

Using Automated Gel Method for Obstetric Antibody Titters: What's the New Normal Range?

EMILY COBERLY, MD

MEDICAL DIRECTOR, TRANSFUSION SERVICES

UNIVERSITY OF MISSOURI HEALTHCARE



Outline

Hemolytic disease of the fetus and newborn

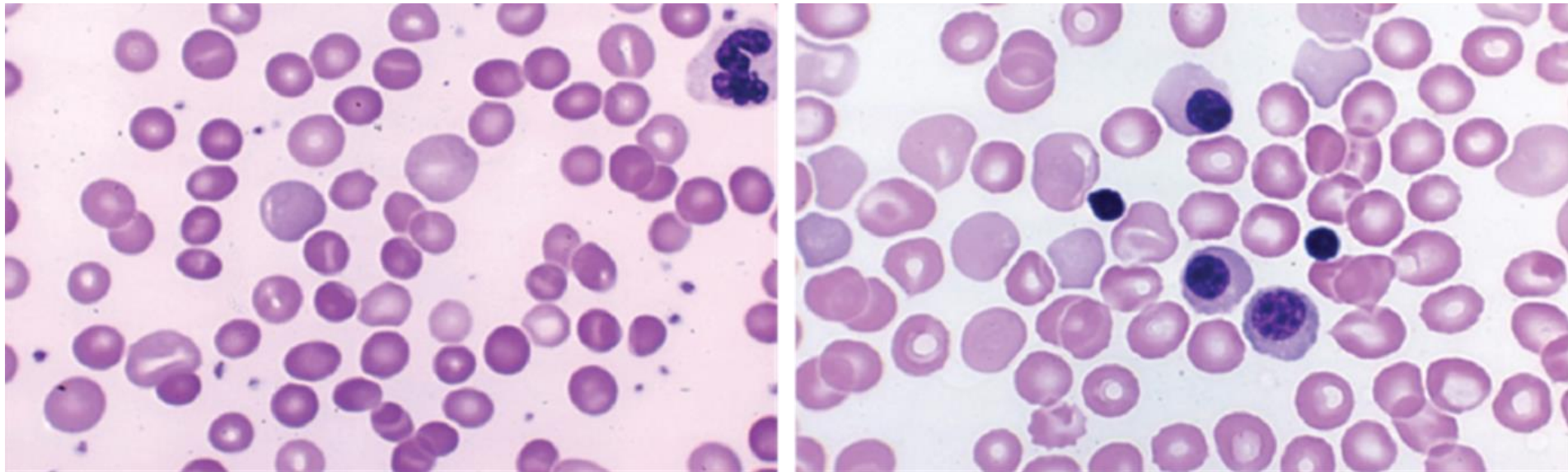
Gel vs tube methodology for titers

What is the “critical” level for automated gel titers in obstetric patients?

Hemolytic disease of the fetus and newborn

HDFN is caused by maternal alloantibodies against paternal antigens that are expressed on fetal red blood cells

Immune destruction of fetal rbc's by the maternal alloantibody leads to anemia and jaundice, and in severe cases hydrops fetalis and fetal death



A

B

ACOG Recommendations

All pregnant women should be screened with an antibody screen at the first prenatal visit

If Rh negative, also repeat the antibody screen prior to the 28 week dose of RhIg, at delivery, and at the time of any event during pregnancy

If antibody screen is positive and paternity is assured, consider paternal rbc phenotyping/genotyping

- If paternal typing is heterozygous or unknown, consider molecular analysis of maternal plasma for fetal DNA (not widely used)



ACOG Recommendations

If there has been a prior pregnancy affected by HDFN, serial antibody titers are not useful

If no prior affected pregnancy, serial titers are recommended

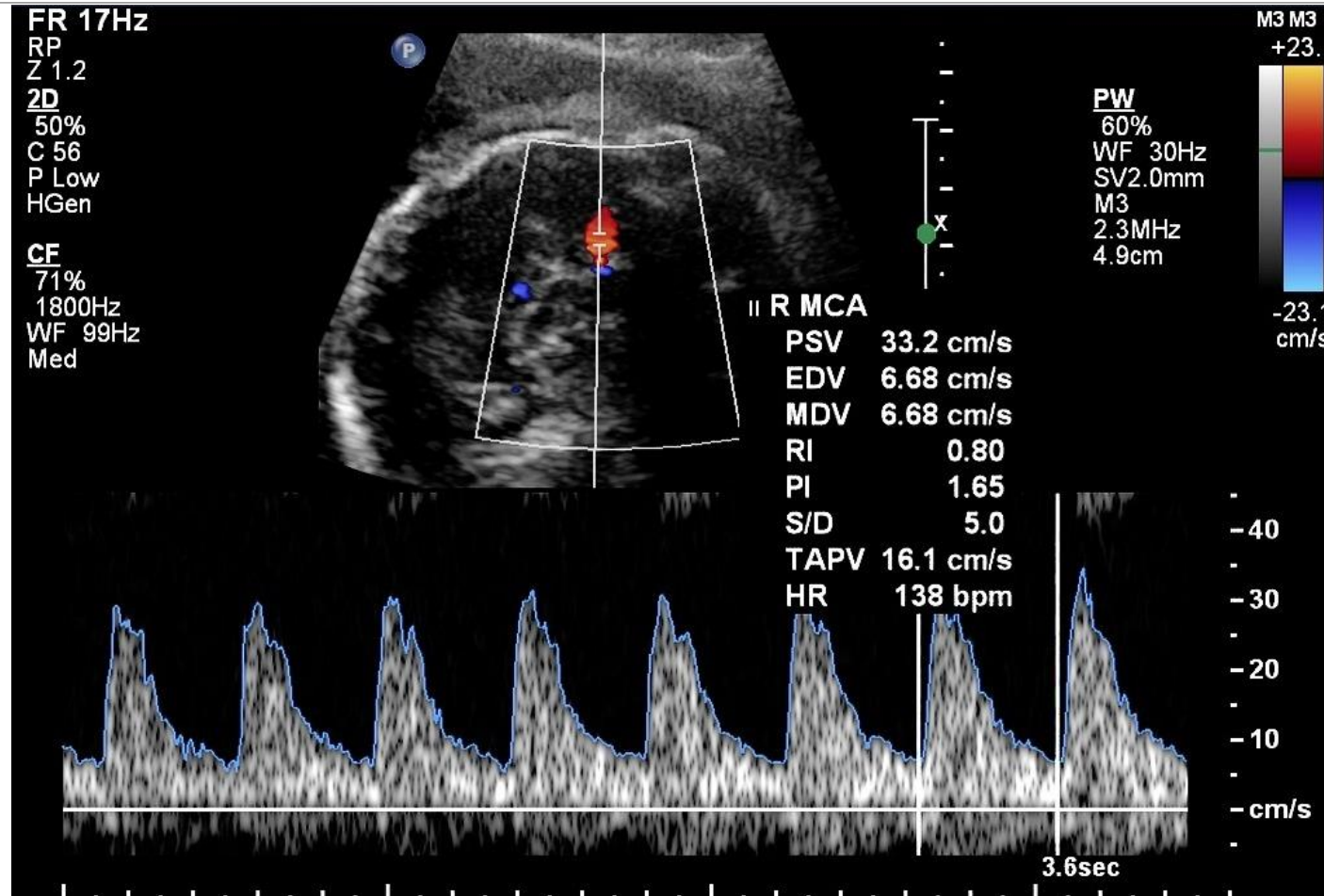
- “Critical” titer is associated with a significant risk for severe erythroblastosis fetalis and hydrops
- Usual “critical” range is a titer of 16 or above (may vary)
 - Anti-K1 titers are less predictive of fetal anemia, so a lower titer (8) may be significant
- Variation in titer results between laboratories is not uncommon
- A change of more than 1 dilution in the same laboratory is also considered significant

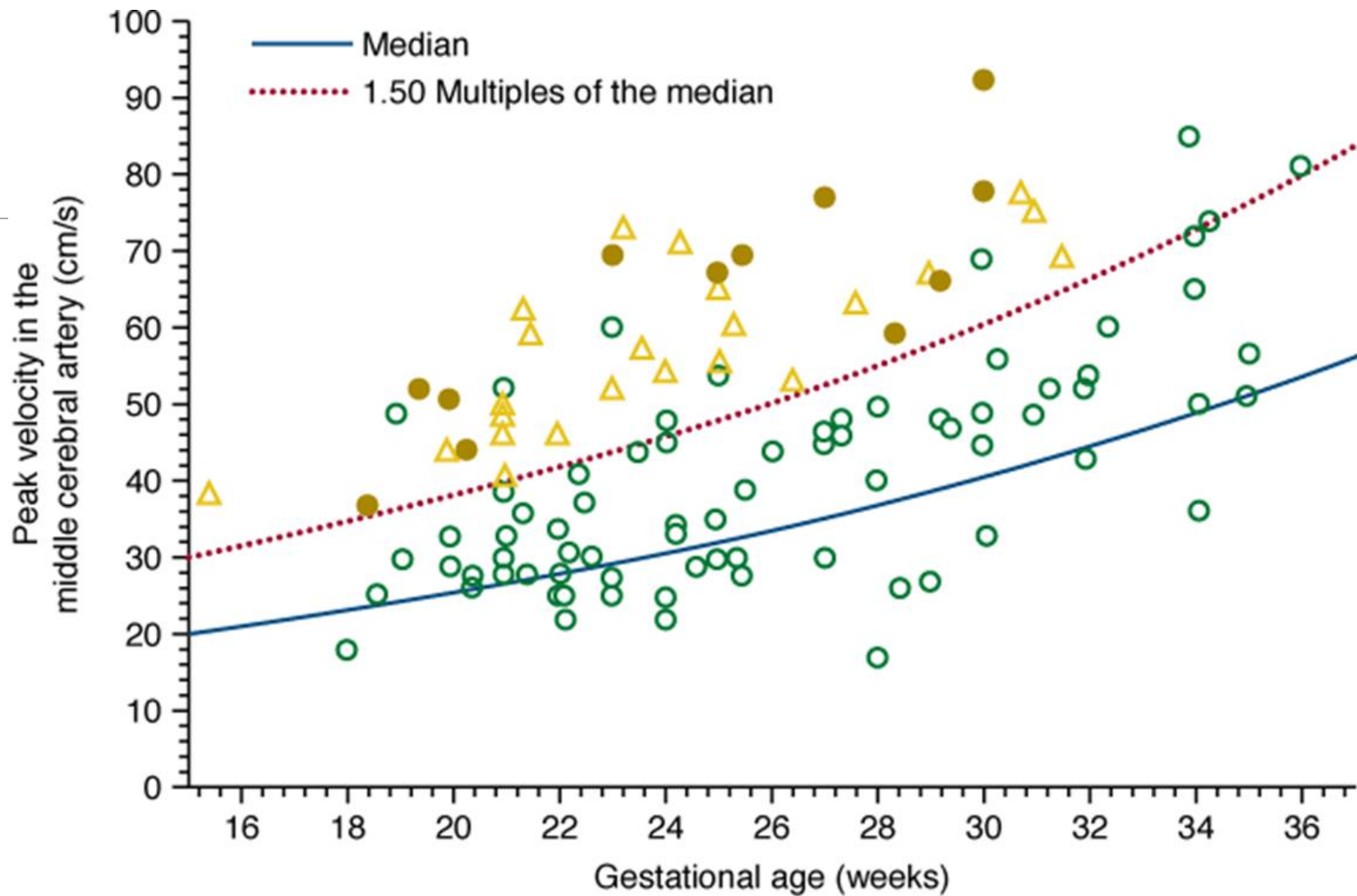


Middle Cerebral Artery Doppler US

If the patient has a prior affected pregnancy or has a “critical” titer, middle cerebral artery (MCA) Doppler ultrasound is indicated to predict the degree of fetal anemia

Middle Cerebral Artery Doppler US



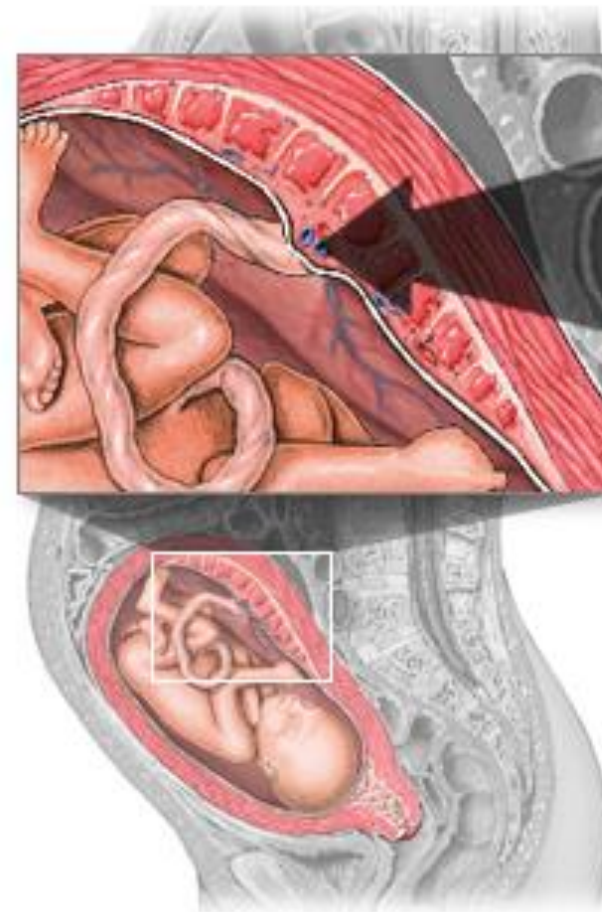


Peak velocity of systolic blood flow in the middle cerebral artery in 111 fetuses at risk for anemia as a result of maternal alloimmunization. Open circles, fetuses with either mild or no anemia; solid circles, fetuses with hydrops; triangles, fetuses with moderate or severe anemia.

Intrauterine transfusion

If the peak systolic velocity of the middle cerebral artery is greater than 1.5 MoM on two occasions

- Deliver the baby or, if too early,...
- Intrauterine transfusion



Intrauterine transfusion



A fetus may receive a blood transfusion through the umbilical vein in the placenta

Obstetric Titers

This entire algorithm is kicked off by the finding of a “critical” antibody titer

AABB Technical Manual:

- Recommended methodology for titers is tube with AHG
- “Other methods, such as using albumin AHG or gel, may result in higher titers than the recommended method and should be validated with clinical findings and laboratory data to ensure appropriate interpretation by the obstetrician and avoid inappropriate referral of patients for high-risk obstetric care.”
- The critical titer for anti-D (the level below which HDFN and hydrops fetalis are unlikely and no invasive procedures are needed) is 16 in the AHG phase. As long as the titer is 8 or lower, except in the case of anti-K, the pregnancy can be followed by titers.”

Despite this, many sites are performing titers using gel methods on the recent CAP PT survey results

Gel vs tube titers

Manual tube testing is known to be associated with variable results (poor reproducibility/precision) and is time and resource intensive

Automated gel is less time intensive, but gel titers are known to be more sensitive and give higher results compared to tube titers

There is a lack of data on clinical outcomes to allow automated gel results to be “validated with clinical findings”

Gel vs tube titers

ABO incompatible renal transplant patients

- Critical level of 16 using tube method is used as the cutoff (same as for HDFN)
- ABO antibody titers (67 samples)
- Gel method was more sensitive, with result approximately 2.5 x higher than tube using the same sample

Obstetric titers

- Vanderbilt University tested 26 patient samples using manual tube and automated gel x 3 different analyzers
- Reproducibility/precision of automated gel was 90%
- Rh antibody titers were 3.2 times higher using gel (1-7), on average
- Non-Rh antibody titers were 1.03 times higher using gel (1-2), on average
- Clinical correlation studies are necessary for clinical implementation

MU Study

40 pregnancies, 84 sets of titers

All titers performed with both tube and automated gel methodology

Clinical outcomes including MCA Doppler results, intrauterine transfusion, neonatal bilirubin and hematocrit levels, phototherapy and transfusion were all recorded to determine the clinical significance of titer results

Results

Automated gel results were equal to or greater than tube method in 100% of cases

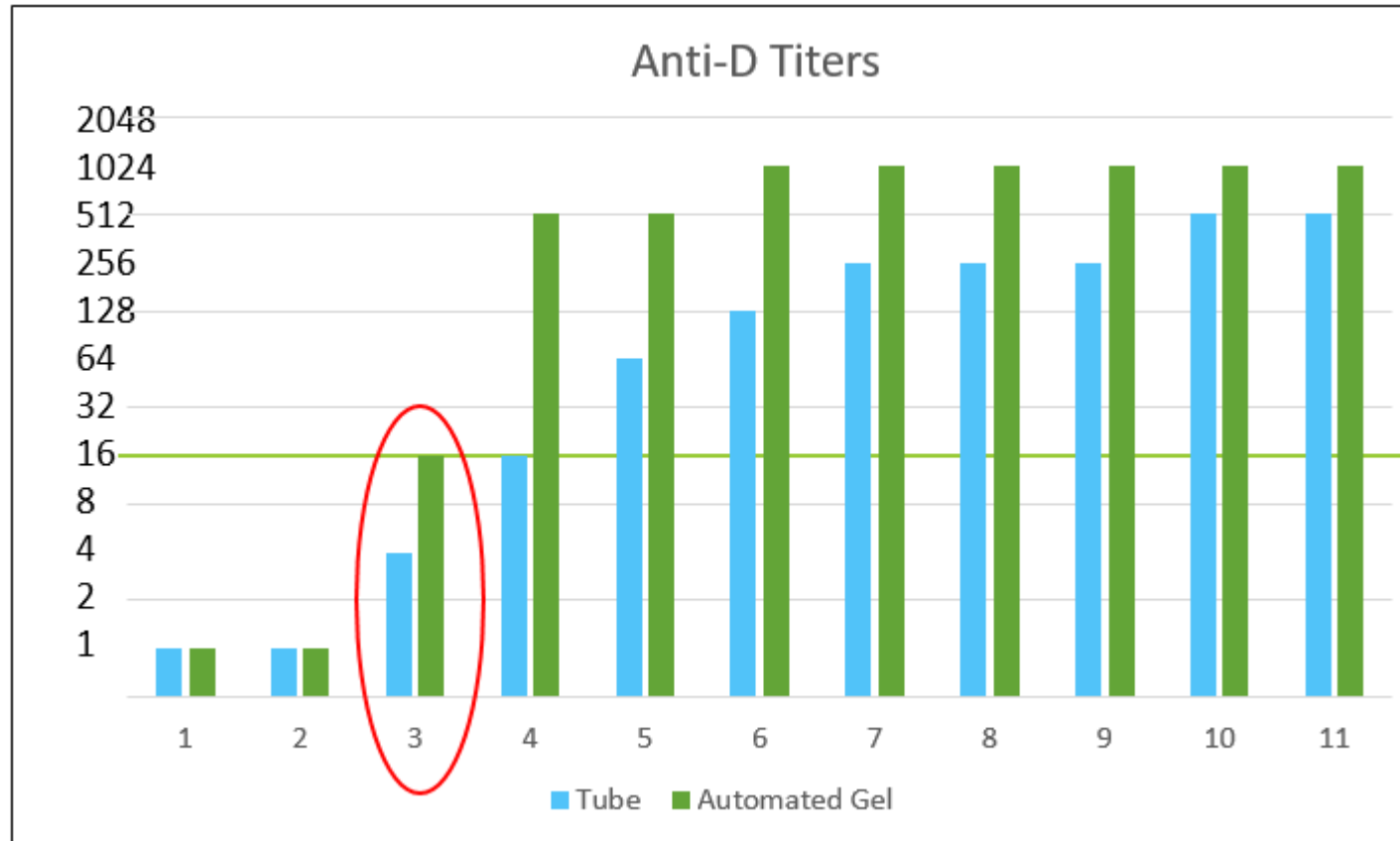
61 sets of titers were for Rh alloantibodies (D, c, C, and E)

- Automated gel results were an average of 3.1 fold higher than tube (range 1-7) *

23 sets of titers for non-Rh alloantibodies (Fya, Jka, K, M, P1, S, and s)

- Automated gel results were an average of 2.3 fold higher than tube (range 1-4)*

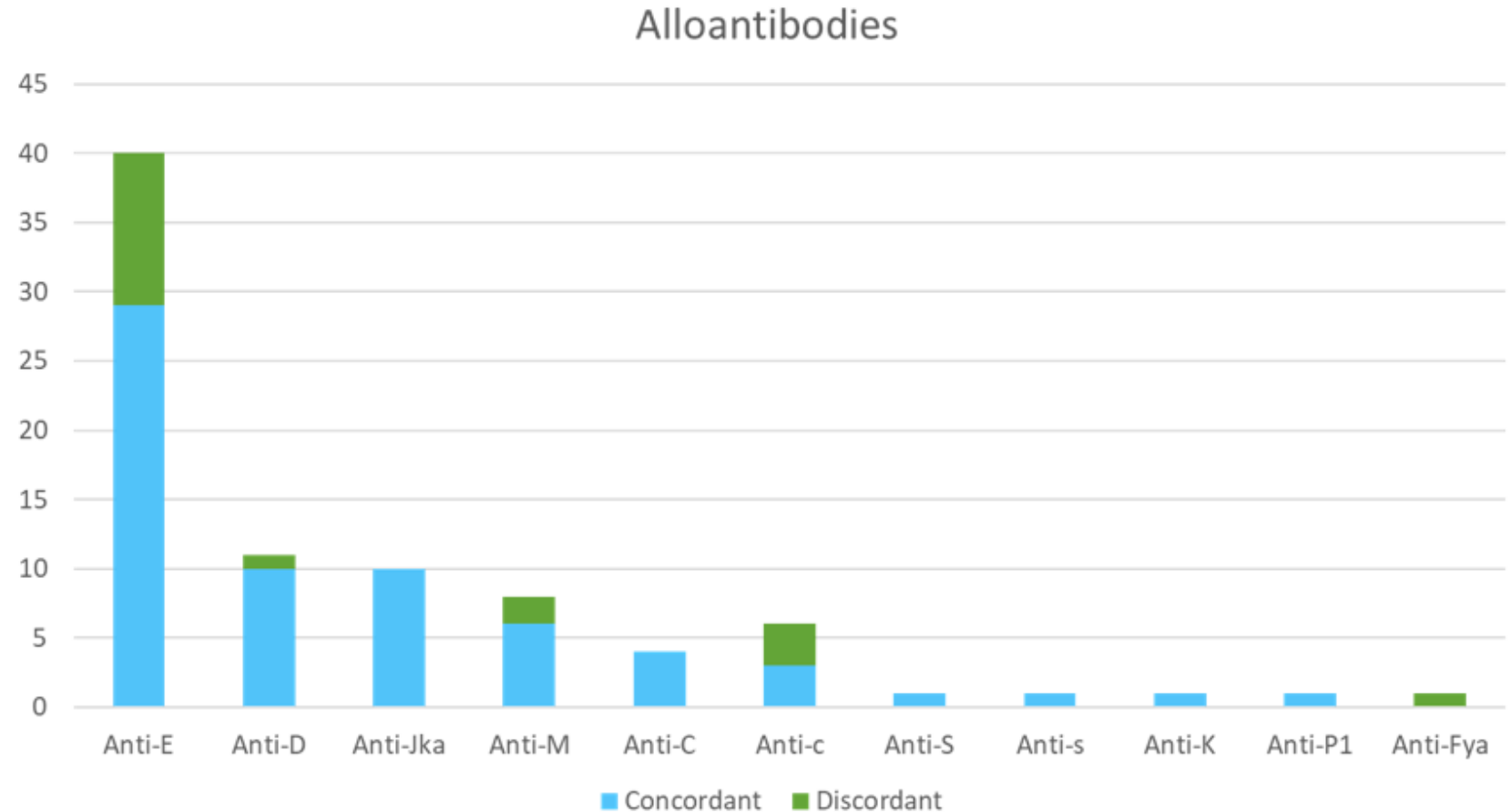
Anti-D



Results

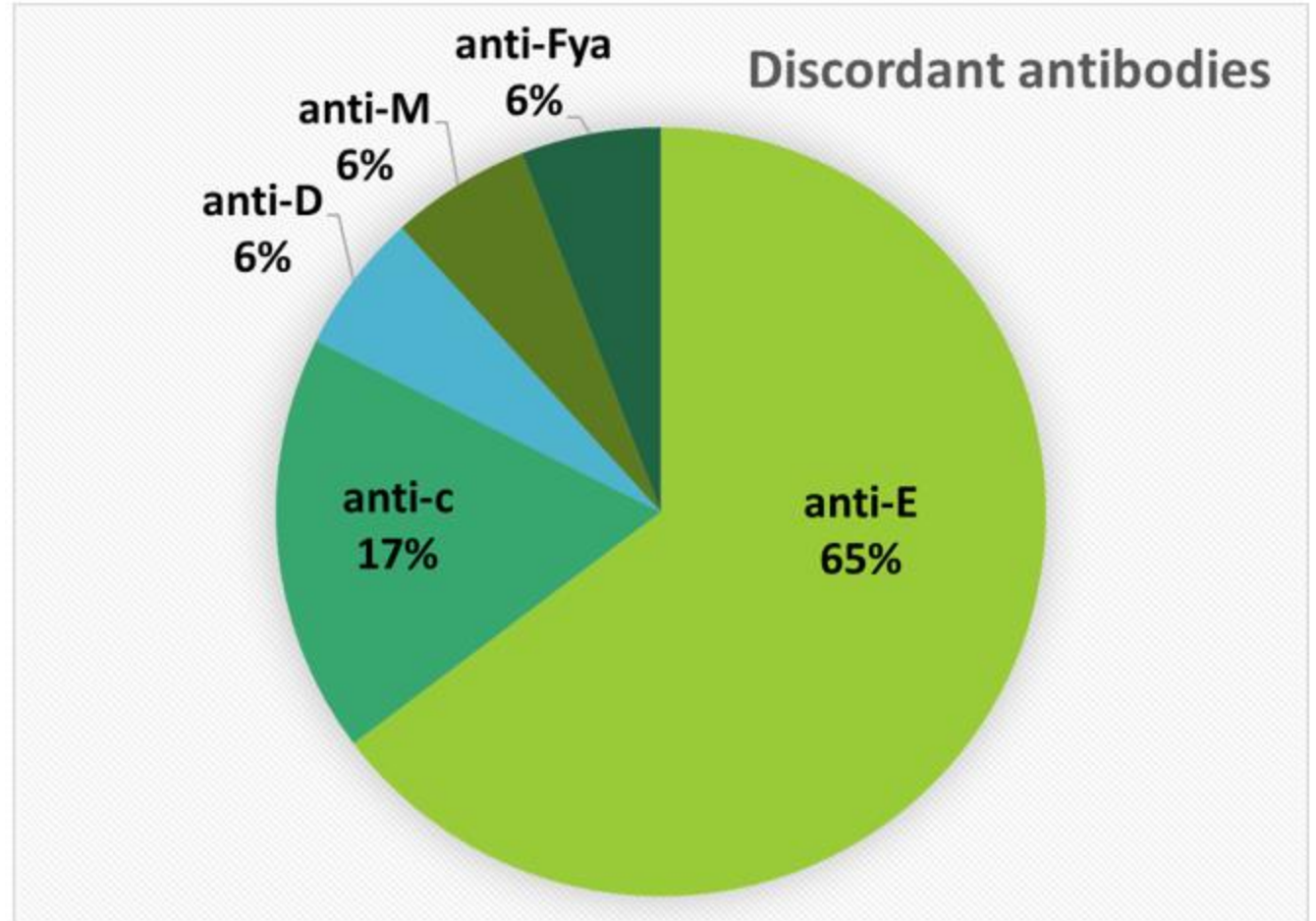
Overall, about 80% of titers were concordant when using 16 as a critical threshold (either both tube and gel were 8 or less, or both tube and gel were 16 or more)

The most common alloantibody was anti-E, followed by anti-D and anti-Jka



Results

Of the ~20% of cases with discordant titers (tube less than 16 and gel greater than or equal to 16), most were anti-E and anti-c



Clinical Outcomes

Of 17 discordant titers (9 pregnancies), only 1 case of clinically significant HDFN

- Anti-D
- Initial titer was 1 in tube and 1 in automated gel (concordant)
- Second titer was 4 in tube and 16 in automated gel (discordant)
 - A 2-fold change in titer performed in the same laboratory is concerning and should
- Patient delivered early at 32 weeks, infant was D+ and required phototherapy for jaundice

Of the remaining 16 discordant titers (8 pregnancies), the automated gel titers ranged from 16-128 with no clinical evidence of HDFN in the infant. If using only automated gel methodology with a critical titer of 16, these 8 patients would have had additional unnecessary testing (Doppler ultrasound).

Summary

In this study, we confirmed that automated gel titer results were equal or higher than tube titers, and Rh alloantibodies tend to have a greater discrepancy between tube and gel than non-Rh alloantibodies

If we continue to use 16 as our critical threshold (excluding anti-K), the majority of the time (80%) the results are concordant with tube

In our hospital, switching to automated gel and keeping the critical titer at 16 would have resulted in 8 patients over 2 years who would have received unnecessary additional testing

- Fortunately, the testing would be a non-invasive Doppler ultrasound

- Unfortunately, some normal babies without anemia will have a MoM above 1.5 and may then receive invasive periumbilical blood sampling!

Can the “critical” titer for automated gel be moved up to 32, 64, or even 128?

- Maybe, and for 8/9 patients this would have been completely safe

- But for one patient, anti-D HDFN could have been missed at a higher titer (although titer changed >2 fold)

Conclusion

We have communicated with our high risk OB group, and are planning to switch our OB titers to automated gel methodology

We will continue to use 16 as a critical threshold for the time being, although for stable gel titers on the border (16, 32) we will allow the OB to request a confirmatory tube titer if it assists with their decision making

If you perform obstetric or transplant titers, consider doing a similar study including clinical evaluations and share your results! More data will allow us all to make the best clinical decision for our patients.

Thank You!

Acknowledgements:

Dr. Jessica Kneib

Krystal Tray

Dena Stroud