1. Will this finding affect the reliability of patient red cell antigen typing?
2. Does this finding have applicability to identification of the antibody(ies)? Many laboratories consider autoantibodies to be a complex investigation, requiring that only selected staff be permitted to perform specialized testing or requiring that samples be submitted to an outside reference laboratory. Certainly special methods are required for final resolution of cold- or warm-reactive autoantibodies. Additional information is given in the section on Unusual Antibody Identification Situations.
3. Does this finding have applicability to the diagnosis and treatment, including RBC transfusion, of the patient? Policy may indicate that medical staff be consulted to determine whether or not further investigation is warranted.

Evaluation of Weak Reactivity with No Pattern Recognized

When no specificity is suggested by initial review, the following information may prove helpful.

1. Look for antigens in common on reactive red cells. Certain antibodies may react with some, but not all, of the antigen-positive red cells. Reasons for this type of reactivity include:
   a. Dosage. In some blood group systems, antigenic expression is influenced by the presence or absence of the antithetical antigen. For most individuals, absence of the antithetical antigen on their red cells indicates the presence of two genes encoding for the antigen, which frequently results in stronger antigen expression on the red cells. Such cells are often referred to as having "double-dose" antigen expression, eg, Jk(a+b–). Red cells that also have the antithetical antigen are described as having "single-dose" antigen expression, eg, Jk(a+b+). Antibodies that give stronger reactivity with double-dose red cells than single-dose red cells are referred to as antibodies demonstrating dosage.

      Careful consideration must be given to the presence of a clinically significant antibody reacting only with double-dose red cells. Antibodies in the Rh, Duffy, Kidd, and MNSs systems may all demonstrate dosage. Understanding the dosing characteristics of commonly encountered antibodies is important in evaluating unidentified reactions.

b. Variation in antigen expression. Variation is most commonly found with Le(a+), Le(b+), and P1+ red cell samples, but may occur with other antigens as well. For example, the D antigen is generally more strongly expressed on R_2R_2 samples than other Rh phenotypes.
c. The phase of testing is not optimal for antibody reactivity. Most laboratories no longer routinely perform antibody detection at temperatures less than 37 C. Antibodies that react best at lower temperatures may react with some antigen-positive red cells in tests performed at 37 C (eg, anti-M or anti-P1).
d. Persistence of IgM antibody into AHG testing. Generally, cold-enhanced, IgM antibodies dissociate from the red cells during the AHG wash phase. However, antibodies of sufficient avidity and/or test methods that do not employ an AHG wash may demonstrate reactivity with some antigen-positive samples because of the presence of direct agglutinating IgM antibody. Examples of frequently encountered cold-enhanced antibodies include: anti-P1, -Le^a, -Le^b , -M, -N, -I, -IH, and anti-i.

2. Certain antibodies are frequently found in pairs. Common examples include: anti-E + anti-c; anti-D + anti-C; anti-C + anti-e; and anti-Le^a + anti-Le^b. Many times one antibody of the pair is less reactive; not infrequently the weaker antibody is not reactive with all antigen-positive red cell samples in a given test method or phase.
3. More than one antibody may be present (see Table 6). A change in methods may destroy or enhance the reactivity of one antibody, allowing multiple antibodies to be separated. Phenotyping of the patient's red cells for the antigens associated with commonly encountered antibodies (see Table 3) may narrow the list of potential alloantibodies.
4. The antibody may be rarely encountered. Some antibodies are directed toward high- or low-incidence antigens that are not required to be confirmed as present on antibody detection red cells. Examples include: C^w, V, VS, Kp^a, Kp^b, Js^a, Js^b, and Xg^a. In some cases, the antigen status may be included only as a special listing or available by contacting the panel manufacturer. Exampes include Co^a, Co^b, Wr^a, U, Vel, Do^a, Do^b, Bg, Ch, Rg, Kn^a, and McC^a. Other rarely encountered antibodies may or may not be associated with clinical importance. For very rare antibodies, the

Table 6. Reactivity Commonly Seen in the Presence of Multiple Antibodies to Common Blood Group Antigens and of a Single Antibody to a High-Incidence Antigen

Type of Antibody	Variation in Reaction Strength	Equal Reaction Strength	All or Most Cells Reactive	Cells Phenotypically Matched to Patient for Common Antigens Reactive
Multiple antibodies	Yes	No	Yes	No
Antibody to high-incidence antigen	No	Yes	Yes	Yes

status of the corresponding antigen on the panel red cells may not be known. The corresponding antigen may be of high, low, or intermediate incidence.[15]

5. The antibody may be relatively weak and not reactive with all antigen-positive cells. Some relatively easy additional testing that may yield antibody identification would include:
   a. Test with a different method. See listing in Table 1.
   b. Test at a different phase. As mentioned previously, cold-enhanced antibodies may give weak reactivity at 37 C and/or AHG. Testing at room temperature may indicate specificity.
   c. Select red cell samples that may give stronger antigen expression, such as
      - Double-dose.
      - Noted as $+^s$ by the panel manufacturer.
      - Effects of gene interactions, eg, R_2R_2 red cells generally express more D antigen than other Rh phenotypes.
      - Fresher dating. Even red cell samples continually stored at 4 C will lose antigens with the passage of time. This loss is generally accelerated by transfer in and out of refrigeration as well as by agitation employed during red cell resuspension.