Time-Resolved Immunofluorometry of Follitropin in Serum, *M. J. Khosravi[1] and E. P. Diamandis[1,2,3]*
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We describe a new time-resolved immunofluorometric assay for follitropin (follicle-stimulating hormone, FSH) in human serum. The assay is based on the sandwich principle, involving (*a*) a solid-phase capture monoclonal antibody immobilized in white-opaque microtitration wells, (*b*) a soluble biotinylated monoclonal used for detection, and (*c*) a streptavidin-based universal detection reagent labeled with a europium chelator [4,7- bis(chlorosulfophenyl)-1,10 phenanthroline-2,9-dicarboxylic acid, BCPDA]. Excess europium is used to form the fluorescent complex. The streptavidin in the detection reagent is covalently linked to a protein carrier agent (thyroglobulin), which has been labeled with multiple BCPDA residues. Because of the use of BCPDA as label, the present assay is insensitive to europium contamination, the principal drawback of time-resolved fluoroimmunoassays in which europium is used as label. The design of the detection system, involving the biotin–streptavidin bridge method, has several additional advantages, which are described elsewhere (*1*).

To perform the assay, add 50 μL of standards or samples and 50 μL of the biotinylated antibody solution to the capture antibody-coated microtiter wells, and incubate for 3 h at room temperature with continuous shaking. After washing, determine the degree of binding of the biotinylated antibody, which is proportional to the amount of follitropin present in the sample, by reacting the sample mixture with 100 μL of the detection reagent. After a second incubation step (30 min, same conditions as above), measure the fluorescence of the final complex (monoclonal antibody–follitropin–monoclonal antibody–biotin–streptavidin–thyroglobulin–BCPDA–Eu^{3+}) on the washed and dried solid-phase support by excitation at 337.1 nm with a nitrogen laser, and monitor the emission at 615 nm with the CyberFluor 615™ Immunoanalyzer.

The detection limit of the proposed assay for follitropin is 0.1 int. unit/L (WHO 2nd IRP 78/549) with a measuring range that extends from 0.0 to 150 int. units/L. The within-run and day-to-day precision at follitropin concentrations of 7, 14, and 32 int. units/L are 4.7–7.3% and 7.8–9.3%, respectively. Lutropin and thyrotropin at concentrations up to 1000 int. units/L and 1000 milli-int. units/L, respectively, do not interfere (cross reactivity <1%). However, cross reactivity with choriogonadotropin is ~2% at concentrations up to 1000 int. units/L. The average analytical recovery of the assay was 97.2 (SD 6.6)%, and results correlated well with those by a commercially available radioimmunoassay kit (Diagnostic Products Corp., Los Angeles, CA 90045) for 255 clinical samples: y(FIA) = 0.982x(RIA) − 0.50 (r = 0.99). The assay design incorporates the speed and sensitivity of fluorescence detection with the specificity of monoclonal antibodies and avoids the well-known disadvantages of radioactive reagents. These advantages, combined with the simplicity of the assay protocol, make the procedure suitable for monitoring follitropin in routine clinical laboratories.

Reference

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