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Reconciling conflicting results for Rh typing using different test reagents

A colleague on the US East Coast reports that his laboratory is trying to resolve an interesting Rh typing problem in a female patient whose initial Rh(D) typing in June 2000 (as part of prenatal testing) was **negative** using a monoclonal/polyclonal blend anti-D typing reagent. The patient was seen a few months later in November 2000 at another hospital, at which time she typed group O Rh negative, but this time with a **"weak Du"** result. In spite of her "weak Du" typing result at the other hospital, she was "treated" as an Rh negative individual, and would have received Rh Immune Globulin at the time of her delivery, if her baby had been Rh positive. The baby apparently was Rh negative. As part of trying to resolve the discordant results from her initial Rh typing, a records review revealed that the two hospitals were using different manufacturers' monoclonal/polyclonal blend anti-D typing reagents during 2000.

The patient returned to the inquiring colleague's hospital for prenatal care during a subsequent pregnancy in December 2002, at which time she showed **weak D** typing results. A Kleihauer Betke stain was negative, which seemed to rule out the weak positive reaction being due to fetal cells. The Rh typing of December 2002 was done with a different manufacturer's monoclonal/polyclonal blend anti-D typing reagent than that used in the June 2000 Rh typing, but interestingly, the December 2002 Rh typing was done with reagents from the same manufacturer as that used by the other hospital in 2000, when a "weak Du" result was reported. To make matters even more complicated, her "Du typing" result is currently **weakly positive** when using an AHG IgG heavy chain reagent, but is **2+** when using a broad spectrum AHG reagent. In addition, her red cells absorb anti-D and the absorbed anti-D can be eluted from her cells.

Has any colleague experienced a similar case? The manufacturer of the AHG reagent apparently has no prior similar experience. Are there QC implications for the AHG reagent that were used in this case? The inquiring colleague reports that his lab will be receiving an alternate source of anti-human globulin to examine what effect that reagent has on the strength of the reactions seen.

The following responses were received.

ADDENDA Feb. 4, 2003

1. **Marilyn Moulds, VP Reference and Education, Immucor Inc./Gamma Biologicals Inc.** (attribution used with permission), refers the e-network forum to the abstract S71-040A in Transfusion on page 20S from this year's AABB meeting, on "Reactivity of FDA-approved monoclonal Anti-D reagents with Partial D RBCs" by Judd, Moulds and Schlanser. In her opinion (verbatim) "I think this reinforces John Judd's suggestion or recommendation that the weak D test NOT be done on prenatal patients. As to the sample mentioned in the article, it could very well be a partial D VI person who could make alloanti-D, or it could be a quantitative weak D that would not produce alloanti-D. As to the antiglobulin sera, IgG vs Polyspecific (broad spectrum), are the reagents made by the same manufacturer? Are they rabbit or monoclonal reagents? If rabbit Anti-IgG, the rabbits used in the Polyspecific reagent are more than likely different than the ones used to make the IgG reagent. If it is monoclonal, then the Anti-IgG is the same as the one in the Polyspecific reagent."
2. **Sheryl A. Kochman, Chief, Devices Review Branch of CBER/OBRR/DBA**, (Phone 301-827-6123), (verbatim) comments: "To understand what is going on here, it is helpful to understand how the monoclonal/polyclonal blend Anti-D reagents function. Current monoclonal/polyclonal blends, as the name implies, consist of material of monoclonal origin (from one cell line) and material of human origin (polyclonal). Each of these components have a

different function. Initially, no licensed monoclonal anti-D were capable of reacting with D category VI partial D cells and reactivity with other partial D cells was also variable. On the plus side, these monoclonal anti-D's are very avid and generally react strongly at immediate spin with cells that are clearly D+ and most weak D (Du cells). However, since no monoclonal antibody alone was suitable for testing donor blood, the initial solution to that problem was to add human polyclonal anti-D, which does detect partial D's and other weak D cells that monoclonal antibodies may miss, at the antiglobulin phase.

The more current solution to the problem of monoclonal antibodies that do not detect partial D cells is to blend them with a new monoclonal anti-D that does react with partial D cells, including D category VI, at the antiglobulin phase. These reagents are named Anti-D (Monoclonal Blend).

It is important to refer to the "Limitations" and "Specific Performance Characteristics" of the manufacturers' package inserts to see what is known about the monoclonal anti-D.

I suspect that in this case, the woman has an extremely weak expression of the D antigen or a weak partial D phenotype. At this time, I cannot explain the difference in reactivity between polyspecific and Anti-IgG anti-human globulin.

Because individuals with the partial D phenotype have been reported to make antibody to the parts of the D antigen they are missing, they should be treated as D negative if a patient or pregnant woman. Donors should be treated as D+, although it is unknown if exposure such a weak expression of the D antigen can cause an immune reaction."

ADDENDA Mar. 25, 2007

- 3. A physician affiliated with a blood bank service at a general hospital in Sparta, Greece** reports a case of an 82-year old man who was admitted April, 2006 because of a hip fracture. At that time and during a follow up admission in May 2006, the patient typed as group **O Rh positive (CcDee, not Du)** using **gel micro-column agglutination** (DiaMed-ID MTS™, ABD/Rh card, human antibodies) and **slide agglutination** techniques with two different blended monoclonal/polyclonal anti-D reagents that reportedly do not detect DVI Rh variant in direct testing (Ortho and DiaMed). Several different experienced technologists reported the patient as Rh positive. The patient was transfused with 4 units group O Rh positive RBCs. During the May 2006 admission the patient was **diagnosed with myelodysplastic syndrome**. The patient had no other admission (or transfusion) at any institution until January 2007 when he was admitted for severe anemia. In **January 2007** he typed as group **O Rh negative (ccdee) using the same methodology** (and same manufacturer's reagents) as were used in April and May 2006. Antibody screening, DAT and Du testing with an indirect antiglobulin test (IAT) were negative and the patient was transfused with group O Rh negative blood. During a **March 2007** admission he again typed as group **O Rh negative (ccdee) but Du testing with IAT conventional tube method was positive** (2+ using polyspecific AHG rabbit IgG, DiaMed) without any mixed-field picture in a micro-column gel test. No elution technique for absorbed anti-D was carried out. The inquiring physician wonders if for practical reasons, such as limited group O Rh negative blood supplies, should management of this patient be based on the previous blood type records which showed the patient to be Rh positive, or should they rely on current Rhesus phenotype results? He wonders if a **molecular analysis would be of clinical value** to define the Rh status.

ADDENDA June 11, 2008

- 4. A Clinical Laboratory Manager of a Transfusion Service in Wisconsin** reports that his laboratory **currently uses a commercial reagent** that consists of a blend of monoclonal IgM and **monoclonal IgG antibodies** (Reagent A) for routine anti-D typing. For **prenatal patients**, however, they **use a different commercial anti-D typing reagent** that consists of a blend of human monoclonal IgM and **human polyclonal IgG** (Reagent B). Their reasoning for using Reagent B for prenatal patients is that it **reportedly does not react in direct tests with Partial D types DVa, DVI.1, DVI.2, DFR, DBT, and R0Har**. The inquiring institution does not want to categorize individuals with such Partial D types as Rh positive, because they **believe those individuals should be potential candidates for Rh immunoglobulin (RhIG) prophylaxis and should be transfused with Rh NEGATIVE cellular blood components**. The inquiring institution has purchased a piece of automated blood bank testing equipment and they would like to include their prenatal testing scheme in the workload run on that instrument. The anti-D reagent strip that the equipment manufacturer recommends states

that "Category VI will not be detected with the anti-D reagents on this strip". The inquiring institution wonders if any other institutions have modified their manual Rh typing reagents or have made **automated testing equipment selections based upon test reactivity with red cells from Partial D type** individuals. If so, what has been the justification and experience?

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Posted: February 3, 2003

Addenda: Feb. 4, 2003; Mar. 25, 2007; June 11, 2008