Weak D Positive. Note: RHIG should be given to Rh Neg mother.
- b. **SF\_CD2:** Unable to interpret Rh due to Positive DAT. NOTE: RHIG should be given to Rh Negative mother.
- c. **SF\_WKD:** Current serologic testing indicates that patient may be Weak D Variant or Partial D Variant. Women of child bearing age should be considered Rh Negative and given RhIg until the Weak D or Partial D status can be confirmed by molecular genotyping.
- d. **SF\_Rh Pos:** Patient is confirmed as \_\_ by genotype performed at VERSITI Wisconsin Inc. on sample dated \_\_. For clinical purposes, this patient is considered as Rh Positive and is NOT a candidate for antenatal/postnatal RhIg and can receive Rh Positive blood.
- e. **SF\_Rh Neg:** Patient is confirmed as \_\_ by genotype performed at VERSITI Wisconsin Inc. on sample dated \_\_. For clinical purposes, this patient is considered as Rh Negative and IS a candidate for antenatal/postnatal RhIg and should receive Rh Negative blood.
- f. **SF\_chgDPos:** Due to change in test grading criteria for females older than 50 years of age and adult males, this patient is now considered Rh Positive.

## F. Computer Results Entry for RhD Negative or RhD IND when Anti-D is Reactive

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

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

1. **RhD Negative**

**ADULTS Only**

- a. To interpret as **RhD Negative when anti-D reaction is positive**, remove the positive reaction and enter 'Not Valid' with **Result Note: anti-D = \_\_\_\_**.
- b. Interpret as **RhD Negative** and add template **SF\_WKD as Result Comments at ABORh interpretation** field. Refer to flowcharts for details.

2. **RhD Indeterminate**

**NEONATES and ADULTS**

**STEP I:**

- a. Enter results for Anti-A, Anti-B, Anti-D, D Con. Leave the following fields blank: Weak D, Con AHG, Wk D CC, Con CC.
- b. Interpret as **RhD Indeterminate** and add template **SF\_DU as Result Comments at ABORh interpretation** field. Refer to flowcharts 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 test for neonates and if performed on adults.
- b. Verify results.

G. **RhD Typing Discrepancy**

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

1. False positive RhD typing can result from warm or cold autoagglutinins, and high plasma proteins. Wash patient's cells (with warm saline if appropriate) and repeat RhD typing.
2. Check identification of all samples. Request for a second sample and repeat test if sample identification is suspect.
3. Perform tube anti-D test. Continue to Weak D test if necessary.
4. Investigate where, when and what methodology or reagent clone was used for the historical typing.
  - a. Weak or variable results with different anti-D reagents suggest the presence of a variant anti-D antigen expressing either quantitative or qualitative antigenic differences.
  - b. In the absence of recent RBC transfusion or HSCT history, compare current anti-D reaction to previous results of the same methodology and reagent clone to determine if sample ID error is the cause of the RhD discrepancy.
5. 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 RhD type from their own.
6. For RhD interpretations, see table below and refer to flowcharts. For bone marrow/stem cells transplant patients, refer to **SFO-WI.1309 TRANSFUSING PATIENTS POST-HSCT**.

**NOTE:** For L&D patients suspected to be Weak/Partial D positive, request additional sample for D Genotype test.



If Rh typing	then
Current and second specimens have the same anti-D results but differ from historical	Historical sample was misidentified. Report RhD based on current anti-D result with the appropriate Result Comments and BB Comments. Enter workup results as Result Note. Update the global RhD type.
Unresolved RhD Discrepancy	If the RhD discrepancy is <b>unresolved</b> after further testing, notify Supervisor (unless not on site) and <b>give RhD negative blood products (add BB Comments). Interpret as RhD 'Indeterminate'</b> . Enter workup results as Result Note. <b>Update the global RhD type to Indeterminate.</b> Supervisor will review and decide if the RhD interpretation should be changed.

H. **Management of Weak D Analysis (D Genotype)**

Weak D Analysis is a test offered by Versiti (formerly Blood Center of Wisconsin) and it includes serological typing with four different anti-D reagents and RhD Genotyping. It is a useful tool to determine a patient’s correct RhD assignment in cases of historical typing discrepancy and RhIg candidacy. **NOTE:** Regional Lab is responsible to send out D Genotype test to Versiti.

1. When the sample is received, order **RhD Geno**. **If DBCK is needed**, order ABORh with collection time 1 minute later to obtain a different Acc#.
2. Affix the ABORh Acc# label on tube and perform testing on Vision.
3. After completion of testing, affix the **RhD Geno** Acc# label over the ABORh label on tube.
4. Give sample to Lab Assistant to send out to **MWS Regional Lab Richmond**.
5. Document send-out in the BB **‘Reference Lab Sendout Tracking Log’** binder.
6. Once the genotype results are received by the Regional Lab, the patient’s demographic (global) RhD type will be updated if needed, to be consistent with the D genotype. **NOTE:** The genotype will be considered the true type and will not be changed for any reason other than potential stem cell transplant.
7. Swing Shift CLS will review the binder every Thursday and follow up with Regional Lab on any pending D Genotype tests.
8. All subsequent RhD type will be reported consistent with the D Genotype result. For the first local ABORh test after the D Genotype, add the appropriate Result Comments and BB Comments (if none).

Result Comments and Blood Bank Comments in PPI:

- a. **SF\_Rh Pos:** Patient is confirmed as \_\_ by genotype performed at VERSITI Wisconsin Inc. on sample dated \_\_. For clinical purposes, this patient is considered as Rh Positive and is NOT a candidate for antenatal/postnatal RhIg and can receive Rh Positive blood.

- b. **SF\_Rh Neg:** Patient is confirmed as \_\_\_ by genotype performed at VERSITI Wisconsin Inc. on sample dated \_\_\_. For clinical purposes, this patient is considered as Rh Negative and IS a candidate for antenatal/postnatal RhIg and should receive Rh Negative blood.

**I. Selection of RhD Negative cellular blood products for RhD Indeterminate and RhD Negative Weak D Positive patients:**

1. **Female patients who are 50 years old and younger (except for neonates)** are reported as RhD Negative with **SF\_WKD** as comments until a final ‘true type’ can be determined through D genotyping.
2. RhD Negative cellular blood products will be selected for routine transfusion.
3. Selection of RhD Positive cellular blood products will generate a LIS warning at the time of crossmatch or dispense.
4. Refer to *SFO-WI-0089 Compatibility Testing* for more information.

**J. 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.

**K. RhIg Candidacy and Dosage**

1. **Candidates for Rh immune globulin (RhIg) are female of child bearing age and RhD Negative (including Rh Negative Weak D Positive) or RhD Indeterminate and not immunized to RhD antigen.**
  - 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 RhD Positive, Weak D Positive or RhD Indeterminate.
  - c. Mother at post-partum, baby is RhD Positive or Weak D Positive and mother has anti-D due to Rh immune globulin.



- d. Females  $\leq 50$  years who received RhD Positive red cells contaminated platelets or granulocytes. Refer to *SFOWI-0078 Platelet Transfusion* and *SFOWI-0115 Granulocyte Transfusion*.

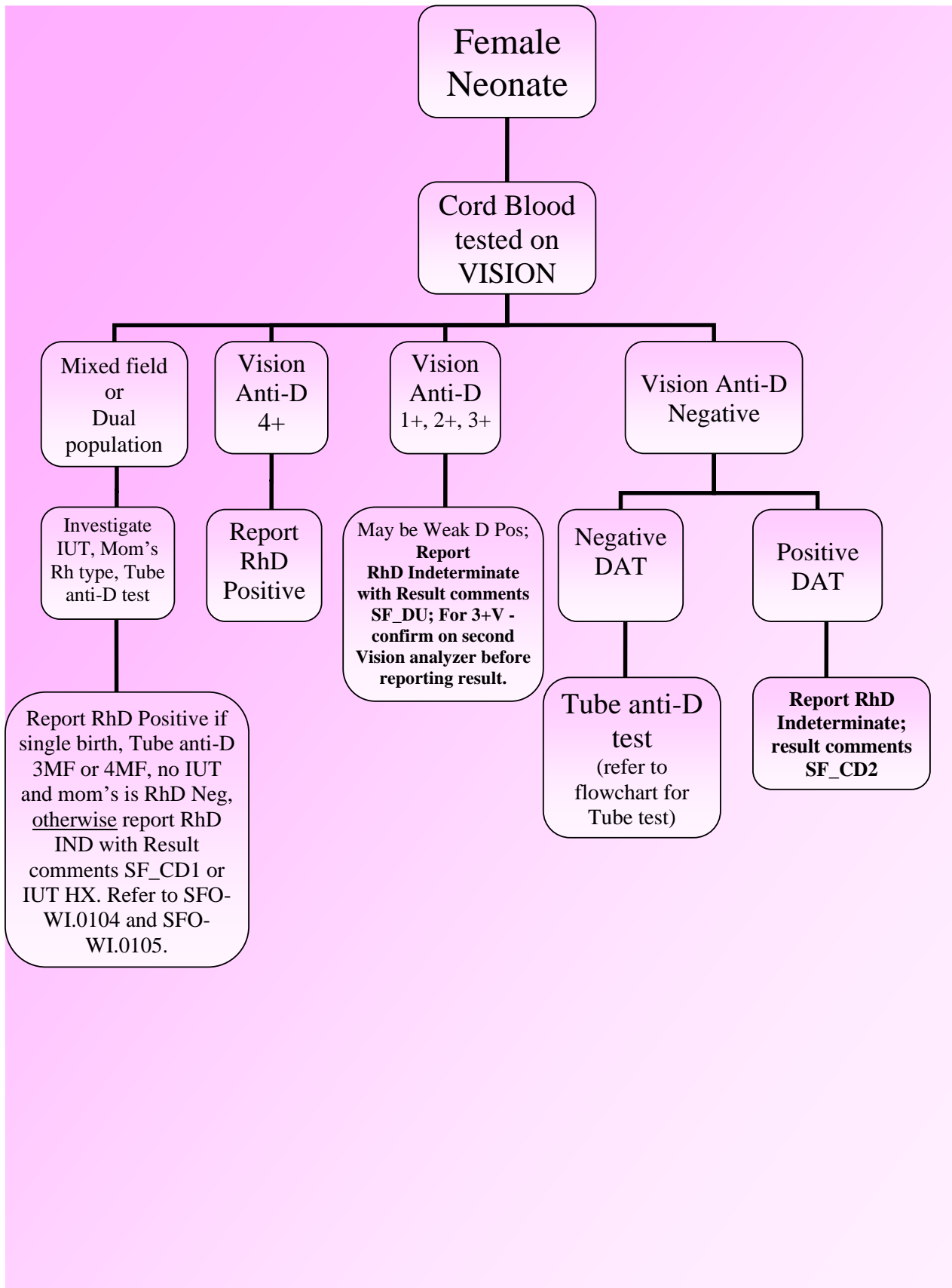
**NOTE:** Be cognizant when a RhD Negative mother who has an apparent anti-D along with anti-C, that it may be anti-G and RhIg administration is recommended as precaution.

2. **Non-candidates for Rh immune globulin** are as follows:
  - a. RhD positive women.
  - b. RhD negative women who have RhD negative babies.
  - c. RhD negative women known to be immunized to RhD antigen.
3. Rh immune globulin (RhIG) should be administered within 72 hours post RhD Positive red cells exposure.
4. **RhIg 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.
  - c. **Postpartum Fetal Screen** for additional RhIg is sent to NCAL Regional Lab.

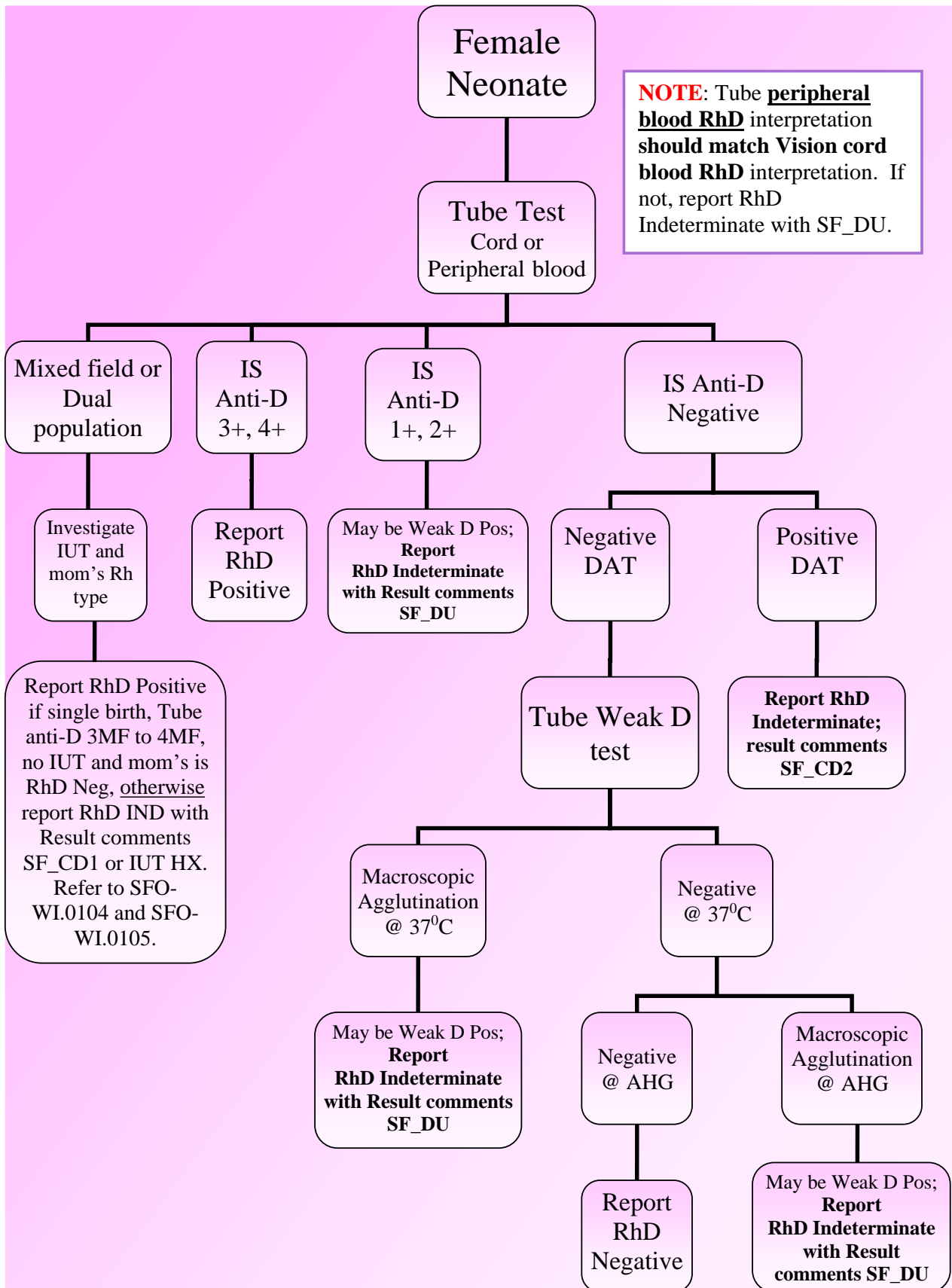
**REFERENCE:**

- A. AABB, Standards for Blood Banks and Transfusion Services, current edition, Bethesda, MD.
- B. AABB, Technical Manual, current edition, Bethesda, MD.
- C. RWLQC-SOP.0296 Rh D Genotyping Prenatal or Postpartum Workflow.
- D. Lippincott Procedures. Kaiser Permanente Northern California.  
<https://procedures.lww.com/lnp/view.do?pId=1824637&disciplineId=1090>

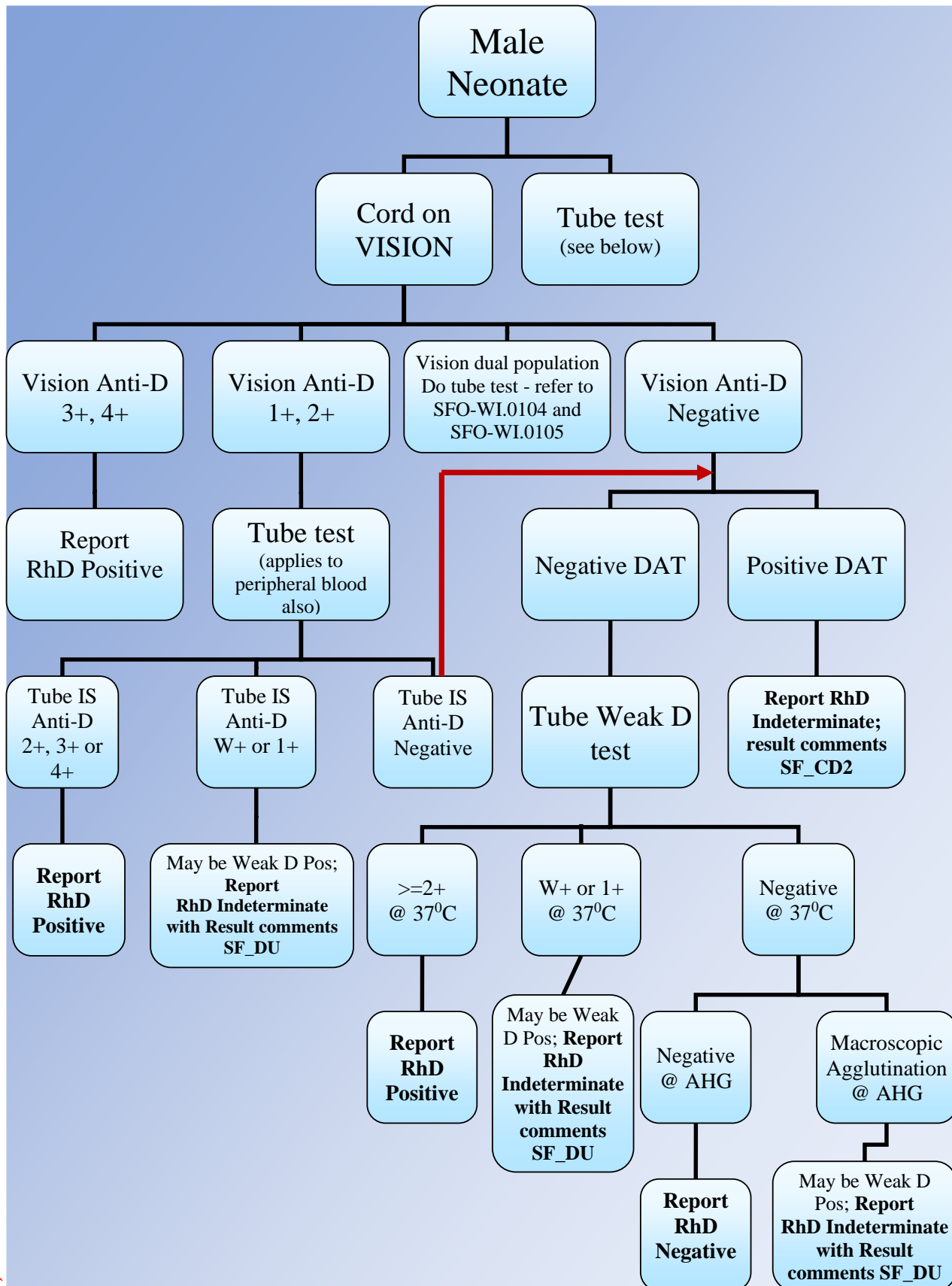
**Female Neonate Rho(D) Typing Flowchart for Vision**



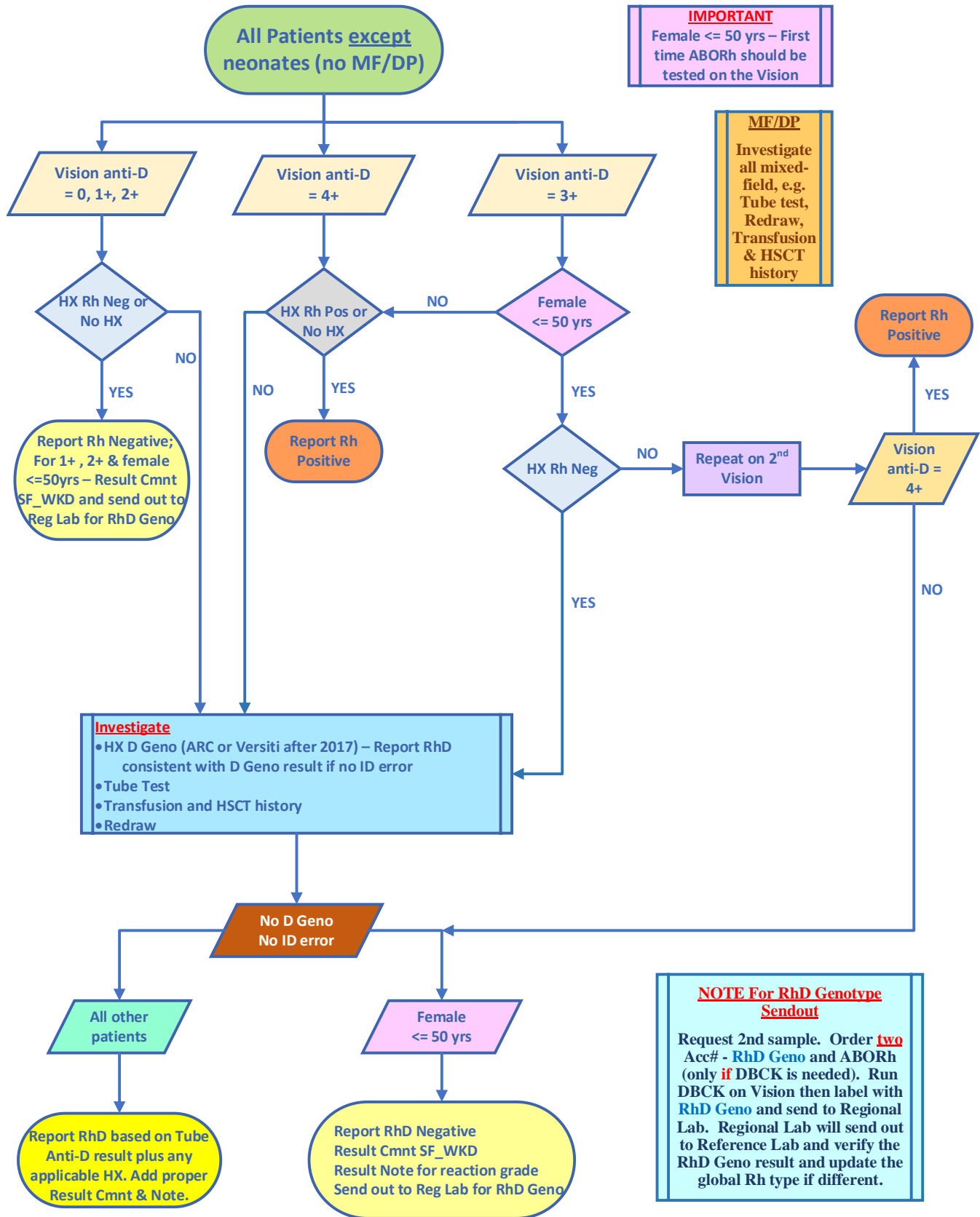
**Female Neonate Rho(D) Typing Flowchart for Tube Test**



**Male Neonate Rho(D) Typing Flowchart**



**Vision RhD Typing Flowchart for All Patients Except Neonates**



**Tube and Weak D Testing Flowchart for All Patients Except Neonates**

