During analysis we found cases in which it was not possible to distinguish between haplotypes (Table 1) because the presence of multiple heterozygous single-nucleotide polymorphisms prevented us from determining which alleles were in cis (same chromosome) or trans (different chromosomes). For example, when we have both the 282 C>T and 803 A>G in the heterozygote form, 2 possible haplotypes appear: NAT2*4/*12B (one chromosome 282C/803A + the other 282T/803G) and NAT2*12A/*13 (282C/803G + 282T/803A).

The observed 9.7% frequency of slow acetylators among the Vietnamese Khin predicts a low incidence of adverse effects caused by overexposure to INH, and potential drug-drug interactions associated with this drug (3). For this minority group of slow acetylators, a decrease in drug dosage would likely reduce the risk of adverse events without compromising the efficacy of the drug, because a dose of INH as low as 3 mg/kg has recently been shown to be sufficient for a successful therapy (4). On the other hand, a dose reduction below 6 mg/kg in fast acetylators can compromise INH efficacy. The observed high frequency of fast alleles may hypothetically affect the success of the standard INH dosing in the overall Vietnamese population, particularly in terms of early bactericidal activity (5).

Limited pharmacogenetic data are available to account for the diversity of the Southeast Asian populations. Future studies should be performed to identify particular population characteristics that might influence pivotal pharmacotherapy in these geographic regions.

References

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DOI: 10.1373/clinchem.2007.092684

C-Reactive Protein (CRP) in Neonates: Comparing VITROS Slide and High-Sensitivity CRP Methods

To the Editor:
C-reactive protein (CRP) is measured on VITROS® Chemistry Systems (Ortho-Clinical Diagnostics) using a “MicroSlide” method. At our hospitals plasma CRP concentrations <10 mg/L are used as an indicator that it is safe to discontinue antibiotic therapy in neonates with known or suspected sepsis (1). After implementing the neonatal sepsis protocol, we found that the VITROS CRP slide gave results up to 33 mg/L higher in neonatal specimens (Fig. 1, open triangles) than the Behring Nephelometer II (BNII, Dade Behring) highsensitivity CRP (hsCRP) assay, a well-characterized method for CRP measurements (2).

According to the manufacturer’s instructions, hemolysis falsely increases VITROS CRP slide results, but the increases observed were not limited to hemolyzed specimens. It has been reported (3) that collecting specimens in serum separator tubes increases CRP values measured by the VITROS CRP slide; however, the differences we saw were much greater than those attributed to serum separator tubes. Because the falsely increased results in neonates were at or near our decision point of 10 mg/L, we concluded that the VITROS CRP slide method was not suitable for use with our neonatal
We began referring neonatal specimens to laboratories able to run CRP on the BNII, the IMMULITE® immunoassay system (Diagnostic Products), or the COBAS Integra® 400 (Roche Diagnostics). We had previously shown (data not included) that the IMMULITE and Integra CRP methods correlated very well with the BNII. At this point we purchased the VITROS® 5,1 FS Chemistry System, which in addition to analyzing CRP by the MicroSlide method, also determines hsCRP by an immunoturbidimetric MicroTip method. According to the manufacturer, specimens with hsCRP greater than the reportable range of 15 mg/L should not be diluted but retested using the MicroSlide method. Because the MicroSlide method was not suitable for neonates, and we were seeking a faster alternative to sending specimens to another laboratory, we evaluated the VITROS hsCRP MicroTip method, including dilutions, for use with neonates (age <6 months). We tested 249 serum and heparinized plasma specimens left over after clinical testing by one of the other analyzers. This sample set included 143 specimens by the BNII (range 0.2–351 mg/L), 95 by the IMMULITE (range 0.1–139 mg/L), and 11 by the Integra (range 1.0–49 mg/L). Specimens with CRP concentrations >15 mg/L were diluted with saline by the instrument and retested. The institutional review boards of the University of Utah and Intermountain Healthcare gave approval for submission of this data.

Our results are shown by the solid squares in Fig. 1. The VITROS hsCRP method showed markedly better agreement with the BNII, IMMULITE, and Integra than the MicroSlide CRP method, especially at our decision point of 10 mg/L. The simple linear regression line between VITROS hsCRP MicroTip and the 3 comparative methods, taken together, had a slope of 1.16 (95% CI 1.14–1.17), an intercept of –0.9 mg/L, and R² = 0.996. Regression analysis data for individual instruments were as follows: BNII slope 1.16, intercept –0.6 mg/L, R² = 0.997; IMMULITE slope 1.12, intercept –0.9 mg/L, R = 0.987; and Integra slope 1.14, intercept 0.6 mg/L, and R = 0.992.

Using specimens from both neonates and older patients, we found that the positive bias was introduced by diluting the specimen, manually or by the instrument, with deionized water, saline, or the 0 calibrator. The bias was 16% using a 10-fold dilution and 21% using a 30-fold dilution. Undiluted specimens showed no bias. We speculate that the dilution bias may be the reason that diluting the specimen is not recommended by the manufacturer. Although the hsCRP method is positively biased when the specimen is diluted, the bias is reproducible and relatively small, and does not preclude using the test to manage neonates with sepsis.

**Grant/funding support:** None declared.

**Financial disclosures:** None declared.

**References**


**Fig. 1. Bland-Altman plot showing the difference between CRP by VITROS and comparative methods.**

- Δ, VITROS CRP Slide vs Behring Nephelometer II.
- ■, VITROS hsCRP MicroTip method vs Behring Nephelometer II.
- DPC IMMULITE, or Roche Integra. Dotted vertical line is the sepsis protocol decision point of 10 mg/L.

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To the Editor:

Whether serum or plasma is the best specimen for determination of matrix metalloproteinases (MMPs) is a matter of debate, as are the influences of sample collection and processing on MMP concentrations (1–3). MMP-2 concentrations do not differ significantly in plasma and serum, whereas MMP-9 concentrations are significantly higher in serum than in plasma (mean 5-fold higher; 

3)

Protein expression did not differ significantly between serum and plasma, nor did it change with silicate treatment in any of the paired sample types. MMP-9 forms were found in significantly higher amounts in serum (mean 5-fold higher; 

P <0.001) than in citrate samples (Fig. 1, lanes 6 vs 4 serum). However, addition of silicate to previously separated serum samples and serum did not noticeably change the zymographic profile of MMP-9 with respect to untreated samples (Fig. 1, lane 4 vs 5, and lanes 6 vs 7).

In addition to silicate, citrate plasma tubes before PB collection increased MMP-9 (Fig. 1, lanes 8 and 9) a mean of 4-fold (P <0.001). Addition of silicate into empty plastic tubes for serum collection before PB addition also significantly increased MMP-9 concentrations (P <0.001) (data not shown). Addition of silicate to citrate and serum tubes before PB addition resulted in similar trends of MMP increase vs silicate concentration: MMP-9 activity (µg/L) = 28.0 × silicate (mg/L) − 9.4, r² = 0.94, and MMP-9 activity (µg/L) = 30.6 × silicate (mg/L) + 91.6, r² = 0.88, respectively.

When samples were collected into empty serum tubes to which buffered silicate (silicate dissolved in PBS containing 137 mmol/L NaCl, 10 mmol/L phosphate, 2.7 mmol/L KCl, pH 7.4) was added before PB collection, all MMP-9 forms were increased, and the zymographic profile was similar to that of Sca (Fig. 1, lanes 10 and 11). Samples collected in the presence of nonbuffered silica (nonsoluble silica particles sprayed into plastic tubes or with silica-gel) (http://catalog.bd.com/ecat/msds/d01/vs60313.pdf) showed MMP-9 release 1.5-fold higher than for buffered silicate (data not shown). Serum collected in plastic tubes with clot accelerators showed the highest MMP-9 activities (Fig. 1, lane 11). The addition of silicate to BCs isolated from citrate PB significantly enhanced MMP-9 release in buffered solution (Fig. 1, lanes 12 and 13). Similarly, silicate addition to U-937 cells cultured in serum-free media significantly increased MMP secretion (Fig. 1, lanes 14 and 15). Thus silicates increase in vitro release of MMP-9 forms from leukocytes. Our observations are consistent with the findings that during silicosis both macrophages and lymphocytes secrete enhanced amounts of MMP-9 forms (5).

To optimize the diagnostic accuracy of PB MMPs as biomarkers, we strongly recommend avoiding the use of serum samples, particularly in serum with clot activators containing silica/silicate. We believe the increased MMP-9 observed in these specimens reflects both the interfering effects of the coagulation/fibrinolysis processes (4) and the induction by silicates of MMP-9 release from leukocytes.