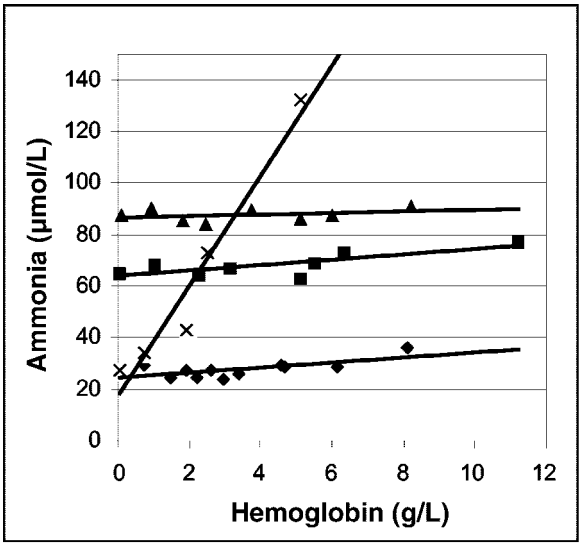

Fig. 1. Effect of hemoglobin concentration on ammonium measurements.

×, sample to which hemolysate was added; ▲, ■, and ♦, samples mimicking a typical sample collection process.

no clinically significant effect on the ammonia result (Fig. 1). By contrast, when the samples were prepared by adding hemolysate, there was a dramatic effect on the ammonia result. The most likely explanations for such increases in ammonia with prepared hemolysates are freezing and aging, which lead to increased ammonia production via deamidation of the cellular proteins in the hemolysate. We suspect that the statement in the Roche package insert was based on experiments in which hemolysate was added to plasma. Therefore, the effect on ammonia is most likely a preanalytical artifact rather than attributable to analytical interference from hemolysis. The data clearly point out the importance of mimicking actual clinical settings as closely as possible when performing interference studies.

We observed no such deamidation of proteins in hemolyzed plasma over a 4-h time period, but to prevent any possible ammonia production as a result of deamidation of the cellular proteins, ammonia samples should be centrifuged immediately and the EDTA plasma separated from cells, sealed, and stored at 4–8 °C, not frozen.

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Editor's Note: A representative of the manufacturer declined an invitation to reply for publication.

Guide to the Expression of Uncertainty of Measurement: Point/Counterpoint

To the Editor:

With great interest I read the "Point/Counterpoint" articles by Drs. Krouwer *(1)* and Kristiansen *(2)*. In this regard, I would like to raise three issues: two analytical and one clinical.

With respect to the first analytical issue: laboratory experts and external quality assurance system organizers advocate that laboratory measurements be highly accurate, i.e., unbiased and precise. Although manufacturers of routine measurement procedures are usually able to solve most of the problems of imprecision, they are often unable to solve problems related to measurement bias. In my opinion, these Point/Counterpoint articles put undue pressure on the manufacturers to "improve the quality of their products". The professionals in laboratory medicine should provide guidance to the manufacturers to help solve the problems of accuracy in a fair and transparent manner.

Quantities measured or determined in laboratory medicine are now divided into two categories: (A) those that are traceable to SI ($n \sim 100$; well-defined chemical compounds) and (B) those not traceable to SI ($n > 500$) *(3)*. For category A, traceability is (or can be) assured, although some experts may argue that this is not yet applicable to all of these quantities. Measurement results of category A compounds in patient samples should be appropriately accompanied by the uncertainty of measurement [preferably according to the *Guide to the Expression of Uncertainty in Measurement* (GUM) to fulfill internationally agreed requirements].

However, very serious problems arise with category B, a very large group of quantities consisting primarily of (glyco)proteins and measured by means of immunochemical techniques. The crucial point is that for category B, we cannot speak of unbiased results of measurement because we have rarely adequately defined the "measurand" in the patients' biological fluids. The "true value or true concentration" of the quantity of category B in patient samples simply is unknown.

The manufacturer responsible for calibration of its product calibrators against "reference materials of higher order", if and when available *(3, 4)*, faces a dilemma. These reference materials of higher order are sometimes available under the aegis of WHO. However, the WHO Expert Committee on Biological Standardization never defined the measurand in biological fluids. Consequently, these materials should be regarded as "surrogates" for the relevant

quantity in human or animal biological fluids.

Nevertheless, manufacturers use these materials for calibration of their "master calibrator lot", but the uncertainty budget (GUM) of the values assigned to these WHO reference materials is unknown. Assuming that these uncertainty budgets become known in the future, that uncertainty should then be further propagated through the calibration hierarchy down to the results reported for a patient's sample *(3)*.

The second analytical issue is the question of whether the available and used reference materials for category A and category B are commutable with the quantity (quantities) in the biological fluids? This question merits attention because the validity of calibration and other measurement exercises depends on it. However, this aspect is hardly ever addressed, for example, in WHO documents.

The biased results of measurements of category B quantities and the issue of commutability for category A as well as for category B analytes have important impacts on the uncertainty of measurement results.

The third issue relates to clinical decision-making: what is the effect on clinicians and the clinical decision process of reporting a measurement result for a patient's sample with the uncertainty budget calculated according to, e.g., GUM? Will clinicians understand it? Will it improve their efficacy and efficiency? GUM was elaborated by representatives of a host of international organizations; it is meant to be applicable to all scientific measurements, be they physical or chemical. The concept of uncertainty of measurement in laboratory medicine was incorporated in the "traceability" document *(3)*, and it plays a role in the obtaining of accreditation by medical laboratories *(5, 6)*. It then is important to ask whether medical associations in, for example, the US, Europe, and Japan have been consulted on this matter? If so, what are the problems perceived by our medical colleagues? As far as I am aware, to date no such attempt has been made. If that is true, are not laboratorians merely satisfying analytical and metrologic requirements? Should we not defer reporting the uncertainty of measurements of patients' samples until it is accepted clinically as useful and beneficial to patient care?

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Genetic Effects on Serum Concentrations of Serum Amyloid A Protein

To the Editor:

MacGregor et al. *(1)* reported a twin-study of the genetic contribution to baseline serum concentrations of two acute-phase proteins, C-reactive protein and serum amyloid A protein (SAA). In their discussion, they stated that no studies had been reported of associations between particular isoforms and different baseline values of SAA. Although twins were not used as subjects, we earlier reported genetic effects on SAA serum concentrations.

Acute-phase SAA is divided into two major isotypes, SAA1 and SAA2, which are coded at different loci. The dominant isotype, SAA1, consists of six allelic variants (SAA1.1 to SAA1.6) *(2)*. In the Japanese population, three major alleles, *SAA1.1* (^{52}Val, ^{57}Ala), *SAA1.3* (^{52}Ala, ^{57}Ala), and *SAA1.5* (^{52}Ala, ^{57}Val), which differ from each other in *SAA1* exon 3 structure, appear with approximately equal frequencies (0.30–0.35). Among 280 healthy Japanese *(3)*, the mean serum SAA concentrations in *SAA1.5* homozygotes, *SAA1.5* heterozygotes, and non-*SAA1.5* carriers were 5.7, 4.1, and 2.2 mg/L, respectively (analyzed after logarithmic conversion of the raw data). The mean SAA concentration (SD range) was 4.5 (2.6–7.8) mg/L in *SAA1.5* carriers, whereas that in noncarriers was 2.2 (1.4–3.6) mg/L ($P < 0.001$). The SAA/C-reactive protein ratio was significantly higher in *SAA1.5* carriers than in noncarriers in Japanese patients with rheumatoid arthritis *(4)*. More recently we reported that human recombinant SAA1.5 protein is cleared from the circulation more slowly than other isoforms in mice *(5)*. Differences in plasma clearance may therefore be one of the possible factors responsible for such genetic effects.

The differences in SAA isoforms are not likely to be attributable to a method effect of the analytical method because we used an assay *(6)* that has been confirmed by polyacrylamide gel electrophoresis analysis *(7)*.

SAA1 allele frequencies in the United Kingdom have been reported to be 0.76, 0.19, and 0.05 for *SAA1.1*, *SAA1.5* (originally considered as *SAA1.2*), and *SAA1.3*, respectively *(8)*. It is predicted that ~35% of the English population (individuals homozygous and heterozygous for *SAA1.5*) have a tendency to have higher SAA serum concentrations.

As MacGregor et al. *(1)* noted, SAA may have some role in atherogenesis. We are also interested to