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# Urine protein concentration estimation for biomarker discovery

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ABSTRACT

Recent advances have been made in the study of urinary proteomics as a diagnostic tool for renal disease and pre-eclampsia which requires accurate measurement of urinary protein. We compared different protein assays (Bicinchoninic acid (BCA), Lowry and Bradford) against the 'gold standard' amino-acid assay in urine from 43 women (8 non-pregnant, 34 pregnant, including 8 with pre-eclampsia). BCA assay was superior to both Lowry and Bradford assays (Bland Altman bias: 0.08) compared to amino-acid assay, which performed particularly poorly at higher protein concentrations. These data highlight the need to use amino-acid or BCA assays for unprocessed urine protein estimation.



## Background

Protein excretion in urine is associated with many pathologies including the pregnancy specific syndrome pre-eclampsia. Characterizing specific proteins in urine is now achievable through advances in proteomic technologies and the use of urine as a source of candidate biomarkers and therapeutic targets is rapidly developing. Recently proteomic techniques have identified potential diagnostic and predictive urinary biomarkers for pre-eclampsia[1–4].

Urine protein estimation of different clinical laboratory techniques has previously been tested but this has not been completed for standard research methods [5]. Proteomic analysis requires precise assessment of total protein concentrations to enable accurate quantitation by subsequent downstream gel-based and tandem mass spectrometry (MS/MS) [6], and is a requisite to confidently explore the role of future biomarkers.

Whilst there is a move to standardize urine collection for urinary proteomic assessment by the Human Kidney and Urine Proteome Project (HKUPP) and the European Kidney and Urine Proteomics (EuroKUP) networks (www.hkupp.org; www.eurokup.org), publications on urinary proteomics use a variety of assays to estimate total protein concentrations (e.g. Bradford and Coomassie Plus assays, [7–11] BCA) [12,13] or assays are not defined. However, these tests were not specifically developed to quantify protein in urine and may suffer inaccuracies due to interference by urinary solutes or pH. High urea concentrations are also likely to interfere with Bradford assay due to the incompatibility of coomassie based protein assays to surfactants, e.g. urea, even at low concentrations, causes precipitation of the reagent [14].

The objective of this study was to assess which protein assay provided the most accurate quantification across a wide range of urinary protein concentrations. We performed three standard assays (Bicinchoninic acid (BCA), Lowy and Bradford) and compared these to the current gold standard amino-acid assay.

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## Methods

### *Sample collection*

Urine samples with a diverse range of protein concentrations were collected from healthy pregnant women at 15 weeks' ($n = 12$) and 20 weeks' ($n = 12$) gestation. Urine samples were also collected from women who had been diagnosed with pre-eclampsia ($n = 8$); according to International Society of Study of Hypertension in Pregnancy Guidelines [15] and from healthy non-pregnant women of reproductive age ($n = 8$). All collections were approved by the St. Thomas' Local Ethics Committee (09/H0802/031) and obtained following informed written consent. Once collected, urine samples were centrifuged at 1400*g* for 10 min at 4 °C and then stored in aliquots at −80 °C until required for protein concentration assays.

### *Protein estimation of urine*

Prior to protein concentration assays, urine aliquots (1.8 ml) were ultracentrifuge concentrated to approximately 170 μL using a 3000 MW filtration column (Millipore Centrifugal Filter Units). Protein concentration was first estimated using the amino acid assay. Subsequent assays using the Lowry, BCA and Bradford assays (Thermo Scientific) were then performed on the same urine samples following manufacturers' protocols after urine dilutions for each sample set and assay were optimized to fit within the recommended standard curve concentration ranges.

### *Statistical analysis*

Initial visual analysis was completed by scatter plots comparing each of the three protein assays with the amino

**Table 1**
Bland–Altman bias and limits of agreement for all assays compared to the amino acid assay.

| Assay comparison | Bias | 95% Limit of agreement | |
|---|---|---|---|
| | | From | Till |
| Bradford assay vs. amino acid assay | −1.7 | −6.9 | 3.5 |
| BCA vs. amino acid assay | 0.08 | −0.7 | 0.9 |
| Lowry assay vs. amino acid assay | −2.4 | −7.0 | 2.3 |

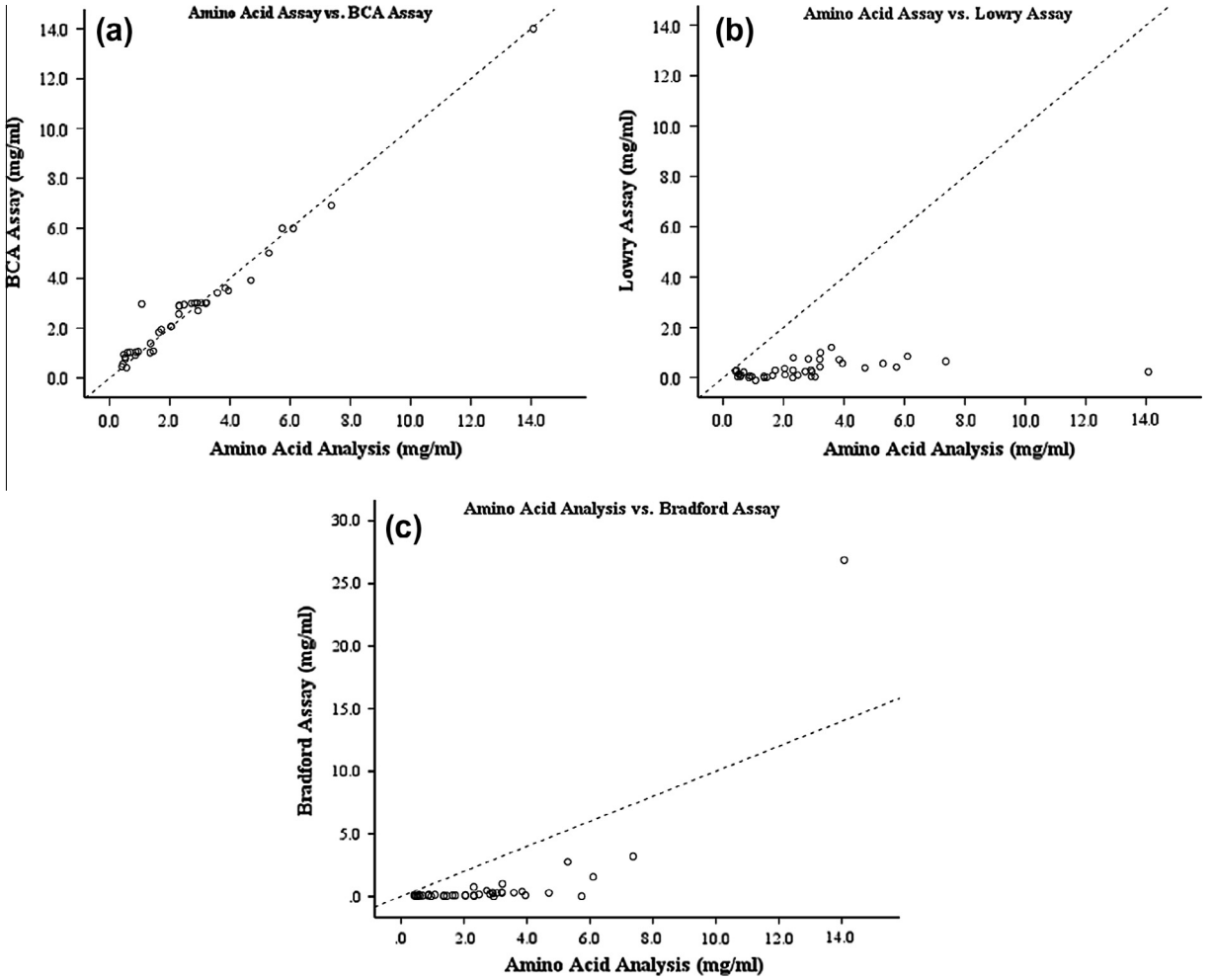


**Fig. 1.** Scatter plots comparing the amino acid assay (AAA) with (a) BCA; (b) Bradford assay; and (c) Lowry assay. Dashed reference lines are $y = x$.