

An Unusual Phenotype

Getting the right type!

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Southern Region

Testing request/Patient Info

Sample referred to IRL for Rh(D) typing on 10-26-2012

- Patient: 4 year old, female
- Diagnosis: Sickle Cell Disease
- Transfusion history: No Record
- Hospital ABO/Rh: group O, Rh pos

Facility testing (tube)

Facility reporting weak typing with anti-D reagent

Reagent in use:

Immucor, Anti-D (Monoclonal Blend),

Lot #506113, exp. 05-04-2013

Reagent	Manufacturer/Lot#	IS
Anti-D (Monoclonal Blend)	Immucor Lot #506113	1+

IRL Initial testing (tube)

Reagent	Manufacturer/Lot#	IS
Anti-D (Monoclonal Blend)	Gamma-Clone® #506091	2+
Rh Control (6% Albumin)	In House	0

So... what's the problem?

- Patient types Rh positive with licensed reagent
- Typing appears valid, controls are appropriate
- ***The patient is Rh positive, right?***

Typical reagent performance

- Although we never anticipate or expect a certain result, we may choose to investigate ‘unusual or atypical’ reactivity
- Goal of testing is to **“assure the identification of all recipients who should be given only D-negative blood”**
- Most direct agglutination with anti-D reacts strongly in patient testing (3+ - 4+). Weaker reactivity may indicate unusual Rh(D) types.

Typical reagent performance

Reagent preparations are proprietary and each may perform differently.

Many FDA-approved commercial Anti-D reagents contain both IgM and IgG components.

- Gamma-clone® is manufactured by blending secretions of 2 human/murine heterohybridomas, grown in fluid culture.
 - the IgM (saline agglutination) component from cell line GAMA401
 - the IgG component is from cell line F8D8

IRL Solid Phase testing (Galileo)

Reagent	Manufacturer/Lot#	Direct Agglutination
Anti-D, series 4	Immucor 504812	0
Anti-D, series 5	Immucor 505622	0
Mono Control	Immucor 492090	0

Weak D testing also performed: Negative (Series 4)

IRL Additional testing (tube)

Anti-D reagent	Lot #	IS	IAT
Ortho BioClone® (Murine Mono-Poly blend)	DB 285A1	0	0 ✓
Immucor/Gamma – series 4 (Monoclonal Blend)	504842	0	0 ✓
Immucor/Gamma – series 5 (Monoclonal Blend)	505642	0	0 ✓

Manufacturer Instructions – Specific Performance Characteristics

Gamma-clone® Anti-D (Monoclonal Blend):

“Certain rare red blood cells that appear to be D-negative with other Anti-D reagents will react unexpectedly with this reagent. Red blood cells of the Crawford phenotype are agglutinated at the immediate-spin test phase and are usually nonreactive at the weak D phase.”

IRL additional testing (tube)

Reagent	Manufacturer/Lot#	IS	IAT
Anti-D (Monoclonal Blend)	Gamma-Clone®	2+	0 ✓
Rh Control (6% Albumin)	In House	0	0 ✓

TABLE 13-4. Reactivity of FDA-Licensed Anti-D Reagents with Some Rh Variant Red Cells

Reagent	IgM Monoclonal	IgG	DVI IS/AHG*	DBT IS/AHG*	D ^{HAR} (Whites) IS/AHG*	Crawford (Blacks) IS/AHG*	ceRT	ceSL
Gammaclone	GAMA401	F8D8 mono-clonal	Neg/Pos	Pos	Pos	Pos [†]		
Immucor Series 4	MS201	MS26 mono-clonal	Neg/Pos	Pos	Pos	Neg	Weak pos	Neg
Immucor Series 5	Th28	MS26 mono-clonal	Neg/Pos	Pos	Pos	Neg	Weak pos	Weak pos
Ortho BioClone	MAD2	Polyclonal	Neg/Pos	Neg/Pos	Neg/Neg	Neg		
Ortho Gel (ID-MTS)	MS201		Neg	Pos	Pos	Neg	Weak pos	Neg
Biotest RH1	BS226		Neg		Pos	Neg		
Biotest RH1 Blend	BS221	BS232 H41 11B7	Neg/Pos		Pos [†]	Neg		
Alba Bioscience alpha	LDM1		Neg		Pos	Neg		
Alba Bioscience beta	LDM3		Neg		Pos	Neg		
Alba Bioscience delta	LDM1 ESD1-M		Neg		Pos	Neg		
Alba blend	LDM3	ESD1	Neg/Pos		Pos	Neg		
Polyclonal			Neg/Pos	Neg/Pos	Neg/Neg	Neg/Neg	Weak pos [‡]	Neg

*Result following slash denotes anti-D test result by the indirect antiglobulin test (IAT).

[†]Test will be negative if IAT is performed.

[‡]Enzyme-treated cells.

IS = Immediate spin; AHG = antihuman globulin.

Source: AABB Technical Manual, Seventeenth Edition

Additional investigative reagent

Reagent	IS
'Anti-Crawford'(Rh:43) IgM Monoclonal Anti-D (research)	3+
Rh Control (6% Albumin)	0

Crawford Antigen

- ISBT symbol: RH43
- Occurrence: Less than 0.1% in Blacks, unknown in other ethnic groups
- Expressed on cord cells

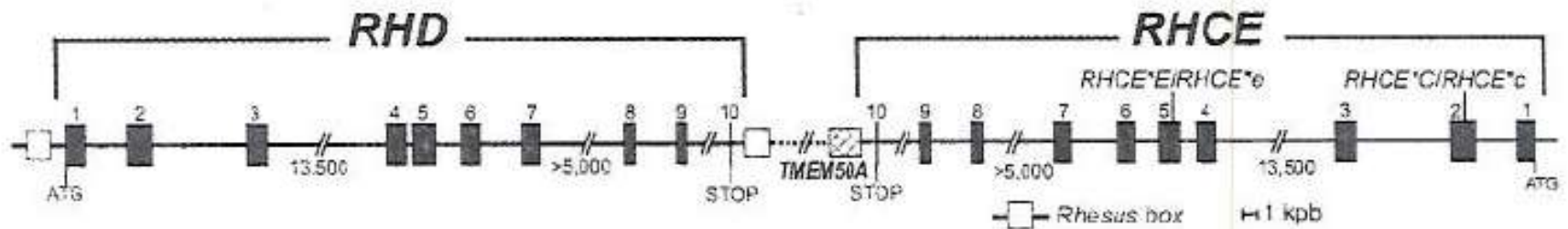
Reported in 1980, the only example of anti-Crawford was found in a reagent anti-D.

Crawford antigen is produced when an amino acid substitution occurs in the *RhCE* gene. This change results in a D-like epitope being expressed on the Rh protein that is detected by some anti-D reagents.

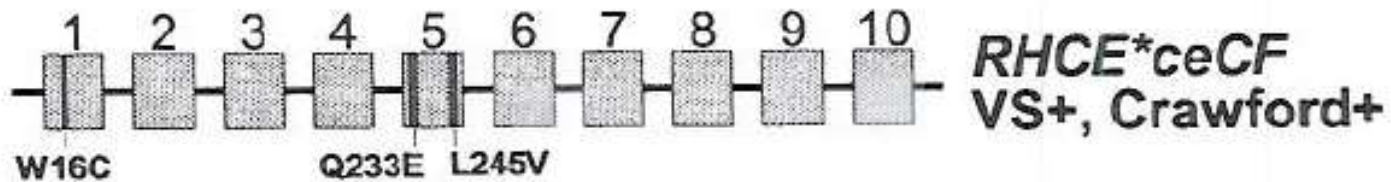
Crawford Antigen (continued)

- Crawford is encoded by an RHCE allele (*RHCE*ceCF*) that also encodes some D-specific amino acids.
- These D-specific amino acids are recognized by several potent anti-D reagents
- (D-) Crawford+ RBC's have been erroneously typed as D+

Gene diagrams



D and D-like Epitopes Expressed on RhCE



Flegel et al. The RHCE allele ceCF: the molecular basis of Crawford (RH43). *Transfusion*, 2006; 46:1334-1342

Summary

- Patient sample reacted (immediate spin only) with 1 of 6 sources of Anti-D reagent tested
- Reactivity with reagent anti-Crawford
- Patient is **D negative**
- Crawford is a low incidence antigen found on red cells of approximately 1 in 900 African-Americans

Most Importantly

Crawford phenotypes can cause D typing discrepancies.

Individuals with this phenotype
lack D antigen

Therefore, they can be sensitized
and produce Anti-D

We must get the type right!



References

AABB Technical Manual, Seventeenth Edition, ©2011, pp.398-399

The Blood Group Antigen FactsBook, Third Edition, ©2012, M.E.Reid,
C.Lomas-Francis, M.L Olsson, pp.243-244

Manufacturers package inserts:

Immucor/Gamma Anti-D (Monoclonal Blend) Gamma-clone®

Ortho Anti-D BioClone® (Murine Monoclonal-Polyclonal Blend)

Immucor/Gamma Anti-D (Monoclonal Blend) Series 4, Series 5

**Sickle Cell
Road Race
2013**

JOIN US!!

Sat. April 13

34th ANNUAL
SICKLE CELL ROAD RACE/WALK
APRIL 13, 2013

**JOIN THE RACE
TO BREAK THE
SICKLE
CYCLE**

7K RUN/4K WALK

A graphic featuring silhouettes of five runners in various stages of a stride, running across a stylized map of Georgia. The map is filled with vibrant, overlapping circular patterns in shades of red, orange, yellow, and green. A red ribbon-like line weaves through the runners, symbolizing the 'Sickle Cell Cycle' mentioned in the text.

RRCA Southern Region 7K Championship
SICKLE CELL FOUNDATION OF GEORGIA, INC.



2012 Sickle Cell Road Race



**American
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Thank You!

