MRDR-HPLC method is more reliable and faster. With these modifications, the MRDR may find wider application for the detection of VAD.

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Effects of Blood Collection Methods on Gelatin Zymography of Matrix Metalloproteinases

To the Editor:

Matrix metalloproteinases (MMPs), zinc-dependent proteinases belonging to the family of matrixins, are involved in the processes of normal development and growth as well as in several pathologic states (1). MMP-2 (gelatinase A; EC 3.4.24.24) and MMP-9 (gelatinase B; EC 3.4.24.35) play crucial roles in the proteolytic cascade leading to extracellular matrix degradation (2). To evaluate the usefulness of MMPs as tumor markers, several authors have measured MMP-2 and MMP-9 in the blood of cancer patients, using serum or plasma samples, and reached contradictory conclusions (3, 4). The preanalytical steps of blood sampling and processing influence the concentrations of MMPs and their inhibitors (5, 6), but no data are available concerning the effect of blood specimen collection methods on the appearance of the full spectrum of MMP isoforms on gelatin zymograms.

We simultaneously collected blood samples from 20 healthy volunteers (median age, 36 years; range, 23–55 years) into plastic tubes with no additive, plastic tubes with a silica gel-coated surface as coagulation accelerator, lithium heparin-coated plastic tubes, and potassium EDTA-coated plastic tubes (Monovette Systems; Sarstedt). The tubes were kept at room temperature and centrifuged immediately after venipuncture (1600g for 15 min at 4 °C), and the supernatants were stored at −80 °C until analysis. Human gelatinases were prepared as described previously (7). Gelatin zymography was performed under nonreducing conditions on 7.5% polyacrylamide mini slab gels (Bio-Rad), copolymerized with 1.5 g/L 90 Bloom gelatin (Sigma) (8). Aliquots containing 50 μg of total protein (Bio-Rad) were used for each zymographic test. To assay gelatin lysis, scaled aliquots of proteins were run in triplicate and submitted to computer-assisted densitometric scans using Image Pro-Plus software (Cybernetics); the semiquantitative results were expressed as a percentage vs control or calibrator.

The gelatin zymography technique allows detection of all MMPs in circulating blood, including the largest isoform (225 kDa; Fig. 1). The four bands showing gelatinase proteolytic activity were fibroblast-derived pro-MMP-2 (72 kDa) and neutrophil-derived pro-MMP-9 (92, 130, and 225 kDa) (7, 8).

As shown in Fig. 1A, pro-MMP-2 is commonly present in both plasma and sera from healthy humans, although in most samples other major proteolytic activities were seen at M_r 92, 130, and 225 kDa. All gelatino-
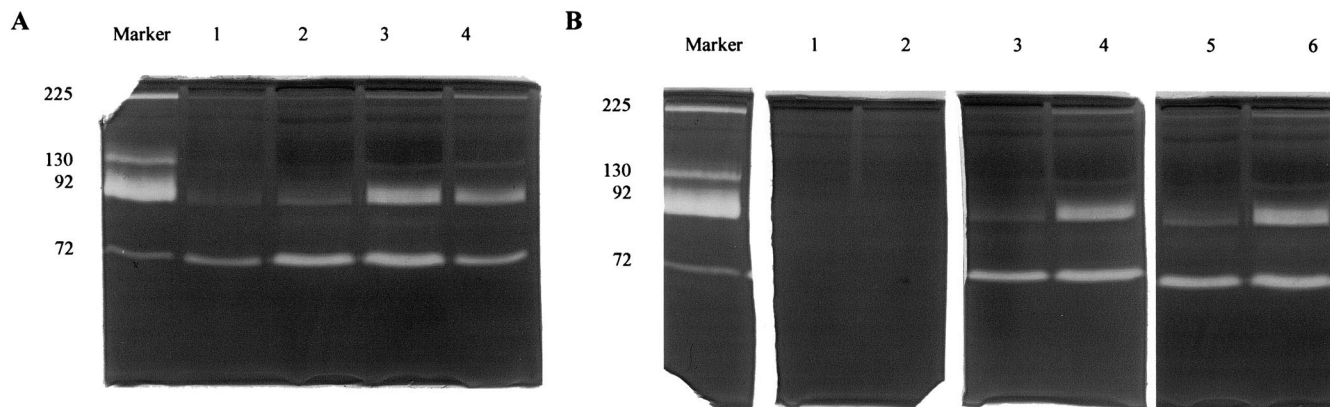

Fig. 1. Gelatin zymograms of plasma and serum MMPs.

Samples (50 μg of total protein) were analyzed on 7.5% gels containing 1.5 g/L 90 Bloom gelatin. The calibrators (*lane Marker*) were capillary whole-blood gelatinases; molecular masses (kDa) are indicated. (*A*), lanes 1 and 2, gelatinases from plasma samples collected into potassium EDTA-coated and lithium heparin-coated plastic tubes, respectively; *lanes 3* and *4*, MMPs from serum collected in tubes with clot activator and with no additive, respectively. (*B*), blood MMP zymograms incubated in the presence of 2 g/L potassium EDTA (*lanes 1* and *2*), 30 kIU/L lithium heparin (*lanes 3* and *4*), or the incubation buffer (50 mmol/L Tris-HCl, pH 7.5, 100 mmol/L NaCl, 5 mmol/L CaCl₂, 1 mmol/L ZnCl₂, 0.2 mL/L Brij-35, 2.5 mL/L Triton X-100, and 0.2 g/L NaN₃) without additives (*lanes 5* and *6*).

lytic bands were confirmed as MMP activity because they were completely inhibited by 5 mmol/L EDTA and 2 mmol/L 1,10-phenanthroline (1).

In accordance with previous quantitative studies, we detected for the first time on zymography that fibroblast-derived MMP-2 did not differ in serum and heparin plasma but was lower in EDTA plasma, whereas the activities of all neutrophil-derived MMP-9 isoforms were 2- to 10-fold higher in serum than in heparin and EDTA plasma (Fig. 1A). To evaluate the effect of anticoagulants on gelatinolytic activity, we added EDTA and lithium heparin to the enzyme incubation buffer at concentrations equivalent to those in the above-mentioned Monovette systems (2 g of EDTA or 30 kIU of heparin/L of sample). The addition of EDTA produced almost complete inhibition of blood gelatinolytic activities (Fig. 1B, lanes 1 and 2), whereas heparin did not affect MMP enzymatic activities (recovery, 95–105%; Fig. 1B, lanes 3 and 4). At present, there is no explanation for this effect, and the inhibitory effect of EDTA could be one reason for the decreased MMP activities in EDTA-plasma samples, although a preanalytical effect of EDTA during blood collection is more likely.

Our results confirm higher MMP concentrations in serum than in plasma, as previously quantified (5, 6), but they also demonstrate that the concentrations of all neutrophil-derived MMP isoforms (92, 130, and 225 kDa) are higher in serum than in plasma, without apparent influence on the proportion of active to latent isoforms. Discrepancies in serum samples prepared in tubes with and without clot activator were also detected: MMP-9 isoforms appeared to be twofold higher with silica-gel coagulation accelerator (Fig. 1A, lanes 3 and 4). Platelet activation or neutrophil mobilization during clotting could produce these differences (9).

The discrepancies between MMP forms in sera and plasma should also be considered to avoid preanalytical misinterpretation. We recommend

that only heparin-plasma samples be used.

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Effect of Sulfamethoxazole on Clinical Capillary Zone Electrophoresis of Serum Proteins

To the Editor:

Capillary zone electrophoresis (CZE) using fused-silica capillaries has become a well-accepted method for the separation of serum proteins and for
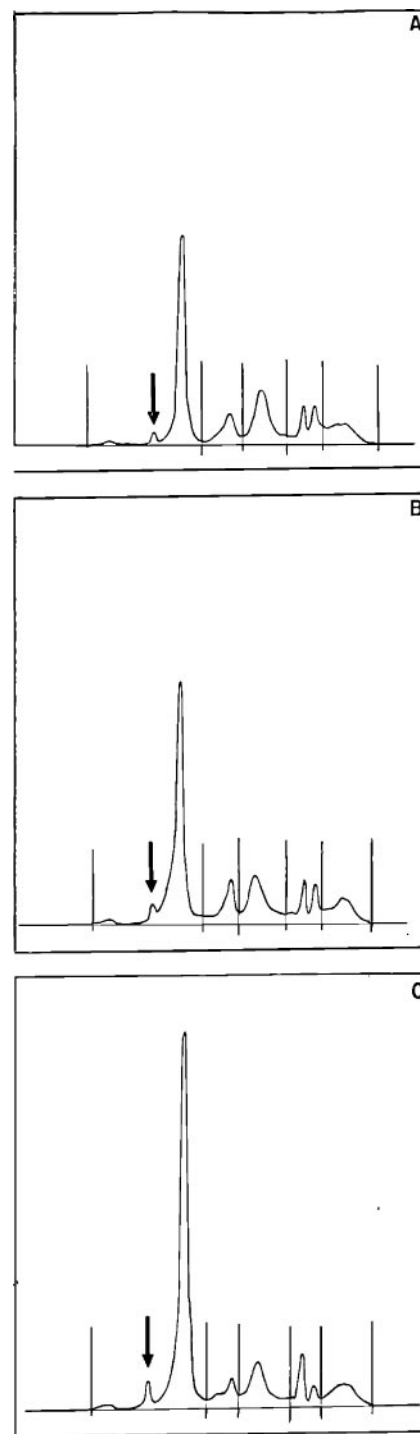

Fig. 1. Effect of sulfamethoxazole on serum CZE.

Panels A and *B* show the CZE electropherograms of samples from two patients receiving intravenous sulfamethoxazole-trimethoprime (400 mg of sulfamethoxazole/80 mg of trimethoprime, 12 ampoules/day for 6 days). *Panel C* shows the CZE electropherogram of a normal sample to which sulfamethoxazole (Roche) dissolved in methanol (final concentration, 240 mg/L) was added. The *arrows* indicate the abnormal peak.