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Mini-RIA: adaptation of conventional ^{125}I-labeled radioimmunoassay to a 96-tube format

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Abstract

Radioimmunoassay (RIA) employing iodinated ligands represents a popular measurement method for small molecules due to its excellent sensitivity and specificity. Yet performing RIAs of large numbers of tubes remains a tedious laboratory chore due to the need to individually handle tubes multiple times. We here present a method in which conventional ^{125}I-labeled RIA ([^{125}I] RIA) is adapted to a microtiter plate format, termed mini-RIA. Tubes are handled in batch for centrifugation or during the separation of antibody-bound ligand from free ligand. A simple draining device for batch decantation of free ligand from 96 minitubes is used. Optimal conditions for the mini-RIA were established using two workup methods—double-antibody immunoprecipitation and direct polyethylene glycol precipitation. Use of the mini-RIA method was found to result in a considerable savings in assay times; in addition, the sensitivity of the mini-RIA was improved over conventional RIA. The mini-RIA is particularly useful for assay of large numbers of samples derived from chromatographic methods, since aliquots can be transferred directly from the fraction collector into the minitubes using multiple channel pipettors. Because the method is flexible with regard to assay workup, we predict that most conventional [^{125}I] RIAs can be adapted to the mini-RIA format.

The technique of isotopic radioimmunoassay (RIA)[1] has enjoyed continued popularity since its introduction in the mid-60s by the team of Berson and Yalow [2], as a consequence of its unmatched combination of sensitivity (due to the use of the high specific activity isotope [^{125}I]) and specificity (due both to antibody affinity as well as competition of cold ligand with radioactive ligand for antibody-binding sites). However, as currently performed in most laboratories, RIAs employing iodinated ligands still involve a great deal of manual labor; 12×75-mm tubes are individually handled during assay setup, workup, centrifugation, and separation of antibody–antigen complexes. For this reason, nonisotopic ELISAs employing 96-well plates have often been substituted in a number of applications. While the ability to use multiple channel pipettors and plate readers makes this an attractive option, ELISAs can be costly in terms of primary antiserum; also, the specificity of the [^{125}I] RIA cannot always be achieved. Two-site IRMAs carried out in 96-well plate formats with spectrophotometric detection [7,10] can provide an additional level of specificity over conventional ELISAs; unfortunately, two antisera to independent epitopes are rarely available for a given compound. Proximity scintillation assays have been developed in a 96-well format which employ special plates (Wallac Microbeta TriLux plates or Packard TopCount plates) coated with fluoromicrospheres—solid-phase supports impregnated with substances such as second antibodies or protein A that emit light in the presence of bound radioactive label-antibody complexes [1,4]; however, these plates are quite expensive for routine laboratory use. Thus to this date commercial and laboratory RIAs are most commonly still carried out using the highly sensitive radiolabel [^{125}I] for ligand labeling and a single polyclonal antiserum in 12×75-mm tubes. This assay format is especially onerous during analysis of column fractions that typically involves many hundreds of assay tubes.

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[1] *Abbreviations used:* RIA, radioimmunoassay; [^{125}I] RIA, ^{125}I-labeled radioimmunoassay; BSA, bovine serum albumin; PBS, phosphate-buffered saline; ACTH, adrenocorticortropic hormone; PEG, polyethylene glycol; CLIP, corticotropin-like intermediate lobe peptide.

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In order to take advantage of the availability of 96-tube batch handling methods as well as multiple channel pipettors, we have adapted conventional [^{125}I] RIA to a microtiter plate format. The repeated handling of plates as a single unit rather than individually saves considerable operator time; for example, decantation in the 96-well format permits separation of antibody-bound and free iodinated ligand without individual tube handling. We estimate that when this method is applied to assay four chromatographic sets of 32 fractions in duplicate with four sets of standards, the overall time saved is reduced by two-thirds: from 4 to 1.5 h per 384 assay tubes. Since most academic laboratories do not possess robotic equipment, this frees technical labor considerably. In addition, miniaturization saves space in the refrigerator and freezer.

Materials and methods

Materials and equipment

Assays are setup in racks of 96 polypropylene 1.2-ml minitubes (Daigger Cat. No. LX23630B, www.daigger.com). A centrifuge capable of handling 96-tube racks is required (such as the Sorvall RT6000B with a PN11093 microtiter plate holder in the H1000B rotor, www.sorvall.com). For collection of chromatography-derived fractions, a collector capable of holding these minitube racks, such as the Gilson FC 230B collector (www.gilson.com), is required. For separation of antibody-bound iodinated ligand from free iodinated ligand, a plastic insert obtained from a deep 96-tube plastic reservoir (Bel-Art Cat. No. 37854-0000, www.bel-art.com; also distributed by Daigger, Cat. No. B7AF37854-0000) is used to restrain the minitubes during draining, as described below. An empty tip rack for 1- to 200-µl tips obtained from USA Scientific (Cat. No. 1120-8810, www.usascientific.com) serves as the reservoir for this assembly. Lastly, an absorbent pad is constructed from common materials, as depicted below. A pulsing vortex mixer capable of handling a 96-tube rack (available from Glas-Col, Cat. No. 107A PVM12, www.glascol.com) is recommended for efficient mixing during the workup step.

Experimental methods

General comments

The standard curve for the mini-RIA is setup in the first three rows of 8; samples obtained from chromatographic methods are initially collected into 96-tube racks and transferred from the originating rack directly to the appropriately labeled tubes next to the standards using an 8-channel pipettor. If hostile volatile solvents are present, these can be removed by drying the tubes prior to assay using vacuum centrifugation (using Savant microtiter plate holders; part number MPTR8-210 for a SC210A Speedvac Plus, www.savec.com). During drying of peptide-containing fractions we routinely add 50 µl/tube of a 5 mg/ml solution of crystalline BSA (Sigma Cat. No. A-7906, www.sigma-aldrich.com) in water to avoid losses. After drying, samples are reconstituted to 100 µl using RIA buffer with the aid of an Eppendorf Repeater pipette (Brinkmann–Eppendorf, Cat. No. 22 26 000-6, www.brinkmann.com).The use of the mini-RIA format is described below for two popular workup methods, the double-antibody precipitation method (in which goat anti-rabbit antiserum is used to specifically precipitate antibody-bound radiolabeled ligand), and the PEG precipitation method (in which PEG is used to nonspecifically precipitate all large protein complexes; used when nonspecific binding is not an issue). ACTH was assayed with the first type of workup; the neuroendocrine marker 7B2 was assayed with the second workup method.

Double antibody precipitation (ACTH RIA)

Solutions

The RIA buffer used for this method consists of 50 mM sodium phosphate, pH 7.6, containing 0.1% Triton X-100 and 0.02% sodium azide. For the workup, a solution of 15% PEG-8000 (Sigma PEG 8000; Cat. No. P-2139), 0.1% Triton X-100 in PBS (with 0.02% sodium azide) is prepared and stored at 4 °C. Carrier γ-globulin (Sigma Cat. No. I-5006) is made up to 10 mg/ml in PBS and stored at −20 °C in 0.5-ml aliquots. Goat anti-rabbit serum (Biogenesis, Cat. No. 5196-4004, www.biogenesis.co.uk) is aliquoted in 1-ml aliquots and kept frozen at −20 °C.

Preparation of standards and samples

The final volume in each tube will be 200 µl. First, the three nonspecific binding tubes (labeled N) receive 150 µl of RIA buffer. One hundred microliters of RIA buffer is added to the three maximum binding tubes (labeled O). The standards are then pipetted in duplicate or triplicate in the appropriate concentration range for the assay; for the ACTH assay, standards consisted of 1, 2.5, 5, 10, 25, 50, 100, 250, and 500 fmol of rat $ACTH_{(1–39)}$ per assay tube. Each tube is then made up to a total of 100 µl of RIA buffer. Samples are either prepared in duplicate or triplicate in the same buffer and are always centrifuged and decanted at the same time, and in the same rack, as the standards. The samples used in our test study consisted of 5 µl of a single high-pressure gel permeation chromatographic fractionation of mouse pituitary in 32% acetonitrile, 0.1% trifluoroacetic acid (using the chromatographic method described in [6]); the sample corresponded to a peak with the size of authentic $ACTH_{(1–39)}$. Five microliters of all fractions from a similar chromatographic separation was used to

generate the data shown in Fig. 2B; samples were transferred directly into the RIA tubes using a 0–20 μl eight-channel pipettor into duplicate rows.

Radioactive ligand is then added to RIA buffer by dilution of stock such that 50 μl yields approximately 10,000 cpm. For this study we used commercially labeled ACTH (Amersham, Cat. No. IM 216, www.apbiotech.com). Two "total radioactivity" tubes receive 50 μl of the radioactive solution and are stored in an independent rack on the bench. Fifty microliters per tube of this radioactive ligand solution is quickly dispensed into the three nonspecific binding tubes, the three maximum binding tubes, the standards, and the samples, using an Eppendorf repeating dispenser with a 2.5-ml disposable syringe (Labsource, P-3521, www.labsource.com). The antiserum is then diluted in RIA buffer at a concentration that yields approximately 35% binding, and 50 μl is dispensed into all tubes except the nonspecific binding tubes. The ACTH antiserum, Kathy (a gift of Dr. R.E. Mains, University of Connecticut), was used in these studies at a working dilution of 1:8000. (Alternatively, the radioactive ligand and antiserum can be combined into a single solution such that a single 100 μl pipetting is done. In this case the nonspecific binding tubes receive 100 μl of a radioactive ligand dilution (yielding 10,000 cpm) prior to the addition of the antiserum to the same solution.) While an 8-channel pipettor can be used for this step, we obtain better reproducibility with the repeating dispenser. The rack is then covered with a soft disposable cap mat (Matrix Technologies, Cat. No. 4411, www.matrixtechcorp.com), manually held on the pulsing plate shaker, mixed at setting 50 for about 5 min, and placed in the refrigerator for 16 h.

Separation of antibody-bound from free labeled peptide

Just prior to use, the workup solution is prepared by mixing for each minitube 4 μl of carrier γ-globulin, 10 μl of goat anti-rabbit antiserum, 0.1% Triton X-100, and 0.5 ml of cold 15% PEG. This solution is normally cloudy and should be mixed to homogeneity by inversion and stored on ice. The Eppendorf dispenser is used to dispense this somewhat viscous solution, with a 12.5-ml disposable syringe (Labsource, P-3524) used to dispense 0.5 ml to each minitube. The rack is covered, attached, and held tightly on the pulse vortex mixer. The tubes are shaken 5 min at setting 50, yielding vigorous agitation. This is a critical step for achieving precipitation of the antibody- ligand complex. Finally, the rack is placed at 4 °C for 3 h. When the incubation time has elapsed, the racks are placed in prechilled microtiter plate holders inside a refrigerated bench-top Sorvall RT6000 centrifuge, and centrifuged for 30 min at 1200*g*.

Once centrifugation is completed, decantation of the workup solution is accomplished by placing a plastic 96-hole barrier grid (the grid removed from a Bel-Art tip box, see Materials and methods) on top of the rack. It is important that the plastic 96-hole grid be placed completely flush with the tops of the tubes; no ridges should be present between the grid and the tubes, or proper drainage will not occur. The 96-hole grid is firmly affixed to the rack of tubes with two $3 \times 1/32$-inch rubber bands, each positioned about a third of the way across the box and not covering any tubes (see diagram); gloves are worn during this process. The assembly is rapidly inverted into a draining reservoir (constructed from a rack for 1- to 200-μl tips previously emptied of its tips and tip holder; see Fig. 1A) where it fits snugly on top of the ridge originally designed for the tip holder. The draining reservoir (but not the tube rack) is then tapped down sharply in order to initiate drainage of the PEG from all tubes. The use of Triton X-100 at 0.1% final concentration in the workup solution reduces the surface tension that will otherwise prevent tubes from draining evenly.

The drained rack of tubes is then immediately transferred to the wicking assembly in order to remove residual PEG clinging to the tube tops (see Fig. 1B). The rack is manually pushed down into a pad of small disposable tissues on top of a plastic bag-sealed pad of gauze wrapped over a rubber sponge, which provides an absorptive, flexible, and soft surface for wicking of the PEG (see Fig. 1B).The sealed bag prevents contamination of the pad from one assay to the next; the tissues are changed twice during each assay. Moderate pressure is applied to the rack in order to generate efficient contact with the tissue pad. The used wipes are disposed of in the radioactive waste and the process is repeated twice with fresh tissues to ensure complete removal of PEG from tube tops prior to counting.

Finally, using forceps, the minitubes are transferred into clear polystyrene 5-ml 12×75-mm holder tubes (Sarstedt, Cat. No. 55.476, www.sarstedt.com) in the gamma counter in the correct order. These holder polystyrene tubes can be reused indefinitely, but should be counted every three or four assays; tubes are replaced when contaminated or when any visible residue is observed.

Polyethylene glycol and carrier globulin precipitation (7B2 RIA)

The setup of the assay is similar to the first method, but the separation of antibody-bound from free labeled ligand differs in that PEG and carrier γ-globulin (rather than a second antibody) are used. The standard range for the 7B2 RIA spanned 1–500 fmol, as described for the ACTH RIA. The 13B6 anti-7B2 antiserum (described in [14]) was diluted such that 50 μl bound 35% of the labeled ligand. Similar parameters were applied to conventional RIA standard curves setup for comparison purposes. However, the final volume in the conventional RIA was 300 μl, and the assay was carried out in 12×75-mm polypropylene tubes.

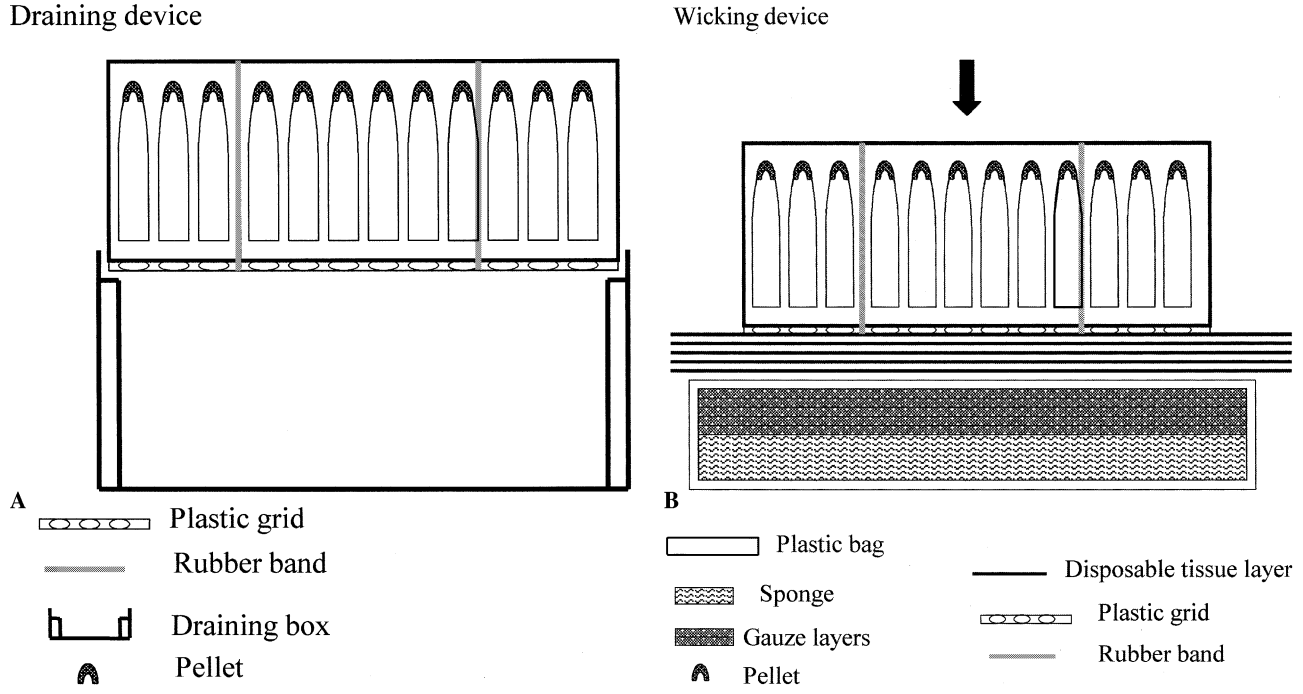

Fig. 1. Draining and wicking devices. (A) A smooth plastic 96-hole grid is affixed with rubber bands to the top of the tubes. The assembly is inverted into the reservoir box where it fits, upside down, on a ridge in the box. (B) The drained rack of tubes with pellets is then immediately transferred to a stack of absorbent tissues placed on a gauze-wrapped sponge inside a plastic bag, and residual PEG allowed to wick into the adsorbent tissues. This step is repeated with fresh tissues two more times, and is required to eliminate all traces of the viscous liquid from the tube tops.

Solutions

The RIA buffer is 0.10 M sodium phosphate, 50 mM NaCl, 0.1% heat-treated BSA, and 0.02% sodium azide. A solution of 25% PEG-8000 in water with 0.02% sodium azide and Triton X-100 at 0.1% is prepared and stored at 4 °C. Carrier γ-globulin (bovine γ-globulin, Cohn fraction II, III Sigma Cat. No. G-5009), at a 0.75% concentration in PBS, is also stored at 4 °C.

Separation of antibody-bound from free labeled peptide

The workup solution is prepared just prior to use by mixing 0.1 ml/minitube of γ-globulin solution to 0.4 ml/minitube of cold 25% PEG/0.1% Triton X-100. The cloudy solution is thoroughly mixed by inversion prior to pipetting 0.5 ml into each minitube, as described above. Once the PEG/γ-globulin solution has been added, the rack is covered, attached, and held firmly on the pulsing plate mixer. The tubes are vigorously shaken at setting 50 for 5 min and incubated at 4 °C for 1 h. The remainder of the workup is as described above.

Results

Important parameters in the mini-RIA

Crucial steps in both types of mini-RIA included the following steps: complete mixing of the precipitant solution with the RIA reaction mixture and decantation.

Decantation of the tubes, accomplished using the draining and wicking devices (Fig. 1), required the use of a grid that blocked egress of the tubes from the rack while still permitting efficient drainage. We found that surface tension was a problem resulting in incomplete and variable drainage unless 0.1% Triton X-100 was included in the workup solution. We tested a number of different grids for retaining tubes and found that this grid could have no ridges near the tubes, but instead had to be closely apposed to the rack of tubes to prevent channeling of the liquid along the ridges. This was accomplished by fixing two rubber bands along the grid at 1/3 intervals. Too-vigorous handling of the tube rack during decantation could dislodge the pellets from the tube bottoms, which resulted in excessive variability. Lastly, replacement of the adsorbent wipes twice was necessary to eliminate all drops of liquid trapped on and in the tube tops. Best results were obtained when only a very small, reproducible meniscus was observed at the surface of each pellet.

We tested various concentrations of PEG ranging from 6 to 25% for the workup solutions; for the double-antibody precipitation method, the optimum concentration of PEG in the working solution was found to be 15% in order to form a firm pellet at the bottom of the tube without an undue increase in viscosity. The presence of firm pellets adhering to the bottom of the tube was associated with better assay reproducibility. Higher PEG concentrations provided good pellet characteris-

tics, but were more difficult to drain evenly; however, we found that the higher concentration of PEG, 25%, was required for the direct precipitation method in order to obtain reproducibly adherent pellets. The salt concentration in the precipitating solution was at physiological strength, although the RIA itself was performed under either isotonic (7B2) or hypotonic (ACTH) conditions. Salt concentrations should be optimized for each individual assay, as salt may affect certain peptide antigen–antibody reactions [13].

Comparison of the mini-RIA with conventional RIA

We performed ACTH assays using the conventional RIA and mini-RIA in triplicate in order to compare the properties of both formats. The conventional RIA was performed using 12 × 75-mm tubes, essentially as described elsewhere [5]; all other conditions, i.e., the workup method (double antibody or PEG immunoprecipitation), the incubation time, and the standard range were identical for both formats. The final antiserum dilution was adjusted to produce approximately 35% binding for each type of assay.

The standard curves obtained for both RIA formats are shown in Fig. 2A. Standard curves were comparable, although the standard curve is shifted to the left in the mini-RIA compared to the conventional assay, indicating greater sensitivity. Increased sensitivity is to be expected given the smaller volume of the assay (200 µl versus 300 µl) and could conceivably be increased further using a 100- to 150-µl total assay volume.

The mini-RIA was also used to measure ACTH-immunoreactive peptides in high-pressure gel permeation fractions obtained from fractionation of a mouse pituitary extract. The data presented in Fig. 2B show the presence of several differently sized peaks of ACTH-immunoreactive peptides; these most likely correspond to POMC/ACTH biosynthetic intermediate, intact ACTH, and an ACTH cleavage product, probably corticotropin-like intermediate lobe peptide (CLIP) (since the antiserum is directed against the C-terminus of ACTH).

Table 1
Interassay variation for the conventional and mini-RIAs for ACTH (double-antibody precipitation method)

Assay type	*n*	Mean ED50, fmol	SE	% error
Conventional RIA	5	31.3	3.8	12.1
Mini-RIA	6	11.1	0.9	8.1

The ED50s of the two assays, performed upon multiple occasions, were compared to assess the interassay variability of the conventional ACTH RIA and the mini-RIA (Table 1). The interassay variation was found to be smaller for the mini-RIA than for the conventional RIA. In agreement with the leftward shift shown in Fig. 2A, the ED50 of the assay was 11.1 fmol in the mini-RIA (compared to 31.3 fmol in the conventional RIA), supporting the idea of increased sensitivity. We conclude that the miniaturization and bulk decantation steps do not impair interassay variability.

In order to compare the intraassay variability of the mini-RIA and the conventional RIA we assayed a pituitary extract with both methods using 10 replicates. The results, expressed in pmol ACTH/pituitary, are shown in Table 2, and indicate that the intraassay variability of the mini-RIA is also comparable to that of conventional RIA. There was in good agreement between the two assays, indicating that the mini-RIA is as accurate as the conventional RIA.

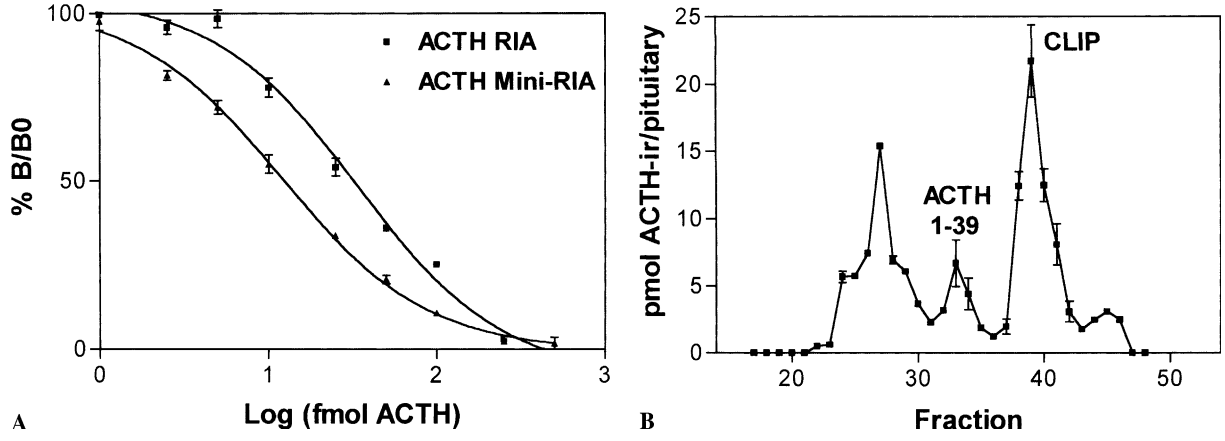

Fig. 2. Comparison of standard curves for conventional versus mini-RIAs. (A) ACTH RIAs (double-antibody immunoprecipitation) were performed using a 1–500 fmol standard range in both assay formats. (Boxes) Conventional RIA; (triangles) mini-RIA. Data are shown as the mean ± SD. (B) A mini-RIA of ACTH-immunoreactive peptides in chromatographic fractions of pituitary extract subjected to size-separation by high-pressure gel-permeation chromatography. About 1/3 of an 129/Sv mouse pituitary was injected onto the column; 5-µl fractions were assayed in duplicate, and the amount of immunoreactivity per tube was back-calculated to the entire pituitary. Values represent the mean ± SD. CLIP, corticotropin-like immunoreactive peptide.

Table 2
Intraassay variation for the conventional and mini-RIAs for ACTH

Sample	*n*	pmol ACTH/ pituitary	SE	% error
Conventional RIA				
5 µl	10	10.9	0.68	6.2
Mini-RIA				
5 µl	10	10.3	0.88	8.5

Table 3
Interassay variation for the conventional and mini-RIAs for 7B2 (direct PEG precipitation method)

Assay type	*n*	Mean ED50, fmol	SE	% error
Conventional RIA	3	19.2	2.2	11.4
Mini-RIA	3	11.8	1.7	14.4

Finally, to further emphasize the general applicability of the mini-RIA to a broad array of assays, we assessed its use in a second workup method: direct precipitation of bound ligand using 25% PEG and γ-globulin as a carrier. Conventional and mini-RIA 7B2 standard curves were performed in triplicate and the ED50s of each assay were compared to each other (Table 3). Again, the mini-RIA exhibits a better sensitivity than conventional RIA; this difference is, however, not as pronounced using the PEG workup method. Interassay variability is again comparable to that of conventional RIA.

We conclude that the mini-RIA method can be used both with the double-antibody workup method as well as with the direct precipitation method, thus increasing its general applicability.

Discussion

The present report describes a 96-tube format RIA method applicable to most if not all conventional [^{125}I] RIAs, including commercial RIAs not involving specially prepared beads or coated tubes. The adaptation of this broadly used technique to the 96-tube format serves multiple purposes. The major advantage of the mini-RIA is the considerable amount of time saved when handling several hundred tubes multiple times during the course of one experiment. In the mini-RIA, tubes are handled only when labeled, for addition of standards, and during placement into the gamma counter, but not prior to and following centrifugation; or during the separation step. Aliquots of chromatographic fractions are added to the assay in batches of 8 using multiple channel pipetting equipment. A total of 384 minitubes (96×4) can be processed at the same time in approximately an hour and a half.

A growing number of techniques in the field of biochemistry share the use of the 96-well format. From the multiple steps of purification of proteins and peptides (FPLC, HPLC fractions) to cell culture and the numerous immune and enzymatic assay applications (spectrophotometer, fluorometer) [11], the 96-well format has become a standard. However, thus far no 96-tube RIA has been described in this format. Evans and co-workers have previously published on a [^{125}I] 96-well RIA in which antibody is prebound to polystyrene wells via protein A prior to addition of radioactive ligand and sample; individual wells are broken off at the end of the assay for placement into the gamma counter [8,9]. This assay has the merits of being both convenient and extremely sensitive; however, in our experience, not all RIAs are adaptable to this technique, and the assay times are much longer than those reported here. Further, samples cannot be concentrated by drying into the wells, since the wells are coated in protein A. Commercial assays such as 96-well immunometric ELISAs exhibit a similar sensitivity (3 fmol for an ACTH ELISA (Immuno-Biological Laboratories, Cat. No. SG51041, www.ibl-hamburg.com) compared to 1 fmol in our method) to our mini-RIA and are very convenient, but are expensive and offer less specificity, since a specific radioactive ligand is not displaced. Other 96-well measurement methods, i.e., 2-site IRMA [12] or proximity scintillation assays [1,3,4] require either specially prepared purified proteins or expensive plates.

In order to adapt conventional [^{125}I] RIA to the 96-well format, we employ racks of 96 minitubes for both collection of fractions from chromatography as well as for the RIA itself. These racks are much smaller than equivalent numbers of 12×75-mm RIA tubes and are easily handled and stored. The draining and wicking devices described above were developed for these particular racks using materials obtained from other 96-well format devices, such as pipet tip racks. The draining device includes an independent 96-hole grid, which was chosen because it precisely matches the top of the tubes and has no ridges that could impair decantation efficiency. This grid is normally reused, but is inexpensive and easy to replace. However, a metal grid machined especially for these 96-minitube racks could perhaps offer better liquid flow. A wicking step was found to be necessary in order to remove all of the viscous PEG solution trapped at the tops of the tubes; our arrangement of absorptive materials was designed to provide rapid wicking with minimal contamination of the benchtop.

Although an outlay of funds for special equipment capable of handling 96-tube racks is initially required to accomplish certain steps—such as the microtiter plate centrifugation adaptors and a 96-tube format fraction collector, if chromatographic samples are to be assayed—the cost of each assay is similar to or lower than

that of conventional RIA in terms of antibody, standards, and radioisotope. The reduction in assay volume in the mini-RIA is potentially associated with a considerable reduction in antiserum consumption.

The mini-RIA has been used for two different RIAs employing two different workup methods; both the double-antibody immunoprecipitation and a direct precipitation method using carrier globulin and PEG yielded successful results. The use of the double-antibody immunoprecipitation method was required for the ACTH RIA due to high nonspecific binding for this particular ligand. Another assay which worked well with this particular workup method was β-endorphin (unpublished data). With regard to the PEG precipitation method, the mini-RIA was used for 7B2 and α-melanocyte-stimulating hormone assays with good results (unpublished data), indicating general adaptability. Steroid assays such as corticosterone would require the use of glass minitubes. These are commercially available in the 96-well format (Hirschmann plates, Scogil Scientific, www.scogilscientificinc.com) but are quite expensive (about $50 compared to $4 per rack of 96 tubes). We have not tested the adherence of PEG pellets to glass tubes but based upon conventional RIAs, we expect this parameter to be comparable or better than plastic.

We noted significantly lower ED50s for the mini-RIA, indicating increased sensitivity of this type of assay, most likely to the use of lower assay volumes. By further lowering the assay volume, the antibody concentration, and the amount of radioactive ligand employed, the sensitivity could most likely be further increased. Delayed addition of radioactive ligand is also a method to increase sensitivity, although our experience indicates that equilibrium assays offer greater reproducibility.

In addition to increased sensitivity, the reproducibility of the mini-RIA, assessed by measuring its intraassay and interassay variations, was comparable to that of the conventional RIA. The tubes are handled in bulk during the mixing of the precipitation solution and also during its removal; this adaptation most likely also contributes to good reproducibility. Good reproducibility depends in part on accurate pipetting of each reagent; in our experience, the antiserum and the labeled ligand can be combined before addition, leading to one less pipetting step (which may further enhance reproducibility). Finally, as noted earlier, the wicking device permits only a small invariant meniscus at the surface of the pellet in each tube to remain. Since assay reproducibility depends greatly upon consistent removal of the solution containing free radioactive ligand, this modification may potentially contribute to superior assay reproducibility over manual solution removal methods such as aspiration.

In conclusion, we here present a method for RIA miniaturization which we expect to be easily adaptable to most, if not all, [^{125}I] RIAs. The method was principally designed to save operator time, but also offers other advantages such as increased sensitivity, decreased space usage, and decreased antibody usage.

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