**CASE #16, ANSWERS**
Case Study by Jim Perkins, MD (©2009)

1. What antibody(ies) appear to be present? Is this identification proven? If not, what hypotheses might you make regarding the cause of the observed reactivity? What steps might be taken to prove or disprove these hypotheses?

   *The reactions of this patient's antiserum do not fit any obvious specificity or combination of specificities. What is more, anti-D, -C, -c, -e, -K, -k, -Fy$^a$, -Fy$^b$, -Jk$^a$, -Jk$^b$,-Le$^a$, -Le$^b$, -S, -s, -M, -N, and –P1 are ruled out by conventional criteria, and lack of reactivity with cell #6 is against the identification of anti-E. Thus there is a non-specific pattern of weak reactivity.*

   *When a patient's plasma or serum shows non-specific reactions certain explanations should immediately come to mind:*

   A. *Although we expect most autoantibodies to react with all cells in a panel, weak autoantibodies may react in a seemingly random pattern. The negative DAT is strongly against the possibility of a warm-reactive autoantibody, but the gel test is sensitive to cold-reactive autoantibodies. The possibility that the reactivity is due to a cold auto- or alloantibody could be investigated by running a "cold panel" by a tube agglutination technique and possibly by performing a cold-autoadsorption if the cold panel is suggestive. At the time of this problem the laboratory was not performing an autocontrol with the initial panel, opting just for a DAT. It would have been useful to have performed an autocontrol in this case.*
   B. *The nonspecific pattern if reactivity could be due to one or more weak antibodies. Antigen expression may vary between different donors, and this extends to expression of membrane protein antigens by donors with similar zygosity. For example, the D antigen is expressed more strongly on R2R2 than on R1R1 cells in spite of the fact that both genotypes produce "double dose" D positive RBCs. The specificity of weak antisera may be clarified by retesting the serum/plasma with a more sensitive method. Of note however, a method that is more sensitive in detecting one antibody may not be more sensitive for another. For example, weak Rh antibodies may react well with enzyme treated cells, but reactivity with anti-M or anti-Fy$^a$ would be lost.*
   C. *Sensitive test methods may show weak, non-specific reactivity which cannot be replicated using other methods. The gel system provides occasional examples of this phenomenon. In this situation lack of reactivity using another indirect antiglobulin test method which has historically provided safe compatibility test results (e.g. tube methods with or without added enhancement media) may reassure the laboratory that there is no hemolytic antibody present.*

2. What antibody(ies) appear to be present now? Is this identification proven? What strategy did the technologist adopt in selecting the additional work performed?

   *The patient has anti-D and anti-K. These alloantibodies are "proven" according to standard criteria with ≥3 D-pos, K-neg cells reacting, ≥3 K-pos, D-neg cells reacting, and ≥3 D-neg, K-neg cells NOT reacting. Anti-C, -c,-E, -e, -k, -Fy$^a$, -Fy$^b$, -Jk$^a$, -Jk$^b$,-Le$^a$, -Le$^b$, -S, -s, -M, -N, and –P1 are ruled out by conventional criteria. (Anti-C is only ruled out on a single dose C-pos cell, but few laboratories would have access to a rare r'r' cell to rule out anti-C in the presence of anti-D.) Finally, the patient lacks the D and K antigens.*

   *In order to resolve this problem the technologist increased the sensitivity of the indirect antiglobulin test by extending the incubation time from 15 to 30 minutes. The manufacturer of the gel test format used in this laboratory requires a minimum incubation time of 15 minutes which can be extended to 40 minutes. This demonstrated reactions between the patient's plasma and R1R1 and K-positive cells that had failed to react in the first panel, leading the technologist to initially "rule out" anti-D and anti-K. Inspection of the antibody detection and panel cells he chose to repeat from the first round of testing suggests that he indeed suspected the presence of these antibodies, noting that although anti-D and anti-K were "ruled out", nevertheless all of the reactive cells were either D or K positive. Also, as mentioned above expression of D is greatest on R2R2 cells and these were all reactive in the initial testing. When seemingly non-specific patterns of reactivity are observed it is important to search for such commonalities among the reactive or non-reactive cells.*

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3. Is there anything anomalous about this result in this particular patient?

*Although anti-D was at one time the most common un-expected antibody seen in patients who had been pregnant or transfused, two developments, the use of Rh negative blood for Rh negative blood recipients beginning in the late 1940's and early 1950's and the use of Rh immune globulin prophylaxis for Rh negative women in the early 1970's, have greatly reduced the incidence of this antibody. In particular, a man born in 1939 would only be expected to have this antibody if he had been transfused as a child, or had been given an emergency transfusion with Rh positive blood. For example, during the Vietnam war wounded American soldiers routinely received Rh positive whole blood regardless of their Rh type, so anti-D is frequently seen in men treated at Veterans' Administration hospitals in the US. In this case the patient had suffered life-threatening trauma in 1952 for which he thought he had been transfused. He probably was exposed to the Rh positive RBCs at that time.*