ELISA Kits Measuring Human IgG Subclasses: Cross-Reactivity against IgG in an IgG ELISA, Sandra L. Nelson and Barbara A. Hynd (Procter & Gamble Pharmaceuticals, Miami Valley Labs., Cincinnati, OH 45239)

Clinical measurement of IgG subclasses is required for identifying numerous disease states (1–3). Measurement of IgG is also frequently required for various investigations of the immune system, including studies of immunoglobulin structure–function (4), autoimmune diseases (5, 6), B-cell function (7, 8), and many others. To study regulation of immunoglobulin synthesis, we evaluated the use of Zymed Laboratories (South San Francisco, CA) ELISA kits to measure IgG1 (cat. no. 99-0001) and IgG4 (cat. no. 99-0004) in culture supernates of human lymphocytes. These are two of the few commercially available kits that provide quantification of human IgG subclasses; most commercial kits for human IgG subclasses provide only positive/negative data on subclass type.

Briefly, the assay follows this format: a coated microtiter plate binds mouse monoclonal antibody against the human IgG subclass (1 or 4); the monoclonal antibodies capture the IgG (1 or 4) from the sample; and the IgG is then labeled with anti-human IgG conjugated to horseradish peroxidase (EC 1.11.1.7). The amount of added colorless substrate that is enzymatically converted to colored substrate is proportional to the amount of IgG subclass in the sample. In this assay, blanks consist of samples containing IgG plus all reagents except the capture antibody (i.e., the conjugated detection antibody and enzyme substrate are added).

The Zymed kits were validated for accuracy, precision, and specificity in our laboratory to allow their use in preclinical studies of new pharmaceutical products. Measuring a human serum sample calibrated against World Health Organization reference standards demonstrated that the kits had acceptable accuracy. The CV within a microtiter plate was determined to be 5% for the IgG, kit and 3% for the IgG2 kit. The CV for assays performed on different days was 9.5% for the IgG, kit and 6% for the IgG2 kit.

Because all of our samples had the same cell-culture media matrix, we at first thought that blank-correction for nonspecific binding due to components (such as rheumatoid factors) in the sera from different individuals was not necessary. However, when testing the Zymed human IgG1 and IgG4 kits for specificity against other human immunoglobulins, we found that it was necessary to use blank wells for the culture supernate samples. (The IgM, IgG1, IgG2, and IgG4 were affinity-purified from myeloma supernates and the IgA and IgG4 were affinity-purified from plasma; all were purchased from Calbiochem, San Diego, CA. The IgG3 with only kappa or lambda chains was produced from myelomas and purchased from Accurate Chemical & Scientific Corp., Westbury, NY). The blank-corrected cross-reaction against other human immunoglobulin classes and subclasses was <0.3% (Table 1). Without using blank-corrected wells, the cross-reactivity against human IgG3 was high: ~85% for the IgG1 kit and 4% for the IgG2 kit. Further testing against monoclonal immunoglobulins demonstrated that IgG3 molecules having a lambda light chain, but not those having a kappa light chain, bound nonspecifically to the capture antibody of these sandwich ELISAs.

Apparently the capture antibody of the Zymed human IgG subclass kits binds to an epitope shared by IgG3 and IgG4, consisting of portions of the lambda light chain and the gamma heavy chain. IgG3 and IgG4 also appear to share an epitope in the lambda and gamma chains that the Zymed capture antibody binds to a lesser degree. Therefore, to obtain accurate results, it is extremely important to use sample blanks for the Zymed human IgG1 and IgG4 ELISA kits.

References