Our aim was to demonstrate the effect of blood sampling on the concentrations of collagenase-1 (EC 3.4.24.7; MMP-1), stromelysin-1 (EC 3.4.24.17; MMP-3), matrilysin (EC 3.4.24.33; MMP-7), and collagenase-2 (EC 3.4.24.34; MMP-8) circulating in blood. Because the preanalytical impact of blood sampling is well known for gelatinase A (EC 3.4.24.24; MMP-2) and MMP-9 (4), we measured these substances for use as comparisons. The study was performed in accordance with the ethical standards of the Helsinki Declaration and was approved by the ethical board of the hospital. All sample donors recruited on a voluntary basis and informed about the objectives of the study provided written informed consent.

During single venipunctures performed in 10 healthy adults [4 female, 6 male; mean (SD) age, 42 (14) years] venous blood samples were collected in plastic tubes (Sarstedt) without additives or with kaolin-coated granulate as coagulation accelerator (serum(–) and serum(+), respectively) and in tubes coated with lithium heparin, disodium citrate, or dipotassium EDTA in that specified sequence and centrifuged within 30 min after venipuncture (1600g; 15 min; 4 °C). The supernatants were removed and stored at −80 °C until analysis. MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-12, and MMP-13 were simultaneously measured using the Fluorokine MultiAnalyte Profiling assays (R&D Systems) on a Luminex 100 Bioanalyzer (Luminex). The assays measure pro-, mature, and tissue inhibitor of metalloproteinase (TIMP)-1–complexed MMPs with <0.5% cross-reactivity between the MMPs.

Fig. 1 summarizes the different MMP concentrations in all samples examined. MMP-12 and MMP-13 could not be determined because the measurements gave signals below the detection limit. ANOVA analyses showed significant differences for all MMPs between the serum(+) and serum(–) samples and among the plasma samples (P < 0.0001). Post hoc paired Student t-tests were used in further statistical analysis of the differences between serum(+) and serum(–), among the plasma samples, and between the plasma and serum(–) samples. We additionally related the MMP values in serum and plasma to the results obtained in citrate-plasma taken as 100%, because citrate was previously recommended as anticoagulant of choice to prepare plasma samples for MMP-9 measurements (5).

The main novel findings of this study were: (a) MMP-1, MMP-8, and MMP-9 showed significantly higher values in serum(+) than in serum(–), but there were no differences for MMP-2, MMP-3, and MMP-7; (b) with a few exceptions, all MMPs were characterized by higher concentrations in serum than in plasma samples, particularly for MMP-1, MMP-8, and MMP-9, which also showed 2- to 4-fold higher concentrations in serum(+) than in serum(–) samples; (c) MMP-2, MMP-3, and MMP-7 measured in citrate-plasma were roughly comparable to the 2 serum-type samples and heparin-plasma, with differences of only 15% for MMP-2 and about 25% for MMP-3 and MMP-7; (d) MMP measurement results in EDTA-plasma samples were more highly variable than those obtained in citrate- or heparin-plasma samples.

Our data demonstrate that in blood the measured concentrations of not only the gelatinases but also of other MMPs are strongly influenced by the sampling procedure. Our study is the first detailed investigation of simultaneous measurements of multiple MMPs in serum with and without clot activator, and in citrate-, EDTA-, or heparin-plasma. Our findings highlight the
important differences between the MMP concentrations in serum collected with and without clot activator as well as the differences among both serum types and plasma samples. It is noteworthy that MMPs such as MMP-1, MMP-8, and MMP-9 are abundantly expressed in platelets and leukocytes. Increased MMP concentrations in serum may be due both to their release during coagulation and to their secretion, which is induced by the clot activator itself, as has been shown for MMP-9 (3, 4).

In conclusion, each MMP to be analyzed as a circulating biomarker in blood requires careful consideration of the preanalytical effects of blood collection methods. Despite the currently available knowledge base, some studies performed on gelatinases and collagenases in serum do not address the concerns raised by reports of pitfalls and misinterpretations linked to preanalytical aspects of blood processing. Our results indicate that the use of serum should be avoided for measurement of MMP-1, MMP-8, and MMP-9. In contrast, MMP-2, MMP-3, and MMP-7 measurements showed comparable values among citrate- and heparin-plasma and the 2 serum types. Citrate-plasma seems to be the sample of choice for the measurement of all MMPs.

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References
Analysis of Urine Benzodiazepines with the Abbott Multigent™ Reagents

To the Editor:

In the January 2008 issue of Clinical Chemistry, Lum et al. (1) reported on their recent experience with the Multigent benzodiazepines assay. We thank Dr. Lum and his colleagues for their comments regarding the assay, and value their insights and expertise in this area. Consistent results from a screening assay that detects multiple compounds belonging to a specific drug class requires an analytical balancing act, because the reagent performance must minimize false negatives while also maximizing confirmation rate by various confirmatory methods. As the manufacturer of this assay, we recognize that this process leads to issues that may concern users of our products.

The Multigent benzodiazepines assay is marketed as a screening assay, and the package insert states that a more specific alternate chemical method must be used to confirm the screening result. This specific Multigent reagent uses polyclonal antibodies, which inherently are subject to changes in specificity and sensitivity. When it became necessary to change antibody preparations, a customer bulletin was sent to users of this product in May 2007 to notify them of this change. The customer bulletin also suggested that a user’s confirmatory method must be able to detect benzodiazepines at concentrations lower than those detected by the Multigent assay.

We are not entirely clear regarding the definition of “false positive” being employed in Dr. Lum’s laboratory. A false positive may be defined in relation to a specific cut-off concentration, in which case detectable compounds may be present, but 2 methods may differ in their limits of detection for these compounds. Alternatively, a false positive may be defined as a result indicating that a drug is present even when a confirmatory method with a lower limit of detection for all compounds present indicates the compounds in question are undetectable. Although we do not dispute Dr. Lum’s findings, we are unsure if some detectable benzodiazepine drugs and related metabolites were observed to be absent by a confirmatory method but might be detected by the Multigent assay. We respectfully suggest the possibility that the Multigent assay might be responding to benzodiazepine-related compounds that were not included in the confirmation, or were not detected with sufficient limits of detection by the confirmatory analysis.

We realize that the antibody pool introduced in early 2007 performed differently from the antibody pool that had been previously used by Dr. Lum’s laboratory and acknowledge the need to return to the earlier performance characteristics. We have initiated a project to reformulate the Multigent benzodiazepines reagent to favor maximum confirmation rates, and we will notify users as soon as the reformulated assay becomes available.

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