

SCINTILLATING MICROTITRATION PLATES IN IMMUNOASSAYS

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ABSTRACT. Immunoassays are standard methods used in clinical laboratories. Applications have increased with the advent of monoclonal antibodies and non-radioactive labels. However, radioactive labels are still widely used in many routine immunoassays. We have developed a novel, easy-to-use format for solid-phase radioimmunoassays (RIA). The assays are based on multiwell microtitration plates manufactured from plastic scintillator for measuring bound radioactivity. These plates are then measured directly in a microplate scintillation counter. We have developed both immunoradiometric and RIAs that can be automated further using the microtitration plate format and existing laboratory equipment. The scintillation plate method gives the user a choice between homogeneous (non-separation) and separation-based assays.

INTRODUCTION

Radioimmunoassays (RIA) are used widely in clinical laboratories, research laboratories and industrial laboratories. These assays can be divided into two categories, competitive RIAs and non-competitive immunoradiometric assays (IRMA) (Wide 1978). In addition to immunoassays, other ligand-binding assays are routinely performed in large quantities, especially in biotechnological and pharmaceutical research laboratories. However, automation of routine RIAs has not been explored as efficiently as for assays employing non-radioactive markers and microtitration plate format. RIAs are often performed in individual tubes that, after a separation step, are measured either in a gamma counter or in a scintillation counter. Complicated assay procedures, assay kinetics, technician time and overall assay automation can be improved by employing microtitration plate format and a counter that can measure the emitted photons directly from the plates.

Coated scintillating microparticles were used previously for homogeneous binding assays (Udenfriend *et al.* 1985; Udenfriend, Gerber & Nelson 1987; Bertoglio-Matte 1983). Their use is based on the fact that the commonly used radiolabels ^3H and ^{125}I emit low-energy electrons that have short ($<12\ \mu\text{m}$) ranges in aqueous solutions. Thus, only the bound label comes close enough to microparticles to produce scintillation light, whereas the unbound label remains too distant to cause significant scintillation. Microparticles are obviously usable in some applications, but they have drawbacks, such as extra handling steps, settling problems and difficult separation. Further, they have a large surface area, which can be beneficial in some assays, but which also increases the risk for a high non-specific binding and variation especially at background level. Generally, microparticles are not handled readily with the existing laboratory equipment, but need additional steps or instruments, *e.g.*, centrifugation or magnetic devices for washing. Using another approach, Burton and Hoop (1983) describe a specially designed reaction chamber with a fluorescing surface for homogeneous competitive binding assays. However, the chamber structure is not compatible with laboratory automation, especially if separation of an unbound from a bound label is required.

Our goal was to develop a ligand-binding assay procedure that would benefit from the widely established and existing microtitration plate format and isotope labels. In this context, a Microbeta™ scintillation counter, which is able to measure light emission directly from plates, plays a key role. Also, the assays will be remarkably simplified by using microtitration plates made of polystyrene containing appropriate scintillators (Oikari, Yrjönen & Lehtinen). These plates have a great advantage because they can be used both in separation and in homogeneous assays with low-energy beta emitters, such as ^3H . The easy separation enables binding assays with high-energy isotopes, *e.g.*, ^{14}C , ^{35}S , ^{33}P , ^{32}P , which otherwise would give high backgrounds due to their far-

reaching radiation. Further, with separation, the user can remove several assay artifacts, such as absorbing colors, turbidity and loose non-specific binding. With existing laboratory equipment, separation is easily accomplished by automatic washers. The whole assay system should be as simple as possible, applicable to a very wide range of assays, and should lend itself to automation.

METHODS

For thyroid stimulating hormone immunoradiometric assay (TSH IRMA), the scintillating plates were coated with a monoclonal anti-TSH antibody ($5 \mu\text{g ml}^{-1}$) in 50 mM carbonate buffer at pH 9.6 by incubating 200 μl of the solution at 25°C for 18 h. The plates were washed three times with tris-HCl buffer, pH 7.8, containing 0.05% Tween-20TM and 15 mM NaCl, then saturated with the same buffer containing 0.5% of bovine serum albumin at 25°C for 2 h. After aspiration, the plates were sealed with a plastic tape and stored at 4°C.

To each well, 100 μl of standard or sample and 100 μl of ¹²⁵I-labeled monoclonal anti-TSH antibody in DELFIA® assays buffer, was added. The plates were incubated for 2 h at 25°C, and measured directly or washed five times with an automatic DELFIA® PlateWash 1296-024 prior to measurement in a 1450 MicrobetaTM. With this instrument, the emitted photons are measured in coincidence, using both a physical barrier (the plate is measured in a 1450-105 cassette) and a software program for crosstalk correction.

The coating procedure for testosterone RIA was essentially the same as above, but anti-rabbit antibody (AB) was used as a secondary AB in the solid-phase, and anti-testosterone AB as a primary AB. In the assay, testosterone was extracted from serum samples with standard procedures using hexane and hexane-ether solutions. The samples were then reconstituted with tris-buffer, pH 8.5. Into the wells, 75 μl of primary AB solution, 100 μl of ³H-labeled testosterone and 50 μl of sample were pipetted. The wells were incubated at 4°C for 18 h and measured directly or washed 4 times, as above, prior to measurement in a MicrobetaTM (Fig. 1).

The method was the same as above for reference testosterone RIA, except that it was performed in 5-ml polystyrene tubes, and after the immunoreaction, the free testosterone was extracted with charcoal in multiple steps. After centrifugation, the supernatant was poured into scintillation vials and 10 ml of scintillant were added. The vials were then counted in a standard liquid scintillation counter for 10 min or 10,000 cpm.

RESULTS

For TSH IRMA, the dose response curve was linear from 0.25 to 324 mU liter⁻¹, and the coefficient of variation (CV%) from 1.3 to 11.6 in the standard curve. The sensitivity was about 0.1 mU liter⁻¹ (Fig. 2). When TSH was measured in a homogeneous assay, the assay was linear but the sensitivity was somewhat lower (data not shown).

With scintillation plate testosterone RIA, we obtained a higher sensitivity than with the reference RIA (Fig. 3A). If we washed the plates before the measurement, we obtained a better precision than with the reference RIA or with the homogeneous assay (Table 1) over the standard range. The counts obtained with the homogeneous assay were somewhat lower with the same counting time, which explains partly the small difference in precision. The correlation between the reference RIA and homogeneous RIA was good when 17 specimens were tested (Fig. 3B). We also obtained a very good correlation between the normal assay, which included a washing step prior to the measurement, and the homogeneous assay (data not shown). In addition to standard immunoassay,

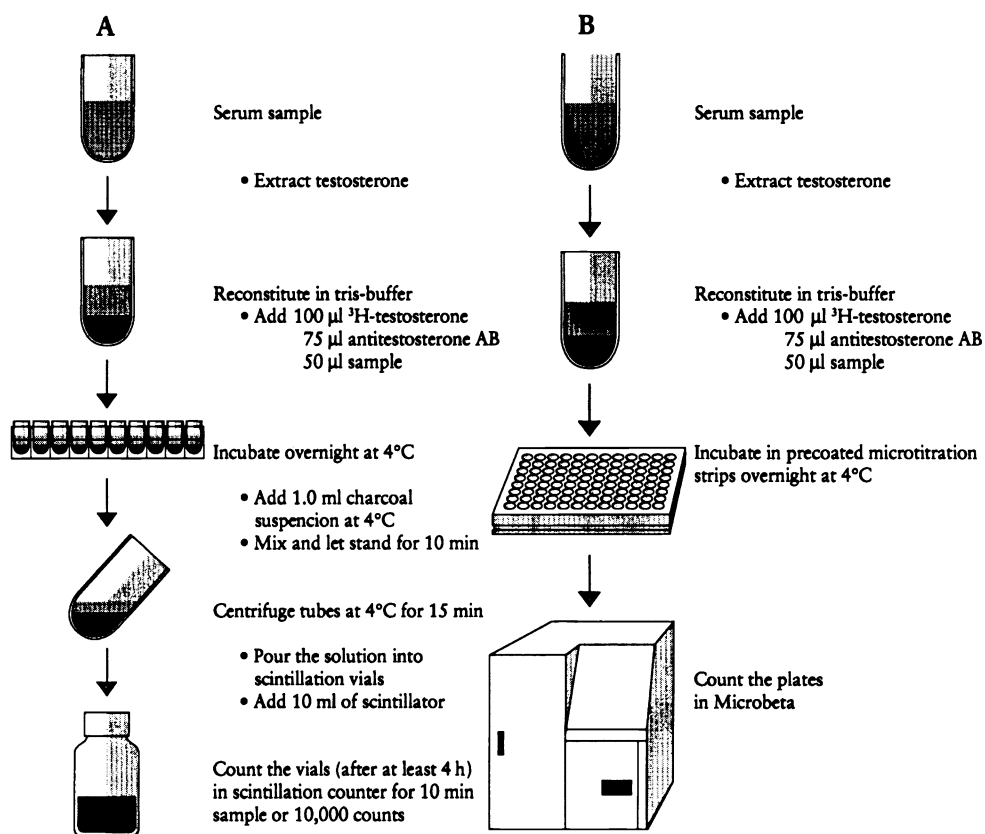


Fig. 1. Assay procedure of A. testosterone RIA and B. homogeneous testosterone

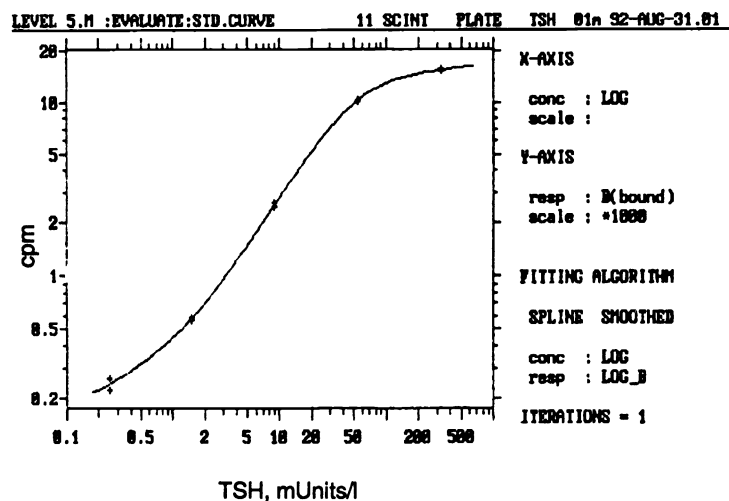


Fig. 2. Standard curve of scintillation plate TSH

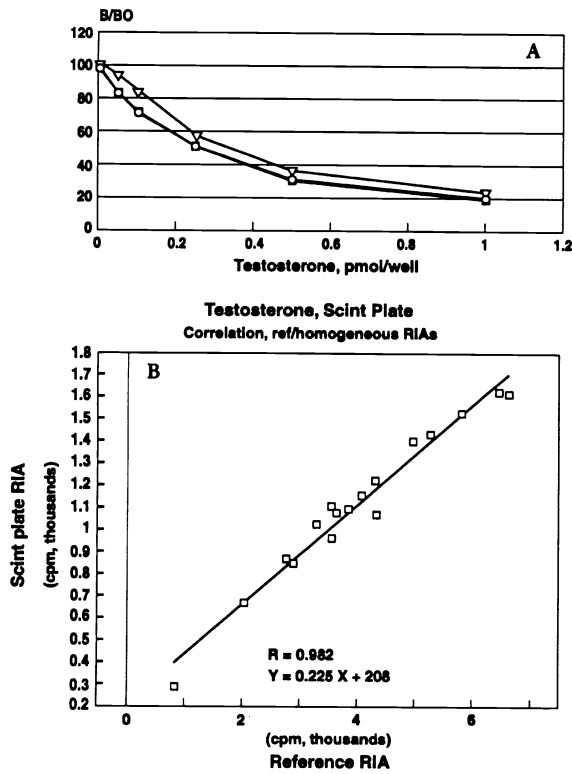


Fig. 3. A. Sensitivity and linearity; B. Correlation of scintillation plate testosterone. ∇ = Reference RIA; \square = Plates washed; \circ = Homogeneous assay

TABLE 1. Example of Standard Curve Precisions of Reference and Scintillation Plate RIAs

Concentration (pmol/well)	Reference (RIA)	Scintillation plate	
		Normal	Homogeneous
0	7.3*	0.4	2.7
0.05	1.5	0.0	2.3
0.10	0.9	1.6	5.4
0.25	0.7	0.6	2.6
0.50	3.6	0.4	4.7
1.00	1.7	0.8	6.7

*Coefficient of variation %, n = 2

the scintillation plate concept can be used in other applications. By using the homogeneous principle, various ligand-binding parameters, e.g., kinetics of a specific interaction, can be studied without interrupting the biological reaction (Fig. 4).

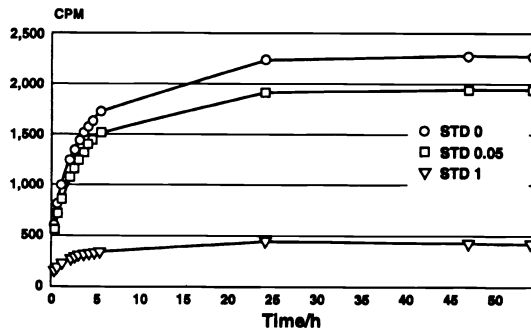


Fig. 4. Kinetics of homogeneous testosterone RIA

CONCLUSIONS

With the assays developed, we were able to obtain the sensitivity needed for laboratory diagnosis of TSH and testosterone-related disorders. The assay had a precision at least equal to that of the reference method, as shown in the representative example in Table 1. Using scintillating microtitration plates, the user can select between homogeneous or separation assays. The unbound label can be separated easily by washing the wells, which also enables assays with high-energy isotopes. Further, separation decreases the potential interference from samples containing absorbing colors, turbidity or loose, non-specific binding.

RIAs and other types of ligand-binding assays can be automated with this assay concept, including the microtitration plate format, scintillating microtitration plates and the scintillation counter. The well-established format, including the existing laboratory instrumentation, further improves the laboratory economy by decreasing reagent consumption and technician time needed for the assays. The amount of waste is decreased dramatically and is in solid form.

These benefits are also applicable in a variety of other biological fields. In research and diagnostic applications of, e.g., cell-cell interactions, receptor-ligand binding studies and nucleic-acid hybridizations, the developed concept would be greatly beneficial for assay performance, assay automation, laboratory economy and waste disposal.

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