capillary, from insulin-dependent diabetic patients attending the Diabetic Clinic, and we compared the results with the current ion-exchange method used in our laboratory. Controls provided by Roche Diagnostics and our own in-house controls were assayed with each run. The immunoturbidimetric reagents were donated by Roche.

The immunooassay uses latex-bound monoclonal antibodies developed against the glycated N-terminus of the β-chain; total hemoglobin was quantified colorimetrically. The assay was performed on a Cobas Mira as recommended by the manufacturer. Pretreatment of the sample is done on the analyzer. The erythrocytes are lysed by low osmotic pressure, and the released hemoglobin is degraded proteolytically to make the β-N-terminal structures more accessible for the immunoassay. In addition, the heme groups are oxidized before the colorimetric determination of hemoglobin. The final result is computed from the HbA1c and the total hemoglobin concentrations. The method is calibrated against an HPLC method that gives values that are, on average, 1.28 ± 1.28 times those of the cation-exchange fast protein liquid chromatography (FPLC) method (Pharmacia, Uppsala, Sweden) used in our laboratory with a MonoS HR5/2 column [1]. For FPLC, the erythrocytes are lysed and the labile fraction is eliminated before chromatography.

Analysis of the 180 whole-blood samples by both methods yielded y = 0.82x − 0.37, Sₓᵧ = 0.39 (Fig. 1). Similarly, when only the capillary samples were compared, y = 0.80x − 0.27, Sₓᵧ = 0.27. Nine samples had increased (1.2% to 1.9%) fetal hemoglobin (HbF), but this did not influence the final result.

HbF was quantified by the Pharmacia FPLC method. Because HbF does not contain the β-chain, HbF<sub>1c</sub> is not quantified in the immunoturbidimetric assay. HbF's contribution to the estimation of total hemoglobin is minimal unless the HbF concentration is >10%. One sample had an abnormal hemoglobin (HbE of 27.3%), and the HbA1c quantification by chromatography was lower than by immunoturbidimetry. This was not unexpected because the point mutation [2] (β26 Glu → Lys) presumably affected the mobility of the hemoglobin on FPLC but would not affect the antigenic epitope, the N-terminal glycoprotein of the β-chain, relevant to the immunoassay. The within-day CV (n = 4) was <2% and the interassay CV (n = 4) was <3% at 5–12% HbA1c.

The immunoturbidimetric method has the advantage, in theory, of specifically detecting all glycated N-terminal forms of HbA unless a point mutation exists in the N-terminal amino glycopeptide region of the β chain. HbF (<2%) did not affect the final result. The ease with which this assay can be performed in both capillary and venous samples makes it an attractive alternative method for determination of HbA1c.

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References

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Antibodies Against [125I]Testosterone in Patient's Serum: A Problem for the Laboratory and the Patient

To the Editor:

Determination of steroids directly in diluted serum samples is widely accepted all over the world, and forms the basis for nearly all commercial steroid immunoassay kits. Provided the reagents include inhibitors against steroid-binding proteins, the kits are reliable and easy to perform. Unless the samples are subjected to extraction before assay, the serum may contain interfering substances that may cause falsely high hormone concentrations. We have recently encountered two such cases with the Spectra Testosterone 125I kit from Orion Diagnostica, Espoo, Finland. The two sera were routine samples sent to the laboratory for diagnostic purposes, and the investigation of the samples, as reported here, is in accordance with the Helsinki Declaration of 1975, as revised in 1983.

Patient 1: A woman, 22 years old, normal ovaries, oligomenorrhea, question of hirsutism, thrombosis, and phospholipid antibodies. Normal female hormone concentrations except for testosterone at 25 nmol/L. The serum was reassayed for testosterone after diethyl ether extraction, giving <0.5 nmol/L.

Fig. 1. Relationship between the FPLC and immunoturbidimetric results for HbA<sub>1c</sub> (%) concentrations in patients' samples, venous and capillary (n = 180).

The line of best fit computed by least-squares linear regression analysis is y = 0.82x − 0.37; Sₓᵧ = 0.39.
The discrepancy between the testosterone concentrations with and without extraction was further investigated. We deduced that the serum could not contain excess testosterone bound to some unknown or modified binding protein, as all protein-bound testosterone should have been extracted with the ether. This point was further elaborated by extracting the sample after acid hydrolysis before assay, again giving <0.5 nmol/L. Accordingly, we assayed for testosterone conjugates that might be present. Serum proteins were precipitated with ethanol, aliquots of the supernatant evaporated, redissolved in water, and either treated with sulfatase or acid hydrolysis to obtain testosterone free of sulfate or other conjugates such as glucuronide. The conjugate-free testosterone was extracted with ether and assayed in the Spectra Testosterone 125I kit. Again <0.5 nmol/L testosterone was found, proving that testosterone conjugates were not present in inordinate amounts in the serum.

The possible presence of antibodies in the patient's serum was further investigated, although we had deduced that these antibodies could not be directed against testosterone as such. However, if the serum contained binding substances for 125I-testosterone, this would also lead to falsely high concentrations in the assay, as less indicator would bind to the solid phase. Accordingly we incubated serum with 125I-testosterone overnight at 4°C and precipitated the IgGs in the sample with protein A-Sepharose (Zymed Laboratories, South San Francisco, CA). Since 83% of the added 125I-testosterone was recovered in the protein A-Sepharose precipitate vs 5% in a control serum, the sample contained an IgG that bound 125I-testosterone. To prove that the IgG was specific for 125I-testosterone, we incubated with 3H-testosterone and precipitated with protein A-Sepharose as before. Only 0.8% of the added 3H-testosterone was recovered with the protein A-Sepharose precipitate, showing clearly that the patient's serum contained an antibody that bound the 125I-testosterone of the Spectra Testosterone 125I kit, but without any activity against testosterone as such.

The 125I-testosterone binding properties of the patient's serum were evaluated by incubating the serum with different concentrations of 125I-testosterone overnight at 4°C and precipitating the IgGs in the sample with protein A-Sepharose. After determining the specific activity of the tracer by comparing the tracer displacement of different doses of 125I-testosterone with the testosterone calibrator of the Spectra Testosterone 125I kit, the binding data were subjected to Scatchard analyses with the IBM PC program Radlig (Biosoft, Cambridge, UK). One binding site with KD = 5.2 (0.5) nmol/L and Bmax = 4.8 (0.1) nmol/L [mean (SE)] was found in the patient's serum.

**Patient 2:** A woman, 45 years old, moderate hirsutism, regular periods, low concentrations of sex hormone binding protein (SHBG) (18 nmol/L), slightly increased concentrations of androstenedione, other examined hormone concentrations normal except for testosterone at >50 nmol/L. The serum was reasayed for testosterone after diethylether extraction, giving 1.2 nmol/L. This serum was tested for 125I-testosterone binding as for patient 1. One binding site with KD = 0.10 (0.03) nmol/L and Bmax = 4.3 (1.0) nmol/L [mean (SE)] was found in the patient's serum.

Both patients were at risk of receiving the wrong diagnosis because of high serum concentrations of testosterone. Patient 1 had in fact already undergone one laparotomy largely on the basis of falsely high concentrations of testosterone. Thus the presence of 125I-testosterone antisera in the patient's blood is not only a laboratory problem, but clearly a diagnostic and therapeutic problem, too.

The prevalence of antibodies against 125I-testosterone is difficult to assess. We detected two patients in 2 months (1 in 800 samples). These sera were detected because of extreme testosterone serum concentrations, and we have no information on whether moderately increased testosterone samples may contain 125I-testosterone antibodies. Likewise we do not know whether the 125I-testosterone of other direct testosterone kits may react against endogenous antibodies. The testosterone tracer from Orion contained testosterone-19-[125I]histamine. We have not tested whether the antibodies bind to 125I-testosterone labeled in other ways, or to histamine. Although we do not know the specificity of the antibodies (except that they do not bind testosterone), our observations are potentially important because testosterone-19-[125I]histamine is a widely used indicator in testosterone immunoadsays. We hope this letter will lead other laboratories to carefully evaluate high testosterone concentrations in women and reassay samples after extraction when in doubt.

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**Clinical Utility of Carbohydrate-Deficient Transferrin to Detect Alcohol Abuse in a General Population**

To the Editor:

An isoform of transferrin, carbohydrate-deficient transferrin (CDT), has been found to be increased in serum in a high percentage of alcoholics [1, 2]. CDT measures an accumulated effect of alcohol consumption, appearing after regular intake of 50–80 g of ethanol per day over several weeks. Decreased glycosylation of transferrin protein before hepatocellular release is the most likely mechanism for the production of CDT. After separation of the different isoforms of transferrin by (e.g.) isoelectric focusing, chromatofocusing, or anion-exchange chromatography, CDT can be quantified by means of binding-assays [1, 2]. We evaluated the clinical utility of a commercially available kit (CDTect™) from Pharmacia (Uppsala, Sweden), which is based on the technique of Stibler et al. [3].

We studied 138 volunteers. Details about alcohol consumption were obtained by a confidential self-report wherein the total amount of alcohol intake (daily number of beverages) during the last 4 weeks was reported. The control group (group 1) consisted of 24 healthy male volunteers with a mean daily alcohol consumption <5 g during the last 4 weeks before blood sampling. Group 2 consisted of 42 clinical patients (18 women and 24 men) with various alcoholic (cirrhosis, fatty liver) and non-alcoholic liver diseases (chronic hepatitis, primary biliary cirrhosis, toxic fatty liver, cholangitis). According to self-report and an interview with one member of their family, they had been abstaining for 1–6 months before blood sampling. Group 3 consisted of 72 men who were regular pub visitors. They were interviewed and