

Chapter 13

The Use of Low-volume High-capacity Scintillation Cocktails in the Automation of Steroid Radioimmunoassays

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INTRODUCTION

The use of iodinated labels for the radioimmunoassay of small molecular weight compounds has received increasing attention, especially in the routine analysis of steroid hormones¹. We have recently reported details of an automated approach for the routine radioimmunoassay of testosterone, oestradiol and progesterone using both iodinated and tritiated tracers². Despite the obvious advantages afforded by the use of γ -emitters, particularly ¹²⁵I, as tracers for routine radioimmunoassays, their use has remained limited to specialist laboratories and other users must rely on manufactured kit assays with their inherent problems. This is mainly due to difficulties in achieving satisfactory radioimmunological activity and yield of the iodinated compound. Furthermore, the short shelf life of iodinated compounds imposes a restriction on their use, and in this respect tritiated tracers which are readily available, may offer some advantage especially in laboratories where research is a major commitment. In this communication, we report on the use of low-volume high-capacity scintillation cocktails (RIALUMA-PBS and RIALUMA-PEG) which are suited for the adaptation of multi-dispensing units for the automation of steroid radioimmunoassays using 3-6 ml insert vials for the counting of tritium-labeled tracers.

MATERIALS

Reagents

Details of specific antibodies for a wide range of steroids, the sources of steroid standards, labeled steroids, as well as the preparation of extraction and chromatography solvents have been reported elsewhere^{2,3}. The dextran-coated charcoal solution was made up with 0.25% Norit A charcoal and 0.025% dextran in 0.1 M PBS buffer. The polyethyleneglycol solution is 30% polyethyleneglycol in distilled water. Scintillation cocktails were supplied by Lumac Systems AG, Aeschengraben 6 CH-4051, Basel, Switzerland.

Equipment

The instrumentation used for the automation of radioimmunoassays was supplied by Ollituote Oy, SF-02320 Espoo 32, Finland. Plastic insert vials were obtained from Sterilin Ltd., Teddington, Middlesex, U.K. (6 ml) and Lumac Systems AG (MILLI-3 and MILLI-6). An LKB/WALLAC 81000 Liquid Scintillation counter was used for the counting of radioactivity, and a Nova 840 computer (System Olli 4000) was used to calculate the results using a modified system based on that of Rodbard and Lewald⁴.

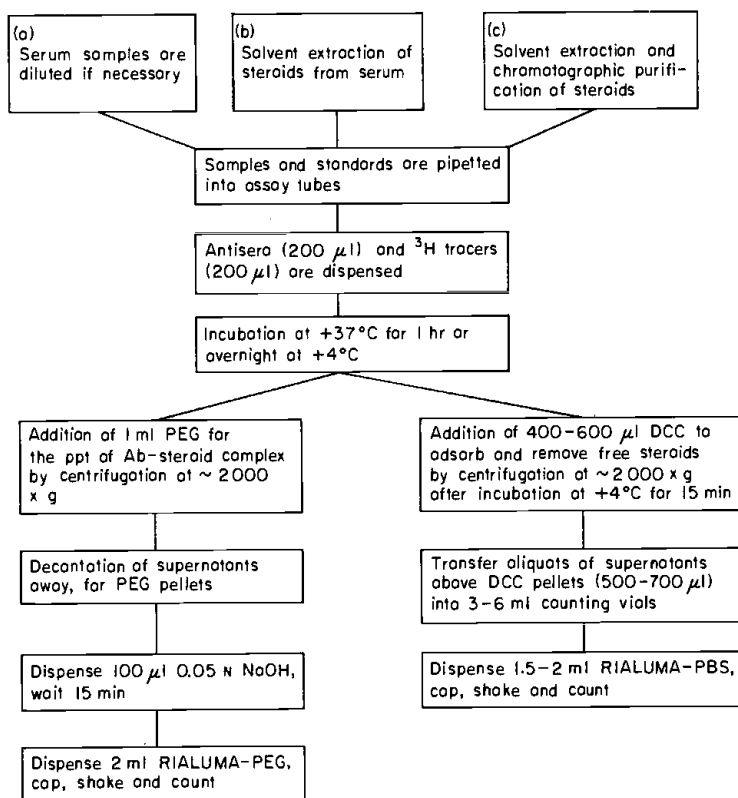


Fig. 1. Flow diagram of steroid RIA methods.

METHODS

Sample preparation

Depending on the steroids and samples under investigation three basic approaches may be employed as illustrated in Fig. 1. (a) Direct measurements of steroids such as dehydroepiandrosterone⁷ in untreated or diluted serum samples may be prepared using the System 011 dispenser 216 which is able to pipette volumes between 50 and 2000 μl with a precision (C.V.) of <1.0%. (b) Simple solvent extraction may be employed for the routine estimation of steroids such as testosterone, oestradiol, progesterone, and androstenedione in human serum. (c) For research purposes, when the concentration of steroids in various body fluids and tissues is known to be extremely variable, it is essential to purify solvent extracts chromatographically prior to radioimmunoassay⁵.

Automation (Fig. 1)

After solvent extraction and chromatography, if necessary, the steroids are taken up in ethanol or buffer and pipetted into assay tubes. Standards are prepared in parallel. Antibodies (200 μl) and ^3H -tracers (200 μl) are pipetted in units of 24 by the dispensing unit. Incubations for 1 h at 37 $^{\circ}\text{C}$, or 16 h at 4 $^{\circ}\text{C}$, are conducted, and the separation of bound and free radioactivities is accomplished by the simultaneous addition of dextran-coated charcoal (DCC) (400-600 μl) or polyethylene glycol (PEG) (1 ml) using the 24-channel dispenser.

In the case of DCC (separation by adsorption) an incubation period of 15 min at +4 $^{\circ}\text{C}$ is followed by centrifugation at $\sim 2000 \times g$ for 3 min in a refrigerated MSE Mistral

centrifuge equipped with a rotor to accommodate up to 6 blocks of 24 assay tubes. Portions of the supernatants are aliquoted, in units of 24, into 3 ml or 6 ml scintillation insert vials by the dispensing unit. RIALUMA-PBS is added by hand dispenser, the tubes are capped, shaken and may be counted immediately.

When PEG is used to separate the antibody-steroid complex by precipitation, the addition of PEG may be performed at room temperature using the dispenser, and mixing is followed by centrifugation at $\sim 2000 \times g$ for 30 min; after which, supernatants may be decanted, 100 μ l of 0.05 N NaOH added by the dispensing unit, and 2 ml of RIALUMA-PEG added to the assay tubes (MILLI-3 vials) which are then capped, shaken and counted. In this way both the radioimmunoassay incubation and scintillation counting may be performed in the same tube.

Results may be recorded on punch tapes and entered by fast paper tape reader into the System Olli 4000 Nova 840 computer, after which standard curve parameters and unknown steroid concentrations are computed.

RESULTS AND DISCUSSION

Automation and tritium tracers

A detailed assessment of System Olli₂3000 for the radioimmunoassay of some steroid hormones has recently been published⁴. The same principle has also been applied for the radioimmunoassay of a wider range of steroids using tritiated tracers, after the microcolumn purification of samples⁵. In practical terms, the use of the dispensing unit has not only reduced the time required for assay preparation and reduced possible errors in pipetting, but has simplified and increased the speed of separation techniques. In the development of the system for use with tritiated tracers, it has been necessary for us to reduce the total sample volume required for counting so that the final separation stages may be automated using the dispensing unit. We have originally used polypropylene 6 ml insert vials for this purpose. However, this requires the use of specially constructed blocks to accommodate the counting vials for use with the dispenser. In order further to simplify the procedure, it has been our purpose to reduce further the total counting volume so that aliquots of supernatants (500 μ l), or PEG precipitates in 100 μ l of 0.05 N NaOH, could be counted in a total volume of approximately 2 ml in 3 ml counting vials which may be accommodated in the conventional Olli incubation blocks. Furthermore, in the case of assays employing PEG as the separation agent these same tubes may be used for both the radioimmunoassay incubation and subsequent counting of radioactivity.

Assessment of insert vials and carrier vials

An experiment was conducted to ascertain the optimum counting volume for both 3 ml and 6 ml counting vials, in which approximately 10 000 cpm of ³H-testosterone were dried in counting vials and to which increasing volumes of scintillator were added. The results are seen in Fig. 2, from which it is apparent that optimum counting volumes are achieved at 2.0 - 2.25 ml and 2.25 - 3.0 ml for the 3 ml and 6 ml counting vials respectively. Furthermore, a comparison between 6 ml polypropylene insert vials and polyethylene insert vials (Fig. 2(a)) revealed that higher count rates are achieved with the latter plastic. This is also seen (Fig. 2) when the same samples were measured in different types of carrier vials; glass carrier vials being superior to the plastics, of which polyethylene was the more efficient. It also seems when

Table 1. Basic characteristics of RIALUMA-PBS

Sample load (0.1 M PBS) max	Flash point/ °C	Counting temp./ °C	Equilibration time	Stability	Penetration of polyethylene
28% as single clear phase at room temp.	47	15 - 30	None	Stable as a single phase for up to 48 h at 25 °C	Very low over 48 h at 25 °C

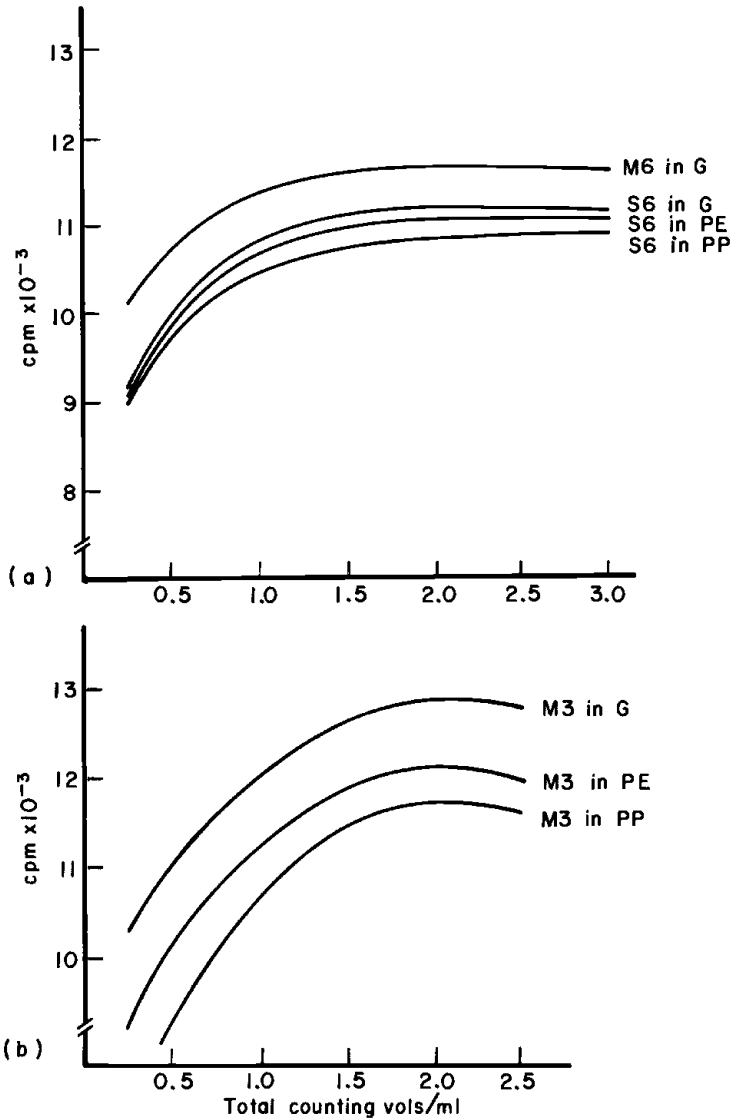


Fig. 2. Optimum counting volumes, insert and carrier vials. M3 = polyethylene MILLI-3 vials; M6 = polyethylene MILLI-6 vials; S6 = polypropylene Steralin 6 ml vials; carrier vials; G = glass; PE = polyethylene and PP = polypropylene.

the results in Figs 2 (a) and (b) are compared that the highest count rates are achieved with the 3 ml insert vials in glass carrier vials. To date glass carrier vials have been used continuously for periods of up to 3 months without appreciable increases in background (<20 cpm), and have been successfully washed and re-used. It is considered that the continuous use of glass carrier vials which remain inside the counter will help reduce the accumulation of dust particles inside the counting chamber and help maintain the condition of the counter.

Assessment of scintillators

A comparison of RIALJMA-PBS with various other commercial scintillation cocktails has been made previously⁷, and the basic characteristics of this scintillator may be

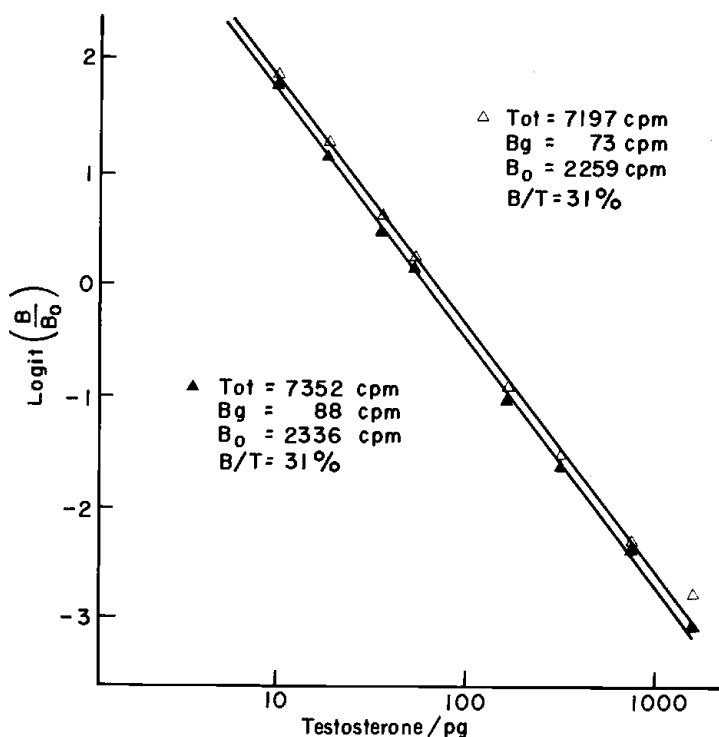


Fig. 3. Evaluation of the use of different scintillator volumes and insert vials on testosterone standard curves. \blacktriangle = 500 μ l RIA supernatants counted with 1.5 ml RIALUMA-PBS in MILLI-3 vials. \triangle = 500 μ l RIA supernatants counted with 2.0 ml RIALUMA-PBS in MILLI-6 vials. Tot = total counts, B_g = background, B_0 = binding at zero testosterone concentration.

summarized in Table 1. RIALUMA-PEG, used for the counting of PEG pellets in 100 μ l 0.05 N NaOH, is similar to RIALUMA-PBS although the maximum sample load tolerated is lower, but in practice this does not have to exceed 5%.

Trials have been conducted using both 3 ml and 6 ml polyethylene insert vials (MILLI-3 and MILLI-6) for the radioimmunoassay of steroids, using DCC (400 μ l) as the separation agent, and removing and counting 500 μ l of the assay supernatants with 1.5 ml and 2.0 ml of RIALUMA-PBS in 3 and 6 ml insert vials respectively. In Fig. 3, it can be seen that the resultant standard curves for testosterone in both cases are practically identical, and as seen by the measurement of total and background counts there is very little difference in the general efficiency of these two counting systems, despite the differences in sample load.

SUMMARY

These results indicate that up to 500 μ l of radioimmunoassay supernatants, after DCC absorption and separation of the free ligands, may be counted satisfactorily in 3 ml insert vials with only 1.5 ml of RIALUMA-PBS. The reduction in scintillator volume and insert vial size not only makes the technique more suitable for automation, but reduces costs and waste disposal problems. Furthermore, the use of the same 3 ml polyethylene vials for both the radioimmunoassay incubation and counting steps, when PEG is used as the separation agent, may be exploited to further simplify the technique and once more reduce costs and waste disposal problems.

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