technical briefs

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Immunochemiluminometric Assays Based on Acridinium Labels with a Microtiter Plate Luminometer, *J. S. Woodhead and I. Weeks* (Dept. of Med. Biochem., Univ. of Wales College of Med., Heath Park, Cardiff CF4 4XN, Wales, U.K.)

Immunoassays based on chemiluminescent acridinium salts are highly sensitive, robust, and simple (*1*) but have not been demonstrated in a microplate format because of the lack of suitable luminometers with "on-board" reagent injection systems necessary for quantifying rapid chemiluminescent reactions.

We report our initial studies on a two-site immunochemiluminometric assay (ICMA) for thyrotropin (TSH) in blood-spot discs used to screen for neonatal hypothyroidism. The assay combines the use of acridinium-labeled monoclonal antibodies and polyclonal antibody-coated microwells. We quantified emission with either a Luminoskan luminometer (Labsystems Ltd., Basingstoke, U.K., or ICN Flow Ltd., Rickmansworth, U.K.) or a ML1000 luminometer (Dynatech Ltd., Billinghurst, U.K.). These machines were equipped with four and three reagent injectors, respectively.

We coated white microtiter plate wells (Dynatech, M11912W) with sheep anti-hTSH IgG (Scottish Antibody Production Unit, Carluke, Lanarkshire, U.K.). We punched 3-mm-diameter blood spot discs into the wells and added 100 μL of acridinium-labeled monoclonal anti-hTSH antibody solution (Magic Lite™; Ciba Corning, Medfield, MA 02052). We incubated the plates at 37 °C for 1 h, and then washed the wells three times with phosphate-buffered saline (phosphate 0.05 mol/L, pH 7.4; NaCl 0.15 mol/L) containing, per liter, 1 mL of Tween 20. We then introduced the plates into the measuring chamber of the luminometer, which was programmed to inject successive 100-μL volumes of peroxide and alkali (Magic Lite Reagents 1 and 2) with a 0.1-s delay between injections. Emission intensity was integrated over 2 s after the second injection and was recorded as relative light units. The dose–response curve for the assay is shown in Figure 1. The intra-assay precision (CV) for replicate determinations (n = 10 at each concentration) at 3.6, 6.3, 26.8, and 43.0 milli-int. units/L was 7.5%, 14.3%, 16.2%, and 5.8%, respectively. The precision of measurement is thus adequate in the clinically important region (~15 milli-int. units/L) between euthyroid neonates and neonates with congenital primary hypothyroidism. Correlation with our routine "in-house" immunoradiometric assay (IRMA) was good (r = 0.990, n = 16): ICMA = 1.04IRMA + 1.9 milli-int. units/L.

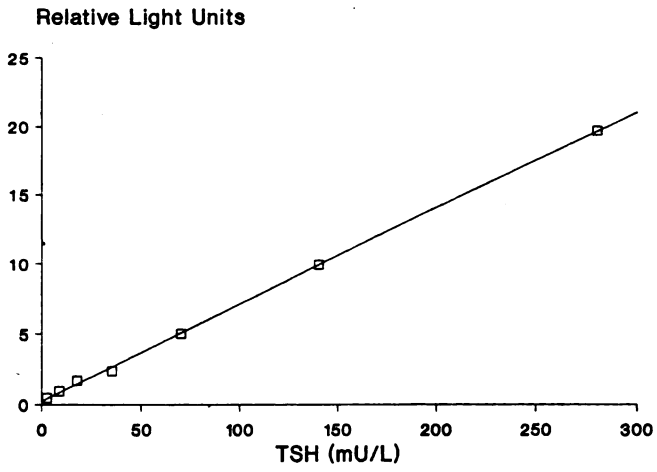

Fig. 1. Dose–response curve for blood-spot TSH-ICMA with antibody-coated microplate

These initial studies suggest that the advantages of chemiluminescent acridinium labels can be exploited in a microplate format. No complex end-point chemistries or incubations are required, in contrast to enzyme-catalyzed systems. The resulting assays are highly sensitive, simple, and robust, making them particularly well suited for screening purposes.

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Reference

1. Weeks I, Sturgess ML, Brown RC, Woodhead JS. Immunoassays using acridinium esters. Methods Enzymol 1986;133:366–7.

Sequential Measurements of Urinary Albumin in Recipients of Renal Allografts, *L. Morelet,[1] C. Legendre,[2] H. Kreis,[2] and B. Lacour[1]* ([1] Lab. Biochimie A, [2] Unité de transplantation rénale, Dépt. de Néphrol., Hopital Necker, 149 rue de Sèvres, 75743 Paris Cédex 15, France)

Various urine components have been suggested as markers for the early diagnosis of rejection—β_2-microglobulin, neopterin, renal tubular enzymes, thromboxane B_2, interleukin-2 (IL-2), and soluble IL-2 receptor—but none has been proved to have either good specificity or good predictive value (e.g., *1–4*). Rejection crises are also associated with increased urinary protein excretion (*5–7*).

We studied for six months 16 renal allograft recipients (mean age 39 years) who were receiving azathioprine (2 mg/kg daily), methylprednisolone (0.25 mg/kg daily), and OKT3 antibodies (5 mg daily for one month) as basic immunosuppressive agents. Ten of them also received low doses of cyclosporin A.

Albumin concentrations were measured daily by immunoturbidimetry of early morning urine samples during the