

TopCount *Topics*

TCA-007

Solid-phase RIA in Microplates

Abstract

Solid-phase radioimmunoassays have traditionally been performed in plastic tubes which have acceptable adsorptive properties and are well suited for gamma counting. However, RIA's performed in this manner are time consuming and costly. These assays can now be performed and counted directly in microplates using TopCount, a microplate scintillation counter which counts 12 samples simultaneously in the 8 X 12 format. Results are presented for competitive (T4, IL-2) and non-competitive (TSH) immunoassays. The adsorptive properties of solvent resistant (Barex) PicoPlates are compared to those of polystyrene plates, and recommendations regarding the use of cocktail with polystyrene plates are provided.

Introduction

Radioimmunoassays (RIA) are typically categorized as competitive (also called RIA) or non-competitive (immunometric or IRMA). Competitive assays utilize a single capture antibody, usually a polyclonal, to which the analyte and tracer (radiolabeled analyte) compete for binding. Maximum sensitivity is achieved using highly diluted antibody and tracer in slight excess; sensitivity is limited mainly by the affinity of the antibody. Non-competitive assays utilize a capture antibody to which the analyte binds. The analyte is then detected with a radiolabeled secondary antibody. The secondary antibody must be in excess; sensitivity is limited by the specific activity of the labeled antibody.

Traditionally, RIA's are performed in plastic tubes using an ^{125}I label with quantification via conventional gamma counting. Recently attempts have been made to adapt RIA to the microplate format using wells which are separable (*i.e.*, Removawell strips; Dynatech, Chantilly, VA). Although this adaptation has several advantages, including decreased sample handling and smaller reagent volumes, one must ultimately transfer the individual wells to tubes for gamma counting. TopCount completes the evolution of RIA by allowing direct microplate counting. Sample handling is dramatically decreased, while throughput is increased.

In order to perform and quantify solid-phase RIA in microplates, the microplates must be compatible with radioisotope counting and possess the adsorptive properties necessary for solid-phase RIA. For scintillation counting in TopCount, the plastic must be pigmented to prevent optical crosstalk. In addition, some cocktails can be quenched by polystyrene. To avoid these problems Packard has developed PicoPlates composed of solvent-resistant Barex plastic (BP Chemical; Cleveland, OH). We show here that white pigmented polystyrene microplates and PicoPlates have very similar adsorptive properties, and either may be used on TopCount. The counting efficiency for ^{125}I in 96-well PicoPlates using MicroScint-20 is 58%.¹ In addition, ^3H labels (38% counting efficiency) may be used.¹

This study demonstrates the efficacy of TopCount for solid-phase RIA. Examples of both competitive [thyroxine (T4), human interleukin-2 (IL-2)] and non-competitive [thyroid stimulating hormone (TSH)] RIA's are included. Some of the assays were performed using Removawell strips so that quantification was possible on both TopCount and a COBRA gamma counter (Packard Instrument Company, Meriden, CT) in order to provide instrument correlation data. The use of a universal solid support (*i.e.*, an ovalbumin-biotin and streptavidin system) is discussed.

Experimental Methods

Universal Solid-Phase.

Plates were coated using ovalbumin/biotin and streptavidin. Wells were incubated for one hour at 37 °C with ovalbumin/biotin conjugate (Dr. Alain Baret, Nantes, France; 50 ug/mL; 300 µL/well) in coating buffer (20 mM boric acid, 50 mM sodium chloride, pH 8.6). After washing four times with water, streptavidin (5 ug/mL; 400 µL/well) diluted in PBS (with 0.1% BSA) was introduced for one hour at room temperature. Plates were then washed and used or stored at 4 °C in PBS/BSA.

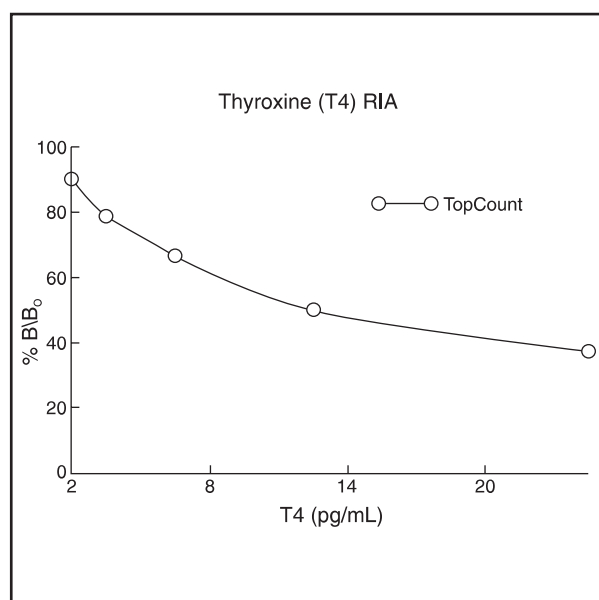


Figure 1.
T4 RIA results using the TopCount.

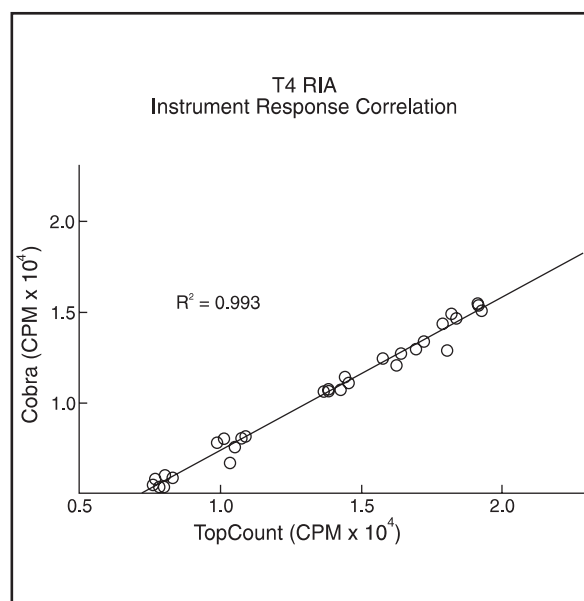


Figure 2.
T4 RIA correlation of TopCount vs. gamma counting (COBRA).

Thyroxine RIA.

In order to facilitate a direct comparison between TopCount and gamma counting, Dynatech Removawell white strips were used instead of PicoPlates. After coating strips with ovalbumin-biotin/streptavidin, the wells were washed four times with wash buffer (25 mM borate, 0.05% BSA, 0.01% Tween 80, 0.1% sodium azide, pH 8.4). Then 300 µL/well of anti-T4 biotin conjugate (provided by Dr. Alain Baret, Laboratoire Trichereau, Nantes, France) at 0.6 ug/mL in PBS/BSA was added and incubated for one hour at room temperature. After washing, 25 µL/well of T4 standards (0, 1.5, 3, 6, 12, and 24 pg/mL) were added. This was followed by 200 µL/well of tracer, [¹²⁵I] T4 labeled conjugate (NEN DuPont, Wilmington, DE; NEX-111, 124.6 uCi/ug, 246.8 uCi/mL), diluted in T4 diluent buffer (50 mM borate, 150 mM sodium chloride, 0.65 mM 8-anilino-1-naphthalenesulfonic acid, 0.1% sodium azide, 0.1% BSA, pH 8.6). Standards and tracer were incubated for one hour at room temperature with agitation on a microplate shaker. Plates were washed, then dried under an infrared lamp. After adding 300 µL/well of MicroScint-20, plates were immediately counted on the TopCount for one minute per well. Afterwards, the individual wells were placed into separate polystyrene tubes for gamma counting (COBRA).

Interleukin-2 RIA.

An RIA kit for human IL-2 (Advanced Magnetics; Boston, MA) was used for this assay. The protocol, originally intended for liquid-phase kinetics and magnetic separation in tubes, was redesigned for the solid-phase microplate format. PicoPlates were coated with polyclonal rabbit anti-human IL-2 (100 μ L/well) diluted in coating buffer. Plates were covered and incubated overnight at 4 °C. After washing four times with wash buffer, wells were blocked with 3% BSA in coating buffer (400 μ L/well) for one hour at 40 °C. Plates were washed, then incubated with 100 μ L/well of Human IL-2 standards (0, 10, 25, 50, 100, 250, 500 pg/0.1 mL) for four hours at room temperature with gentle agitation. Without washing, 100 μ L/well of 125 I-IL-2 tracer was added and the incubation was continued overnight at 4 °C. After washing, 300 μ L/well of MicroScint-20 was added and plates were sealed with TopSeal P (Packard Instrument Company), mixed on a microplate shaker, and counted on TopCount. Microplates should always be sealed with TopSeal, a heat sealing film, in order to facilitate mixing and prevent detector contamination.

TSH IRMA.

PicoPlates, Dynatech Microlite 1 microplates, and Dynatech white strips were coated with the universal solid support as described above. After washing four times with wash buffer, 300 μ L/well of monoclonal anti-TSH biotin conjugate (Dr. Baret; Nantes, France) diluted to 5 μ g/mL in PBS/0.1% BSA was added and incubated for one hour at room temperature. Wells were washed and then incubated for two hours (with agitation) with 150 μ L/well of TSH standards (0, 0.2, 1, 5, 40, 200 μ LU/mL; Amersham, Arlington Heights, IL) and 200 μ L/well of [125 I] anti-TSH antibody diluted in TSH diluent buffer (100 mM citrate, 100 mM NaCl, 0.1% BSA, 0.2 mg/mL mouse immunoglobulin (Ig), 0.2 mg/mL bovine Ig, 0.01% Tween 80, pH 5.5). Plates were washed and dried, and 300 μ L/well of MicroScint-20 added for counting on TopCount. Individual wells of the Dynatech strips were subsequently counted on the COBRA for one minute/well.

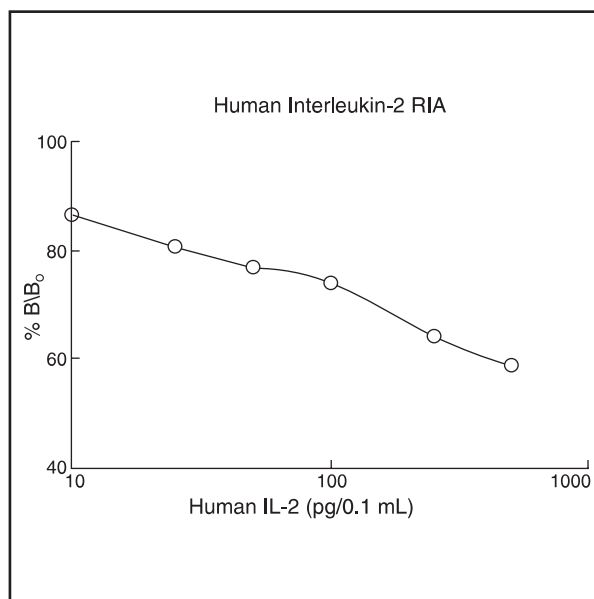


Figure 3.
IL-2 RIA in PicoPlates.

Adsorption Comparison.

For this assay a constant concentration of ovalbumin/biotin was bound to the wells using the above procedure. The wells were then incubated for one hour at room temperature with 300 μ L/well with serial dilutions of [125 I] streptavidin (Amersham; first dilution 5 uCi/mL, then 1:2 thereafter). Nonspecific binding was determined by coating wells with ovalbumin only.

Quench Study.

Ten microliters of [3 H]-palmitic acid (1 mCi/mL in toluene) was added to 250 μ L of MicroScint-O and 10 μ L of 3 H-uridine (1 mCi/mL in water) was added to 250 μ L of MicroScint-20 in separate wells (and in triplicate) of PicoPlate, Dynatech Microlite 1, and white Dynatech MicroFLUOR microplates. Plates were mixed on a plate shaker for ten minutes and then counted on TopCount for three minutes. The same plates were then recounted after three, eight, 24, 48, and 72 hours.

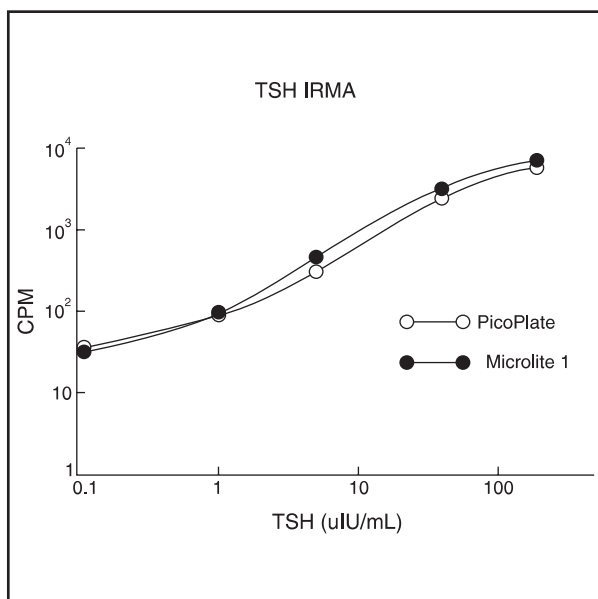


Figure 4.

TSH IRMA standard curves of Packard PicoPlates vs. Dynatech Microlite 1 microplates.

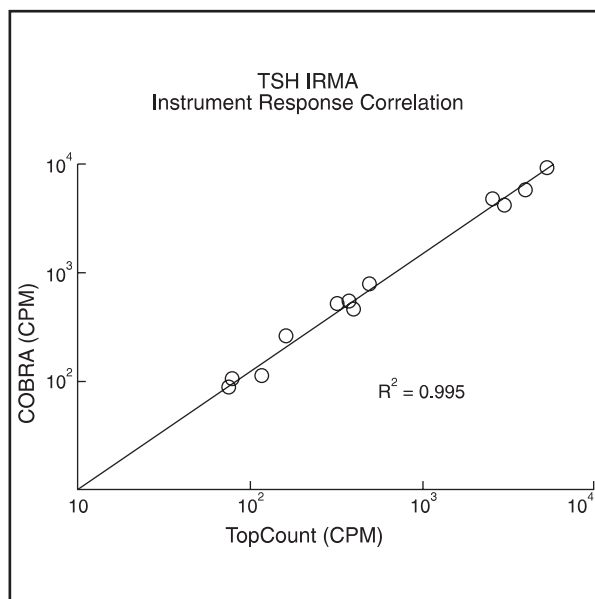


Figure 5.

TSH IRMA correlation of TopCount vs. gamma counter (COBRA).

Results & Discussion

Thyroxine RIA.

The results from a T4 RIA are shown in Figure 1. Dynatech white strips were coated using the universal solid support, and a standard curve was generated using T4 standards and an ^{125}I -labeled tracer. The assay was clearly sensitive at the highest T4 dilution (2 pg/mL) used in this study. Standards were prepared in replicates of six and had an average CV of 2.5%. After the strips were counted on TopCount, the individual wells were placed into plastic tubes for counting on a COBRA gamma counter (Figure 2). The two instruments correlate well ($R^2 = 0.993$).

IL-2 RIA.

Another competitive RIA was performed for human IL-2. However, this time the assay was performed in PicoPlates (Figure 3). The assay was performed twice (standards in triplicate) yielding an intraplate CV of 3.2% (data from Figure 3) and an interplate CV of 4.2%.

TSH IRMA.

An IRMA for TSH was performed on PicoPlates and Dynatech Microlite 1 microplates (Figure 4) and on Dynatech white strips (Figure 5) using the universal solid support. Figure 4 represents a standard curve generated using TSH standards and an iodinated

monoclonal anti-TSH antibody. Essentially identical results were obtained with the two types of microplates. Once again, good instrument correlation ($R^2 = 0.995$) was observed for the IRMA performed in microwell strips (Figure 5).

Adsorption Comparison.

An experiment was performed to compare surface adsorption of PicoPlates with a white Polystyrene microplate, Microlite 1. This experiment was essentially an adaptation of the procedure used for the universal solid support. As shown in Figure 6, both microplates exhibit equivalent binding of serially diluted ^{125}I streptavidin. Background binding was subtracted by counting wells coated by ovalbumin.

Quench Study.

Organic solvents may dissolve polystyrene over time, and since some applications may require the use of polystyrene microplates, the effect of solvent contact with pigmented polystyrene microplates was investigated by measuring counts over time (exposures of three, eight, 24, 48, and 72 hours). As shown in Figure 7, MicroScint-20 induced no quench over time. The ^3H counts were constant for 72 hours with MicroScint-20, whereas counts in MicroScint-O decreased by 73% in Microlite 1 and 43% in MicroFLUOR. Optical crosstalk will vary depending on the pigment load used by the manufacturer.

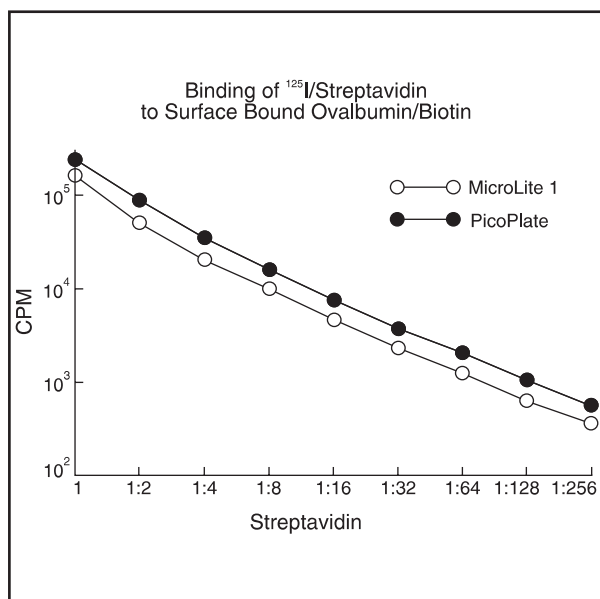


Figure 6.
Binding of ¹²⁵I/Streptavidin to surface bound ovalbumin/biotin.

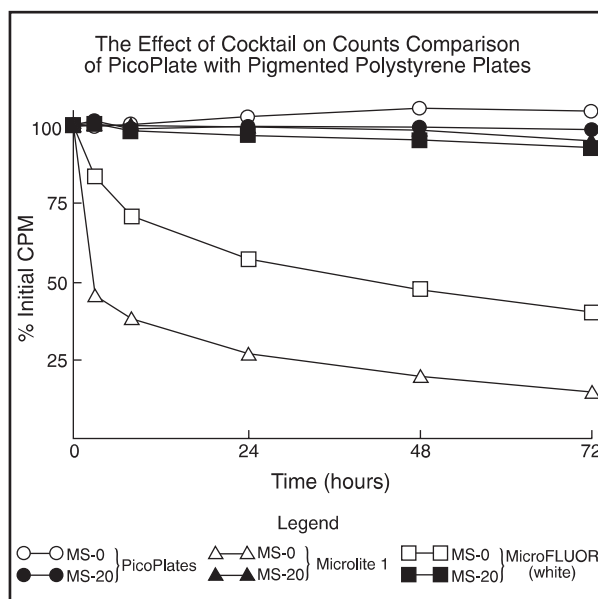


Figure 7.
Effect of solvent contact with pigmented polystyrene microplates.

For example, in the above experiments both PicoPlates and MicroLite 1 had < 0.001% crosstalk, while MicroFLUOR had 0.02-0.07%.

Conclusion

Direct microplate counting offers significant savings in labor and waste disposal costs by decreasing sample handling, sample volumes, and disposal costs. TopCount successfully merges the sensitivity of RIA with the convenience of microplate-based solid-phase immunoassays, which has been largely restricted to enzyme immunoassays (EIA). Unlike EIA, RIA does not suffer from a limited dynamic range or high background. Furthermore, kinetic studies or the additional step required to stop the reaction is not required.

The ovalbumin-biotin and streptavidin solid-phase is highly recommended for increasing the functional capacity of primary antibodies. This is particularly important when using monoclonal antibodies which tend to denature during adsorption to hydrophobic surfaces.² Studies have shown that the antigen capture capacity of monoclonal antibodies is significantly higher when adsorption is via an avidin-biotin link versus direct surface adsorption.³

When performing solid-phase RIA or any other applications which require the use of organic solvents, such as the MicroScint series, Packard recommends using PicoPlates due to their prolonged solvent stability and excellent sealing capacity. Only the emulsifier-based MicroScint-20 or -40 cocktails should be used to count samples in white polystyrene microplates. It is recommended that these plates be counted within 72 hours since the seal will weaken with time.

References

- TopCount Topics: Counting Aqueous Samples with the TopCount Microplate Scintillation Counter. Packard Instrument Co. One State Street, Meriden, CT.
- Suter, M. and Butler, J.E., The immunochemistry of sandwich ELISA's. II. A novel system prevents denaturation of capture antibodies, *Immunol. Lett.*, 13,313, 1986.
- Suter, M., Butler, J.E., and Peterman, J. H., The immunochemistry of sandwich ELISA's. III. The stoichiometry and efficacy of the Protein-Avidin-Biotin Capture (PABC) system, *Mol. Immunol.*, 26, 221, 1989.

