patterns of BRCA1 mutations. In fact, the SSCP patterns did not differ from the pattern obtained with 1× TE, suggesting that strong denaturants are not necessary for sample denaturation. A similar result using a low ionic strength buffer for denaturation before SSCP analysis has also been reported (7). It should be noted that at high concentrations of methylmercuric hydroxide and DMSO over-denaturation of the DNA occurred and caused smeared bands.

The final variable that we investigated was snap-chilling after denaturation. This step is found in 99% of all SSCP protocols published, suggesting that it is essential for successful SSCP analysis. We tested this hypothesis, using six known BRCA1 mutations. For each mutation, the product was either heated for 3 min at 100 °C and snap-chilled on ice or heated and loaded directly onto a polyacrylamide gel. Contrary to popular methodology, we found that snap-chilling the denatured sample on ice was unnecessary. No effect on SSCP patterns was seen in the six BRCA1 mutations tested (Fig. 1B). Similar results were also found when we examined p53 and cystic fibrosis mutations. These results suggest that the refolding of single-stranded DNA occurs rapidly and that it is more stable than previously thought. In addition, we tested the effect of leaving the samples at room temperature for 2.5 h after denaturation in 24% formamide with no snap-chilling. No change was seen in the SSCP band patterns, providing further support that the refolding of single-stranded DNA occurs rapidly and is quite stable.

On the basis of our findings in this study, we analyzed 11 DNA samples containing known BRCA1 mutations. We were able to detect mobility shifts in all 11 samples (Fig. 1C). In seven of the samples, we could also detect heteroduplexes. Because some double-stranded DNA molecules usually remain in samples prepared for SSCP analysis, they can be used as a confirming feature when both forms of DNA are present in the same sample.

Our data demonstrate that the concentration of primers in the PCR reaction has the largest effect on the formation of specific SSCP patterns and that the effect is sequence-dependent. All of the other factors tested had minimal effects on SSCP pattern reproducibility. Thus, our results suggest that variations in primer concentrations in PCR reactions are the most likely cause of nonreproducible SSCP patterns.

**References**


**Measurements of Total and Desialylated Sex Hormone-binding Globulin in Serum by ELISA, Jenny Vaysse,**

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The sex steroid-binding protein, also called sex hormone-binding globulin (SHBG), is an androgen- and estradiol-carrier protein in human serum. The two identical subunits of this glycoprotein both bear three oligosaccharide chains, two N-linked at Asn^51^ and Asn^56^ and one O-linked at Thr^7_. The role of this carbohydrate moiety in the biological function of SHBG is not known; however, the complete enzymatic deglycosylation of human SHBG has very little effect on the steroid-binding activity (1). Because estrogens regulate SHBG synthesis, this protein is usually measured to evaluate estrogen status, especially in the biochemical assessment of hirsutism. Other indications for SHBG measurement are promising because high SHBG concentrations are associated with a decreased risk of breast cancer in postmenopausal women (2) and with an increased incidence of hepatocellular carcinoma in patients with cirrhosis (3). Furthermore, in the latter situation, it has been suggested that increased androgen transport through the hepatocyte membrane, via the asialoglycoprotein receptor, might be involved in hepatic carcinogenesis (3). Determination of desialylated SHBG (dSHBG) is needed to investigate this hypothesis and could be useful in tracking this disease.

Total SHBG is usually measured with a radiometric method using tritiated dihydrotestosterone or with immunoradiometric assay (4, 5). To our knowledge, procedures for dSHBG determination have never been described. The present study was thus designed to propose two easy ELISA methods for the parallel measurement of SHBG and dSHBG.

Total SHBG was determined with a classical ELISA sandwich assay. Calibration was performed with purified human SHBG obtained from Calbiochem or Eurodiagnostica. Controls were run with sera from Eurodiagnostica and with dSHBG prepared by treatment of SHBG obtained from Calbiochem with agarose-linked Clostridium perfringens neuraminidase (EC 3.2.1.18) from Sigma (6). The sera, collected from in-patients included in a screening program (3) and from healthy donors, were stored at −25 °C. Informed consent was obtained, and blood sampling was performed in accordance with ethical standards. The microtiter plates (Nunc) were coated overnight at 8 °C with 100 μL of a 1.500 dilution of a 3.4 g/L purified
immunoglobulin fraction of rabbit anti-SHBG serum (Dako) in 17 mmol/L phosphate buffered saline, pH 7.4 (PBS). The contents of the wells were removed and saturation of each well was achieved with 400 μL of 10 g/L bovine serum albumin (BSA) in PBS (BSA/PBS) incubated for 1.5 h. After the wells were washed (for each wash session, the wells were washed six times with PBS containing 0.5 mL/L Tween 20), 100 μL of each specimen diluted in BSA/PBS was added. Calibrators in concentrations ranging from 0 to 60 μg/L were used. For serum samples, two dilutions were tested: 1:546 and 1:1066. The plates were incubated for 1 h, and then washed. After the addition of 100 μL of 1.3 g/L peroxidase-conjugated anti-SHBG immunoglobulins (Dako) diluted 1:2000 in BSA/PBS, the plates were incubated for 1 h. After the wells were washed, 100 μL of substrate (0.1 g/L o-phenylenediamine in sodium citrate, pH 4.5, with 30 mg/L H202) was added, and the plates were incubated for 30 min. The reaction was stopped with 100 μL of 0.5 mol/L sulfuric acid, and the absorbances were measured at 492 nm with a nonspecific estimate at 620 nm on a LP400 microplate reader (Sanofi Pasteur). Except for the coating step, all incubations were done at room temperature under gentle continuous shaking. Each determination was performed in duplicate. The absorbances of blanks without coating antibody run simultaneously were subtracted. The data were analyzed after fitting with the Levenberg-Marquardt algorithm and processed with the KaleidaGraph (Ver. 3.0) package from Synergy Software.

Blank absorbances were <0.02. Within-batch imprecision was established on 70 samples analyzed in duplicate. The CV in the concentration range 0–75 μg/L was 4.9%. The between-batch CV, determined with controls over 10 assays, was 8%. The range of values found for the healthy donors was similar to that reported by other authors (4, 7). A comparison of the concentrations obtained with the described ELISA-SHBG and with RIA-SHBG (Eurodiagnostica) showed a linear regression: y = 1.17x + 0.165, where y is the ELISA value and x is the RIA value; r = 0.994, n = 30, S_y|x=0.81 μg/L. The sialylation rate of SHBG did not affect the immunological reactivity of this protein because no substantial differences could be seen in the calibration curves obtained either with native SHBG from both origins or with dSHBG (Fig. 1A.).

The determination of dSHBG, or more accurately of sialic acid-deficient SHBG, was initiated according to Tertov et al. who recently described a procedure for desialylated LDL determination (8). Because removal of sialic acid leads to the exposure of galactosyl residues, these authors first captured asialo-apolipoprotein B with Ricinus communis (RCA120; Sigma), an immobilized lectin that has affinity for galactose, then measured the protein with a peroxidase-conjugated apolipoprotein B antibody. In our first attempt, we applied this procedure to the determination of dSHBG. Experimental conditions were similar to those described for SHBG, except that (a) the plates were coated with a 18 mg/L RCA120 solution instead of an anti-SHBG immune serum, (b) 17 mmol/L phosphate buffer without saline (PB), pH 7.4, was used, and (c) in vitro neuraminidase-treated SHBG was used as a standard. This low ionic strength PB was suitable because of the relatively low lectin-binding affinity. When applied to dSHBG calibrators, this method gave absorbances that correlated well with dSHBG concentrations. However, when the method was used with sera, the absorbances were too low to give reliable results, probably because dSHBG is only a minor component among the bulk of serum compounds able to bind to RCA120. Although this procedure is not suitable for dSHBG determination in serum, it can help evaluate the efficiency of the neuraminidase treatment on SHBG standard solutions; complete desialylation is obtained when the difference of absorbances between SHBG and dSHBG reaches a maximum, i.e., after 30–60 min incubation at 30 °C. Thus, a 1-h treatment with neuraminidase was carried out to prepare the dSHBG standard.

Because the Tertov et al. procedure proved to be unsuitable for the dSHBG measurement in serum, a reverse sandwich assay was performed that involved coating the wells with 100 μL of 1:500 anti-SHBG in PB

Fig. 1. Representative calibration curves for SHBG (A) and dSHBG (B).

(A) SHBG prepared from Calbiochem (●); Eurogenetics (○). The desialylation of SHBG (☐) did not modify the signals. (B) dSHBG prepared from Calbiochem (◼).
and additional detection of the bound dSHBG with 100 μL of 0.5 mg/L peroxidase-linked RCA120 in BSA/PB. Because this lectin can bind to immobilized immunoglobulins through their carbohydrate moiety, we ran a blank test in which no serum was added; the absorbances of these blanks, always <0.15, were acceptable. Concentrations of the dSHBG standard solutions ranged from 0 to 500 μg/L (Fig. 1B). Serum samples were usually diluted 1:286 and 1:546 in BSA/PB; however, for some patients, higher dilutions had to be tested. All determinations were done in duplicate, and data were analyzed as previously described for total SHBG.

The within-run CV for duplicates was ~7% (n = 173), and mean analytical recovery (load = 30 μg/L) was 105% (SD = 17%, n = 40). Because the in vivo desialylation process may affect the glycan chains differently, serum probably contains several SHBG isoforms with various sialic acid or terminal galactosyl residues contents and thus with different reactivities toward RCA120. Because of this heterogeneity in the isoform profile, the use of in vitro neuraminidase-treated SHBG as a standard is questionable. Serum dSHBG concentrations were thus expressed as arbitrary units, one unit being defined as the absorbance obtained with a 1 μg/L dSHBG calibrator. In healthy donors, values range from 9 to 12 U/L, whereas in some patients with hepatic diseases, concentrations may rise to 100 U/L.

SHBG and dSHBG can be determined in parallel easily using the described procedures because most of the steps are similar: coating with anti-SHBG antibody, BSA saturation, conditions for washing and incubation, enzymatic amplification, photometric measurement, and data analysis. Because total SHBG determination can be measured in a low ionic strength buffer, i.e. PB, the two assays differ only by (a) the nature of the peroxidase-conjugate (anti-SHBG for total SHBG and RCA120 for dSHBG), (b) the serum dilution, and (c) the standard for calibration. The convenience of this coupled procedure allows extensive studies of various pathological conditions, such as hepato-cellular carcinoma, and may thus be useful for a better knowledge of the physiopathology of this disease.

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The Use of a Whole-Blood Benchtop Analyzer (Nova 16) in a Cardiac STAT Intensive Care Unit

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The Nova 16 STAT Chemistry Analyzer is designed to provide quick laboratory results of a chemistry profile consisting of glucose, blood urea nitrogen (BUN), creatinine, sodium, potassium, chloride, carbon dioxide, and hematocrit. The analyzer may be of use in the emergency department, the STAT laboratory, or as a mobile laboratory on a movable cart. We obtained the instrument to provide immediate results to the cardiac STAT intensive care unit for the above chemistry constituents. These analytes were previously performed on serum with a Beckman Instrument CX3 analyzer with an ~45 min turnaround time. The major components of the 45 min required to provide these laboratory results were the time to allow specimens to clot, time for centrifugation, and internal laboratory transport times. The Nova 16 whole-blood analyzer eliminates the times required for specimen clotting and centrifugation. Bringing the analyzer physically closer to the patient treatment area or locating it in close proximity of a pneumatic tube system provides further reduction in the turnaround times. We evaluated the performance of the Nova 16 to determine the suitability of this instrument for use in the cardiac STAT intensive care unit and to ascertain the effect of the Nova 16 on turnaround times.

The Nova 16 uses biosensors to determine the concentrations of glucose, BUN, and creatinine (1). The glucose sensor consists of glucose oxidase bonded to a polymeric membrane substrate. β-D-glucose is then oxidized to gluconic acid and hydrogen peroxide. The hydrogen peroxide produced is oxidized at the platinum electrode where the hydrogen peroxide is detected. The BUN assay uses an enzyme, urease, that has been chemically bonded to a membrane. The ammonium ion produced is detected with an ammonium ion-selective electrode. The creatinine sensor consists of three enzymes, creatinine amidohydrolase, creatinine amidinohydrosase, and sarcosine oxidase. Creatinine from the sample diffuses through the membrane where the creatinine is converted to hydrogen peroxide. The hydrogen peroxide is oxidized at the platinum electrode. The total carbon dioxide electrode consists of a gas permeable membrane and a pH electrode.