Heat elution: a modification of the Landsteiner-Miller method

C. Dean-El and N. Quraishy

Elution removes antibodies coating red blood cells and allows for serologic testing of the recovered antibodies. Heat elution may be used in the investigation of ABO hemolytic disease of the fetus and newborn, in the detection of weak A and B antigens in combination with adsorption–elution using polyclonal antibodies, and for the resolution of interfering IgM agglutinating antibodies. *Immunohematology* 2019;35:45–47.

**Key Words:** DAT, direct antiglobulin test, elution, heat elution, ABO, IgM antibodies, HDFN

**Principle**

Antibodies and antigens fit together in a “lock and key” mechanism, stabilized by van der Waals forces, hydrogen bonds, and electrostatic and hydrophobic interactions. The *avidity* of an antibody correlates with its binding strength—for example, the IgM pentamer with its 10 antigen-binding sites has high antigen avidity in contrast to the monomeric IgG. The *affinity* of an antibody for a given antigen depends on the binding capacity of a single antigen-binding site. The interaction between the antibody and its antigen is reversible and is affected by the ionic strength, temperature, and pH of the testing environment. The process of removing an antibody attached to red blood cells (RBCs), either in vivo or in vitro, is termed *elution*, and the recovered, concentrated antibody in solution is called an *eluate*.

Heat elution, the first RBC elution procedure, was described by Landsteiner and Miller in their studies on chimpanzees. The original method involved a 5-minute incubation at 56°C of antibody-coated RBCs in normal saline, but resulted in eluates with low antibody levels. Currently, a modified Landsteiner-Miller heat elution method described by Judd and discussed in the AABB Technical Manual is used.

The exothermic reaction for formation of antigen (Ag)–antibody (Ab) complexes is represented by the following equation:

\[ \text{Ag} + \text{Ab} = [\text{AgAb}] + \text{calories} \]

An increase in temperature on the Ag–Ab complex results in intensification of the thermal motion of atoms and molecules driving the reaction in reverse and causing dissociation of the Ag–Ab complex.

Additionally, heat causes a conformational change in proteins, resulting in a loss of the “lock and key” interaction between antigen and antibody.

**Indications**

Elution studies may be performed on patient samples having a positive direct antiglobulin test (DAT) due to auto- or alloantibodies, due to drug-induced antibodies, or in the investigation of hemolytic disease of the fetus and newborn (HDFN). They may also be used in combination with in vitro adsorption studies to identify weakly expressed blood group antigens and for the identification of multiple alloantibodies.

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**Reagents/Supplies**

<table>
<thead>
<tr>
<th>Reagents</th>
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<tr>
<td>Saline (ice-cold for IgM cold agglutinins)</td>
<td>56°C water bath or heat block</td>
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<tr>
<td>6% bovine albumin</td>
<td>12 × 75 mm test tubes</td>
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<tr>
<td>Reagents for testing, including group A, B, and O RBCs, if applicable</td>
<td>Centrifuge</td>
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<td>Optical aid</td>
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<td>Timer</td>
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**Procedural Steps**

- Wash patient RBCs four to six times with saline. Use ice-cold saline washes for IgM cold agglutinins.
- Remove and place the final wash in a clean, labeled test tube.
- Place an equal volume of washed RBCs and bovine albumin in another clean, labeled test tube. Cap the tube, and mix the contents thoroughly by gently inverting the tube.
- Incubate the tube at 56°C for 10 minutes. Periodically, invert the tube, and mix the contents during incubation.
- After incubation, centrifuge the tube at 900–1000 g for 2–3 minutes (use a heated centrifuge if available).
- Transfer supernatant eluate into a clean, labeled test tube.
- Test the eluate and final wash in parallel, using the appropriate test phase(s) in a manner that is best for detection of the antibody, following the laboratory’s standard operating procedure.

RBCs = red blood cells.
Indications for heat elution include:

- Investigation of ABO HDFN. Elution is rarely required, however, because the diagnosis is generally made from clinical findings consistent with HDFN in an ABO-incompatible mother and newborn.²
- Identification of weak A and B antigens by a combination of adsorption and elution methods. Polyclonal anti-A or anti-B is adsorbed onto the RBCs, and heat elution is used to harvest the adsorbed-bound antibody to determine the presence of a weak A or B antigen.⁸
- Identification of IgM cold autoantibodies. Heat elution has been described as an effective method for the identification of IgM cold autoantibodies when compared with acid stroma (digitonin), organic solvent, and Lui freeze–thaw elution methods.

Procedure

Preparing the Eluate

Before performing the elution, the sensitized RBCs must be washed thoroughly four to six times with large volumes of saline. Use ice-cold saline to prevent dissociation of cold-reactive IgM antibodies. The wash step is critical for removing cell-free antibody while retaining RBC-bound antibody. After the last wash is performed, harvest the supernatant wash into a clean, labeled test tube.

Place an equal volume of washed packed RBCs and 6 percent bovine albumin in a labeled 12 × 75 mm test tube. A preferable starting volume is 1 mL or 20 drops of each. Cap the tube, and mix the contents well by gently inverting the tube. Immediately place the tube in a 56°C water bath or heat block, ensuring complete submersion of the tube in the heated water. Incubate for 10 minutes, with periodic agitation of the tube. Centrifuge the tube at 900–1000 g for 2–3 minutes. If available, use a centrifuge that is heated to 56°C.

Transfer the supernatant eluate into a clean, labeled 12 × 75 mm test tube. The eluate is now ready for testing using standard antibody identification methods. Perform testing immediately after elution, since eluates are not stable.⁵

Testing the Eluate

Test the eluate using the appropriate test phase(s) in a manner that is best for detection of the antibody, including a reading at room temperature if applicable. Include group A, B, and O cells if testing for ABO antibodies. Test the recovered last wash in parallel with, and in the exact same manner, as the eluate.

Use clean test tubes during the elution procedure. Contaminated test tubes carry the risk of antibody adhering to proteins on the contaminated test tube surface and being reintroduced to the last wash or eluate.²

Quality Control

The last wash must be tested in parallel with the eluate and must be negative for the eluate results to be valid. A positive last wash indicates the presence of unbound antibody due to inadequate washing of the sensitized RBCs. In that case, the elution procedure must be repeated after more thorough washing of the RBCs.

Another option for quality control is to perform a DAT on the RBCs after the heat treatment, and compare it with the pre–heat treatment DAT. A negative DAT post–heat treatment indicates successful elution.


Limitations

The heat elution method is simple to perform but has limited applications, as described under Indications. Drawbacks of heat elution include the following:

- There is the possibility of marked hemolysis of the eluate. The red color of the eluate can make agglutination difficult to read during testing.
- Heat can denature the RBC membrane and affect clinically significant blood group antigens, weakening or destroying them; thus, the RBCs used in the elution procedure should not be used for phenotyping.¹⁰
- Heat elution is not an effective method for removal of warm autoantibodies or alloantibodies, other than ABO.²,¹¹

As with any elution procedure:

- Inadequate washing of the RBCs before the elution method will result in interference from unbound antibodies.
- There is a risk of the Matuhasi-Ogata phenomenon.²,⁵
References


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