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When should maternal testing for red cell antigens by cffDNA be considered?

Dr. Barbra de Vrijer
Maternal Fetal Medicine
London Health Sciences Centre, Ontario

Faculty Disclosure

In compliance with CPD policy, Temerty Faculty of Medicine requires the following disclosures to the session audience

- This program has received no external financial support
- This speaker has received no external financial support

Objectives

- Discuss the benefits of routinely performing cffDNA testing for Rh negative pregnant patients to assess the fetal Rh status to allow targeted RhIg prophylaxis
- Discuss the situations when alloimmunized patients should have cell-free DNA testing performed
- Discuss practical aspect of cell-free DNA testing including samples required, cost, how to access coverage for patients
- Discuss recommendations for routine testing and plans for the future

Statement 21

STATEMENT 21

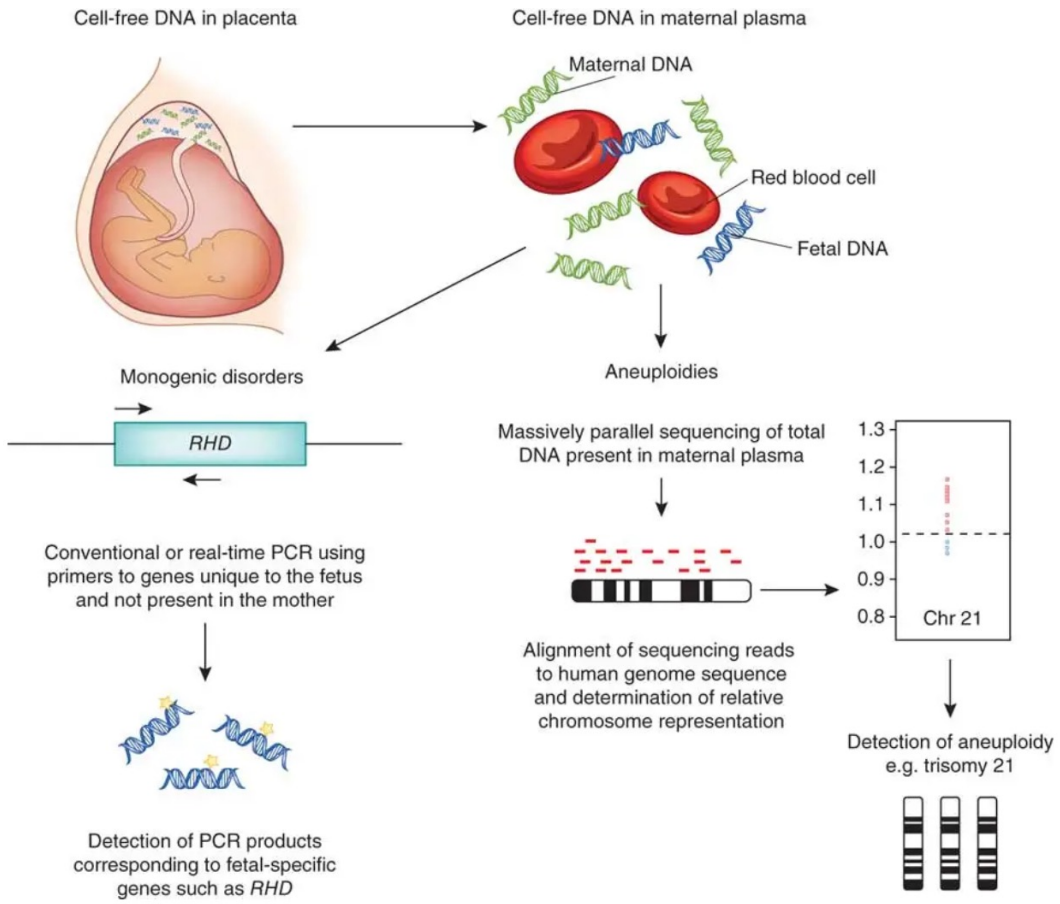
For patients with clinically significant antibodies (to RhD, C/c, E, or K), cell free fetal DNA testing (non-invasive perinatal testing) should be performed when feasible. Optimal timing of such testing will be determined by the testing program.

References:

de Haas M, Thurik FF, Koelewijn JM, van der Schoot CE. Haemolytic disease of the fetus and newborn. Vox Sang. 2015;109(2):99–113.

Scheffer PG, Van Der Schoot CE, Page-Christiaens GCML, De Haas M. Noninvasive fetal blood group genotyping of rhesus D, c, e and of K in alloimmunised pregnant women: Evaluation of a 7-year clinical experience. BJOG An Int J Obstet Gynaecol. 2011;118(11):1340–1348.

Non-invasive Fetal RhD genotyping



- Cell-free fetal DNA in maternal circulation
- Same technology as NIPT for aneuploidies
- Detectable as early as 7weeks GA
- As sensitive as postnatal serology
- Reduction of antenatal RhIg administration by 40% (22,000 individuals in Canada)

Non-Invasive Fetal RhD genotyping

- Successfully implemented nationally for RhD negative pregnancies in several European countries (Denmark, England, Finland, Netherlands, Sweden)
  - health system cost-savings
  - conservation of human blood product resources
  - reductions in unnecessary exposure to human blood products, overtreatment and associated burdens for pregnant individuals.

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ONTARIO GENETICS ADVISORY COMMITTEE

Noninvasive Fetal RhD Blood Group Genotyping: A Health Technology Assessment

Noninvasive Fetal RhD Blood Group Genotyping: A Systematic Review of Economic Evaluations

Olga Gajic-Veljanoski, MD, PhD;[a] Chunmei Li, MMI;[a] Alexis K. Schaink, MPH;[a] Jennifer Guo, MA
Caroline Higgins, MISt;[a] Nadine Shehata, MD, MSc;[b] Nanette Okun, MD, MHScAdmin;[c]
Barbra de Vrijer, MD;[d] Petros Pechlivanoglou, PhD;[e] Vivian Ng, MSc, PhD;[a] Nancy Sikich, RN, M

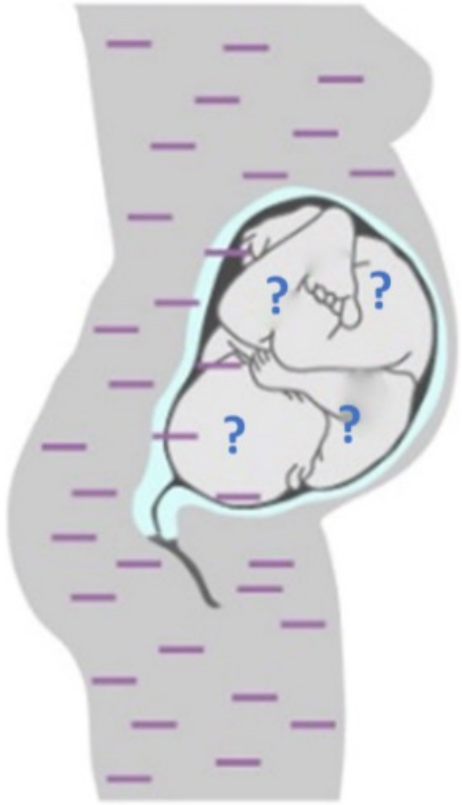
Cost-effectiveness of noninvasive fetal RhD blood group genotyping in nonalloimmunized and alloimmunized pregnancies

Olga Gajic-Veljanoski[1] | Chunmei Li[1] | Alexis K. Schaink[1] | Jennifer Guo[1] | Nadine Shehata[2] | George S. Charames[3,4] | Barbra de Vrijer[5] | Gwen Clarke[6] | Petros Pechlivanoglou[7] | Nanette Okun[8] | Rita Kandel[3] | Joseph Dooley[9] | Caroline Higgins[1] | Vivian Ng[1] | Nancy Sikich[1]

Assess the accuracy, clinical impact and cost effectiveness of non-invasive fetal genotyping in non-alloimmunized and alloimmunized pregnancies

Non-alloimmunized pregnancy

(RhD negative pregnancy with negative antibody screen)



Adapted from Encyclopaedia Britannica, Inc.
https://www.britannica.com/science/Rh-blood-group-system

Prevention of alloimmunization

Prevention of development of antibodies against RhD (anti-D) in RhD negative pregnancies:

No RhIG: 12-16%

Only postnatal RhIG: 1.6-1.9%

Postnatal *and* antenatal RhIG: 0.02%

Non-alloimmunized testing:

cfDNA for RhD prevents unnecessary antenatal administration of RhIG to 40% of RhD negative pregnancies

Alloimmunized pregnancy

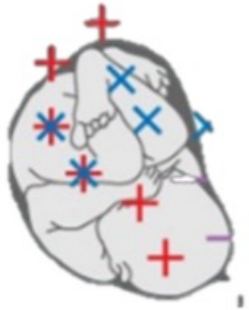
(Presence of anti-C, anti-c, anti-D, anti-E and/or anti-Kell antibodies)

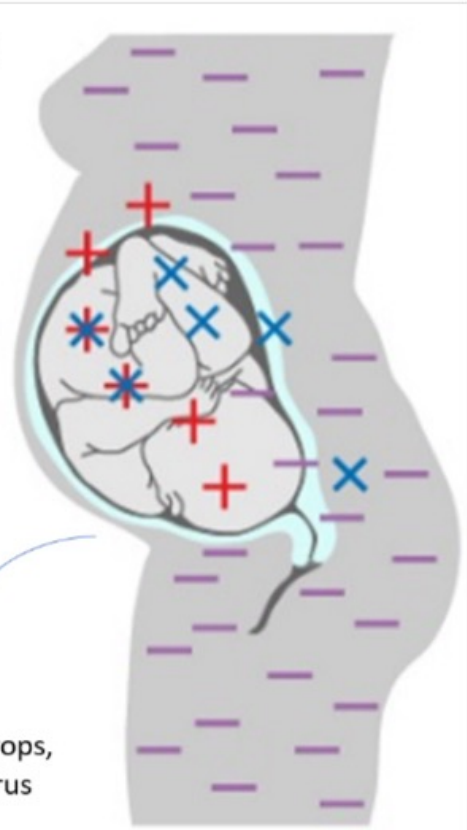
Screening for fetuses at risk for HFDN

- Assessment of antibody levels until critical threshold is reached
- Biweekly ultrasound screening for fetal anemia

Alloimmunized testing:

- cfDNA for RhC, Rhc, RhD, RhE and Kell prevents unnecessary screening for HFDN in pregnancies carrying a fetus that is negative for the antigen





Fetus with HFDN

- Fetal breakdown of blood: anemia, hydrops, neonatal anemia and jaundice, kernicterus
- Fetuses with severe anemia can receive intrauterine transfusions to prevent long-term morbidity and death

Alloimmunized pregnancies

NHS
Blood and Transplant

International Blood Group Reference Library
Bristol, UK

- Maternal EDTA blood must be processed within 48 hours of venepuncture for fetal Kell genotyping, within 72 hours of venepuncture for fetal RhD/C/c/E genotyping.
- https://ibgrl.blood.co.uk
- Involves paternal genotyping

Fetal Genotyping from Maternal Blood	Pregnancy must be at least 16 weeks for RhD/C/E/c Pregnancy must be at least 20 weeks for Kell typing.	16ml EDTA blood per Rh test plus 3ml paternal blood if available (for RhD only)	FRM4674 (Word 113KB) FRM4674 Guidance (PDF 143KB)	Within 7 business days

Non-alloimmunized Pregnancies: Commercially Available Tests and Kits

Name (Manufacturer)
Cell3 Direct Rhesus D Fetal Blood Group Genotyping Kit[22] (Nonacus)
SensiGene Fetal RHD Genotyping[a] (Sequenom)
Free DNA Fetal Kit RhD[23] (Institut de Biotechnologie Jaques-Boy under Bio Rad label)

- Not offered by NIPT laboratories in Canada
- No public funding
- Test only accessible for alloimmunized pregnancies

Abbreviations: cffDNA, cell-free fetal DNA; PCR, polymerase chain reaction; RhD, rhesus D blood group.
[a]Considered investigational by the following US health insurance providers: Blue Cross Blue Shield of North Carolina, AmeriGroup RealSolutions in healthcare Medical Policy & Technology Assessment Committee, Regence of Oregon and Utah, Premera Blue Cross.

Diagnostic Test Accuracy

Table 3: Diagnostic Accuracy Summary Estimates From Systematic Reviews of Noninvasive Fetal RhD Genotyping

Author, Year	N Samples	Accuracy % (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	PPV or LR+ (95% CI)	NPV or LR− (95% CI)
Mackie et al, 2017[36]	10,290 tests[a]	—	99.3 (98.2–99.7)	98.4 (96.4–99.3)	LR+ 61 (22–167)	LR− 0.007 (0.003–0.186)
Zhu et al, 2014[20]	All:11,129	95.3	—	—	—	—
	Conclusive: 10,777[b,c]	98.5[b,c] (98.2–98.7)[d]	98.9 (98.6–99.1)[d]	97.7 (97.2–98.1)[d]	98.7 (98.4–98.9)[d]	98.0 (97.5–99.0)[d]
Geifman-Holtzman et al, 2006[34]	All: 3,261	91.4 (NR)	—	—	—	—
	After some exclusions: 3,184[e]	91.7 (NR)	—	—	—	—
	After all exclusions: 3,078[f]	94.8[a] (NR)	Random effects 95.4 (90.6–97.8) Bayesian model 96.7 (92.5–98.9)	Random effects 98.6 (96.4–99.5) Bayesian model 98.9 (96.7–99.9)	Random effects 99.0 (97.9–99.6) Bayesian model 99.4 (98.4–99.9)	Random effects 92.1 (80.9–97.0) Bayesian model 92.7 (81.8–97.9)

High acceptance of the test

Author, Year	Testing Uptake, % (n/N)
Haimila et al, 2017[46]	Year 1: 69.7% Year 2: 97.3%
de Haas et al, 2016[44]	Overall: at least 98% First 4 weeks: 91.1% Year 1: 96.3% End of study period: 97.5%
Clausen et al, 2014[41]	84.2%[a] (581/690)[a]
Tiblad et al, 2013[50]	89% (8,374/9,380)
Grande et al, 2013[45]	94% (284/302)
Damkjær et al, 2012[42]	90% (216/239)

Abbreviations: n, number of people tested; N, total number of pregnant people; RhD, rhesus D blood group.
[a]Reported only for Region 1 after 15 months of Danish screening program.[41]

cfDNA to guide targeted RhIg administration: Improved outcomes?

Table 11: Relative Risk of Alloimmunization With Targeted RhIG Prophylaxis by Fetal RhD Genotyping Compared With Universal Prophylaxis

Author, Year	Genotyping Cohort Incidence, % (95% CI)	Reference Cohort Incidence, % (95% CI)	Risk Ratio (95% CI)	Absolute Risk Difference, % (NNT)
Tiblad et al, 2013[50]	0.26 (0.15–0.36)	0.46 (0.37–0.56)	0.55 (0.35–0.87)	0.20 (500)

Abbreviations: CI, confidence interval; NNT, number needed to treat; RhD, rhesus D blood group; RhIG, Rh immunoglobulin.

- No studies assessed relative risk of alloimmunization in scenarios with routine antenatal RhIg administration

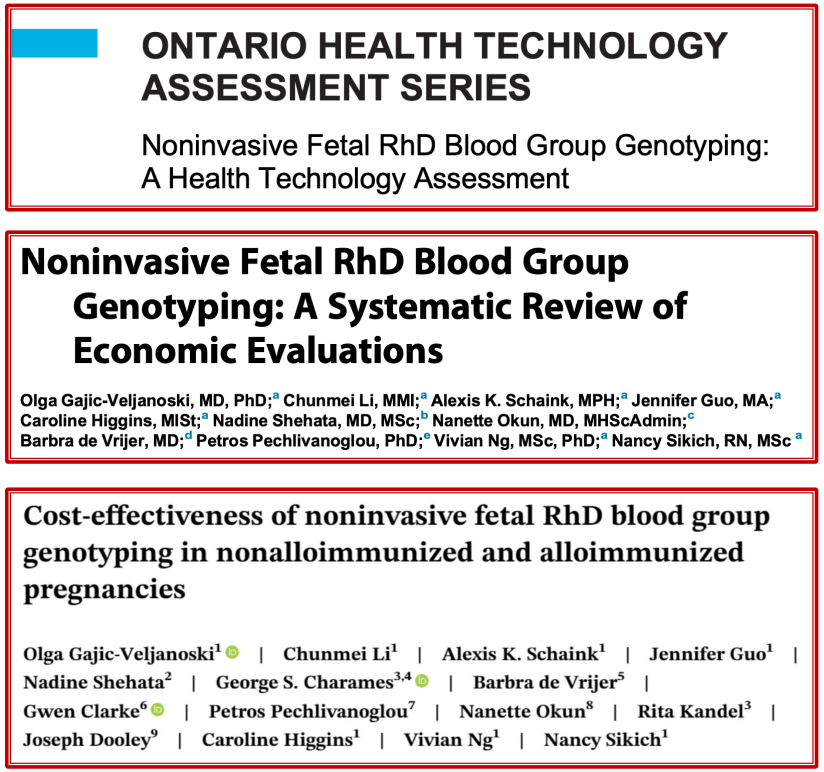
cfDNA to guide targeted RhIg administration: Improved outcomes?

Clinical Utility

The evidence suggests that noninvasive fetal RhD blood group genotyping using cell-free fetal DNA from maternal blood to guide care of RhD− pregnancies:

- Largely avoids unnecessary RhIG prophylaxis in the vast majority of nonalloimmunized RhD− pregnancies not at risk of alloimmunization (GRADE: Low)
- May lead to high compliance with targeted RhIG prophylaxis programs for nonalloimmunized RhD− pregnancies at risk of alloimmunization, but the evidence is very uncertain (GRADE: Very low)
- Leads to large uptake rates of fetal RhD genotyping of 84% or higher as part of care for nonalloimmunized RhD− pregnancies (GRADE: Low)
- May reduce the risk of alloimmunization when fetal RhD genotyping is used to target prenatal RhIG prophylaxis, compared with RhIG prophylaxis administered only after birth or potentially sensitizing events, but the evidence is very uncertain (GRADE: Very low)
- May avoid unnecessary invasive procedures in alloimmunized RhD− pregnancies not at risk of hemolytic disease of the fetus and newborn, but the evidence is very uncertain (GRADE: Very low)

Ontario Health Technology Assessment



ONTARIO HEALTH TECHNOLOGY ASSESSMENT SERIES

Noninvasive Fetal RhD Blood Group Genotyping: A Health Technology Assessment

Noninvasive Fetal RhD Blood Group Genotyping: A Systematic Review of Economic Evaluations

Olga Gajic-Veljanoski, MD, PhD;[a] Chunmei Li, MMI;[a] Alexis K. Schaink, MPH;[a] Jennifer Guo, MA;[a] Caroline Higgins, MISt;[a] Nadine Shehata, MD, MSc;[b] Nanette Okun, MD, MHScAdmin;[c] Barbra de Vrijer, MD;[d] Petros Pechlivanoglou, PhD;[e] Vivian Ng, MSc, PhD;[a] Nancy Sikich, RN, MSc [a]

Cost-effectiveness of noninvasive fetal RhD blood group genotyping in nonalloimmunized and alloimmunized pregnancies

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Nonalloimmunized pregnancies:

Adopting fetal RhD genotyping will lead to an increased budget:

- $2.6 million in year 1 (80% uptake)
- $3.4 million in year 5 (100% uptake)
- $14.8 million over 5 years.

Alloimmunized pregnancies:

Adopting fetal RhD genotyping will lead to a possible cost savings:

- $9 million in year 1
- $12 million in year 5
- $51.5 million over 5 years.

Ontario Health Technology Assessment

Recommendations:

- Public funding of fetal RhD genotyping by cfDNA technologies for alloimmunized RhD negative pregnancies based on clear cost-effectiveness
- Public funding of fetal RhD genotyping by cfDNA technologies for non-alloimmunized RhD negative pregnancies, conditional on attainment of reasonable cost-effectiveness

Cell-free DNA Antigen Genotyping for Non-alloimmunized and Alloimmunized Pregnant Individuals in Ontario: An Advisory Report

IMPLEMENTATION RECOMMENDATIONS

ONTARIO FETAL RHD GENOTYPING TASK FORCE

Submitted to MOH, July 2021

Co-chairs

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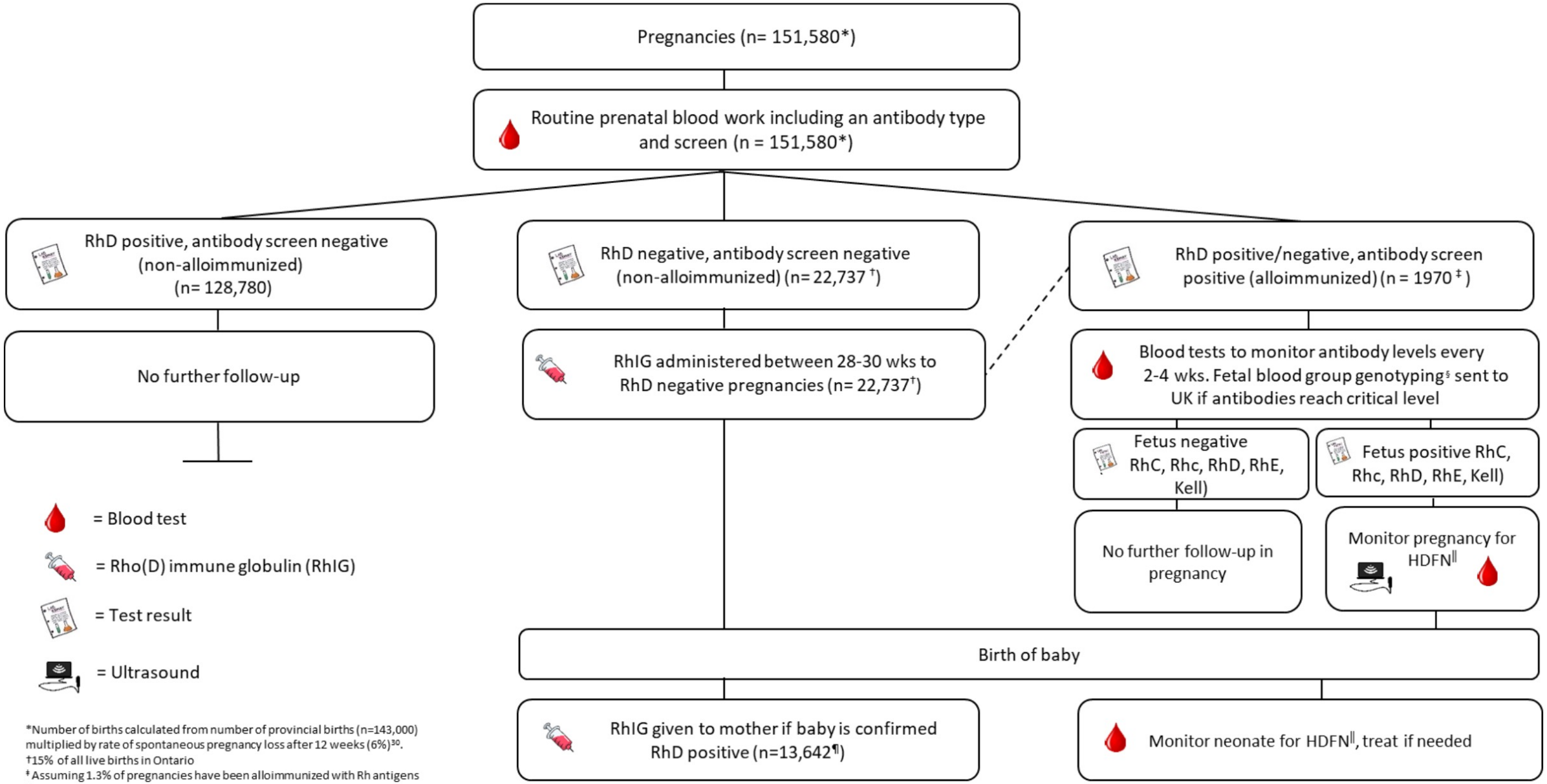
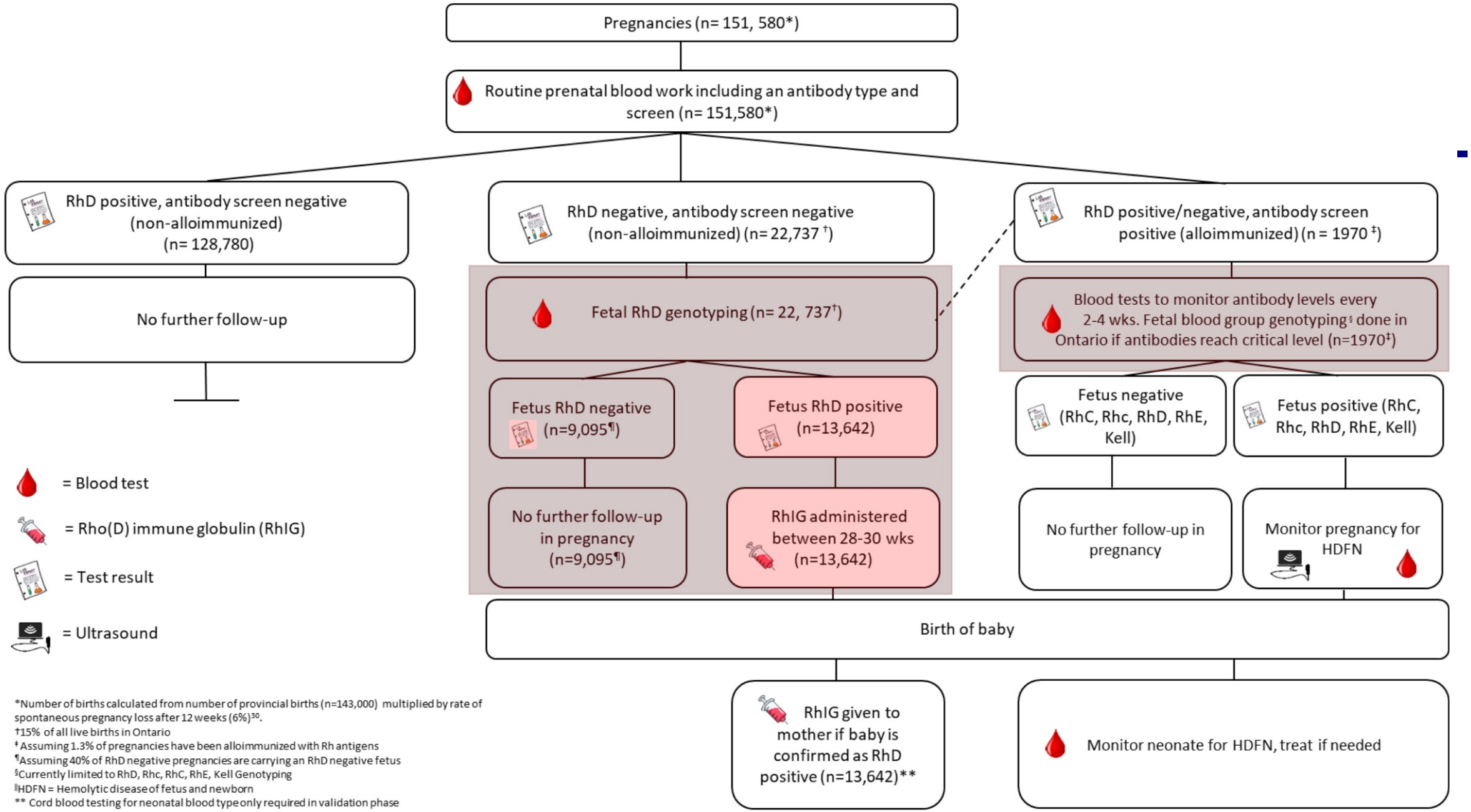


*Number of births calculated from number of provincial births (n=143,000) multiplied by rate of spontaneous pregnancy loss after 12 weeks (6%)[30].
†15% of all live births in Ontario
‡ Assuming 1.3% of pregnancies have been alloimmunized with Rh antigens
¶Assuming 40% of RhD negative pregnancies are carrying an RhD negative fetus
§Currently limited to RhD, Rhc, RhC, RhE, Kell Genotyping
‖HDFN = Hemolytic disease of fetus and newborn



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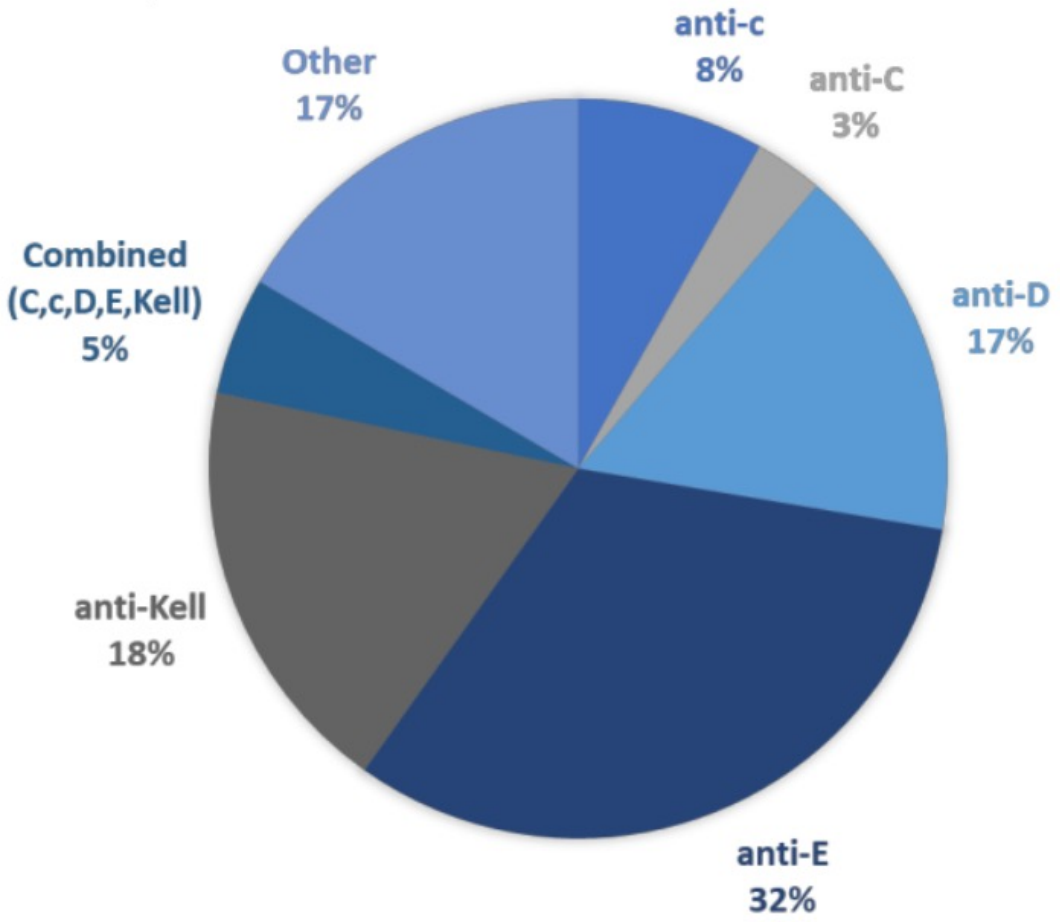
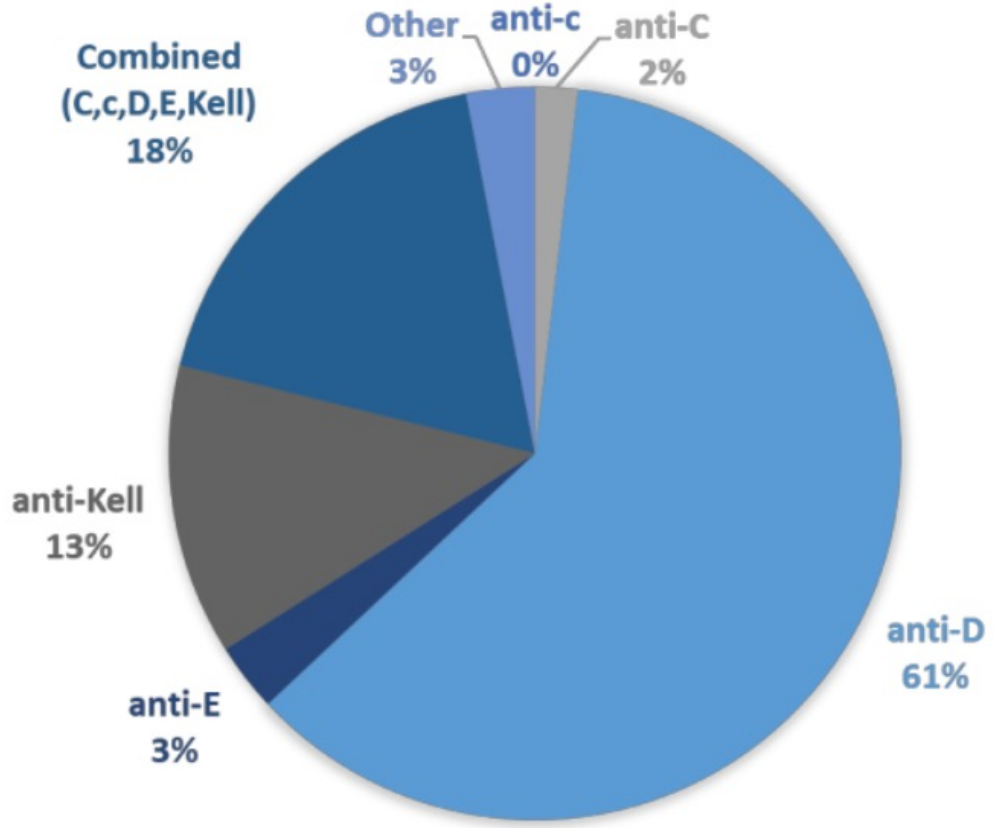
¶Assuming 40% of RhD negative pregnancies are carrying an RhD negative fetus
§Currently limited to RhD, Rhc, RhC, RhE, Kell Genotyping
‖HDFN = Hemolytic disease of fetus and newborn
** Cord blood testing for neonatal blood type only required in validation phase

Alloimmunized pregnancies:
Fetal blood group genotyping for clinically significant antibodies



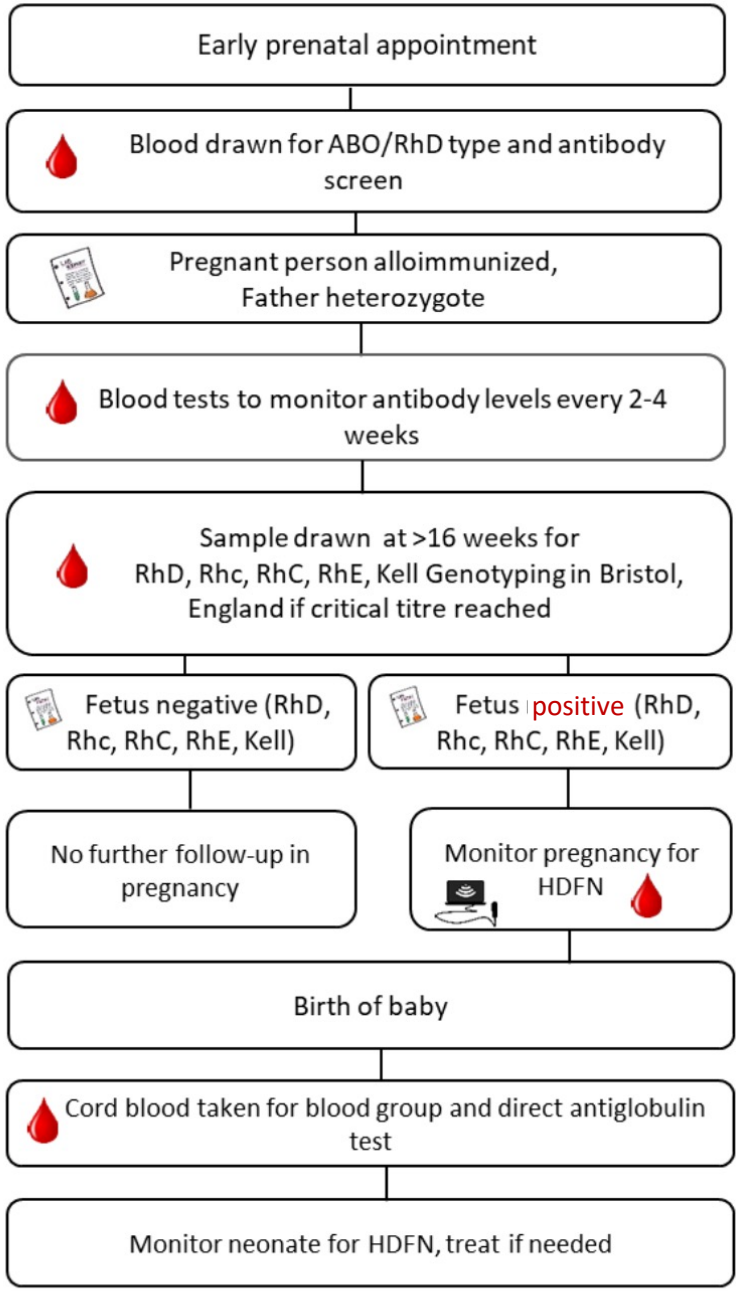
Alloimmunization by antibody type



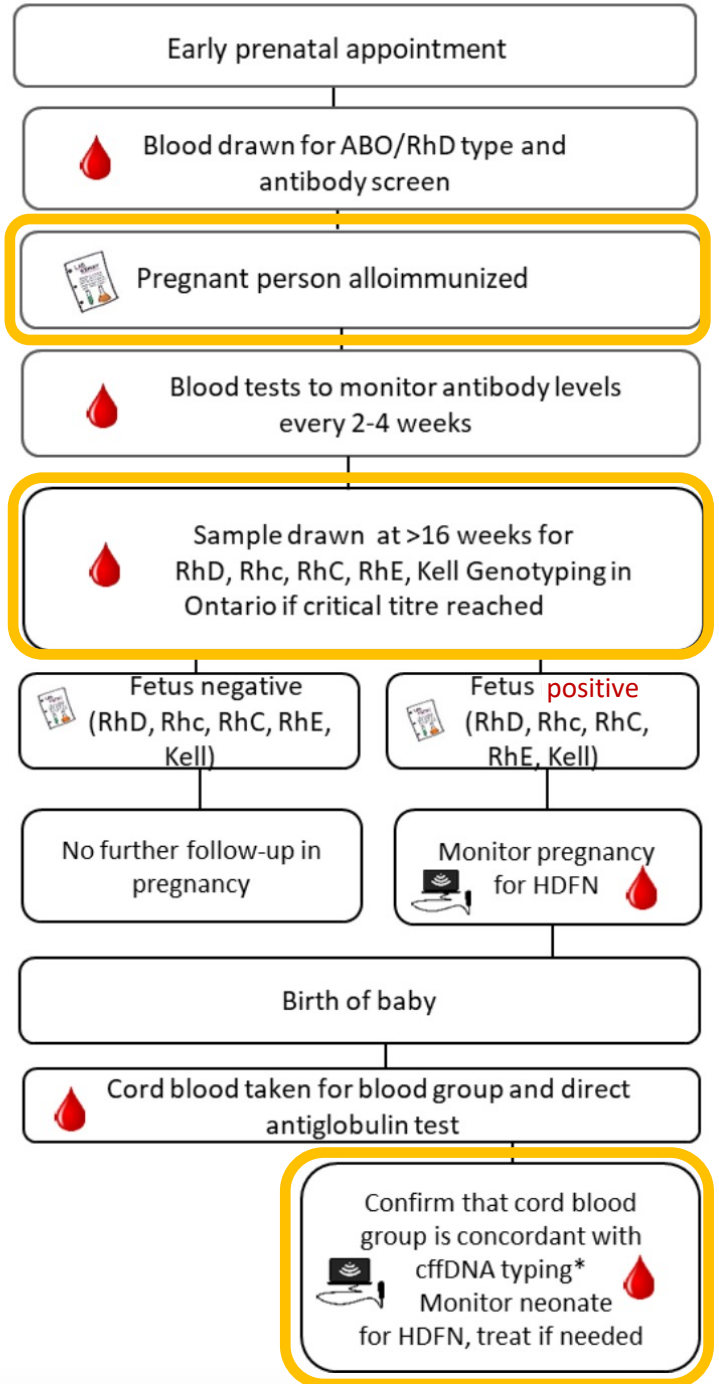
HFDN by antibody type

- If pregnant person is suspected or has a bleed before 11 weeks, recommend blood type and screen

Current state



Future state



= Blood test

= Test result

= Ultrasound

* Only required in validation phase

Alloimmunized pregnancies

Current situation

- Antibody titres are not always comparable
- Experience with Trisomy 21 NIPT: continuous evolution of the test, expertise, quality improvement

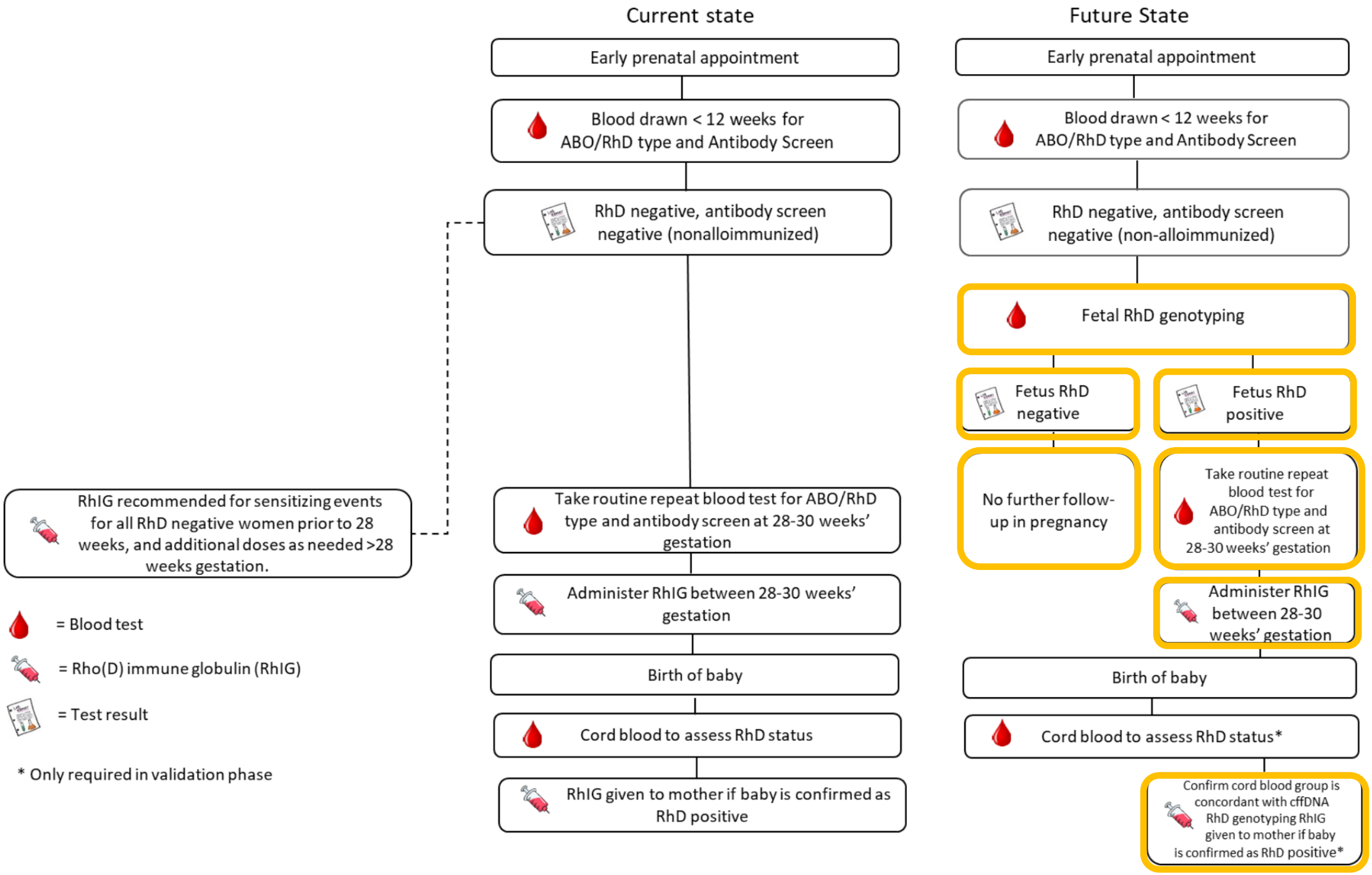
Recommendation

- Centralized laboratory for standardized antibody titration
  - clinical expertise (transfusion medicine, hematology)
  - connections to maternal-child health care providers
- Centralized laboratory for fetal blood group phenotyping

Non-alloimmunized pregnancies

Recommendation

- Nation-wide program
- Inconclusive test results should be treated as RhD positive
- Modification of pregnancy blood group test documentation and forms should include EDD
- Single, centralized laboratory would facilitate high-throughput testing, reducing cost and improving efficiencies and test performance
- Responsibility for education

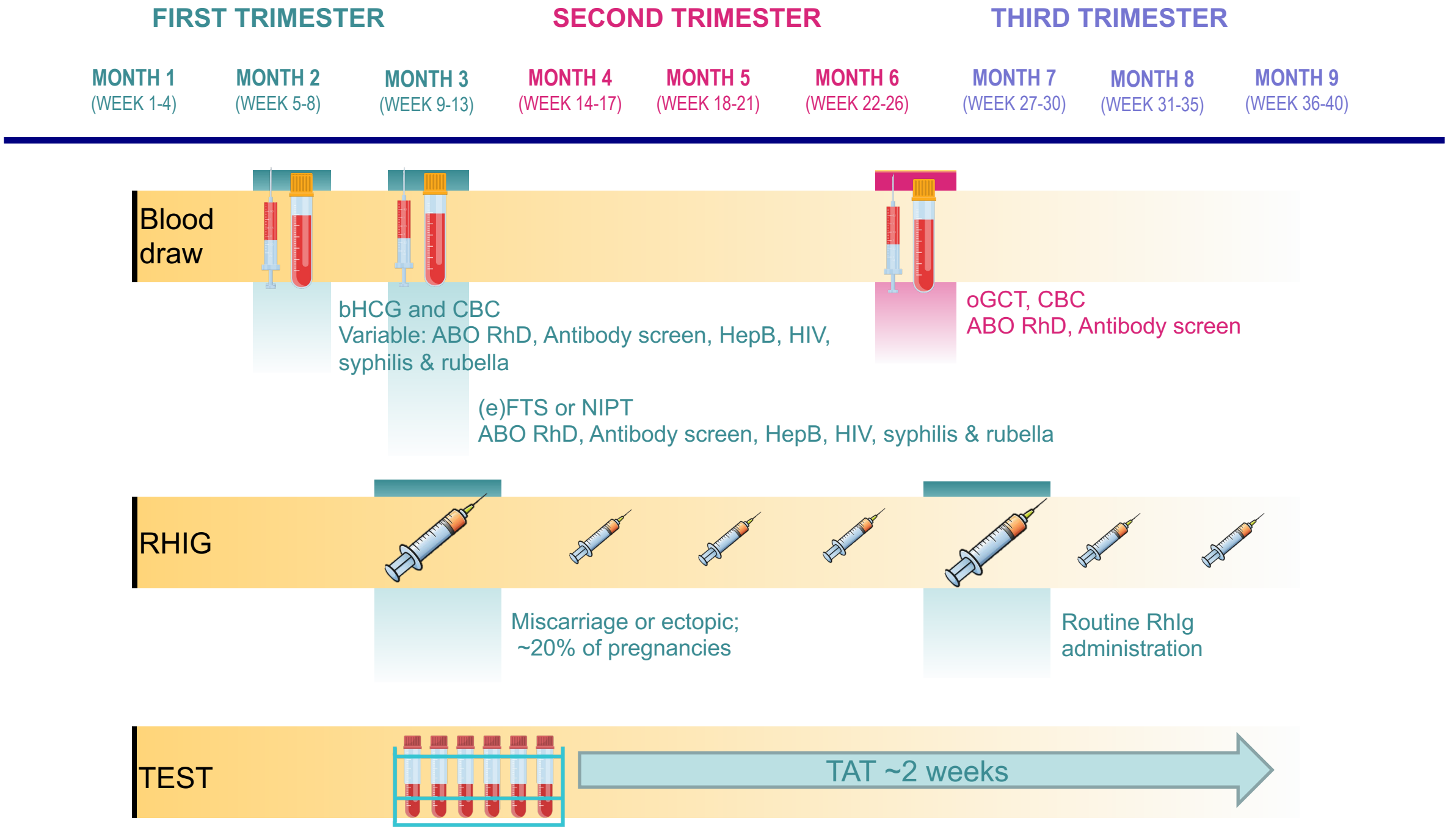

Current state
Early prenatal appointment
Blood drawn < 12 weeks for ABO/RhD type and Antibody Screen
RhD negative, antibody screen negative (nonalloimmunized)
RhIG recommended for sensitizing events for all RhD negative women prior to 28 weeks, and additional doses as needed >28 weeks gestation.
Take routine repeat blood test for ABO/RhD type and antibody screen at 28-30 weeks' gestation
Administer RhIG between 28-30 weeks' gestation
Birth of baby
Cord blood to assess RhD status
RhIG given to mother if baby is confirmed as RhD positive
= Blood test
= Rho(D) immune globulin (RhIG)
= Test result
* Only required in validation phase
Future State
Early prenatal appointment
Blood drawn < 12 weeks for ABO/RhD type and Antibody Screen
RhD negative, antibody screen negative (non-alloimmunized)
Fetal RhD genotyping
Fetus RhD negative
Fetus RhD positive
No further follow-up in pregnancy
Take routine repeat blood test for ABO/RhD type and antibody screen at 28-30 weeks' gestation
Administer RhIG between 28-30 weeks' gestation
Birth of baby
Cord blood to assess RhD status*
Confirm cord blood group is concordant with cffDNA RhD genotyping RhIG given to mother if baby is confirmed as RhD positive*

Non-alloimmunized pregnancies: timing of the test

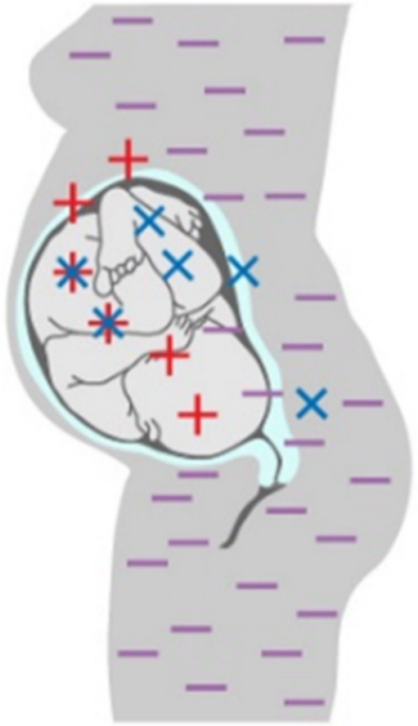
Table 5: Diagnostic Accuracy of Noninvasive Fetal RhD Genotyping by Trimester of Pregnancy

Author, Year	1st Trimester Accuracy, % (95% CI)	2nd Trimester Accuracy, % (95% CI)	3rd Trimester Accuracy, % (95% CI)
Zhu et al, 2014[20]	99 (NR)	98.3 (NR)	96.4 (NR)
Geifman-Holtzman et al, 2006[34]	90.8 (86.3–94.0)	85.0 (81.1–88.2)	85.3 (80.4–89.2)

Abbreviations: CI, confidence interval; NR, not reported; RhD, rhesus D blood group.


FIRST TRIMESTER
SECOND TRIMESTER
THIRD TRIMESTER
MONTH 1 (WEEK 1-4)
MONTH 2 (WEEK 5-8)
MONTH 3 (WEEK 9-13)
MONTH 4 (WEEK 14-17)
MONTH 5 (WEEK 18-21)
MONTH 6 (WEEK 22-26)
MONTH 7 (WEEK 27-30)
MONTH 8 (WEEK 31-35)
MONTH 9 (WEEK 36-40)
Blood draw
bHCG and CBC
Variable: ABO RhD, Antibody screen, HepB, HIV, syphilis & rubella
(e)FTS or NIPT
ABO RhD, Antibody screen, HepB, HIV, syphilis & rubella
oGCT, CBC
ABO RhD, Antibody screen
RHIG
Miscarriage or ectopic; ~20% of pregnancies
Routine RhIg administration
TEST
TAT ~2 weeks

Summary



STATEMENT 21

For patients with clinically significant antibodies (to RhD, C/c, E, or K), cell free fetal DNA testing (non-invasive perinatal testing) should be performed when feasible. Optimal timing of such testing will be determined by the testing program.

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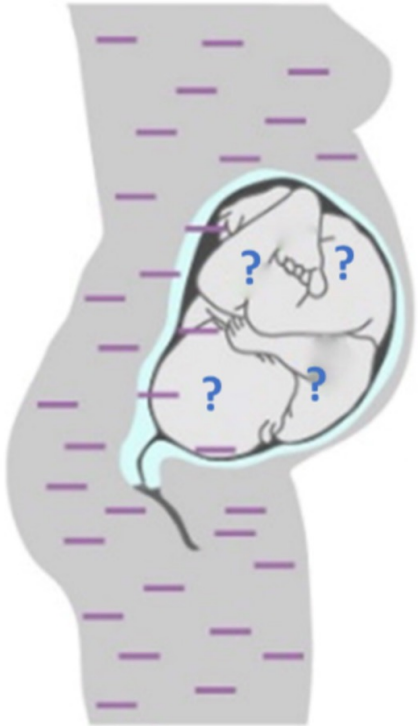

Alloimmunized testing:

- cfDNA for RhC, Rhc, RhD, RhE and Kell prevents unnecessary screening for HFDN in pregnancies carrying a fetus that is negative for the antigen

Non-alloimmunized testing:
cfDNA for RhD prevents unnecessary antenatal administration of RhIG to 40% of RhD negative pregnancies