Radioimmunoassay of Prolactin in Blood Spotted on Filter Paper

Fiona Bassett, Barbara A. Gross, and Crewe J. Eastman

In this method for estimating prolactin, 50 μL of whole blood obtained by finger puncture is spotted onto filter paper and blood-spot samples are "punched out" with a 3-mm (diameter) paper punch. The blood is extracted with aqueous buffers and the prolactin measured in large batches by radioimmunoassay. Results were identical with those for prolactin in serum. Prolactin in blood spots is stable at room temperature for up to one week and for several months at −20 °C. This simple technique for obtaining blood samples for prolactin estimation has particular potential for field studies of lactating women.

Additional Keyphrases: lactation • fertility • pregnancy • contraception • screening

Measurement of prolactin concentrations in serum of lactating amenorrheic women provides a sensitive predictive index of returning menstruation and fertility (1–3). Although the duration of breast feeding differs widely from one society to another, in many developing countries it is prolonged for one to two years, with a concomitant prolongation of postpartum amenorrhea. Because about 10% of mothers may become pregnant while still breast feeding but before menstruation begins again, timing of the introduction of contraception, other than reliance solely on lactation as the guide, is important. Thus there is a great need for a valid, simple method to predict the return to fertility in breast-feeding mothers who wish to delay or prevent a pregnancy.

Estimates of prolactin in serum may help determine the ovulatory status of an individual breast-feeding mother. Such concentrations are usually measured by RIA of plasma or serum separated from whole blood obtained by venipuncture. Collection of serial venous blood samples from lactating mothers is difficult, time consuming, and expensive, and in many countries and cultures women are reluctant to provide these samples. To overcome these problems, we have developed a simple, inexpensive method for measuring prolactin in serum by adapting a commercial RIA kit (Prolactin RIA Diagnostic kit; Abbott Laboratories, North Chicago, IL 60064) for use with filter papers spotted with whole blood.

Materials and Methods

Materials

Phosphate-buffered isotonic saline (0.1 mol/L, pH 7.4). Dissolve 81 mmol of Na₂HPO₄·12 H₂O, 19 mmol of NaH₂PO₄·2 H₂O, 9 g of NaCl, 20 g of bovine serum albumin, and 1 g of sodium azide per liter of distilled water.

Radioimmunoassay and antibodies (Abbott Laboratories). Dilute both ¹²⁵I-labeled prolactin (human) reagent solution (maximum radioactivity 0.1 mCi/L (4 mBq/L)) and rabbit-anti-human prolactin antiserum fivefold with phosphate-buffered saline for the assay. Dilute goat anti-rabbit IgG in phosphate-buffered saline, supplied in the Abbott kit, 80-fold in a 150 g/L solution of polyethylene glycol 6000 (BDH Chemicals) in isotonic saline.

Human prolactin standards and controls. We dissolved one ampoule of the World Health Organization's International Reference Preparation (IRP) of Prolactin, Human 75/504 (20 μg of prolactin) in 500 μL of phosphate-buffered saline (4), and stored 10-μL aliquots at −40 °C. Whole blood with known low concentrations of prolactin, collected from women who either had hypopituitarism or were being treated with bromocriptine to suppress prolactin secretion, was used to prepare zero standards as follows. Pipet 50 μL of the whole blood from an individual woman onto filter papers and store them as described below in Procedures. Prepare prolactin standards of 160, 80, 40, 20, 10, and 5 μg/L by serially diluting the IRP aliquots with the low-prolactin whole blood, then spotting these onto filter paper as for the zero standards. For controls, prepare samples covering a range of prolactin concentrations (15–240 μg/L). These are also used to assess inter- and intra-assay variability (as we did over a six-month period).

Procedures

Sample collection. Sample blood by finger puncture (we used an "Autolet"; Owen Mumford Ltd., Woodstock, Oxford, U.K.) and spot it onto filter paper (No. 903; Schleicher and Schuell, Dassel, F.R.G.) to fill 15-mm (diameter) preprinted circles (5). Allow the spots to dry at room temperature and store the samples in air-tight plastic bags at −20 °C. Obtain samples for assay by punching 3-mm (diameter) discs from the center of the circles.

Radioimmunoassay. We measured the total count, standards, and nonspecific binding in triplicate, and the unknown samples and the controls in duplicate. The RIA procedure was as follows. Add blood spots (two 3-mm discs per tube) to each of the series of 75 × 10 mm tubes with the exception of the total-count tubes. Add 100 μL of the diluted first antibody (rabbit) to the standards, controls, and unknown samples, then add 100 μL of phosphate-buffered saline. To all tubes, including those for total count and nonspecific binding, add 100 μL of the diluted ¹²⁵I-labeled prolactin and mix well. Take care that all the filter paper pieces are at the bottom of the tubes. Cover the tubes with Parafilm and incubate for 18 to 24 h at room temperature...
(20 to 27 °C), shaking the tubes continuously for the first 2 h to ensure that the blood is completely eluted from the filter paper.

Next, pipet 1 mL of the second antibody (goat) solution into all tubes except the total-count tube, vortex-mix, and allow to stand at room temperature for 15 min. Centrifuge the samples at 4100 × g for 30 min and aspirate the supernate to within 0.5 cm of the filter-paper pellet. Count the radioactivity of the pellet in a gamma counter and plot the results as the usual dose–response curve: prolactin concentrations in serum vs % (bound/total counts). We used a standard curve prepared from results for eight replicates at each point, including results for nonspecific binding.

Analytical recovery. To assess recovery of prolactin, we added three concentrations of prolactin to whole blood, spotted aliquots onto the filter paper, dried the samples, and assayed them by the above protocol.

Stability study. We exposed a series of blood spots with known concentrations of prolactin to various environmental conditions for up to three months, to assess the stability of prolactin for the blood spot assay. We studied the effects of storage at −20, 4 °C, room temperature (20 to 27 °C), and 37 °C, and also at 37 °C and room temperature in the presence of high (saturated) humidity.

Method-comparison study. From 19 women (six pregnant, eight breast-feeding, and five nonlactating and nonpregnant) we collected blood by venipuncture for estimation of prolactin in serum, and by finger prick for collection onto filter paper.

Prolactin in the serum samples was measured with the Prolactin RIA Diagnostic kit, and the results were compared with those from the blood-spot assay of the finger-puncture samples. We also compared the latter results with prolactin concentrations in blood taken by venipuncture and spotted onto filter paper.

Results

Precision. Figure 1 illustrates a typical standard curve. Sensitivity, defined as twice the standard deviation of the zero point of the standard curve, was 2 μg/L. The inter assay variability (CV) was 15.9, 12.0, 10.6, and 10.4% for prolactin concentrations of 15, 30, 60, and 120 μg/L; the respective intra-assay CVs for these concentrations were 6.4, 8.7, 7.0, and 7.2%.

Analytical recovery. Mean recoveries of added prolactin (20, 60, and 240 μg/L) from whole blood were 99, 99, and 91%, respectively.

Comparison of methods. There was a highly significant linear correlation (r = 0.984) between prolactin concentrations measured in 19 sera and the corresponding finger-puncture whole-blood spots (corrected for hematocrit). The regression equation was $y = 3.399 + 0.635x$ (SE = 2.977). Prolactin values ranged from 8 to 66 μg/L for the spots on filter paper. There was also a highly significant linear correlation (r = 0.996) between prolactin estimates from 17 blood spots taken by finger puncture and by venipuncture. The corresponding regression equation was $y = 1.474 + 0.962x$ (SE = 1.955).

Environmental conditions. Figure 2 shows the effects of different storage temperatures and humidity on observed values for blood-spot prolactin concentration. Prolactin was most stable when samples were stored at −20 °C. However, samples were stable for up to one week without significant loss at room temperature (20 to 27 °C), and for several weeks in the refrigerator (4 °C). At high temperatures (37 °C) or high humidity prolactin was unstable. High humidity promoted mold growth and invalidated the results.

Discussion

Samples on filter paper have been used for many blood components (6, 7) but this is the first report, to our knowledge, of its application to prolactin estimation. This simple, accurate method for this assay shows excellent linear correlation between prolactin concentrations in sera and blood spots (corrected for hematocrit values), confirming its validity. There was no dilution of capillary finger-puncture samples by interstitial fluid, because the concentrations of prolactin in whole-blood samples obtained by either finger-puncture or venipuncture spotted onto filter paper were highly linearly correlated. Moreover, the sensitivity of the RIA for prolactin in filter-paper spots was comparable with that of the RIA for prolactin in serum.1

This blood-spot technique is suitable for large-scale longitudinal studies of breast-feeding women. It is more acceptable to women who object to venipuncture. It can be self administered at low cost or performed by semiskilled field workers after a little training (5), with little discomfort or risk to the subject. The technique minimizes the need to provide facilities for separating and storing blood samples in the field. Samples can be mailed to a central laboratory with no loss of immunoassay potency from adverse environmental conditions. Relative humidity at the time of collection has been associated with errors in phenylalanine elution (8) and may be of importance in field studies. Rapid drying of the samples with a hair dryer or fan minimizes such errors.

Abbott Diagnostics Division kindly provided the Prolactin RIA Diagnostic kits free of charge. We are grateful for the assistance of the Nursing Mothers Association of Australia in recruiting subjects, and thank those mothers who participated.

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1 Recently we have used a modified assay procedure of a new solid-phase RIA (Abbott Prolactin Riabea). This method has given identical sensitivity and precision.
Fig. 2. Stability of prolactin in dried blood spotted onto filter paper
The results are expressed as percentage of prolactin concentration on day one over various time periods under different environmental conditions

References