RhD typing Challenges

Advancing Clinical Practice with RHD genotyping

Who needs Rh negative blood and Rh immune globulin ?

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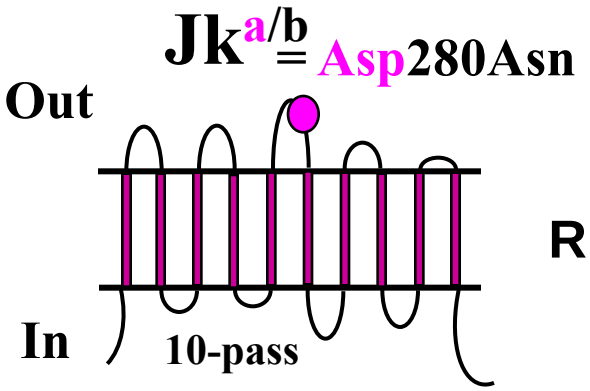
Why is typing for RhD sometimes problematic?

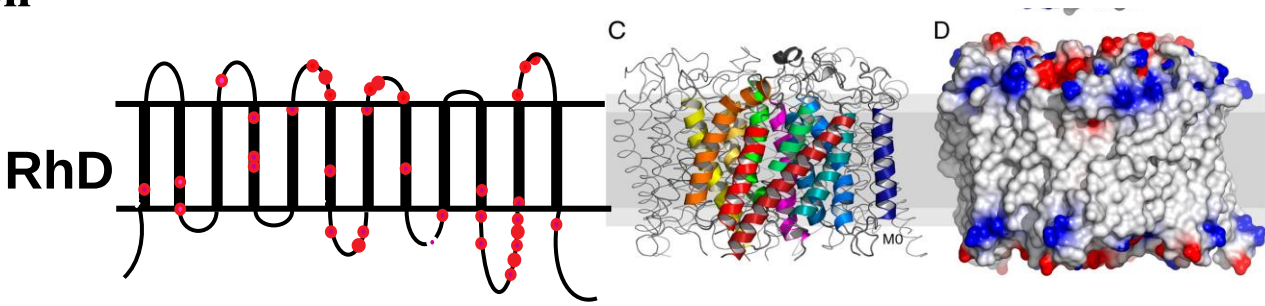
- **Large number of variables**
  - **Variation in D strength of antigen expression on some RBCs**
  - **Variation in test methods**
  - **Variation in the specificity of antibody clones and reagent formulations**
  - **Variation in interpretation**

Why is typing for RhD sometimes problematic?

- **The D antigen is NOT a single epitope on a red cell protein, unlike for example, Jka/b**
- **D typing detects the presence or absence of a entire red cell protein**

Example: Most blood group antigens are single change



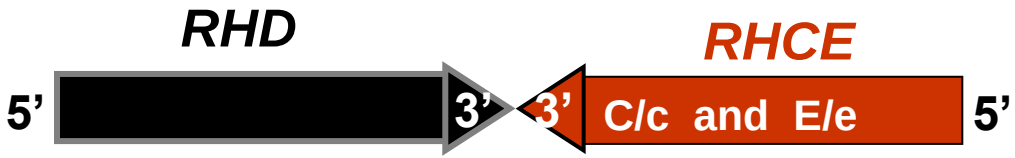


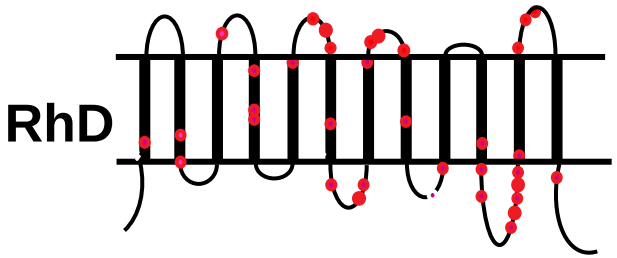
35 amino acid changes from Rhce

Aspartic acid at position 280 = Jk(a+)
Asparagine at position 280 = Jk(b+)

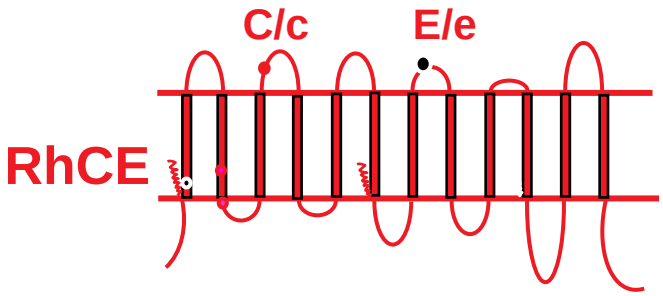
RH Blood Group Locus – 2 Genes

Rh "positive"





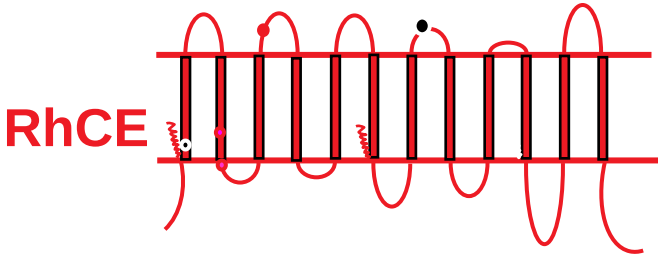
D antigen
32-35 amino acid changes



C or c and E or e antigens

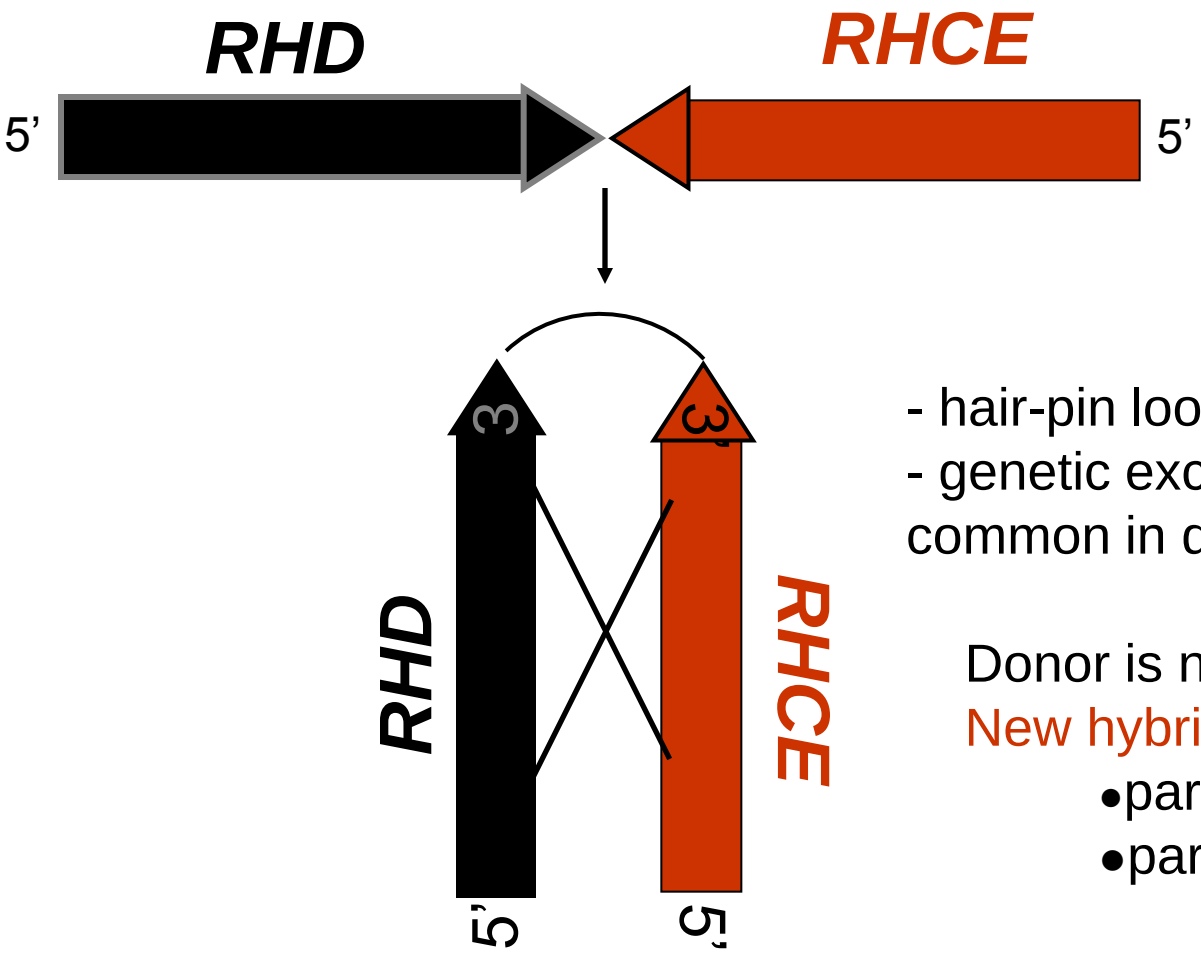
Rh "negative" x ***deletion x***

No RhD



RH LOCUS

Gene conversion and rearrangement



- hair-pin loop structure
- genetic exchange

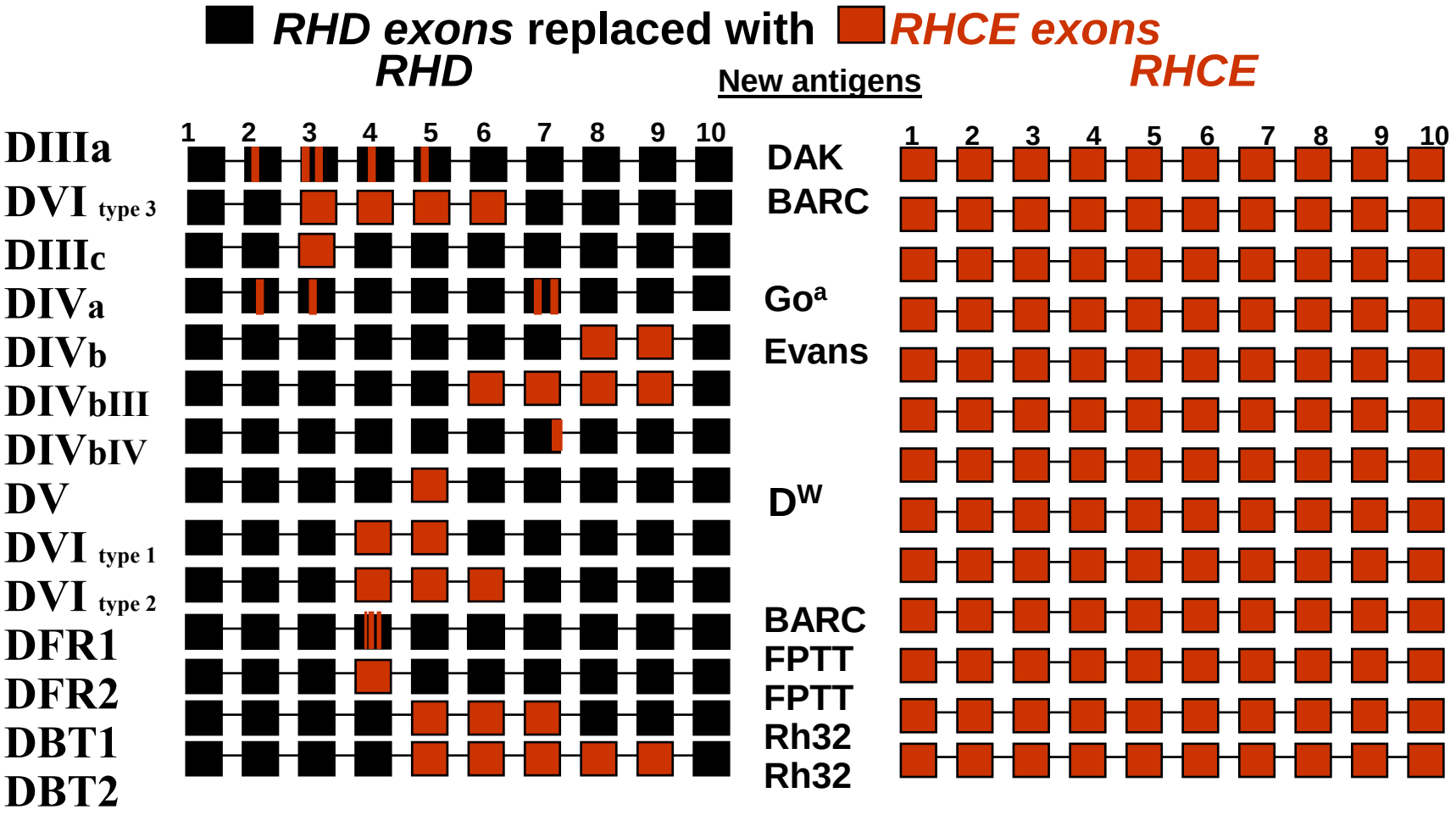
common in duplicated genes/linked

Donor is not changed

New hybrid alleles and proteins

- part of RhD into RhCE
- part of RhCE into RhD

Partial D examples: *RHD*/*RHCE* hybrid alleles



Many type as D+ but patients can make anti-D

Partial DVI – associated with majority of cases of fatal HDFN (Caucasians)

Females (under age of 50) should receive Rh- blood; are RhIG candidates

I. Variation in D antigen expressed on RBCs

- More than 200 different RHD alleles in populations
  - single or multiple amino acid changes in RhD
  - could potentially be >200 different antigens or “D subgroups”

- Two Primary Categories
  - **Weak D**
    - changes **decrease antigen expression** level
    - definition: react weaker than expected (≤2+ OR require IAT)
    - **Not at risk for anti-D** (rare exceptions, but no HDFN or HTR)
      - weak D types 1, 2, 3 most common
  - **Partial D**
    - changes **alter the epitopes or epitopes are missing**
    - **At risk for clinically significant anti-D**

Cannot be distinguished by routine serologic D typing

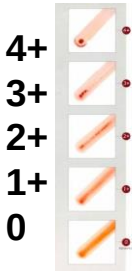
How many patients have altered *RHD* gene?

- **0.5%- as many as 4% of patients carry *RHD* genes with mutation(s)**
  - incidence depends on ethnic group
- ~25% of sites in CAP survey reported they had seen at least one patient in the past 12 months with a serologic weak D phenotype who made alloanti-D
- Literature: >30 reports of D+ persons, presumed to have partial D, who made anti-D associated with HDFN

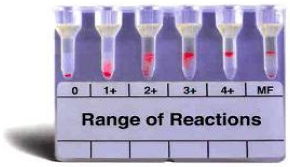
Sandler SG, Roseff S, Domen RE, et al. Policies and procedures related to testing for weak D phenotypes and administration of Rh immune globulin: results and recommendations related to supplemental questions in the Comprehensive Transfusion Medicine survey of the College of American Pathologists.
Arch Pathol Lab Med 2014;138:620-5.

II. Variation in Test Methods to type for D

- **Manual tube** – with or without IAT (AHG) for serologic weak D
- **Gel card**
- **Solid phase**
- **PK** – enzyme treated cells for donor testing



Manual Tube



Solid Phase Capture

Donor centers
PK 7600

Automation – ABO & Rh, antibody screening, identification

Gel Card

BioTest TANGO™
Benchtop Blood Bank Analyzer

ImmucorGamma's
Capture solid phase
Echo and Neo

II. Variation in Test Methods to type for D

- **Donors**
  - **Goal: prevent anti-D by detecting any D expression as positive**
  - **AABB requires "method designed to detect weak expression of D"**
    - U.S. – use of enzyme treated cells and two anti-D (PK instrument)
    - OR use of indirect antiglobulin test (IAT)
- **Patients**
  - **Goal: to prevent anti-D alloimmunization**
  - **AABB "testing for weak D expression by IAT not required/optional"**
    - exception: newborns when evaluating D- mothers for RhIG

II. Variation in Test Methods to type for D

- **Donors**
  - **AABB requires "method designed to detect weak expression of D"**
- **Patients**
  - **AABB "testing for weak D expression by IAT not required/optional"**

Why differ?

- Patients
  - **some partial D are only detected in the IAT**
    - females and OB's "may be better served as Rh negative"
    - Partial DVI only detected by IAT
  - **concern for "false positive"** (RBCs with +DAT, rouleaux, etc)

CAP survey: majority of hospitals do not do IAT for weak D for patients

History of anti-D typing in U.S.

- **Long recognized that donor and patient typing goals may differ**
  - Donor: need more sensitive testing to avoid stimulating anti-D
  - Patient: no harm in treating patient as Rh negative

- **1960-70's Polyclonal anti-D reagents (detect multiple epitopes)**
  - **Peter Issitt "**tradition in blood banking demands that before a donor can be regarded as Rh negative he/she be shown not only to lack D antigen, but also C and E"
  - based on fact that weak D antigen expression is often inherited with C+ or E+
  - **anti-CDE reagent was in wide use for donor testing**

- **1980's - Monoclonal anti-D reagents**
  - Increased sensitivity IgM clones **– many RBCs D+ at IAT - now reactive initial spin**
  - Could select clones to specific D epitopes
  - Proposed different reagents: one for typing donors; one for patients
    - Too confusing
    - **FDA: anti-D reagents for U.S. market MUST be non-reactive with DVI on initial testing** (so these patients type as Rh negative)
    - **Must react with DVI on IAT** (so donors type as Rh positive)

III. Variation in FDA licensed anti-D reagents

Reagent	IgM monoclonal	IgG
Gammaclone	GAMA401	F8D8 monoclonal
Immucor Series 4	MS201	MS26 monoclonal
Immucor Series 5	Th28	MS26 monoclonal
Ortho BioClone	MAD2	Polyclonal
Ortho Gel (ID-MTS)	MS201	
Bio Rad RH1	BS226	
Bio Rad RH1 Blend	BS232	BS221, H41 11B7
Alba Bioscience alpha	LDM1	
Alba Bioscience beta	LDM3	
Alba Bioscience delta	LDM1/ ESD1M	
Alba blend	LDM3	ESD1

- **majority contain different clones**
- often differ in reactivity with RBCs with partial D or weak D
- even the same clone can react differently
  - different potentiators are added
- reactivity with anti-D may differ depending on C or E status of the RBCs

III. Variation in FDA licensed anti-D reagents

Reagent	IgM monoclonal	IgG
Gammaclone	GAMA401	F8D8 monoclonal
Immucor Series 4	MS201	MS26 monoclonal
Immucor Series 5	Th28	MS26 monoclonal
Ortho BioClone	MAD2	Polyclonal
Ortho Gel (ID-MTS)	MS201	
Bio Rad RH1	BS226	
Bio Rad RH1 Blend	BS232	BS221, H41 11B7
Alba Bioscience alpha	LDM1	
Alba Bioscience beta	LDM3	
Alba Bioscience delta	LDM1/ ESD1M	
Alba blend	LDM3	ESD1

Alba delta:

FDA – this reagent for donor testing only
detects partial DVI at initial spin

"is not recommended for patient testing for transfusion"

III. Variation in FDA licensed anti-D reagents

Manufacturer instructions & cautions vary

EXAMPLES:

- "Reactions less than 2+ should be evaluated since they may be false positive"
- "Agglutination <1+ at IS should be tested using alternative reagent by IAT prior to final determination"
- "Patients should not be classified as D+ on basis of a weak reaction with a single anti-D"
- "If a clear positive not obtained it is safer to classify the patient as D-"

IV. Variation in interpretation and practice

2014 CAP Survey of ~3,100 laboratories

- Reporting **(1992 D^u was renamed weak D and should no longer be used)**
  - **47% as D positive** - **30% as "weak D"**
  - **11 % as D negative** - **females or OB's as D negative**

- Treatment
  - **D positive**
    - Rh positive blood and no RhIG
    - risk for anti-D
  - **D negative**
    - conservative approach; avoids risk for anti-D
    - females- avoid risk for possible HDFN
    - results in excess use of Rh negative blood
    - results in excess use of Rh immune globulin

RHD genotyping (DNA testing) can distinguish

- **Weak D alleles**
  - **Types 1-76** (76 different point mutations)
  - Weak D type 1, 2, or 3 most common in Caucasian (~95%)
    - **ARE NOT AT RISK**
- **Partial D alleles**
  - >100 alleles with multiple changes
  - appear to lack epitopes
    - **AT RISK**

Weak D alleles more common in Caucasians

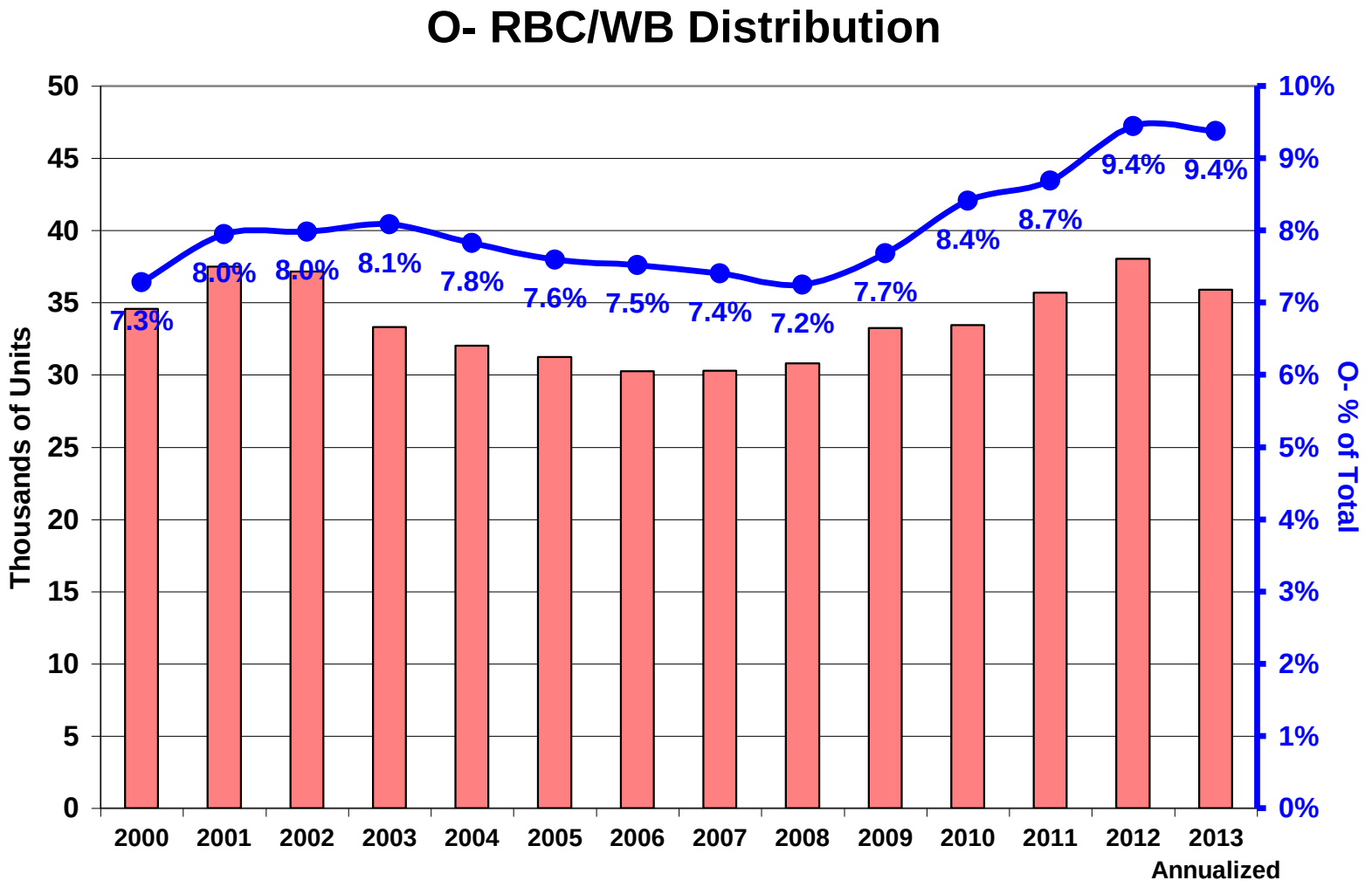
Partial D alleles more common in African-Americans

2014 Charge to Rh workgroup

Members: CAP, AABB, ACOG, ABC and ARC

- **Develop a recommendation for *RHD* genotyping when a serological weak D phenotype is identified**
- **Goal: to begin phase-in the use of *RHD* genotyping**
- **A recommendation should help**
  - **clarify clinical issues related to RhD blood typing in pregnant women and transfusion recipients**
  - **while helping to avoid the unnecessary use of Rh immune globulin and transfusion of Rh-negative Red Blood Cells**

Impact on the Blood Supply



Number of Rh negative units needed to meet demand
Overall blood use declining, Rh negative usage increasing

Brigham and Women's *RHD* genotyping for OB's

- To guide RhIG prophylaxis and selection of blood for transfusion
  - **OB women with D typing discrepancies**
    - positive previously and now negative: or the reverse
    - Rh type from physician office different than hospital
  - **D typing weaker than expected**

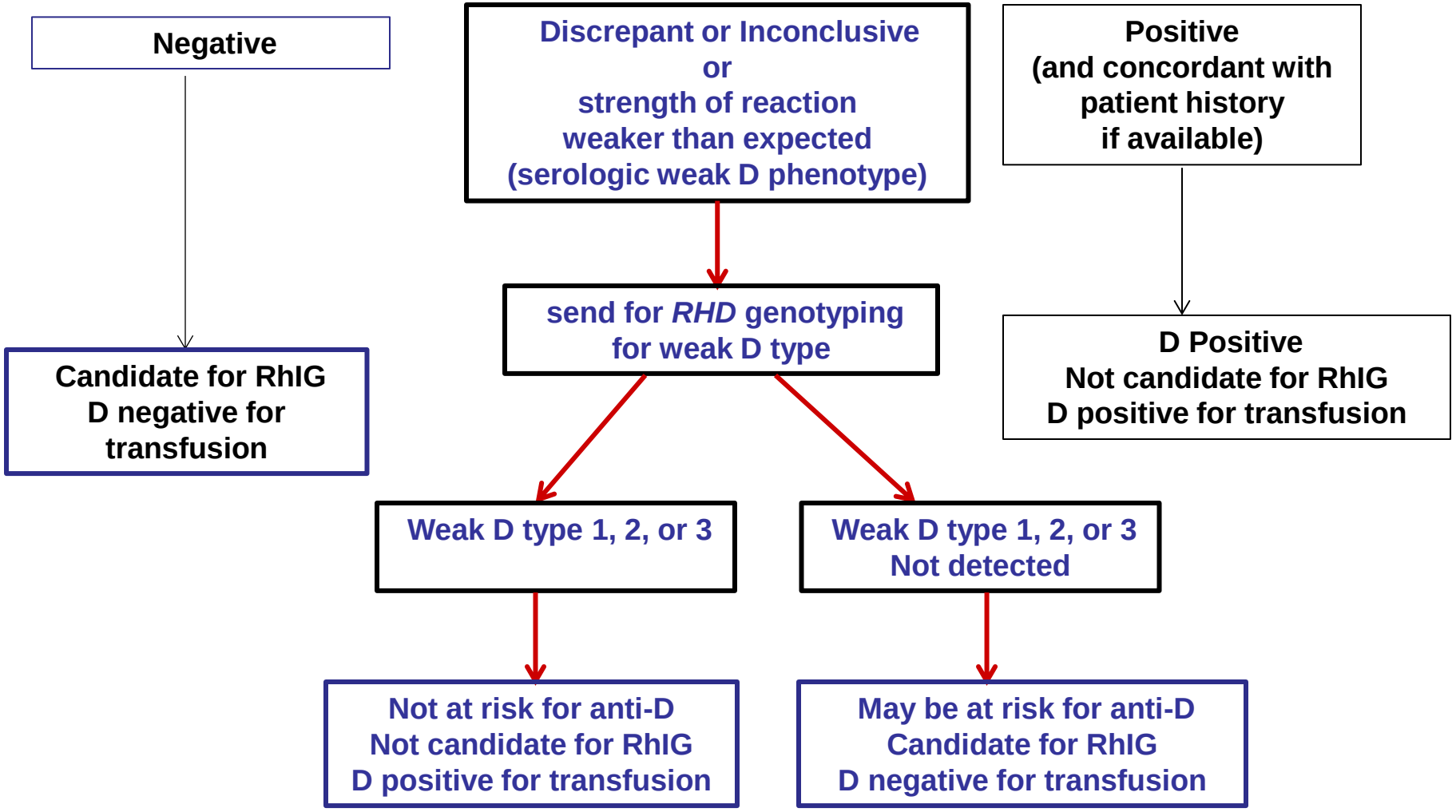
*RHD**	weak D type 1	weak D type 2	weak D type 3	weak D type 4.0	Partial *DAR*	No RHD *RHCE*ceCF*	New alleles	Total
# OB patients	16	9	2	2	4	1	2	36
% of total tested	44%	25%	5.5%	5.5%	11%	2.8%	5.5%	100%
Risk for anti-D	NO			Majority not at risk	YES	YES	UNKNOWN	
RhIG	Not candidate for RhIG				Candidate for RhIG	Candidate for RhIG	Candidate for RhIG	

75 % **25%**

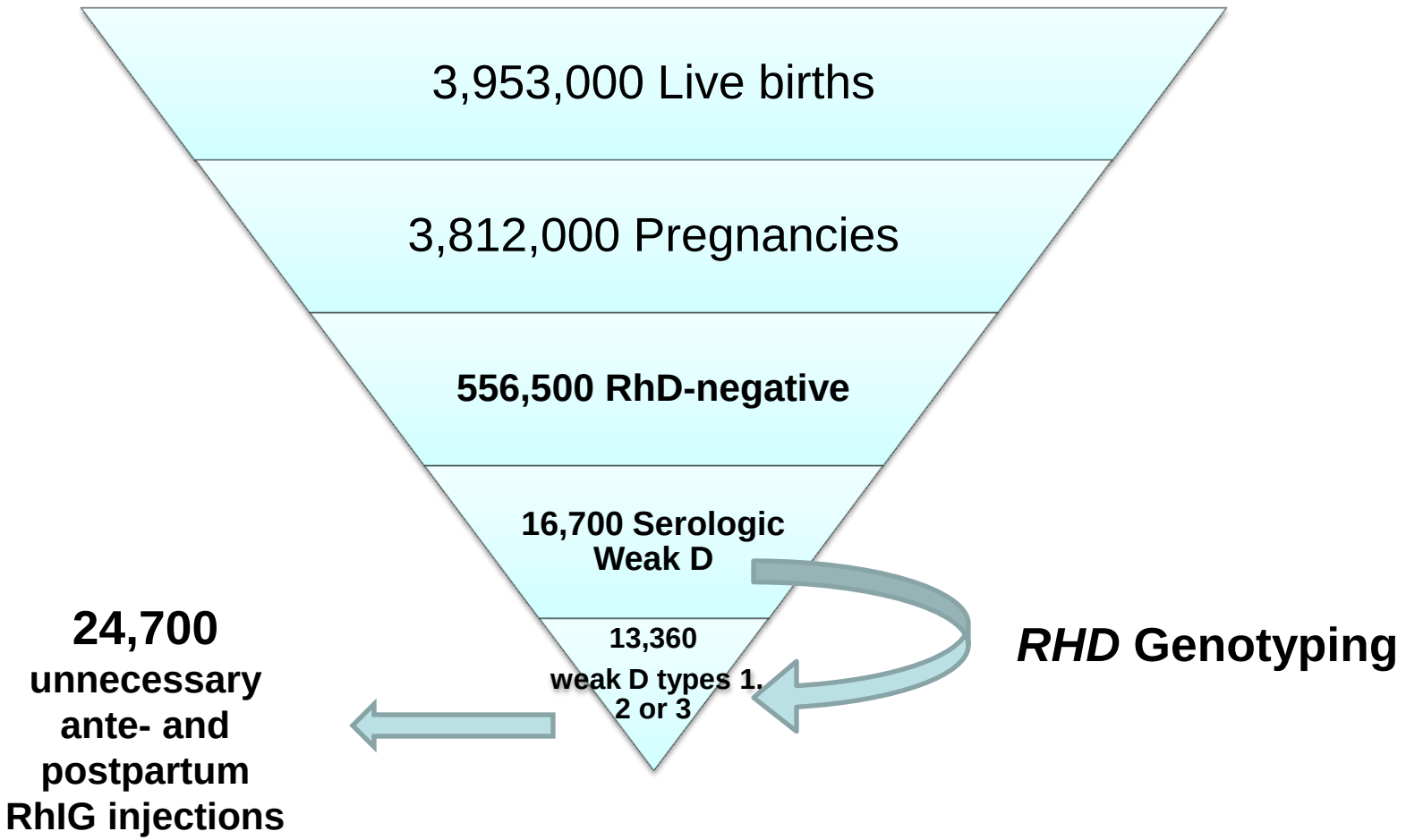
Patients are managed based according to their *RHD* genotype

Algorithm for Resolving Serologic Weak D Test Results by RHD Genotyping for Determining Candidacy for RhIG and Rh Type for Red Cell Transfusions

Result of RhD typing by Manual Tube or Automated Methods



Potential Benefits of *RHD* Genotyping Pregnant Women



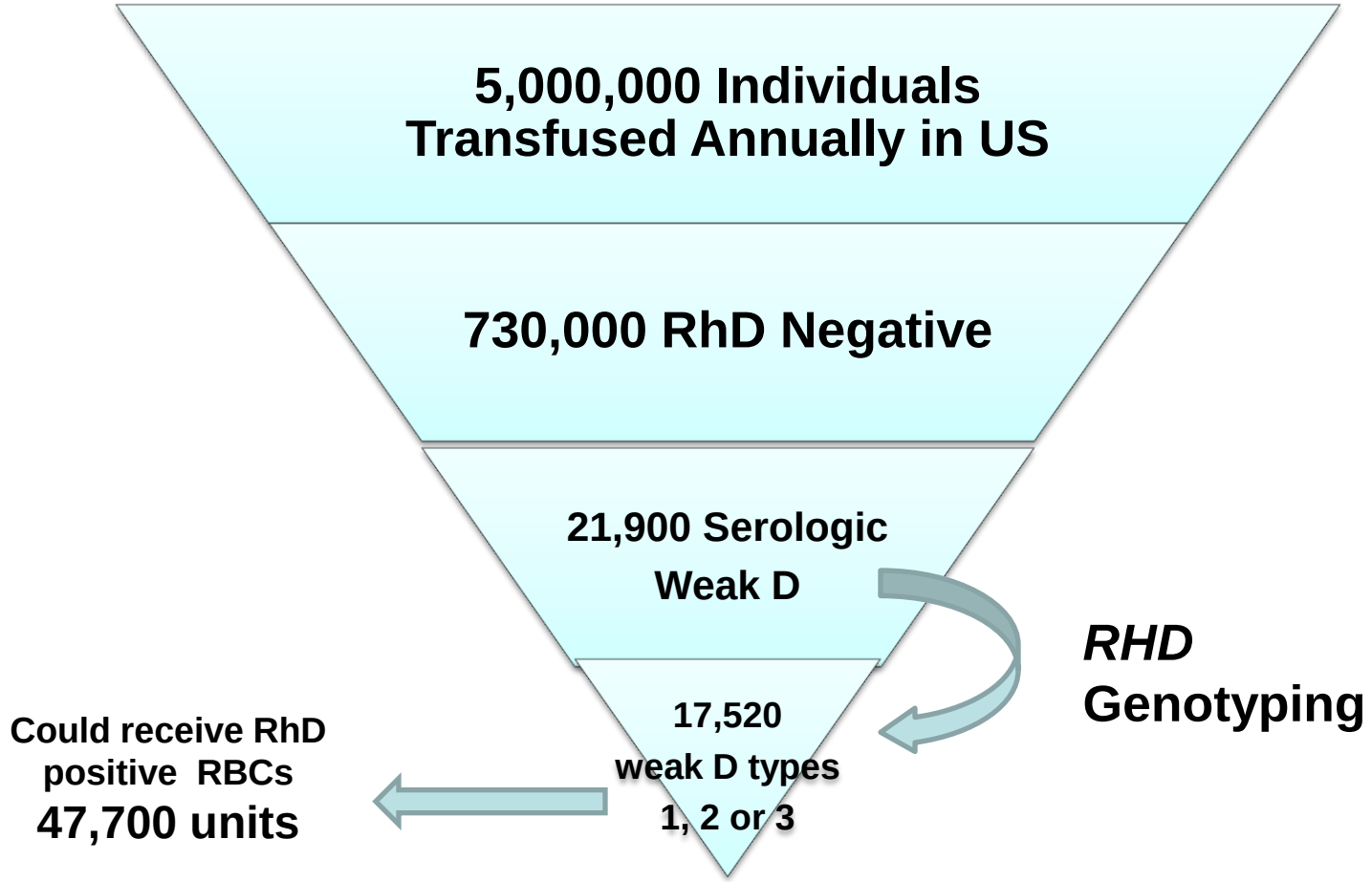
Why be concerned about excess usage of RhIG?

- one of the greatest medical advances of the 1960's
- **Very safe product**

BUT

- a human blood product
- manufactured from pooled plasma from paid donors
- must be actively immunized
- ethical issues when biologic products are administered unnecessarily
- are no reports of transmission of hepatitis B virus, hepatitis C virus, or HIV caused by RhIG manufactured in the United States……..
- always potential for emerging agents

Potential Benefit of *RHD* Genotyping Transfusion Recipients



Rh Workgroup Recommendations

- Definition of weak D serologic result
  - **weaker than expected reactivity (≤2+)**
  - **depends on method, reagent, and local population being tested**
  - **institution should have policy**
- Are not indicating institutions must change methods of typing or do an IAT on all female patients
- Use *RHD* genotyping to resolve
  - **D typing discrepancies**
  - **weaker than expected reactivity**
- Use *RHD* genotyping to manage clinical decisions
  - **Determine candidates for Rh immune globulin**
  - **RhD status for blood transfusion**

Rh Workgroup Recommendations

For women with a serological weak D phenotype associated with an *RHD* genotype ***other*** **than weak D type 1, 2 or 3**, the work group recommends conventional prophylaxis with RhIG at this time.

Reference laboratories performing RBC genotyping services should offer tiered services, beginning **with affordable first-tier testing**, so that the most prevalent and clinically relevant *RHD* genotypes can be detected.

Clinicians and investigators are encouraged to **publish outcomes of pregnancies and transfusions** of individuals with *RHD* genotypes for which the risk of RhD alloimmunization is unknown.

Phasing-in *RHD* genotyping will apply modern genomic methods for more precise decision making in obstetrical practice and transfusion medicine.

Financial Implications of *RHD* genotyping for OB's

- **Cost-Benefit Analysis**
  - RHD genotyping is an LDT – Laboratory Developed Test
  - Research Use - RUO testing (CPT code)
  - Performed in CLIA regulated laboratory
  - Cost of testing has not "stabilized"
- **Goal: evaluate the costs** of RHD genotyping for *pregnant females with serologic weak D phenotypes*
  - using a **comparison strategy of managing as D–**
  - ***RHD* genotyping done at first visit/first pregnancy** when Rh typing done and results made part of medical record
  - direct medical costs assessed over 10- and 20-year periods for a simulated population of US women
  - one-way and probabilistic sensitivity analyses used to assess the robustness of conclusions

Cost Input Parameters – CMS reimbursement

Testing and Product	Cost $	Range
Initial Testing		
ABO Group	12.12	(9.09-15.15)
RhD Type	12.12	(9.09-15.15)
Additional RhD Testing		
RHD Genotyping Assay	250	(100-500)
Cord Blood RhD Typing	30.33	(22.75-37.91)
Blood Products		
Rh Immune Globulin (300 µg dose)	162	(121.50-202.50)
Rh Immune Globulin Administration	9.60	(7.20-12.00)

Cost-savings over treating as Rh negative when *RHD* genotyping is ~ $256

Financial implications of RHD genotyping of pregnant women with serologic weak D phenotype
Kacker S, Vassallo R, Keller M, Westhoff CM, Frick K, Sandler S, Tobian A *Transfusion 2015 Early View*

Limitations

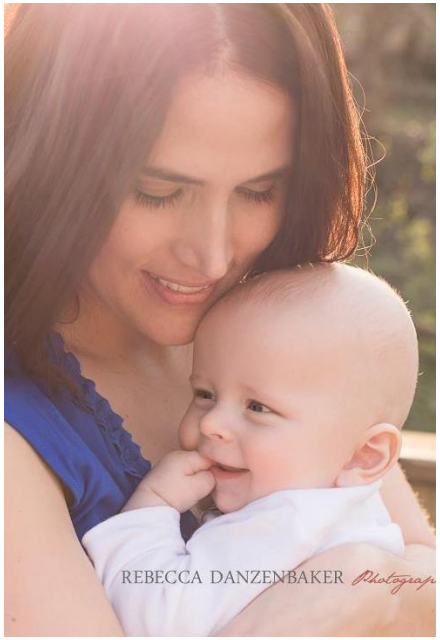
- Did not address ***detection*** of partial D phenotypes
  - workgroup focus on clinical management of patients with a serologic weak D phenotype
  - **some women with weak D+ will not be detected by method used**
    - are typed as D negative
    - get unnecessary RhIG

    Will require <u>testing all Rh negative women</u> by *RHD* genotyping
  - **women with partial D who type strongly D+** (partial DIIIa, DIVa)
    - are typed as D positive
    - do not get the needed RhIG
      - no cases associated with fatal HDFN in literature
      - but results in costly monitoring of an “at risk pregnancy”

    Will require <u>testing all Rh positive women</u> by *RHD* genotyping

Future for all pregnant women

Rh status will be determined by RHD genotyping



Summary Recent Publications in *Transfusion*

1. It's time to phase in RHD genotyping for patients with a serologic weak D phenotype Sandler S, Flegel W, Westhoff CM, Denomme G, Delaney M, Keller M, Johnson S, Katz L, Queenan T, Vassallo R, Simon C. *Transfusion 2015:55:680-689*

- Commentary from RhD workgroup (ABC, AABB, CAP, ARC, ACOG)
- Goal to BEGIN standardization of practice

2. How do I manage Rh typing in obstetric patients? Haspel R, Westhoff CM *Transfusion 2015 55:470-74*

- 25% of women with discrepant or weak D typing were at risk
- 75% were weak D type 1, 2, or 3 and NOT at risk

3. Financial implications of RHD genotyping of pregnant women with serologic weak D phenotype Kacker S, Vassallo R, Keller M, Westhoff CM, Frick K, Sandler S, Tobian A *Transfusion 2015 Early View*

- Rather than managing as D-
- Cost-savings when cost of RHD genotyping is below $256

Thank You !

New York Blood Center

Immunohematology and Genomics Laboratory