Despite the high correlation, the results differed numerically. Two differences must be considered: the anticoagulant itself and the dilution of sample by the anticoagulant. The difference between results depended mainly on sample dilution by citrate solution; the EDTA sample was diluted only minimally. When the PT test is calibrated with normal EDTA plasma, these two differences disappear, so that it is possible to use EDTA plasma to measure PT with the Owren procedure.

The coagulation reagent contains sufficient Ca^{2+} to allow coagulation with either sample type. The physiological concentration of Ca^{2+} in plasma is ~1.3 mmol/L. The Ca^{2+} concentrations in the reaction mixtures are higher for both sample types (1.525 mmol/L for citrate and 1.708 mmol/L for EDTA).

The main reason for the lack of a problem with EDTA as anticoagulant may reside in the assay procedure used. In the Owren method, only ~5% of the reaction mixture is sample, whereas it is 33% in the Quick PT method. Difficulties in correctly balancing the Ca^{2+} concentration are thus much smaller with the Owren PT method than with the Quick PT.

In an EDTA sample, blood cells preserve their cell morphology well (or the changes in morphology are known and do not cause diagnostic confusion), and there are minimal nonphysiologic effects on the cells *(8)*. When tubes with solid EDTA are used, there is no dilution of the sample. In this study, tubes were used in which EDTA was in liquid form, but the dilution was ~2.34%.

It is important that the calibrators (for results in percentages and INR units) have the same dilution as the samples; calibrator manufacturers should inform the user of the diluent and dilution rate. When seconds are the unit, no calibration is involved. According to the regression equation, coagulation times are shorter with EDTA plasma because of the dilution. EDTA sample results (in percentages and INR units) can be converted by equation to citrate plasma results or vice versa. If we calibrate (percentages and INR units) our coagulation analyzer with normal EDTA plasma, we immediately obtain the correct result, and the citrate-plasma reference and therapeutic range for oral anticoagulant treatment are the same as for EDTA plasma.

Use of a common tube for hematologic and coagulation assays is a good aim. Hematological analyzers use ~150–250 μL of a blood sample. After hematological measurements are made, the sample can be centrifuged to obtain plasma for PT testing. This order of proceeding does not alter the relationship between plasma and cells. Use of the same EDTA sample for hematological and coagulation estimations has many advantages: sampling is faster in one tube, material and waste costs are lower, and the overall number of tubes needed in the laboratory is smaller, which saves natural materials. Moreover, the small sample volume needed is important for different patient groups and children. In addition, laboratories usually have an adequate supply of normal EDTA plasma for calibration.

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Stability of Cannabinoids in Hair Samples Exposed to Sunlight, *Gisela Skopp,[1*] Lucia Pötsch,[2] and Martin Mauden[1]* ([1] Institute of Legal Medicine, University of Heidelberg, Vossstrasse 2, 69115 Heidelberg, Germany; [2] Institute of Legal Medicine, University of Mainz, Am Pulverturm 3, 55131 Mainz, Germany; * author for correspondence: fax 49-6221-565252, e-mail gisela_skopp@med.uni-heidelberg.de)

It has been well recognized that hashish and marihuana lose potency during storage because of a decrease in the content of tetrahydrocannabinol (THC), which is the major psychoactive constituent of cannabis *(1)*. The effect of oxygen on stored plant and resin materials or solutions of pure cannabinoids seems much less significant than that of higher temperatures (≥37 °C) or light *(2–7)*. A few data are available on the stability of THC and major metabolites in blood *(8–11)*. However, the stability of cannabinoids in the hair shaft has not been addressed, although scalp hair represents one of the most exposed parts of the body. Therefore, a study was performed to elucidate whether cannabinoids such as THC, cannabinol (CBN), or cannabidiol (CBD), which usually are determined from hair samples, would exhibit similar instability in this particular biological matrix when exposed to solar radiation.

Clipped hair bundles (n = 11) suggested to be cannabis positive were washed twice with dichloromethane (10 mL

each time for 10 min) and divided into four strands each. Two of the strands were kept in the dark at ambient temperature. Under these storage conditions, the analytes and their concentration remained unchanged as ascertained in a preliminary experiment (data not shown). The other two strands were stored outside in quartz glass vials (Pyrex) and exposed to natural sunlight for 10 weeks from August 1, 1999 to October 10, 1999 (114 m above sea level, 40° 30″ degree of latitude).

CBN is suggested to be produced from THC by oxidation and might, therefore, be used as a degradation marker of THC. To monitor CBN production from THC and to test its stability in the presence of active oxygen species, an accelerated degradation experiment (40 °C; observation period, 3.5 h) was performed by adding hydrogen peroxide (~300 mL/L; 40 μL of Roth) to 2 mL of a solution of THC in ethanol (0.1 g/L; Sigma). Aliquots (1 μL) were injected directly into a gas chromatograph equipped with a flame ionization detector (Shimadzu).

The cannabinoid content in the hair samples was determined by gas chromatography–mass spectrometry. Each strand was pulverized in a ball mill, and ~50 mg of the hair powder was weighed; 25 ng of THC-d_3 (Radian International) was added as internal standard, and the sample was further processed according to Cirimele et al. *(12)*. A 1-μL aliquot of the extract was injected into a gas chromatography–mass spectrometry system [HP 6890 gas chromatograph and HP 6890 mass spectrometer, both from Hewlett Packard; column, CP-Sil 5, 12.5 m × 0.53 mm (i.d.), from Chrompack]. Detection was by ionization in the electron impact mode (70 eV), with the scan mode set at single-ion monitoring (THC, *m/z* 299, 314, 271; THC-d_3, *m/z* 302, 317, 274; CBN, *m/z* 295, 310, 238; CBD, *m/z* 231, 246, 314). Measurements were taken twice from each strand. The THC concentrations determined from a particular sample differed by at least 15% and are given as mean values (n = 4; Table 1). Deuterated standards for CBN and CDB are not yet commercially available; hence, these compounds were not quantified, and triplicate measurements of six drug-free hair samples were used to estimate their particular detection limits (mean + 3 SD). The limit of detection was 0.05 ng/mg of hair for all analytes.

Table 1. Changes in cannabinoid content in hair strands exposed to global solar radiation for 10 weeks.

		Original concentration			Findings after exposure to light		
Sample	Color	THC[a]	CBN[b]	CBD[b]	THC[a]	CBN[b]	CBD[b]
1	Brown	0.11	−	+	−	−	−
2	Dark brown	1.72	+	+	0.35	+	+
3	Brown	0.18	+	+	−	−	−
4	Light brown	0.10	+	−	−	−	−
5	Blonde	0.30	+	−	0.11	−	−
6	Dark blonde	0.20	−	−	−	−	−
7	Light blonde	1.20	+	−	0.10	−	−
8	Blonde	0.76	+	−	−	−	−
9	Middle blonde	0.95	+	−	−	+	−
10	Brown/gray	1.18	+	−	−	−	−
11	Red	0.87	+	−	−	+	−

[a] In ng/mg of hair, mean value (n = 4).
[b] +, above the limit of detection (0.05 ng/mg of hair); −, not detectable.

Upon gross inspection, all hair samples had faded slightly in color during the 10 weeks of exposure. The results obtained from the particular strands kept in the dark and those exposed to global solar radiation are summarized in Table 1. The original concentrations were within the ranges reported for Caucasian hair in the literature *(13)*. Exposure to light produced marked decreases in THC, CBN, and CBD concentrations. THC was detectable in small amounts in only 3 of the 11 samples, and CBN and CBD could still be found in 3 and 1 of 8 and 3 formerly positive samples, respectively. Obviously, the decrease in cannabinoid content was not connected to the hair color. Initially, THC might have been converted to CBN, which could be further degraded in the presence of an oxidizing agent as already indicated by the stress test. In this experiment, THC decreased to 77% of its initial concentration 10 min after the experiment started. The THC concentration then decreased more slowly, to 68% of the starting concentration after 3.5 h. CBN was formed and reached peak concentrations after 1.5 h (12%, relative to THC), but its concentration continuously declined to 4% (relative to THC) after 3.5 h.

This study clearly demonstrated that cannabinoids usually measured in hair analysis are more affected by solar radiation than other drugs of abuse detected in hair, such as 6-acetylmorphine, dihydrocodeine, or cocaine *(14)*. CBN is thought to be a chemical degradation product of THC, and the compound concomitantly appeared during storage in plant materials or in solution, whereas the THC concentration decreased *(1, 7)*. However, in keratinized hair exposed to sunlight, CBN was obviously further degraded. A review of the literature on the photodegradation of THC in solution and the present stress test tentatively support the suggestion of a light-induced radical reaction as the underlying mechanism *(6)*. Melanin is a photoreactive biopolymer. At visible and long ultraviolet (UV) wavelengths, it releases most of its energy as heat, whereas at short UV wavelengths, reactive intermediates may be formed *(15)*. Both eumelanin and pheomelanin have the ability to generate active oxygen species, such as $O_2^{\cdot-}$, $HO^{\cdot}$, and H_2O_2, in various amounts *(16, 17)*. These reactive species have been shown to degrade both THC and CBN as demonstrated by the hydrogen peroxide experiment. In addition to the deleterious effect of sunlight on the stability of cannabinoid constituents in hair, the weathering of hair, which damages the hair fiber at the ultrastructural level, may cause additional changes in drug concentrations in hair *(18–20)*.

The present findings may have implications on both sample collection and the interpretation of analytical results in hair analysis. In general, only a sample below the top hair should be collected, and information should be acquired on the hair donor's outdoor activities as well

as exposure to artificial UV light (such as visits in the solarium). The presence of fading in single hairs of the strand or its ends should also be noted. Because of the rapid decrease in cannabinoid constituents in hair exposed to UV radiation, the result from a particular sample might be rather of a qualitative nature, and a negative finding may not conclusively indicate that there was no cannabis use. At present, it seems a real challenge to identify degradation products of THC, CBN, and CBD as markers for a positive cannabis test in hair. Such work is in progress.

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Evaluation of a Homogeneous Direct LDL-Cholesterol Assay in Diabetic Patients: Effect of Glycemic Control,

Brian D. Ragland, Robert J. Konrad, Carolyn Chaffin, C. Andrew Robinson, and Robert W. Hardy[*] (Department of Pathology, University of Alabama at Birmingham, Birmingham, AL 35294; * address correspondence to this author at: The University of Alabama at Birmingham, Department of Pathology, LHRB 573, 1530 3rd Ave. S, Birmingham, AL 35294-0007; fax 205-975-9927, e-mail hardy@path.uab.edu)

Increased serum LDL-cholesterol (LDL-C) is associated with increased risk for ischemic heart disease, and lowering of LDL-C has been shown to decrease mortality in patients with known coronary heart disease *(1–3)*. Persons with diabetes have a greatly increased risk for atherosclerosis and its complications. Many of these patients have increased plasma cholesterol (including LDL-C) and triglycerides (TGs). Additionally, glycation and oxidation of circulating LDL particles may further increase the risk for atherosclerotic disease in patients with diabetes *(4, 5)*.

Currently used methods for LDL-C include calculations [based on total cholesterol (TC), HDL-cholesterol (HDL-C), and TG concentrations], ultracentrifugation, and most recently, direct LDL-C assays. Calculation methods, such at the Friedewald formula *(6)*, are well known to have a prominent negative bias in patients with TG concentrations >4.5 mmol/L (400 mg/dL) *(6, 7)*. In addition, because TGs are measured, fasting blood samples are preferred. This can present a problem for certain patient populations, including many people with diabetes. Ultracentrifugation methods are time-consuming and expensive and generally are performed only in reference laboratories.

Direct LDL-C assays have been developed recently and have been shown to provide accurate and precise measurements of LDL-C *(8–12)*. They overcome the TG and fasting limitations of calculation methods, are readily adapted to clinical laboratories, are less expensive than ultracentrifugation methods, and provide greatly improved turnaround time. Direct LDL-C assays make use of either an immunoseparation step or specific detergents to separate LDL particles from other lipoproteins, followed by measurement of cholesterol by conventional enzymatic reactions. The N-geneous™ LDL-C assay is a solute-based homogeneous assay that agrees well with ultracentrifugation *(11)*. The purpose of the present study was to evaluate the effect of glycemic control (as gauged by hemoglobin A_{1c}) on the accuracy of the N-geneous assay. Additionally, we compared this direct LDL-C assay to density gradient ultracentrifugation *(13)* and to the Friedewald calculation *(6)*, and assessed its usefulness in determining a calculated VLDL-cholesterol (VLDL-C) concentration.

Overnight fasting plasma samples were obtained from 52 patients. Direct LDL-C was measured using the N-geneous LDL-C assay (Genzyme Diagnostics) performed on the Beckman SYNCHRON LX 20 (Beckman-Coulter).