Q&A, 8/15

Editor: Frederick L. Kiechle, MD, PhD

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Submit a Question

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Q. Our laboratory is adding urine total protein to its Siemens Dimension EXL test menu. The test is being performed now at our reference lab on the Siemens Advia 1800. Our Dimension EXL method validation studies have revealed an average 40 percent positive bias over the Advia method. This bias is also evident in peer group evaluations for the quality control product we are using. The test system peer mean for the Dimension EXL averaged 16.48 for level one, which is almost 54 percent higher than the test system peer mean for the Advia 1800 at 7.59. The bias suggests essential differences in the two methods; however, the method principles (pyrogallol red), expected values/reference ranges (0–150 mg/day), and units of measurement (mg/dL) are the same. How is the significant difference in results explained to clinicians using this information to diagnose preeclampsia in pregnancy?

A. The questioner raises an interesting, although not totally new, laboratory issue. Urine has been studied for millennia in an effort to diagnose disease, and uroscopy, now termed urinalysis, as a clinical tool dates back to Hippocrates and Galen. An interesting and at times humorous account of urine testing can be found in the January 2014 issue of the *Cleveland Clinic Journal of Medicine*, in an editorial1 by editor in chief Brian Mandell, MD, PhD, who summarizes much of an earlier article on the topic.2 These articles relate a proclamation made by the Royal College of Physicians in 1601: “It is ridiculous and foolish to divine the…course of disease…from the inspection of urine.” Luckily, most modern clinicians do not heed this warning. Nevertheless, urine protein measurement procedures do have some problems and historically have yielded inconsistent results depending on the exact measurement procedure used. Harmonizing results across urine protein measurement procedures has been further complicated by the fact that urine may contain many different types of proteins that can give different quantitative responses with the various protein measurement procedures. This lack of harmonization has often led to substantial confusion and seemingly conflicting information in the medical literature about the clinical utility of urine protein measurements in various diseases.

The most widely used method for “urine protein” measurement is the semiquantitative dipstick. This technique relies on the property that certain pH indicator dyes change their pKa when bound to a protein. Dipstick methods primarily detect urine albumin and show relatively little response to urines containing mainly globulins. Different methodological approaches are used for quantitative total urine protein measurements. Most current quantitative measurement procedures include trichloroacetic or sulfosalicylic acids or benzethonium chloride precipitation with turbidimetric detection and dye binding, using primarily Coomassie Brilliant Blue, pyrogallol red-molybdate, or pyrocatechol violet-molybdate with spectrophotometric detection. Unfortunately, the amount of protein measured varies depending on the specific precipitating agents and the dyes used. Some urine protein procedures, notably

those using sulfosalicylic acid and Coomassie Brilliant Blue, tend to show substantially higher recoveries of urinary albumin compared with most globulins, while others show higher response to most, but not all, globulins.

The major mechanisms leading to proteinuria are increased leakage of plasma proteins through damaged glomerular capillary walls (glomerular proteinuria) or decreased reabsorption of low-molecular-weight proteins by the proximal tubules (tubular proteinuria). In the first mechanism, albumin is the major protein found in the urine. In the second, globulins from low to higher molecular weight predominate. A less common cause of elevated urine protein is often termed overflow proteinuria. In this mechanism, extremely high concentrations of proteins not normally found in plasma are passed into the urine via the glomerulus. Examples include hemoglobinuria with severe intravascular hemolysis, rhabdomyolysis leading to myoglobinuria, and light chain secreting multiple myeloma leading to immunoglobulin light chains in the urine, also called Bence-Jones proteinuria.

In healthy individuals, very little protein ever reaches the excreted urine (less than about 20 to 30 mg/day). However, the exact reference range and amount measured depends on a laboratory's analytical procedure and the "normal" population being studied. Very little albumin, probably less than 5 mg/day, is excreted by the vast majority of healthy individuals. In normal individuals, albumin represents less than 15 percent of the total urine protein. The other normally excreted urinary proteins are largely low-molecular globulins such as beta-2-microglobulin, cystatin C, retinol-binding protein, and uromodulin, also called Tamm-Horsfall glycoprotein. However, individuals with no other evidence of renal disease can intermittently excrete albumin in their urine after periods of exercise and during febrile illnesses, with amounts well above the normal urinary albumin cut points of 30 mg/g creatinine (3 mg/mmol creatinine) that has been recommended for staging chronic kidney disease.3 There is also an apparently benign but somewhat poorly understood syndrome of postural albuminuria, which is the most common cause of excessive proteinuria in children and adolescents. In this condition, there is excessive passage of albumin through the glomerulus when upright and ambulatory, but little albumin passage when supine and sleeping.

Based on the variation in types of proteins in the urine in different pathological states and the large variability in responsiveness of the various clinical measurement procedures used to quantify them, the measured "urine protein" result on a given patient's sample can differ widely. As the questioner suggests, one would think two procedures using the same general methodological principle (pyrogallol red-molybdate) might be expected to give similar quantitative results. However, this is not always the case. Some of the early literature on using pyrogallol red as a dye for measuring urine protein noted that the procedures in which it was used were far more sensitive to albumin than to globulins. It was found that adding sodium dodecyl sulfate (SDS) to the reaction mixture tended to reduce the assay mixture's responsiveness to albumin as compared with various globulins.4 The product literature for the Dimension EXL's urine protein procedure lists "surfactant" as a reagent component while the Advia's product literature does not. Thus we suspect the relative differences in sensitivity to albumin versus globulins and what proteins are present in each procedure's calibrator and in the different patient samples led to the discrepancies observed. Although the Dimension EXL results were 40 to 50 percent higher than the Advia results in the patient specimen method comparison studies and with at least one control material, we would not be surprised that if urines from different kinds of patients were tested—for example, from patients with monoclonal light chain disease or other causes of globulin-predominating proteinuria—then the two analyzers might give more equivalent results or perhaps even reverse the relative bias.

Considering the pitfalls in urine protein measurements, why do we still measure total urine protein? Nephrologists have largely abandoned use of total urine protein, and now most guidelines recommend measuring and reporting urinary albumin for early detection of renal damage in type 1 or type 2 diabetes and hypertension.3 However, some clinicians seem not to fully understand the limitation and inherent variability in quantitating urine total protein using different measurement procedures. There is an excellent review of proteinuria in pregnancy available online that discusses in far more detail many of the topics touched on in this reply.5

The short answer to the question about bias is that different urine protein measurement procedures simply give different answers due to their calibration and sensitivity to various proteins that can be found in urine. This rather unsatisfactory answer is probably just as frustrating to obstetricians in general as it is to the person who submitted

the question. In fact, the American College of Obstetricians and Gynecologists' Task Force Report on Hypertension in Pregnancy actually recommends abandoning the use of urinary total protein for diagnosis of preeclampsia altogether and states: "…accumulating information indicates that the amount of proteinuria does not predict maternal or fetal outcome. It is for these reasons that the task force has recommended that new-onset of hypertension together with new-onset of any one of the following: thrombocytopenia, elevated or rising serum creatinine, elevated serum transaminases, pulmonary edema, or cerebral or visual symptoms can fulfill the diagnosis of preeclampsia even in the absence of proteinuria."6 As nephrologists concluded more than a decade ago for diagnosis of chronic kidney disease related to diabetes and hypertension, perhaps changing to measuring urine albumin rather than measuring urine total protein in suspected preeclampsia might improve the test's diagnostic utility, but further research is needed to explore this option.

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John H. Eckfeldt, MD, PhD
Ellis Benson Professor
Department of Laboratory Medicine and Pathology
University of Minnesota Medical School
Minneapolis
Member, CAP Chemistry Resource Committee

Glynnis B. Ingall, MD, PhD
Professor, Department of Pathology
University of New Mexico School of Medicine
Albuquerque
Member, CAP Chemistry Resource Committee

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Dr. Kiechle is medical director of clinical pathology, Memorial Healthcare, Hollywood, Fla. Use the reader service card to submit your inquiries, or address them to Sherrie Rice, CAP TODAY, 325 Waukegan Road, Northfield, IL 60093; srice@cap.org.