Alloimmunization

[October 2025 (replaces December 2023)]

Author: **Dr. Carey Eppes**, Dr. April Adams
Editor: Dr. Ingmar Bastian

Rhesus antigens were added to the tables; a critical titer for Anti-Kell and Anti-c (little c) was changed to ≥1:4. and cfDNA section was updated with an example of pretest counseling. The figures have been updated to reflect these changes and for increased visibility.

Highlights

- The threshold titer at which there is an increased risk of development of fetal hydrops is 1:16 for almost all antigens. The critical titer for Anti- Kell and Anti-Little c is 1:4.
- Titers should only be monitored for the first affected pregnancy (the pregnancy in which the antibodies are first detected). Titer monitoring is not recommended in subsequent affected pregnancies and MCA Doppler studies should be performed.
- Paternal Antigen status should be obtained (if possible) in the first affected pregnancy
- cfDNA for RhD status is a not recommended as the first line evaluation in an alloimmunized pregnancy.

Background and Definitions

Red cell alloimmunization refers to an immune response following exposure to foreign red cells, resulting in formation of red cell antibodies. It has become a rare event during pregnancy in the United States in recent years, with the prevalence reported for rhesus alloimmunization as 6.8 per 1,000 live births.[1,2] Other reported blood groups associated with mild to severe risk of fetal anemia include those listed in Table 1 and Table 2.

The formation of maternal RBC antibodies may lead to various degrees of transplacental passage of these antibodies into the fetal circulation; **note that IgG phase antibodies can cross the placenta, IgM do not to a large degree.** Lewis (Le^a and Le^b) and I antibodies do not cause HDFN because they are predominantly of the IgM type, and thus do not require additional evaluation and management. Depending on the degree of antigenicity, amount and type of antibodies involved, this transplacental passage may lead to hemolytic disease in the fetus and neonate.[3] The terminology used in determining fetal risk involves discussion of **antigen status** (i.e., phenotype) and genotype and dosage of the implicated antigen (or zygosity) in the father's genome.

Titer threshold

Titers of antibodies are determined with indirect coombs tests to determine the degree of alloimmunization and therefore the risk of hemolytic disease in the fetus. Variation between laboratories is common, but in the same laboratory, the titers should not vary by more than one dilution. A critical titer is defined as the titer associated with a significant risk for fetal hydrops**. In our hospitals, 1:16 is a critical titer for all antigens except anti-Kell and anti-little c, which is critical at 1:4**. Notably, a rise of three or more titers can signify active immune sensitization.

Kell Alloimmunization

The Kell blood group is one of the most common of the minor RBC antibodies. Unlike RhD, the titer of anti-Kell antibodies may not correlate well with the degree of fetal anemia. Therefore, most experts recommend increased surveillance once a Kell titer of 1:4 is reached.[1,2,4,5] Anti-K antibodies are most commonly acquired through blood transfusion, therefore paternal testing is very important in determining fetal risk (i.e., if the father is Kell negative, then the fetus is not at risk as long as paternity is assured).

Table 1. Blood Groups with Mild Risk for Hemolytic disease of the fetus/neonate (HDFN)

Blood Group System	Antigens Related to Hemolytic Disease	Hemolytic Disease Severity
Kell	k	Mild
Duffy	By^3	Mild
Kidd	Jk^b	Mild
	Jk^3	Mild
Rhesus (Rh)	C	Mild
	e	Mild
MNSs	N	Mild
Lutheran	Lu^a	Mild
	Lu^b	Mild
Xg	Xg^a	Mild
	Yt^b	Mild
	Lan	Mild
	Ge	Mild
	Jr^a	Mild
	Co^{1-b-}	Mild
Private Antigens	Batty	Mild
	Becker	Mild
	Berrens	Mild
	Evans	Mild
	Gonzales	Mild
	Hunt	Mild
	Jobbins	Mild
	Rm	Mild

	Ven	Mild
	Wright[b]	Mild

Adapted from ACOG Practice Bulletin and Creasy (3-4).

Table 2. Blood Groups with Moderate or Severe Risk for HDFN

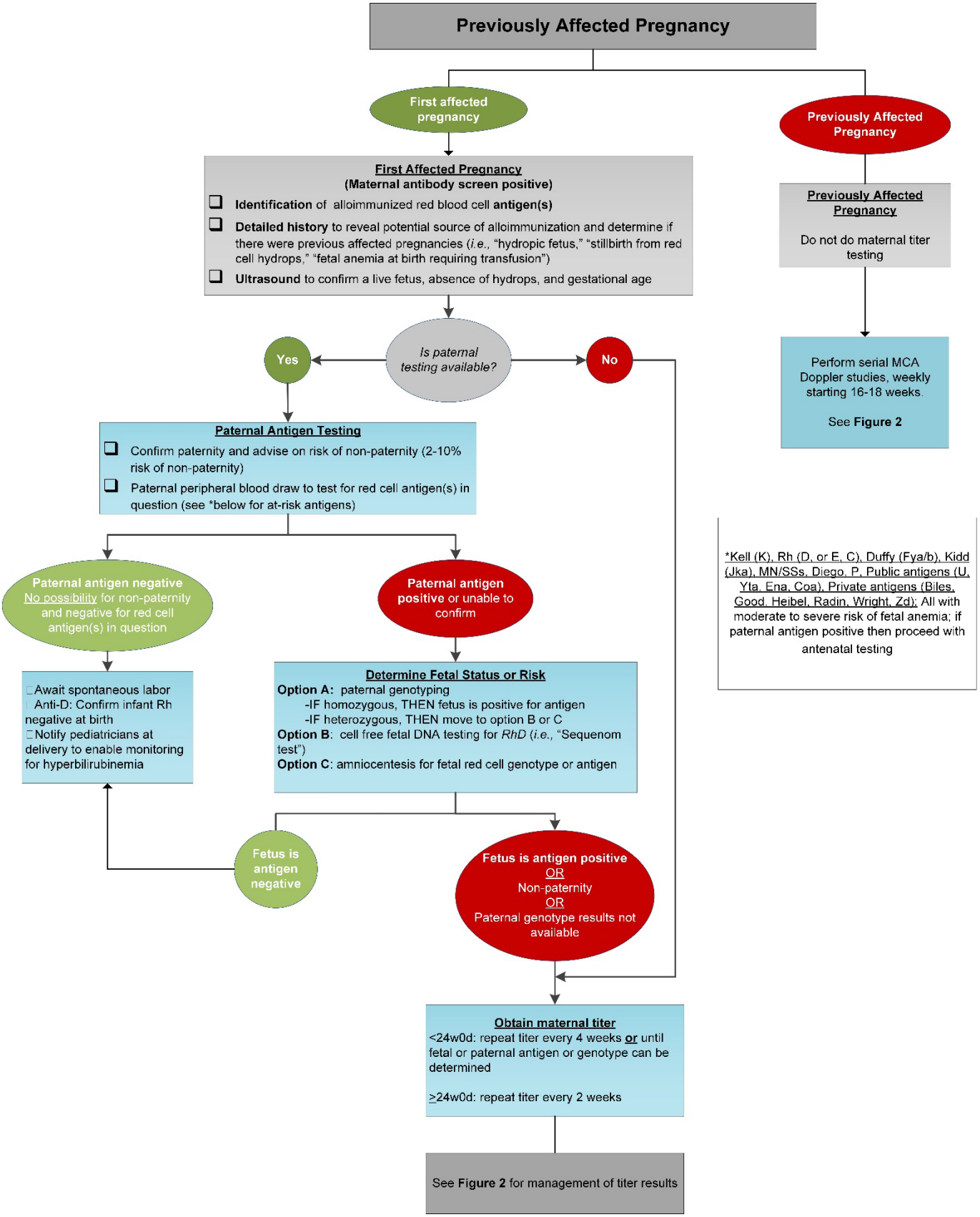
Blood Group System	Antigens Related to Hemolytic Disease	Hemolytic Disease Severity
Kell	K	Mild to Severe
Rhesus (Rh)	D	Severe
	c	Mild to Severe
	E	Mild to Severe
	C	Mild to Severe
Duffy	Fy[a]	Mild to severe
Kidd	Jk[a]	Mild to Severe
MNSs	M (only if IgG phase)	Mild to Severe
	S	Mild to Severe
	s	Mild to Severe
	U	Mild to Severe
	Mi[a]	Moderate
MSSs	Mt[a]	Moderate
Diego	D1[a]	Mild to Severe
	Di[b]	Mild to Severe
P	PP_{1pk}	Mild to Severe
Public Antigens	Yt[a]	Moderate to Severe
	En[a]	Moderate
	Co[a]	Severe
Private Antigens	Biles	Moderate
	Good	Severe
	Heibel	Moderate
	Radin	Moderate
	Wright[a]	Severe
	Zd	Moderate

Adapted from ACOG Practice Bulletin and Creasy (3-4).

Management[6]

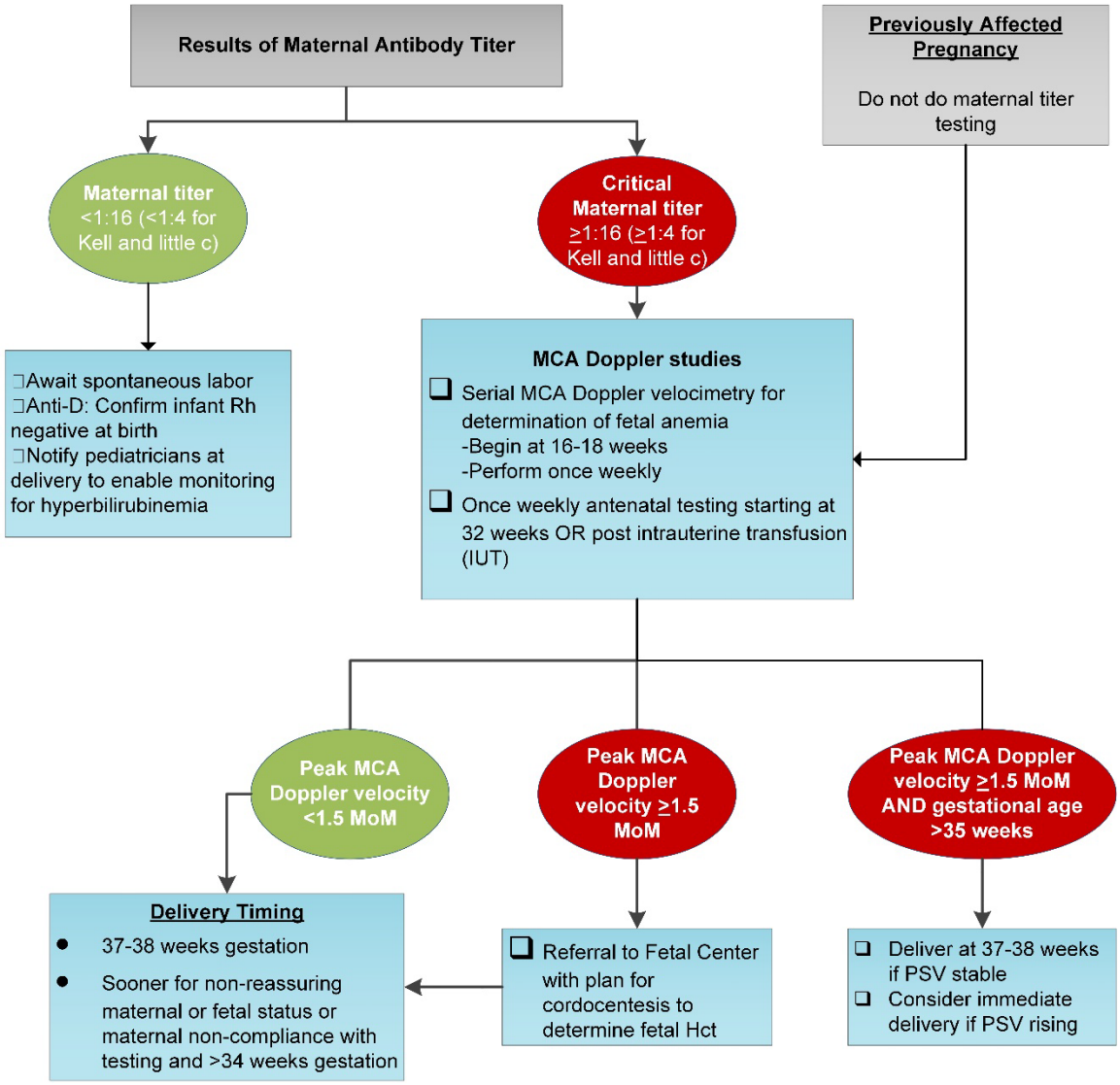
See Figure 1 and Figure 2 for an overview of management.

Figure 1. Management of Alloimmunization



[1] The first affected pregnancy is the pregnancy in which the anti-red cell antibody is first detected. A pregnancy does not require adverse sequelae or HDFN to be categorized as affected.

Figure 2. Management based on maternal antibody titers and in previously affected pregnancies



Testing

If the assessment of maternal type and screen reveals evidence of alloimmunization (i.e., a positive type and screen), the blood bank will reflexively report the red blood cell antigen and titers.

History and non-paternity

A detailed maternal history is important to identify potential sources of alloimmunization (i.e., previous blood transfusions, paternity, and previously affected pregnancies). Details such as a previous hydropic fetus, stillbirth, or fetal anemia requiring red cell or exchange transfusion indicate a previously affected pregnancy. It has been reported that there is a 2-10% risk of non-paternity, and as this is a sensitive topic patients should be counseled carefully on the fetal risks of such. The history can help determine the likelihood of non-paternity as well: if the patient has no prior history of transfusion and states she has never been pregnant with another partner, yet this partner is antigen negative, then non-paternity must be suspected.

Paternal antigen testing

Once maternal type and screen reveals evidence of alloimmunization, paternal antigen testing is recommended. In the case of RhD sensitization, the first step should be paternal antigen testing followed by paternal genotyping for RhD. Genotyping is recommended over paternal antigen status to provide dosage (homo- versus hetero-zygous) and due to the possibility of the RhD pseudogene. If the paternal antigen is positive, then genotyping is recommended for RhD zygosity. If the father is homozygous, then the fetus is presumed to be at risk. If the father is heterozygous, then the next recommended step is cell free fetal DNA testing. If this is not possible, then amniocentesis for fetal RBC RhD genotype should be offered/recommended. If the father is not available for testing, then we recommend proceeding directly to maternal antibody titers (if there is no history of previously affected pregnancies) and initiation of MCA Dopplers once a critical titer is reached.

For other RBC antigens, if the paternal antigen is positive for the RBC antigen in question, then genotype testing should be done. Homozygous paternal genotype indicates the fetus has a 100% chance of inheriting the antigen. Heterozygosity indicates a 50% risk and therefore the next steps include evaluation of the fetal antigen status via amniocentesis and/or MCA Dopplers.

Fetal non-invasive diagnostic testing

Cell Free Fetal DNA (cfDNA, aka NIPT for fetal RHD genotyping: This technique determines the fetal RhD status in maternal plasma. A recent meta-analysis[7] demonstrated that NIPT specifically for fetal *RhD* genotyping was estimated highly sensitive/specific beyond 11-weeks gestation. Two thoughts were of note: (1) amplifications from ≥2 exons are optimum to increase accuracy, and (2) the diagnostic accuracy of fetal RhD genotyping in non-white populations is unknown. This latter statement is supported by a second meta-analysis from 2019 (7) and follows the prior discussion in this guideline highlighting that the presence of the *RhD* pseudogene can vary by African descent. Ergo, prenatal detection of fetal RhD type from maternal blood could lead to higher rates of false positive results in this particular population; more research is needed in non-white populations.[7,8] Thus, while cfDNA permits cost-effectiveness, precious resources sparing, and low emotional stress testing, due to limited evidence, the accuracy of cfDNA in non-white people and multiple pregnancies is unclear at present.[7,8] When available and interpreted with confidence, this test replaces the need for amniocentesis or cordocentesis. It also circumvents issues with self-reported paternity assessment by directly assessing fetal status.[7] **While single gene cfDNA testing is available, there is currently not enough evidence to recommend it as a replacement for the current screening and testing algorithm.** Pre-test counseling is recommended before the use of the test and shared decision making regarding the clinical management of the result (Box 1).

Box 1. *Sample Counseling for Use of cfDNA for RhD genotyping*

We discussed the limitations of the cfDNA screening test and that amniocentesis is required for definitive testing. Alternatively, when paternity is certain, paternal testing can be performed. We also noted that cfDNA can yield false-negative results (indicating an RhD-negative fetus when it is actually positive) and false-positive results (indicating an RhD-positive fetus when it is actually negative).

In cases of false-negative testing, there is the risk of undetected fetal anemia and its associated risks. Conversely, a false-positive test poses a risk of unnecessary intervention. Another limitation discussed is that cfDNA is less accurate for certain RhD variants, especially in individuals of non-European ancestry who may have different genetic variations. Additionally, there is a relative lack of large-scale clinical validation studies demonstrating the test's effectiveness for detecting fetal RhD status in widespread clinical practice. Therefore, while NIPT is an excellent screening test, some potential limitations exist.

Lastly, we reviewed the standard management for women with RhD alloimmunization. Anti-D antibody levels are serially measured, usually monthly until 24 weeks of gestation, and then every two weeks. When a critical titer is reached, weekly antenatal testing is performed, and delivery planning is based on the outcome of this testing.

cfDNA is a good option for patients who feel comfortable with its limitations and wish to avoid the intensive surveillance involved. The patients' questions were answered, and she decided to proceed with standard surveillance.

Middle Cerebral Artery (MCA) Doppler Studies

Middle cerebral artery peak systolic velocity (MCA-PSV) Doppler >1.5 MoM have been found to have 100% sensitivity and a 12% false positive rate for predicting fetal anemia before 35 weeks. After 35 weeks, MCA Doppler appears to have reduced sensitivity and there are no evidence-based recommendations to guide management. **The SMFM algorithm for management of fetuses at risk for anemia includes continuation of MCA-PSV Doppler interrogation after 35 weeks, with delivery if the Dopplers are ≥1.5 MoM and the PSV trend is increasing.**[6]

Indications for delivery

As outlined in Figure 2, with the need to employ best clinical judgment.

References

References

1. Castleman JS, Kilby MD. Red cell alloimmunization: A 2020 update. *Prenat Diagn*. Aug 2020;40(9):1099-1108. doi:10.1002/pd.5674
2. Moise KJ, Jr., Argoti PS. Management and prevention of red cell alloimmunization in pregnancy: a systematic review. *Obstet Gynecol*. Nov 2012;120(5):1132-9. doi:10.1097/aog.0b013e31826d7dc1
3. ACOG Practice Bulletin No. 192: Management of Alloimmunization During Pregnancy. *Obstet Gynecol*. Mar 2018;131(3):e82-e90. doi:10.1097/AOG.0000000000002528
4. van Wamelen DJ, Klumper FJ, de Haas M, Meerman RH, van Kamp IL, Oepkes D. Obstetric history and antibody titer in estimating severity of Kell alloimmunization in pregnancy. *Obstet Gynecol*. May 2007;109(5):1093-8. doi:10.1097/01.AOG.0000260957.77090.4e
5. Moise KJ, Jr., Abels EA. Management of Red Cell Alloimmunization in Pregnancy. *Obstet Gynecol*. Oct 1 2024;144(4):465-480. doi:10.1097/aog.0000000000005709
6. Moise KJ, Jr., Queenan J. Hemolytic Disease of the Fetus and Newborn. *Creasy and Resnik's Maternal-Fetal Medicine*. 9th Edition ed. Elsevier; 2023:634-649.e3:chap 35.
7. Alshehri AA, Jackson DE. Non-Invasive Prenatal Fetal Blood Group Genotype and Its Application in the Management of Hemolytic Disease of Fetus and Newborn: Systematic Review and Meta-Analysis. *Transfus Med Rev*. Apr 2021;35(2):85-94. doi:10.1016/j.tmrv.2021.02.001
8. Yang H, Llewellyn A, Walker R, et al. High-throughput, non-invasive prenatal testing for fetal rhesus D status in RhD-negative women: a systematic review and meta-analysis. *BMC Med*. Feb 14 2019;17(1):37. doi:10.1186/s12916-019-1254-4