Time-Resolved Fluorescence Immunoassay System Especially Suited for Research Applications, *E. P. Diamandis* (CyberFluor Inc., 179 John St., Toronto, Ontario, Canada M5T 1X4, and Dept. of Clin. Biochem., Univ. of Toronto, 101 College St., Toronto, Ontario, Canada M5G 1L5)

Researchers interested in setting up in-house immunological methodologies for measuring analytes in biological fluids find many current nonisotopic immunoassay systems not easily accessible for research because of the complete automation involved ("black box" approach) and the dedicated reagents used. Also, the available menu of analytes on such automated systems is usually limited, making it impractical for users to measure infrequently requested analytes, e.g., as part of a specific project or of interest in a particular hospital setting.

Here, I describe briefly a new time-resolved fluorescence immunoassay system (*1*), focusing on the merits of the system for in-house assay development and outlining briefly why the system is especially suited for research use. The essential parts of the system (all from CyberFluor Inc., Toronto, Canada) are:

(*a*) A time-resolved fluorometer/analyzer (Model 615) for measuring solid-phase fluorescence at the bottom of white microtiter wells. All the assay protocol variables are easily modifiable.

(*b*) The solid-phase: white, opaque microtitration wells in 12-well strips. Any number of wells can be cut from the strips for use in a specific run, and can be coated with the specific antibody (two-site immunoassay approach) of the researcher's choice. Antigen coating for competitive-type immunoassays is also possible, as described in detail elsewhere (*1*).

(*c*) Biotinylated detection antibody, for both competitive and noncompetitive immunoassays. The biotinylation procedure is very simple, takes about 1 h, and yields labeled antibodies that are stable for many months at 4 °C.

(*d*) Two tracers. The first incorporates streptavidin labeled with ~15 molecules of the europium chelator 4,7-bis(chlorosulfophenyl)-1,10-phenanthroline-2,9-dicarboxylic acid (BCPDA) (*1*). The second incorporates streptavidin covalently linked to thyroglobulin labeled with ~150 molecules of BCPDA (*2*). These tracers can detect as little analyte as 10^{-10} to 10^{-11} or 10^{-11} to 10^{-13} mol/L, respectively.

The researcher either purchases or produces the antibodies desired for a specific application and prepares the standard analyte solutions for calibration curves.

If the antibodies and standards are available, all necessary reagents and materials can be prepared and calibration curves obtained in one working day. All reagents are stable, nonisotopic, and either commercially available as universal systems (tracers) or quickly and easily prepared in the laboratory. Moreover, the detection reagent is added in a second step and thus is never in contact with serum, so avoiding nonspecific interactions between serum and label. The final fluorescent complex is stable for many months and can be read at any convenient time. Sensitivities achieved can exceed those obtained by using radioactivity.

References

1. Diamandis EP. Immunoassays with time-resolved fluorescence spectroscopy: principles and applications. Clin Biochem 1988;21:139–50.

2. Diamandis EP, Morton RC, Reichstein E, Khosravi MJ. Multiple fluorescence labeling with europium chelators. Application to time-resolved fluoroimmunoassays. Anal Chem 1989;61:48–53.