Practical Application of Genotyping for D Variants

When is a Positive Truly So?

Meet Mrs. G...

- 31 year old female in labour
- Sample tested with automated geltube testing (ProVue)

  O Positive

  Negative antibody screen

- Floor questions these results as patient has received 28 week RhIG based on community lab results

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History Review

- 1st pregnancy (2 years ago)

  At delivery: O RHD Positive

  No antibodies detected

- O RHD Positive baby boy delivered

- No RhIG was given

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History Review

- <u>This pregnancy</u> 28 weeks: tested at other lab (presumed tube)

  O RHD Negative

  No antibodies detected
- Received 28 week RhIG
- Patient at delivery: RHD positive (in-house automated gel testing)

  Patient may test as RHD negative in other labs

  due to variability in antisera

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Upon Review

Considerations:

1) 2+/3+ reaction with RHD antisera
2) Patient has previously delivered an RHD pos infant with
   NO RhIG prophylaxis and did not seroconvert after RHD confrontation

Decision:

O RHD Positive

RhIG NOT recommended

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Genotyping Results

- Partial D (DAR-E) ☹
- Decreased antigen expression AND structural variation
- Patient may recognize wildtype D as foreign and is at risk for alloimmunization
- BUT report received 32 days post birth
  Too late for RhIG!

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Outcome:

Will there be tolerance or sensitization?

Allo Anti-D identified

(5 months post delivery)

Procedure change….

Step in the right direction!

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Next Red Flag..

- Patient presented to RhIG clinic for 28 week RhIG. Valid grouping results not available so sample collected at our facility.
- RHD positive in gel with **4+** RHD reaction.
- Due to the identified discrepancy, sample sent for genotyping.

Weak D Type 68

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Retrospective Review:

- This spurred a retrospective review of known discrepancies
- 1 y period (July 2014 – June 2015): 18 variants identified
  (2 potentially alloimmunizable)

Genotyping Result	Total
Weak D Type 1	8
Weak D Type 2	5
Weak D Type 3	3
Weak D Type 5	1
Weak D Type 68	1
Total	18

11%

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Moving Forward..

- After looking back with some dread… it was time to formally look forward, in an attempt to define the scope of our problem

- At CSTM we presented the results of a more systematic approach to these Rh+ women that we were worried about

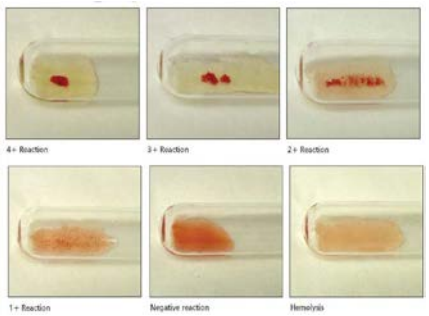
When is a Positive Truly So?

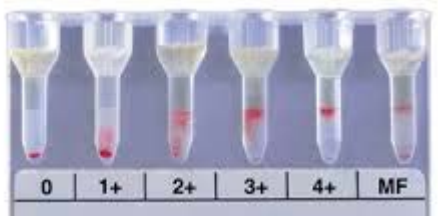
Defining a New Cut-Off in a Precautionary Approach to **D** Typing in Child-Bearing Age Females

Lisa Richards, Irene Skinner, Allan Lupish, Grant Johnson, Sally Balmer, Lani Lieberman, Yulia Lin, Jeannie Callum, Jacob Pendergrast, Christine Cserti-Gazdewich

RHD Background

- Correct RHD typing in child bearing age females (CBAF) dictates who warrants Rh immunoprophylaxis and restriction to D- products.
- Traditional "D+" defined as $\geq$ 2+ reactivity (regardless of test modality) and assumes wildtype configuration/D tolerance.
- Grading and interpretation of agglutination is the foundation of blood transfusion testing.
- Grading has well documented criteria specific for each test modality.





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RHD Background

- Tube test package insert Interpretation of Results:

  Agglutination of the red blood cells is a positive test result and indicates the presence of the D antigen.

- Gel package insert Interpretation of Results:

  Agglutination and/or hemolysis of the red blood cells is a positive test result.

- Origin of ≥ 2+ reactivity represents RHD positive?
- Evidence to support same interpretation irrespective of test modality?

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Purpose

- To determine our rate of modality-related D discrepancies, because inter-reagent discrepancies may suggest sensitization-vulnerable variants.
- To determine if ≥2+ agglutination accurately reflects Rh positive status.
- To determine the clinical meaning/significance of the D discrepancies as defined by their genotyping results.

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Methods

- Routine RHD grouping uses automated geltubes (ORTHO ProVue)
- Anti-D in gel card is *monoclonal human IgM (MS-201)*

- Tube testing used 2 Anti-D reagents
- D1: *monoclonal human IgM (MAD2)* + *polyclonal human serum IgG* BioClone (OrthoClinical Diagnostics)
- D2: *monoclonal IgM D7B8* + *monoclonal IgG H1121G6/LORIFA* BioClone (OrthoClinical Diagnostics)

Methods

- 1000 consecutive Rh positive (as established in gel) CBAF samples then tested in tube
- CBAF defined as female ≤ 45 years *(Transfusion, November 2012)*
- Rh positive defined as ≥2+ *(AABB Technical Manual)*
- 01 August 2015 – 18 November 2015
- Patients only enrolled once, no duplicate samples
- Dissimilar tube results were sent for genotyping (Immucor RHD BeadChip) to determine D classification

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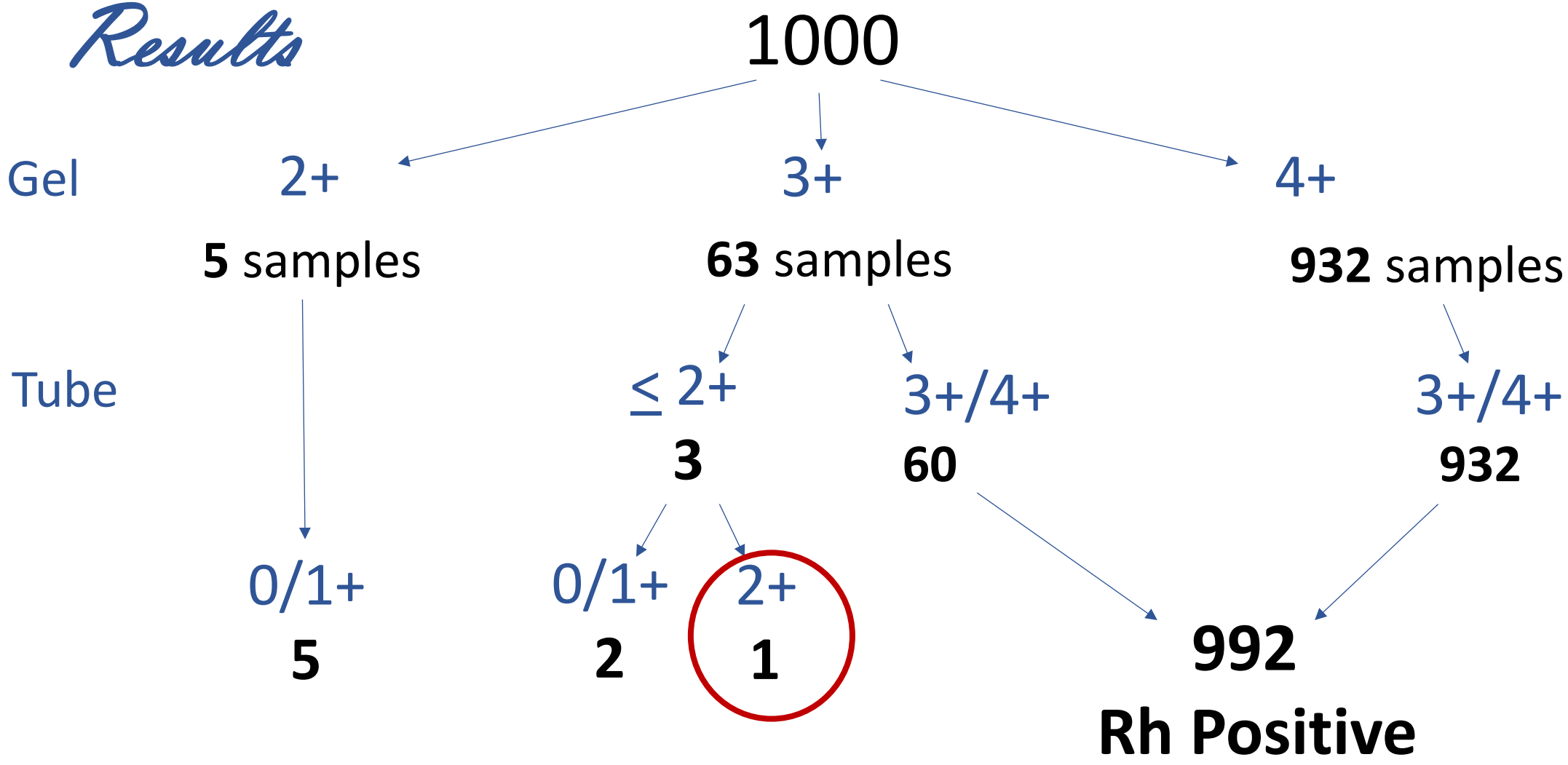
Results

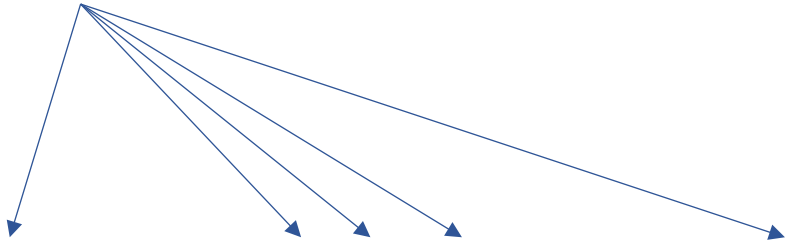

1000
Gel
2+
3+
4+
5 samples
63 samples
932 samples
Tube
≤ 2+
3+/4+
3+/4+
3
60
932
0/1+
0/1+
2+
5
2
1
992
Rh Positive

2+ Gel

5/5 variants

3+ Gel

3/3 variants



Type 1

1

Type 2

3

DAR

1

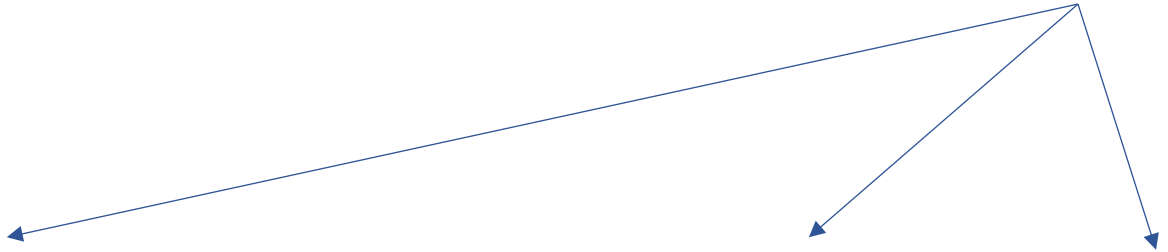
Type 4.1

2+ Gel

5/5 variants

3+ Gel

3/3 variants



Type 1

1

Type 2

DAR

1

Type 4.1

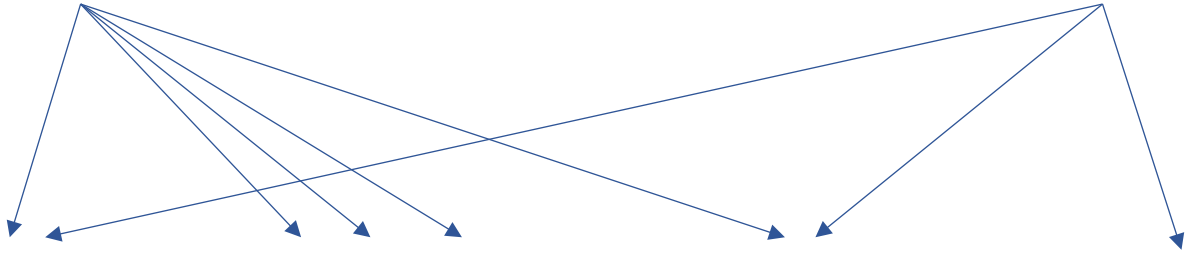
1

2+ Gel

5/5 variants

3+ Gel

3/3 variants



Type 1

2

Type 2

3

DAR

2

Type 4.1

1

Tolerant

5

At Risk

3

Results

- 0.8% of gel RHD+ samples associate with weaker tube results
- 100% of gel>tube discrepancies had RHD variants on genotyping
- 38% of gel>tube discrepancies were potentially alloimmunizable
  - Weakest gel cases (2+): 1 in 5 at risk
  - 3+ gel cases with tube discrepancies: 2 in 3 at risk

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Limitations

- Tube agglutination grading is subjective, infrequently performed and therefore possibly inconsistent. Training and visual aids should be available for consistent reporting.
  - Study testing was performed by one experienced technologist using grading chart to eliminate inconsistencies.

- The consistency between 4+ gel and high-strength tube (3+/4+) left these patients genotypically uncharacterized.
  - It is possible that some of these, too, are at risk of being variants, but
    - only a handful have arisen in tens of thousands
    - genotyping to prove this point may be cost-prohibitive

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Prospective Study Conclusions

- The ≥ 2+ definition of Rh positive status may be misleading.
- By using this historic cut-off, **three** women would have missed Rh immunoprophylaxis. (That's one in every 333 consecutive D+ CBAFs….)
- Strong 4+ agglutination in gel more assuredly identifies Rh + CBAF
- We now scrutinize gel reactions ≤ 3+

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Our Process

CBAF's

- 4+ on ProVue resulted as RH positive (unless any evidence of Rh discrepancy)

Yes 3+!

- ≤3+ on ProVue continue to have confirmatory tube test
- TUBE 3+/4+ resulted as RH positive
- If TUBE 2+ or less patient managed as Rh negative until genotyping results define accurate RHD status

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Our Process...an Integrated Approach

- Begins with sorting patients by clinical demographics
- Symbiotic relationship of ‘old’ and ‘new’!
- Initial testing with a ‘new’ more expensive, highly sensitive gel reagent
- Followed with the ‘old’ gold standard cheap tube reagent
- Used in combination, we are able to identify the women that will benefit from genotyping
- Helping to steward an expensive resource

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Genotyping

- One time testing that ensures patients with D variants are identified and dictates future product requirements.
- Eliminates the need for Rh negative product and RHIg overuse on non-alloimmunizable variants (weak D 1,2,3)

- Physician notified and report faxed
- Genotyping results and interpretation added to patient history

- **But** if information is not disseminated, then NO improvement to patient care or cost savings will be achieved…

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D Cards!

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905 576-8711 ext.3440
Transfusion Medicine

NAME: Test Patient	**DOB:** 12/08/85	**ABO/RH:** B Neg

Significant Information: **Genotyping results**
This patient is a PARTIAL D type. She **DOES** require RHig during pregnancy and **RH NEGATIVE** blood products, despite the variable D typing results.

Date: 01October, 2015

PRESENT THIS CARD AT ANY HOSPITAL OR LAB VISIT

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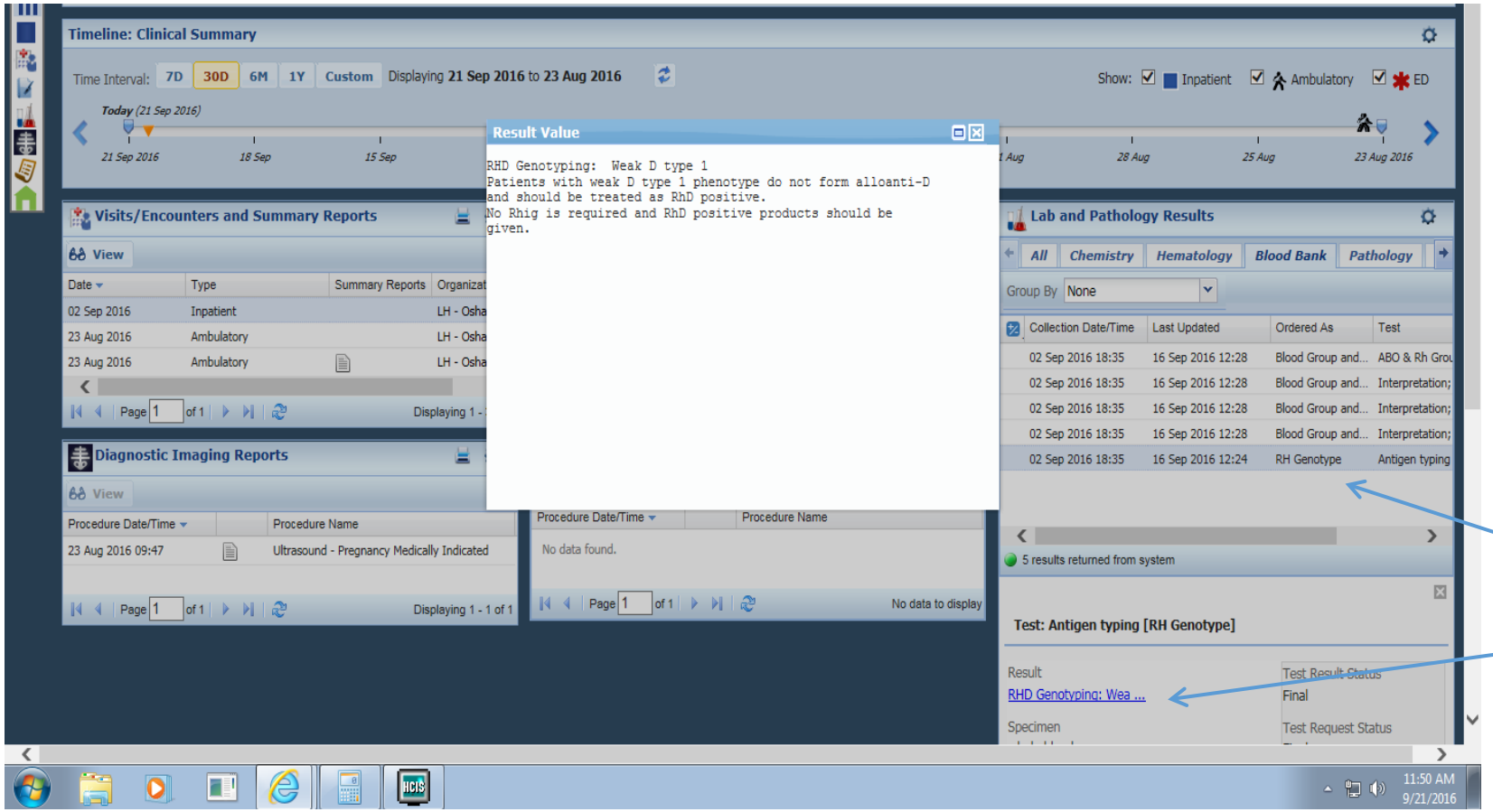
CGTA Viewer



Click on RH Genotype
Then click on blue link
RHD Genotype

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Alert to OBS

To: Lakeridge Obstetric Team

Determining the RhD status of obstetric patients is very important to ensure Rhogam prophylaxis is provided appropriately.

A correct RhD determination is challenging in some patients due to a weakened D expression and/or a partial D expression on their red cells.

Many of our obstetric patients have prenatal blood work tested in the community. Please be aware that private laboratories do not have the same policies regarding weak D/partial D testing as Lakeridge Health. We have found reports from local private laboratories that are unclear/misleading.

This is only one example:

Blood Group: B POSITIVE

Antibody Screen:
No clinically significant antibodies detected.
A weak D antigen was detected.
Results have been repeated and verified.

Routine laboratory testing is NOT able to differentiate between a partial D requiring RhIg prophylaxis and a weak D that does NOT require Rh immune globulin.
We recommend a follow-up maternal type and screen be drawn and tested at Lakeridge Health laboratory if you encounter prenatal Rh results that are ambiguous/unclear.

Ambiguous test results may include a patient with the following:

1) Discrepant results -eg Rh pos in one lab, Rh neg in another
2) Any comment regarding 'Weak D antigen' regardless of blood group interpretation.

Please order T/S for Rh confirmation and book the appointment through Central Booking at 905-576-8711 ext 4717.
This testing will ensure the best care for the present pregnancy and for all future pregnancies and transfusion needs.

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Post Study

Genotyping results (01Sept 2015 – 31 Aug 2016)

Genotyping Result	Number identified	Tolerant or At Risk
Weak D Type 1	5	Tolerant
Weak D Type 2	4	Tolerant
DAR	3	At Risk
Weak D Type 4.1	1	At Risk
Weak D Type 4.0 or 4.3	2	At Risk
D variants (other than 1,2,3)	2	At Risk
Totals	17	

47%

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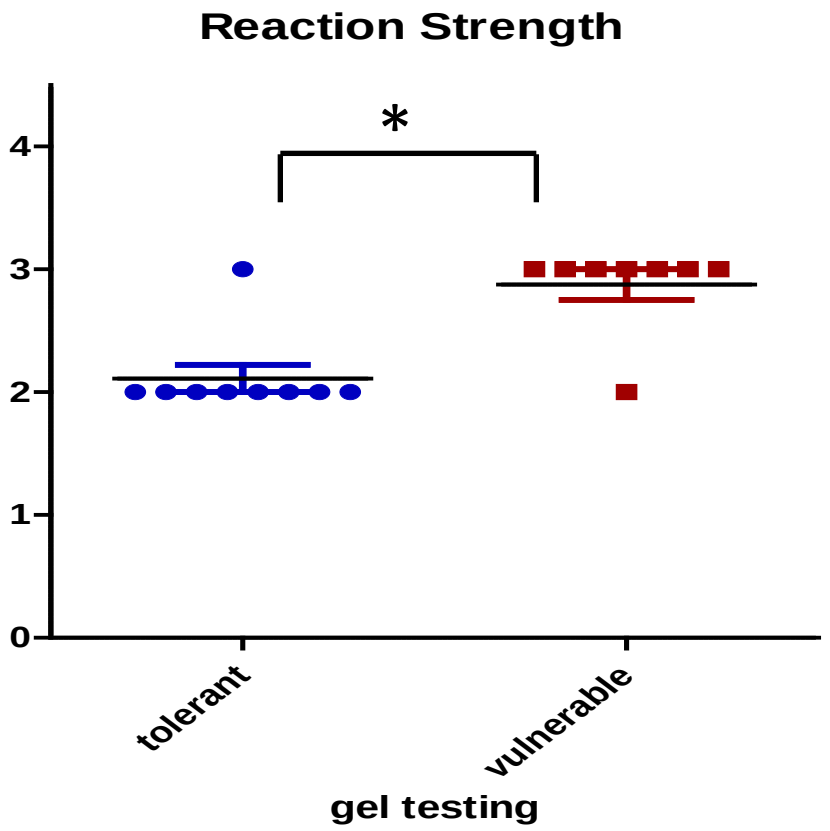
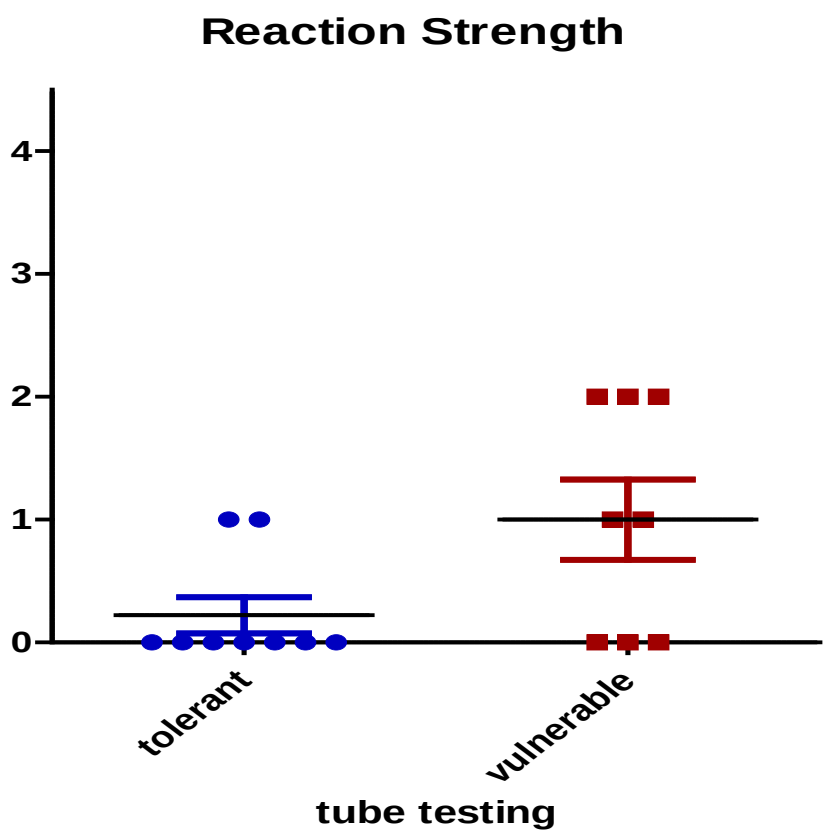
Gel-Test Subjects with Strength <4+:



* P-value 0.0027

	tolerant	vulnerable
Median	2+	3+
95% CI	1.9 – 2.4	2.6 – 3.2

Corresponding Tube Strengths:



P-value 0.0641 (NS)

	tolerant	vulnerable
Median	0	1+
95% CI	0 – 0.6	0.2 – 1.8

Practicalities

- Based on the study population: 68/1000 samples would have warranted a tube test, at an additional reagent cost of $13.60
  - only $4.63 per vulnerable woman discovered

- This does not include the savings of:
  - Preventing confusion/wasted time caused by investigating discrepancies
  - Overuse of RHD negative blood and RHIG
  - 3 potential HDFN cases averted

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Final Thoughts

- Gel is advantageous in females >45 and males, (preventing RHD- blood overuse), because RHD+ overcall is not as dangerous as in CBAF.

- Our take: Gel <4+ (7% of positives), with Tube <3+ (12% of <4+ in gel), justifies RHD genotyping and D- precautions until evidence of D tolerant molecular types are identified.
  - We are uncomfortable calling them weak & tolerant:
    - 38% were at risk of alloimmunization
  - Depending on testing era – went from 10% to 47%
  - All depends on how you look

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Thank you!

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