One of the most difficult concepts to explain when training immunohematology staff involves the investigation of hemolytic anemias. In the transfusion service and in the immunohematology reference laboratory setting, rapid and efficient investigation can be extremely important for patients with critical anemia requiring transfusion.

The flow charts presented here provide possible patient scenarios and a logical sequence for initial and subsequent serologic testing for investigation. A clinical assessment of anemia of unknown origin or the finding of an unresolved positive antibody screen in pre-transfusion patient testing begins the investigational flow process. The testing sequence is predicated on the fact that performing a direct antiglobulin test (DAT) on all patients has a low predictive value and should be reserved for patients with unexplained anemia. The process begins with assessing the results of DATs with anti-IgG and anti-C3. Subsequent charts A, B, and C aid in investigating these results. Flow Chart A is for the investigation of warm or cold autoantibody, Chart B is for the investigation of cold-agglutinin or drug-induced immune hemolytic anemia, and Chart C is for the investigation of alloantibody in the transfused patient.

While the most common approaches to the initial and subsequent test results are in these flow charts, the charts are not inclusive of all possible diagnoses or presentations. These flow charts are meant to be a guide to assist the laboratory in developing a standard approach to efficient investigation and resolution in patients with unexplained anemia.
Investigation of warm or cold autoantibody

1. All RBCs negative, consider:
   • Ab to a drug if unexplained anemia. See B
   • Anti-A or anti-B coating RBCs

2. Some RBCs reactive, suspect alloAb or autoAb with specificity
   If alloadsorption does not remove reactivity:
   1. If the reactivity is weakened, test adsorbed plasma with treated adsorbing RBCs
      • If positive, perform additional adsorptions
      • If negative, consider presence of alloAb that are difficult to adsorb (anti-e-like, HTLA-like)
   2. If no decrease in reactivity, consider that the target Ag was destroyed by pre-adsorption RBC treatment

Ab = antibody; RBCs = red blood cells; DAT = direct antiglobulin test; — = negative; Ag = antigen; □ = decision; ■ = process step.

End
**Investigation of cold agglutinin or drug-induced hemolytic anemia**

- Investigate for drug Ab
  - NO
    - Cold Ab screen positive? YES → Perform thermal amplitude studies (37°C, 30°C, 22°C, 4°C)
    - NO → Cold Ab screen positive? YES → Perform thermal amplitude studies (37°C, 30°C, 22°C, 4°C)
    - NO → History of viral illness? YES → High thermal range or high titer?
  - YES → Drug solution test positive?
    - YES → Donath-Landsteiner Test positive?
      - YES → END
      - NO → Drug adsorption studies positive?
        - NO → END
        - YES → Medical consult
  - NO → Patient history implicates drug therapy?
    - YES → Drug solution test positive?
      - YES → END
      - NO → Drug adsorption studies positive?
        - NO → END
        - YES → Medical consult

**Ab** = antibody; ■ = decision; □ = process step.
Investigation of alloantibody in the transfused patient

- **History of alloAbs?**
  - YES: Test serum and eluate with selected RBCs
  - NO: History of alloAbs?

- **All RBCs reactive?**
  - YES: Consider multiple antibodies present in plasma and/or eluate
  - NO: Ab identified in serum and/or eluate?

- **Ab identified in serum and/or eluate?**
  - YES: Consider variant allele if patient Ag positive with concomitant Ab
  - NO: Consider routine genotyping

- **Chronic transfusion likely?**
  - YES: Perform genotyping
  - NO: Patient has African ethnicity?

- **Patient has African ethnicity?**
  - YES: Consider RHD and RHCE or variant allele genotyping
  - NO: Ab identified?

- **Ab identified?**
  - YES: END
  - NO: Consider routine genotyping

- **If eluate negative, consider drug Ab (see B) or ABO Ab**

- **Phenotypically similar RBC positive with plasma and/or eluate?**
  - YES: Perform adsorption with phenotypically similar RBCs and elute presumed Ab to HPA
  - NO: Test adsorbed plasma for Abs to common Ags

- **Test RBCs lacking HPAs, negative for other Abs (if needed) using eluate from adsorbing RBCs**

- **Identify Ab(s) in eluate from patient’s RBCs based on plasma Ab identification**

- **Test RBCs with selected RBCs**

- **Consider routine Ab identification**

- **Test serum and eluate with routine Ab panel**

- **History of alloAbs?**

- **Ab identified?**

- **Ab identified?**

- **YES**: END

- **NO**: Consider variant allele if patient Ag positive with concomitant Ab

- **Phenotypically similar RBC positive with plasma and/or eluate?**

- **All RBCs reactive?**

- **Ab identified in serum and/or eluate?**

- **Chronic transfusion likely?**

- **Patient has African ethnicity?**

- **Consider RHD and RHCE or variant allele genotyping**

**Ab** = antibody; **RBCs** = red blood cells; **Ag** = antigen; **HPA** = high-prevalence antigen; **rBGA** = recombinant blood group Ag;

**mf** = mixed field; **IRL** = immunohematology reference laboratory; **△** = decision; **□** = process step.