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# The problems of proteinuria measurement in urine with presence of Bence Jones protein

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ABSTRACT

**Objectives:** Protein concentration measurement in the urine can be problematic in the presence of Bence Jones protein. We have carried out an external quality control assessment with the participation of 79 clinical biochemistry laboratories from the Czech Republic and Slovakia.

**Design and methods:** The laboratories received a reference urine sample obtained from a patient with multiple myeloma and lambda free light chain proteinuria and were asked to type the paraprotein using immunofixation and to measure total urinary protein using their established method, most commonly turbidimetry, pyrogallol red assay, and biuret assay.

**Results:** There was a very wide inter-laboratory variability in the protein concentration readouts with up to three-fold difference in some cases. High-resolution two-dimensional electrophoresis and linear mass spectrometry showed that a high proportion of the urinary paraprotein was composed of lambda light chain fragments with molecular weight of 12 kDa.

**Conclusions:** Our results highlight the challenges of reliable and reproducible measurement of urinary protein concentration in the presence of Bence Jones protein.



## Introduction

Although the presence of proteins with unusual thermoprecipitation pattern in the urine of multiple myeloma (MM) patients was first described in 1845, it was not until 1962 that the groups of Edelman and Solomon proved that this so-called Bence Jones protein (BJP) is composed of monoclonal free light chains (FLCs) of immunoglobulins [1,2].

Measurement of protein concentration in urine is challenging because none of the current methods fully meets analytical and clinical requirements. The problems only become more obvious if monoclonal FLCs are present in the analyzed urine sample.

An external quality control assessment for monoclonal gammopathies is carried out regularly for clinical biochemistry laboratories in the Czech Republic and Slovakia. In this report, we have used the data from the 2008 quality control assessment to highlight the most common problems in quantification of proteinuria in the presence of FLCs.

## Materials and methods

The control urine sample was obtained from a 77-year old female MM patient with free lambda light immunoglobulin chains after acquisition of her informed consent. Sodium azide 0.02% was added to the control urine sample as a preservative before shipment. Seventy-nine laboratories (64 from the Czech Republic and 15 from Slovakia) participating in the external quality control assessment program for monoclonal gammopathies received this control urine sample and were asked to carry out immunofixation to type the paraprotein and to measure its concentration using their established method. The following methods were used for urinary protein concentration measurement: pyrogallol red assay in 26 laboratories (using kits manufactured by Pliva Lachema, Czech Republic—4 laboratories; Olympus, Germany—7 laboratories; Beckman, Great Britain—4 laboratories; BLW, Czech Republic—3 laboratories; Randox, Great Britain—3 laboratories; Thermo Fisher Scientific, Finland; BioVendor–DiaSys, Germany; Roche, Germany; Siemens–DADE, Germany; and Human,

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Germany—1 laboratory each), biuret assay in 9 laboratories (kits by Olympus, Germany—5 laboratories; Roche, Germany and Abbott, Germany—2 laboratories each), turbidimetric measurement in 36 laboratories (kits by Roche, Germany—22 laboratories; Skalab, Czech Republic—9 laboratories; Abbott, Germany—3 laboratories; Siemens–Bayer, Germany; and DOT Diagnostics, Germany—1 laboratory each). Further 8 laboratories used other methods for urinary protein concentration measurement.

High-resolution two-dimensional electrophoresis with subsequent Western blotting [3] with an antibody specific for lambda chains (Sebia, France) was used as the reference method for the characterization of proteins in the urine sample.

Regarding MALDI analysis the urine sample (0.8 μL) without any pretreatment was directly spotted on MALDI target sample plate and allowed to air-dry at room temperature. Matrix solution (0.8 μL) containing sinapinic acid (10 mg/mL) in aqueous 30% acetonitrile with 0.5% trifluoroacetic acid was dropped onto sample spot. MALDI analysis was performed on a 4800 MALDI-TOF/TOF instrument (Applied Biosystems, Framingham, MA, USA) in a linear positive ion mode. Mass spectrum was acquired across the mass range of 2000–20,000 m/z using 500 laser shots per spectrum, further methodology details are described in publication [4]. The concentration of free light chains in the urine sample was determined using the FREELITE kit (The Binding Site Ltd., Birmingham, UK).

## Results

Widely ranging results were reported by the participating laboratories (Table 1). Measurement methods based on pyrogallol red resulted in readouts approximately three times higher than those obtained with turbidimetry. The reference FLC concentration measurement was carried out using the FREELITE kit with the result of 8.531 g/L.

The closest readouts from the tested laboratories were obtained using the Urinary/CSF Protein kit (Olympus) used in seven laboratories, with the mean readout of 7.02 g/L ± 0.57 g/L. The concentration on repeated measurement using a diluted urine sample was 8.30 g/L.

About 99% of urinary M-spike given by the densitometric scan was represented by FLC lambda and its fragments (albumin quantity was <1%; exactly 0.63%); no intact immunoglobulins were present. Lambda chain monomers, fragments with molecular weight (MW) of 12 kDa, and smaller fragments with MW of 6–8 kDa were identified by high-resolution-two dimensional electrophoresis with subsequent Western blotting (Fig. 1) and linear mass spectrometry (Fig. 2).

Applicability of methods for measurement of urinary protein concentration in the absence of BJP was tested in another Czech external quality control system cycle (24th of October 2008, AM2/08; mean protein concentration in the control urine sample was 0.99 ± 0.235 g/L). Results of all used methods including biuret assay, pyrogallol red assay, and turbidimetry with coefficients of variation of 6.4% ($n = 26$), 5.8% ($n = 119$), and 8.0% ($n = 70$) were satisfactory.

**Table 1**
Results of protein concentration measurement in a reference sample from a patient with lambda free light chains in urine.

| Method | | Number of laboratories | Total protein in urine ± SD [g/L] |
|---|---|---|---|
| Biuret assay | | 9 | 5.97 ± 3.23 |
| Pyrogallol red assay | All kits | 26 | 6.21 ± 0.98 |
| | Olympus kit | 7 | 7.02 ± 0.57 |
| Other chromogenic methods | | 2 | 3.26 ± 0,48 |
| Turbidimetry (BTC) | | 36 | 2.23 ± 0.54 |
| Other methods | | 6 | 3.98 ± 1.91 |
| FREELITE assay | | 1 | 8.53 |

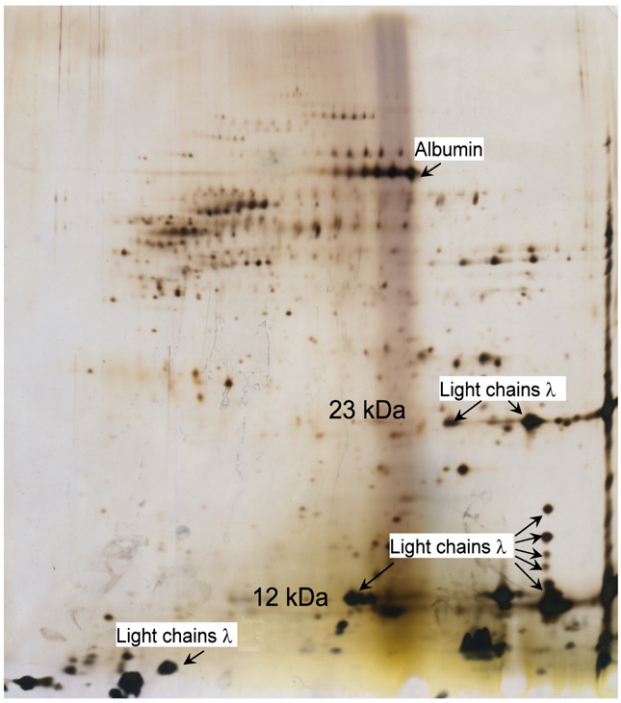


**Fig. 1.** Silver-stained two-dimensional gel electrophoresis of urine sample with labeled positions of lambda free light chains and albumin. The location of free lambda chains is derived from immunoblotting study; the location of albumin was determined in previous proteome urine analyses.

## Discussion

Monoclonal immunoglobulin FLCs are frequently found in the urine of patients with MM, Waldenström macroglobulinemia, AL amyloidosis, light chains deposition disease, and occasionally, also in non-Hodgkin lymphomas and chronic lymphocytic leukemia. In monoclonal gammopathy of unknown significance (MGUS), the presence of BJP is associated with higher risk of progression [5].

Immunofixation electrophoresis of urinary proteins is the basic method for the detection and typing of monoclonal immunoglobulin FLCs. Of the many laboratory methods for proteinuria quantification, none provide reliable results if BJP is the dominant urinary protein. The thermo-precipitation test is of historical interest only, as it is unreliable and has poor sensitivity. Proteinuria indicator strips are based on the so-called protein-error reaction of some pH indicators, such as tetrabromophenol blue that turns yellow in acidic protein-free environment but blue-green if proteins (especially albumin) are present. These diagnostic strips are inexpensive but usually not sensitive enough to the presence of BJP which is often not detected at all [6].

Standard methods for the quantification of total urinary protein such as turbidimetry with benzethonium chloride, colorimetric methods based on protein affinity of pyrogallol red, Coomassie blue or other indicators, and the biuret assay are all imprecise for the measurement of microprotein concentrations, including those of monoclonal immunoglobulin FLCs [6].

The results of our external quality control assessment underline the difficulty and high variability of protein concentration measurement in the presence of BJP. The high variability in BJP characteristics among patients with monoclonal gammopathies

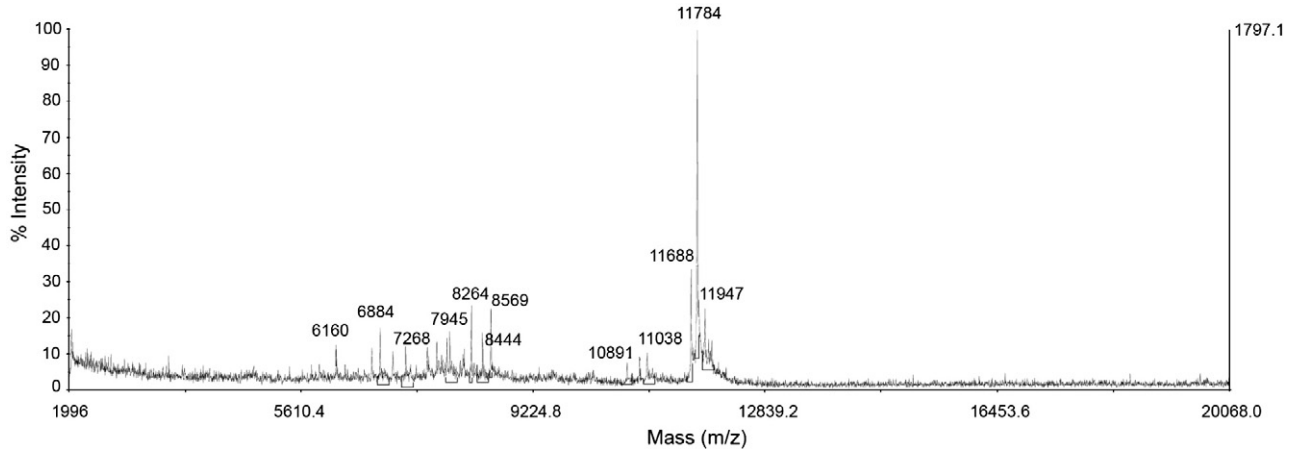


**Fig. 2.** The mass spectrum of urine was measured in positive linear mode of MALDI/TOF/TOF mass spectrometer. Mass range was set from 2000 to 20,000 m/z.

may be responsible for some of the problems of FLC concentration measurement using current analytical methods. Molecular weight of BJP varies widely as it can be composed of light chain monomers (MW of approximately 22 kDa), dimers (MW of approximately 44 kDa), or fragments with low MW (5–18 kDa) [6–8]. BJP in some cases has a pronounced tendency to polymerization [9,10]. The range of urinary BJP concentrations is also very wide, from a few mg/L to tens of g/L [11].

The optimal method for urinary monoclonal FLC concentration measurement has not been established [12–14]. Some authors propose the quantification of FLC concentration in serum as an alternative or complementary method [15,16]. Serum and urine FLC concentrations do not necessarily correlate and urinary BJP concentration is influenced by renal functions and therefore is not a reliable measure of tumor volume or activity [11]. BJP fragments may lack certain epitopes and thus escape detection by some antisera. The common presence of several gradients on electrophoresis of urinary proteins in monoclonal gammopathies and the extreme concentration variability of BJP together with the lack of an international reference calibrator pose a formidable challenge to reliable and reproducible analysis [11,17]. However, our data also show that urinary protein concentration measurement is challenging only in the presence of BJP, as in the absence of FLCs all of the methods used by the participating laboratories performed well.

For the time being, the assessment of BJP in urine remains an integral part of the initial evaluation of patients with monoclonal gammopathies [13,14]. The guidelines of the College of American Pathologists recommend the following procedure for the investigation of possible urinary paraprotein: total proteinuria quantification from a 24-hour urine sample, electrophoresis and immunofixation of concentrated urine, densitometric detection of possible peaks after urinary protein electrophoresis, and the calculation of FLC concentration based on total protein concentration and FLC percentage [6]. However, as evidenced by our results, these recommendations may not be sufficient because of high analytical variability of different methods for the measurement of protein concentration in the presence of BJP. Monitoring of BJP concentration may be of clinical value in some cases of paraproteinemia but it is necessary to always use the same reagents, calibration, and reaction conditions for samples from a given patient.

## Conflict of interest

All authors declare no financial/commercial conflicts of interest.

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