Enzyme Linked Antiglobulin Test

**What is ELAT?**

ELAT or Enzyme Linked Antiglobulin Test is an enzyme linked immunosorbent assay (ELISA) for the detection of immunoglobulin bound to the surfaces of red blood cells. The direct antiglobulin test (DAT or direct Coomb's) depends on visual inspection and subjective grading of agglutination of immunoglobulin- or complement-sensitized erythrocytes by the addition of antiglobulin reagent. In contrast, the endpoint of the ELAT is a change in optical density (OD) read spectrophotometrically. Red cells are washed, and then incubated with antihuman IgG conjugated to alkaline phosphatase. The reaction is developed by the addition of substrate solution (para-nitrophenyl phosphate, or PNPP) and measurement of absorbance at 405 nm. A standard curve plotting OD versus quantity of IgG per erythrocyte (IgG/RBC) is constructed by testing Rh positive red cells which have been sensitized by incubation with various known concentrations of Rh0(D) immune globulin (anti-D). Spectrophotometric readings on test cells are then plotted against the linear standard curve for quantification of red cell sensitization. An ELISA for the detection of complement components on red cells has also been developed recently (10). Results are compared to a calibration curve constructed using in vitro complement coated erythrocytes. Platelet associated immunoglobulin also may be quantified by an enzyme-linked assay similar to the ELAT in the diagnosis of immune thrombocytopenia.

**When is ELAT clinically useful?**

In the two years since Leikola and Perkins (7) described the test, ELAT has been utilized in the diagnosis of warm autoimmune hemolytic anemia (AIHA) in cases in which the DAT is negative. It has also been reported to be useful in following such cases of DAT negative AIHA and some cases of DAT positive hemolytic anemia (1).

Red cell sensitization by IgG and/or complement is characteristic of warm reactive autoimmune hemolytic anemia, many drug-induced hemolytic anemias, and many delayed transfusion reactions. In the vast majority of these cases the immunoglobulin or complement coated red cells can be detected by the DAT. However, DAT negative AIHA is a well-documented phenomenon accounting for up to 2-4% of cases of AIHA (6). Greater than 200-500 IgG molecules per RBC must be present for detection by the DAT. But sensitization by a much smaller number of molecules has been shown to be sufficient to cause significant hemolysis in some cases (9). Gilliland found 76-434 molecules IgG/RBC in a series of cases of DAT negative acquired hemolytic anemia, while normal individuals generally had less than 35 IgG/RBC (4,5). In most cases of DAT negative AIHA, the red cells probably fail to agglutinate with addition of antihuman globulin because the quantity of IgG/RBC is below the sensitivity limits of the DAT. A small percentage of cases of AIHA give false negative DAT's due to technical difficulties, even though greater than 500 molecules IgG/RBC can be demonstrated by quantitative techniques. A
more sensitive procedure employing different methodology from the DAT, such as
the ELAT, should facilitate diagnosis and possible treatment of DAT negative AIHA.

A number of assays for RBC bound IgG have been developed which are considerably
more sensitive than the DAT. These include radioimmunoassay, erythrocyte
antibody rosette formation, an automated antiglobulin test using an autoanalyzer,
and complement fixing antibody consumption techniques. Each of these methods has
proved to be too time consuming and technically difficult for utilization by most
clinical laboratories, and to require expensive equipment. An antiglobulin test
employing the biotin-avidin system might be a particularly sensitive means of
assaying membrane bound IgG, although perhaps technically cumbersome. Results
of an immunoradiometric assay (IRMA) for red cell associated IgG have recently
been reported but offer no clear advantage over the ELAT (13). The ELAT has the
advantages of being easy to perform without requiring elaborate instruments, while
being considerably more sensitive than the DAT. In addition, it allows
quantification of IgG/RBC on sensitized red cells, and for this reason has been
recommended for following cases of AIHA for decreases or increases in
immunoglobulin coating red cells as a predictor or monitor of hemolysis.

What are the clinical and technical limitations of ELAT?

The ELAT is clearly a sensitive test, able to demonstrate elevated quantities of
RBC bound surface immunoglobulin in many DAT negative patients. Gilman (6)
calculated the sensitivity of ELAT as nine times greater than the DAT. The
quantitative result must be interpreted in conjunction with other data on the patient
(reticulocyte count, hemoglobin, bilirubin, LDH, haptoglobin, etc.) because the
significance of slightly elevated amounts of IgG/RBC has not been definitively
determined. Any technique of increased sensitivity introduces the problem of
increased numbers of false positives (decreased specificity). If the ELAT is to be
used in the diagnosis of AIHA, false positives represent cases in which the number of
IgG molecules per RBC exceed the "normal range" in the absence of laboratory or
clinical evidence of hemolytic anemia. The definition of the normal range is
obviously critical. Gilman et al. (6) tested 100 "normal" DAT negative subjects and
set the upper limit of normal at the arithmetic mean plus 1.645 SD. Values between
this level and the mean plus 1.96 SD were considered borderline, while levels higher
than this range were considered positive. Some laboratories performing ELAT have
roughly defined the normal range on the basis of Gilliland's (4,5) results using the
complement fixing antibody consumption test for quantification of IgG/RBC. Thus
less than 40 IgG/RBC is considered "normal," 40-75 IgG/RBC borderline, and greater
than 76 IgG/RBC positive. Using the normal range of Gilman et al., 5% of 100
normal DAT negative subjects had elevated IgG/RBC by ELAT. Approximately ten
percent of hospitalized patients have been reported to have positive DATs, while
only a small fraction of these have clinical evidence of hemolytic anemia or an
historical reason, such as ingestion of certain drugs, for red cell sensitization (9).
Recent data by Toy et al. suggests that many positive DATs in the absence of
hemolysis may be associated with hypergammaglobulinemia (12). What percentage
of hospitalized patients without evidence of hemolytic anemia have a positive
ELAT? Until such statistics are available, the clinical utility of ELAT in the
diagnosis of AIHA will be limited. The meaning of a negative ELAT result also
needs definition. The presence of less than 10 molecules of anti-D on red cells has
been shown to cause hemolysis in some cases (9). Such low levels of IgG/RBC would not exceed the normal range although hemolytic anemia could theoretically be present. Thus the ELAT may define a new subgroup of DAT negative AIHA whose members are also ELAT negative, and a negative ELAT can not be used to definitively rule out the possibility of AIHA.

Bodensteiner et al. (1) recommended that the ELAT be used for following DAT negative hemolytic anemias. They demonstrated a close correlation between the quantity of sensitizing IgG on the surfaces of RBC's and the hemoglobin and reticulocyte count. As the reticulocyte count returned to normal in these patients, the IgG/RBC decreased. Although not technically difficult, the ELAT is very time consuming (2-1/2 hours for 12 samples as compared to 30 minutes for 12 DAT samples (6)) compared to the performance of a reticulocyte count, and the ELAT price exceeds that of a reticulocyte count by a factor of ten. Since the results correlate well with each other, it appears to be more practical to follow the reticulocyte count and hemoglobin in cases of DAT negative AIHA than to follow the ELAT.

Many of the technical limitations reported by Bruner and Kissling (3) in 1978 in their report of an ELISA for detecting IgG sensitized red blood cells have been overcome. Hemolysis presented a considerable problem in the early reports due to release of endogenous alkaline phosphatase activity and to the release of oxyhemoglobin which has a maximal absorbance peak at the same wavelength as nitrophenol, the end product of hydrolysis of PNPP. Interference by hemolysis can be minimized by optimizing buffers and temperature (7) and by comparing OD readings to hemolysis blanks (6). Phosphatase associated with red cell membranes presents a theoretical problem, however the quantity is insufficient to significantly alter the test result. Some authors have warned of nonspecific absorption of alkaline phosphatase conjugate to RBC's and glassware, but the problem is substantially overcome by rigorous washing of red cells before addition of substrate (3). Nonspecific binding of nonimmune IgG to RBC surfaces presents a potential interference, but an actual interference could not be demonstrated by Bodensteiner et al., even in patients with elevated serum IgG levels (1). In their description of an immunoradiometric assay for red cell associated IgG, Jeje et al. (13) stress the importance of removing all granulocytes, monocytes, and platelets from the RBC preparation, since these cells have relatively large amounts of surface bound IgG. Purification of red cells using Ficoll-Hypaque might increase the specificity of the ELAT but the test would be considerably more time consuming.

In summary, the ELAT is a sensitive means of detecting elevated quantities of IgG bound to the surfaces of RBC's. While the standard antiglobulin test is adequate for the detection of red cell sensitization by IgG in most cases, false negatives occur due to insufficient sensitivity. A positive ELAT result is helpful in supporting a clinical suspicion of AIHA, but is not sufficient data alone to justify such a diagnosis. A negative result also does not definitively rule out the possibility of red cell sensitization by a relatively small number of IgG molecules per RBC. These limitations plus the relative costliness and time demanding aspects of the test limit the ELAT's clinical utility to a select group of DAT negative cases with clinical and laboratory data strongly suggestive but not diagnostic of AIHA.
References


