EDUCATIONAL COMMENTARY – RH TYPING

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Learning Outcomes

Upon completion of this exercise, the participant will be able to:

- Identify the test used to detect a Weak D antigen.
- List two advantages of monoclonal reagents.
- Explain how controls can be used to detect false test results.

Rh antigens, which are composed of polypeptides, occur on the surface of human red blood cells (RBCs). Currently, there are 56 distinct antigens in the Rh Blood Group system. Rh antigens are expressed on two different proteins, the RHD protein (see Figure 1) \(^1\) that expresses the Rh D antigen, and the RHCE protein (see Figure 2) \(^1\) that expresses other common Rh antigens such as C, c, E, and e. Because commercial Rh antisera are available only to 5 antigens, routine typing is generally limited to: D, C, E, c, and e.

![Figure 1](image1.png)

![Figure 2](image2.png)


Rh antigens are considered “antigenic” because they often cause a specific antibody to be produced when they are introduced into an animal that lacks the antigen. These antibodies are proteins, produced by the animal’s immune system, and they are capable of combining with the antigen. This is the basis for Rh typing. To determine whether a red blood cell expresses a particular antigen, the cell is mixed with an antigen-specific antibody (typing serum), and the mixture is observed for a reaction, usually agglutination. For example, if a red cell is agglutinated by anti-D typing serum, the cell is said to be Rh D positive. However, some individuals have a very weak expression of the Rh D antigen, and they are nonreactive...
with anti-D typing serum by direct agglutination. To detect a weak expression of the D antigen, a more sensitive technique, the indirect antiglobulin test, is needed.

Antibodies used for producing typing sera are either polyclonal or monoclonal in nature. Polyclonal antibodies are derived from more than one antibody-producing cell, and they recognize multiple sites or epitopes on the antigen. Polyclonal antibodies, used in making typing sera, are produced after repeated immunization of an animal that results in the production of high-titered IgG immunoglobulin.

Monoclonal antibodies are derived from a single antibody-producing parent cell, and they react with a single epitope on the antigen. Monoclonal antisera are produced by hybridoma technology, in which an antibody-producing cell is fused with a rapidly proliferating myeloma cell. The resulting hybridoma is cloned and maintained in an in vitro culture for many years. Monoclonal antibodies may be either an IgM or IgG immunoglobulin; however, most monoclonal antiserum is produced from IgM antibodies. Monoclonal antibodies have exquisite sensitivity for their corresponding epitope, but sometime this can be a disadvantage.

Variant antigens may possess most, but not all, of the epitopes that exist in the wild type (common form) antigen. Negative typing results may occur when cells with variant antigens are typed with a monoclonal antiserum that reacts with the epitope they lack—even though the cell possesses the rest of the expected epitopes. To overcome this problem, several monoclonal antibodies may be blended together or monoclonal antibodies may be combined with polyclonal antibodies to broaden the specificity of the typing reagent.

IgG antibodies sensitize or coat red cells, but rarely agglutinate cells directly. Direct agglutination is an advantage because typing results can be determined immediately (one step) without performing an antiglobulin test (two steps). By adding bovine albumin and high molecular weight potentiators such as dextran polyvinylpyrrolidone to IgG antibodies, it is possible to make IgG antibodies agglutinate red cells directly. Bovine albumin and potentiators have the disadvantage of causing cells that are coated with immunoglobulin, i.e. direct antiglobulin test positive, to agglutinate spontaneously and give a false-positive typing result.

To avoid false-positive results, testing must be performed in parallel with positive and negative controls. Typically, control material contains antibody-free pooled human sera, sodium chloride solution, bovine albumin, and a high molecular weight potentiator. These controls are available from the manufacturers of typing sera. When performing a test, it is essential that the typing serum and the control be products of the same manufacturer. The formulation of antisera and controls are proprietary, and may differ from manufacturer to manufacturer. For example, bovine albumin used in antisera production may or may not contain the antibacterial chemical, sodium caprylate.
IgM antibodies agglutinate red blood cells directly, and no additives such as bovine albumin or high molecular weight potentiators are needed to produce low protein IgM antiserum. Natural source IgM antibodies, produced by immunized animals during a primary sensitization, are in short supply. By contrast, monoclonal antibodies, produced by clone technology, secrete nearly unlimited quantities of high titer antibodies. It is critical to follow the manufacturer’s directions when using monoclonal antisera. Monoclonal antibodies have optimal reactivity conditions. Failure to perform tests at the recommended temperature or incubation time may cause false test results. Monoclonal antibodies also have an optimal association time, and the antibody may dissociate from the antigen if the incubation time is extended, resulting in a false-negative result.

Some manufacturers recommend using controls for monoclonal antisera, and others do not. Manufacturers who do not offer a clone control reason that monoclonal reagents are in a low protein medium, and if a cell gives a negative result in one or more low protein tests (e.g. ABO grouping), this result serves as a negative control for other monoclonal reagents. Other manufacturers supply the clone diluent as a clone control. When a clone control is available, it should always be used.

However, such a control is not infallible. Clone technology produces consistency in the antibody produced, but there may be some batch-to-batch variation in amount of antibody secreted. Strong antibodies are diluted to standardize the reagent, resulting in differing amounts of diluent in the final products. When a red blood cell is heavily saturated with antibodies, as in a strongly positive direct antiglobulin test, the cells may spontaneously agglutinate. A patient auto control should be performed when the direct antiglobulin test is positive. This control could consist of a suspension of the patient’s red cells with a clone control, with autologous serum, or with 6% or 8% bovine albumin. If the patient auto control is reactive, the typing result is invalid.

In summary, Rh typing antibodies may be polyclonal or monoclonal. Polyclonal antibodies are produced by immunizing a susceptible animal, and monoclonal antibodies are produced by hybridoma technology. IgM and IgG immunoglobulins may be produced by either technology, and each of these forms has both advantages and disadvantages. Controls must be used for all types of Rh typing sera to ensure that the test results are valid.

References
1. Figures 1 and 2 are available from Google™ Images