caution, we routinely use vials of glucose standards stored at 4 °C in small aliquots (e.g., 1 mL). In two years we have never identified contamination of glucose by the SMI pipette as a source of error.

We estimate that substitution of the SMI pipette and the modified reagent decreases direct costs by $84.07 per 1000 tests, exclusive of labor. This calculation compares purchase of Beckman reagent kit no. 671664, which has reagents and tips for 1000 tests ($126.25) with the purchase in bulk of sufficient glucose oxidase from Sigma coupled with the appropriate number of SMI tips ($42.18). Recycling the reagent would provide additional savings. However, because Case and Phillips (3) reported the feasibility of recycling Beckman reagent, we have not added this adjustment to our comparative calculations. The reagent may be prepared in less than an hour. In our laboratory, the reagent quality-control effort required is the same whether the material is prepared on site or purchased. We concur with Fischl et al. that we can make substantial savings by preparing rather than purchasing reagent. We suggest that other laboratories may, depending on their testing volume and purchasing power, derive some financial benefit from using the reagent we describe and from substituting SMI pipettes for Beckman pipettes.

Analytical accuracy, precision, specificity, and instrument performance have not been affected by the substitutions.

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References

Discrete Modular System for Automation of Radioimmunoassay of Serum Choriomammotropin

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We describe a discrete automated radioimmunoassay system for determining choriomammotropin (placental lactogen) in human serum. With the present system it can be measured in as many as 37 unknown sera (50 μL) and three quality-control sera, in duplicate, within 1.5 h. The time required for sample preparation, incubation (15 min), and separation of free and bound radioactivities (a 150 mL/L polyethylene glycol solution is superior to a twofold volume of absolute ethanol) is less than 45 min. The remaining time required is for counting and data processing. Intra-assay precision is 4.6% (CV). The modular approach endows the instrumentation with much flexibility, and consequently is suitable for automation of a wide range of assay protocols.

Additional Keyphrases: System Olli 3000 • monitoring high-risk pregnancies • values during pregnancy

The increasing variety of radioimmunoassays available for routine clinical use has necessitated the introduction of automated techniques designed to increase assay capacity, speed, and precision. Many of the systems currently available are rather inflexible and slow. We have therefore adapted components of an existing clinical chemical analyzer (System Olli 3000) to automate our radioimmunoassays. The use of this instrument for the automation of radioimmunoassays for steroid sex hormones has been described (1), and we describe here how it may be used to automate the determination of human choriomammotropin (placental lactogen, HPL), as an example of how the instrument can be applied for the analysis of serum proteins.

Materials and Methods


Reagents. The assay diluent was a 40 mmol/L phosphate-buffered (pH 7.4) saline containing 20 mmol of ethylenediaminetetraacetate and 1 g of bovine serum albumin per liter. The antisera used was raised in rabbits (Nordiclab Oy, SF-90120 Oulu 12, Finland) against purified HPL (ICN Pharmaceuticals Inc., Cleveland, OH). The titre used was 1/1000 (B0 = 80% of total 125I-labeled HPL). Purified HPL (5 μg) was radioiodinated with 1.5 mCi of Na125I (Radiochemical Centre, Amersham, U.K.) by the Chloramine T

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method (2) and purified by single Sephadex G-100 column chromatography (bed volume 5 mL). A 150 mL/L polyethylene glycol solution made up in saline (9 g/L) served as the precipitating reagent for separating antibody bound from free 

\[ ^{125}\text{I}-\text{HPL} \]

Standards (0–16 mg/L) of the purified HPL used as antigen were prepared in normal human serum from males and checked against the HPL 70/144 reference standard (National Institute for Biological Standards and Control, Holly Hill, London NW3, U.K.).

**Assay protocol.** Standards and samples were arranged in blocks of 24 on pre-labeled code plates. Duplicate aliquots (50 μL) were dispensed into duplicate blocks of 24 assay tubes arranged on pre-labeled code plates, with use of the appropriate size of pipette tips. These pipette tips are available in prepasted units of 24, which are compatible with the 24-unit block matrix, and may be rapidly (5 s) changed between different pipetting steps when required.

Aliquots of 

\[ ^{125}\text{I}-\text{HPL} (80,000 \text{cpm}/200 \mu\text{L}) \]

were dispensed into assay tubes in units of 24, then 200-μL aliquots of antisera were added in the same way. The contents of the tubes were then mixed (10 s) in units of 24.

After a 15-min incubation at room temperature, 500 μL of the polyethylene glycol solution (150 mL/L) was dispensed into the duplicate blocks of assay tubes and mixed for 10 s. The tubes attached to the code plates were then transferred to the centrifuge and immediately centrifuged at 2000 × g for 10 min.

After centrifugation, the supernates were decanted in units of 24 by inverting the code plates, left to drain for 5 min on soft paper towels, then removed from the code plates and placed in duplicate sequence in the LKB/Wallac Rack Gamma and the radioactivity was counted until 10,000 counts had accumulated (approximate mean counting time, 20 s/tube). Results were computed by the counter, and duplicates which differed by more than 5% from the mean were identified automatically, and low, medium, and high control sera were used to monitor assay quality.

**Results**

**Evaluation of the HPL radioimmunoassay method.** The influence of various incubation times was assessed at room temperature (23 °C); there was little difference between standard dose–response curves generated after 15, 30, or 45 min. Therefore we used a 15-min incubation.

Absolute ethanol (3) and a 150 mL/L polyethylene glycol solution were both tested as potential selective precipitants of HPL-antisera from the incubation mixture. We consistently found that use of the latter gave a steeper dose–response curve than did ethanol (Figure 1) and also resulted in less nonspecific background counts in the precipitate.

Precision was evaluated in terms of the precision of the instrumentation, as well as the overall assay characteristics. To test the precision of the instrumentation, we compared the counts obtained for 16 replicate low (0.5 mg/L), medium (4.0 mg/L), and high (9.0 mg/L) control sera obtained in one assay series; the respective coefficients of variation were 2.6, 3.4, and 4.6%. The overall intra-assay precision for the same control (n = 16), estimated after the counts were converted to mg/L after logit/log transformation of the standard curve, was 3.9, 5.2, and 6.5% for the low, medium, and high controls, respectively.

**Reference values of HPL.** Serum samples were collected weekly from eight women between 27 weeks of pregnancy and delivery: all were singleton pregnancies and had proceeded quite normally. Figure 2 shows the values, which display an even distribution between 3.2 and 9.8 mg/L, with an increase towards the end of gestation. The best-fit mean concentration and two standard deviation limits, also shown, are similar to those generally reported in the literature (4, 5).

**Discussion**

We describe the adaptation of dispensing, mixing, and centrifugation components of a clinical chemical analyzer (System Olli 3000) for direct radioimmunoassay of HPL in 50-μL serum samples. The system is based on a discrete modular concept; therefore carryover is non-existent and incubation conditions (time and temperature) may be selected according to the requirements of a particular assay protocol. In the case of protein hormones, this may vary from 15 min for HPL to several hours in the case of many others, particularly if it is desirable to maximize sensitivity and (or) achieve
complete equilibration between assay components. On the other hand, the addition of reagents in units of 24 is so rapid that it may well be feasible to conduct many assays at non-equilibrium, especially when maximum sensitivity is not an essential requirement. Moreover, the actual time required for sample/assay preparation when using the System Olli 3000 dispenser 216 is trivial as compared to the incubation times, and more than one analyst may utilize the instrument at any one time. With the present system, 96 assay tubes may be analyzed from samples to results within 1.5 h; counting requires about half of this time. However, the introduction of gamma counting instruments with multiple counting wells will inevitably reduce the total time of such an assay to less than 1 h.

Unlike the previously described system for the analysis for steroid hormones, the protocol used for determining protein hormones such as HPL requires no pre-purification of samples, such as extraction, and the serum samples may be assayed directly. Although this means that the instrumentation is used for every procedural maneuver, it also means that the instrument must be able to dispense small volumes of serum accurately. At present the smallest volume which could be reliably dispensed is 50 µL.

We conclude that use of 150 mL/L polyethylene glycol solution results in a superior standard curve as compared to the use of absolute ethanol as the precipitating agent, and also less 131I-labeled HPL was precipitated. The most important advantage of the increased slope of the standard curve for HPL is that no dilution is required when high serum concentrations of HPL are to be measured.

The precision achieved with the present system in terms of the instrument error (CV, 2.6–4.6%) is comparable with that achieved by a highly skilled technician. However, it must be appreciated that manipulative errors, which inevitably follow the tedious of pipetting large numbers of samples and reagents, are eliminated with the present system.

It has been suggested that serum HPL assay not only should assume an important place in the screening of high-risk pregnancies (6), but should also be included as part of a routine screening program during the third trimester (7). In the case of high-risk pregnancies, it is essential that results be obtained with the least delay. The automated system described fulfills the requirements set by these needs. In addition to capacity and speed, it is apparent that the system described is highly precise and is both simple to operate and flexible in nature.

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References